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PRINCIPAL INVESTIGATOR: Harrington, Laura C.

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14. ABSTRACT While Lyme disease (B. burgdorferi) is the nation's most prevalent vector-borne zoonotic disease, the increasing suite of human pathogens transmitted by the blacklegged tick, lone star tick and the recent introduction/establishment of the Asian longhorned tick in the Western Hemisphere, increases occupational health risk for soldiers training in the field, for military dependents and civilians who live, work or recreate on military installations and wild lands. In tis project, wee utilized multiple approaches to assess the efficacy of new pesticide formulations and reservoir targeted vaccines for the control of tick species. In addition, we conducted repellency assays with botanical formulations including duration of repellency for a variety active ingredients where information is lacking. We also conducted baseline monitoring of pesticide susceptibility and resistance to inform integrated tick management. Our results demonstrated the high efficacy of 10% permethrin topical treatment of white-tailed deer via 4-poster devices in reducing host seeking tick densities and Ehrlichia infection rates of A. americanum. Fipronil-laced bait was readily accepted by white footed mice and other rodent tick borne pathogen reservoirs and reduced juvenile I. scapularis burdens on rodents. Over 90 outdoor contained					
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INTRODUCTION: For this project we sought to utilize multiple approaches to assess the efficacy of new pesticide formulations and reservoir targeted vaccines for the control of tick species; conduct repellency assays focusing on the Asian longhorned tick and duration of repellency for a variety of products, where information is lacking; conduct baseline monitoring of pesticide susceptibility and resistance, make integrated tick management recommendations.

BODY:

This report summarizes our project accomplishments for the entire funding period.

Accomplishments

Objective 1. Utilize multiple approaches to assess the efficacy of new acaricide formulations and reservoir targeted vaccines for the control of tick species which potentially pose a significant medical threat to military personnel operating in the eastern United States; namely, *I. scapularis*, *A. americanum*, and *H. longicornis*.

1.a. Evaluate the 4-poster system for environmental control of multiple tick species.

We evaluated the effectiveness of topical treatment of 10% permethrin delivered via 4-poster devices to white-tailed deer, *Odocoileus virginianus*, in the management of a newly discovered *A. americanum* population in Norwalk, Connecticut. Using a high-density deployment of one 4-poster device/12.7 ha, we were successful in significantly reducing densities of host-seeking adults (93% reduction), nymphs (92%), and larvae (96%) from 2018 to 2020. We also documented a significant reduction (87%) in parasitizing adults and nymphs on white-tailed deer from 2018 to 2019. The prevalence of *Ehrlichia chaffeensis* and *Ehrlichia ewingii* combined in host-seeking adults declined significantly from 47% at the time the *A. americanum* population was discovered in 2017 to 7% in 2020. However, the prevalence in nymphs remained static (~9%) throughout the study period. These data demonstrate that, when properly deployed in a density-dependent manner in terms of deer abundance, 4-poster devices can effectively manage parasitizing and host-seeking *A. americanum* populations and reduce the prevalence of two *Ehrlichia* species of public health importance. A manuscript reporting these results has been published (Williams et al. 2021).

1.b. In combination with four poster assessments, evaluate Reservoir-Targeted Vaccines (RTV) as a second complementary strategy. The RTV was not available in time, so we sought to field deploy a commercially available fipronil-laced bait (Kaput® Flea Control Bait, Scimetrics LLC., Wellington, CO, USA; 0.005% fipronil; Environmental Protection Agency Reg. No. 72500-28), in an alternate use targeting white-footed mice (*Peromyscus leucopus*) to determine bait acceptance and potential impacts to juvenile *I. scapularis* burdens. Bait was readily accepted by wild *P. leucopus* and other rodent reservoirs. An *ad libitum* distribution strategy as well as placing smaller volumes of fipronil-laced bait within individual Sherman traps both resulted in significant reductions (57-94%) in juvenile *I. scapularis* burdens as compared to control over two years. The oral delivery of systemic acaricides shows promise in reduction of *I.*

scapularis burdens on *P. leucopus* and should be further explored to determine effectiveness on host-seeking tick abundances, associated pathogen infection, and potentially incorporated into integrated tick management programs. A manuscript reporting this work has been published (Williams et al. 2023).

1.c. Test alternate compounds to be used for area wide tick control and localized barrier applications. *Arena Construction and Installation.* Arena enclosures were constructed of SDR35 Green Gasketed PCV pipe (30.5 cm diameter and 12.7 cm height) with a (0.3 cm deep and .24 cm height) channel 1.8 cm below the top edge of the PVC ring. The channel allows a fine polyester mesh to be secured with two stretch silicone bands, which nest into the channel, keeping the mesh taut and secure. A second piece of SDR35 Green Gasketed PCV pipe (30.5 cm diameter and 2.3cm wide) was covered with .6 cm x .6cm galvanized hardware cloth and secured with six galvanized (1.2 cm long) Phillips head, Mod Truss self-drilling lath screws. The arena bases were fitted with two (5 cm long) zinc plated hook and eye latches with spring locks while the cover had two (1.1 cm) diameter stainless steel eye hooks allowing attachment. Prior to field installation, the inside walls of the arenas were coated with Insect-A-Slip Insect Barrier – Fluon® a fluoropolymer resin (PTFE-30, DISP30). Tape was applied to the top and bottom edge of the arenas to create a temporary edge. While the arena was on its side, a small amount of Insect-A-Slip was added, and the arena was slowly rotated to yield an even coat of Insect-A-Slip on the interior edges. The arena was gently placed on its side and the lower tape barrier removed to allow excess coating to drain. The coating was allowed to dry a minimum of 24 hours prior to installation in the field. The Teflon coating inhibits the climbing abilities of the ticks. Arenas were installed in the field location by placing the PVC ring down and cutting along the outer edge with a hatchet through the leaf litter and into the soil. The hatchet allowed easy movement through small roots and other matter. The PVC arena was pressed into the newly cut channel and evenly tamped with a hammer to securely set it into the soil (3.5 cm to 5 cm deep). Soil was gently compacted around the inner edge of the PVC arena and leaf litter was returned to a non-compacted state. Soil was compacted along the outer edge to further secure the PVC arena.

Tick Collections, Marking and Placement. Adult and nymph *I. scapularis* and *A. americanum* were collected from various Suffolk County properties and parks in the spring and fall of 2018 and 2019. Ticks were placed within a tightly closing polystyrene petri dish (50mm wide and 9mm height) with a small amount of ultraviolet fluorescent powder (average particle size of 15 microns). The petri dish was then gently rotated rolling the ticks into the powder for 30 seconds. Ticks were then transferred to a clean container with a moistened strip of filter paper until placement into the arenas. Adult *I. scapularis* and *A. americanum* were color coded in cohorts of 10. A total of 40 adult ticks (20 male and 20 female) were placed in each arena. Nymph *I. scapularis* and *A. americanum* were color coded in cohorts of 20 with a total of 40 nymphs in each arena. Marked ticks were placed within installed arenas and fine polyester mesh was secured tautly with two stretch silicone bands. Ticks acclimated to the arenas for a

minimum of 24 hours prior to acaricide treatments. Ticks were kept in humidified vials for no more than 3 week prior to marking and placement in the field between early June and July.

Application of acaricides. Acaricide treatments were applied to plots in a randomized complete block design. Products were applied using gas powered Maruyama backpack blowers using 1.89 L of water per plot. Reference plots were treated with 1.89 L of water. Backpack blower tanks were triple washed between products. Separate tanks were utilized for EPA minimum risk and synthetic pesticide products to eliminate cross contamination potential. We applied Nature Cide - 25(b) exempt, Essentria All Purpose - 25(b) exempt, Suspend Polyzone EPA: 432-1514, and DeltaGard (granular) EPA: 432-836 Nature Cide active ingredients consist of 0.5% clove oil and 0.5% cotton seed oil. Essentria ALL Purpose active ingredients consist of 20% rosemary oil, 5% geraniol oil, and 2% peppermint oil. Suspend PolyZone has 4.75% deltamethrin as an active ingredient. DeltaGard G has 0.1% deltamethrin as an active ingredient. Application rates were as follows: Nature Cide 8fl oz per 1000 sq/ft, Essentria All Purpose 6 fl oz per 1000 sq/ft, Suspend Poly 1.5fl oz per 1000 sq/ft, and DeltaGard 3/lbs per 1000 sq/ft.

Post treatment tick sampling. Sampling took place 24 hours and, if possible, 48 hours after acaricide application with additional sampling every 2 to 3 days, depending on weather. For the longevity trials initial sampling was done 24 hours and, if possible, 48 hours after the ticks were placed within the pre-treated plot arenas. After 10 to 12 days, the arenas were removed from the field, soil core intact, and transferred to the lab where leaf litter and fine duff material was transferred into a white bin and placed in a ziplock bag. The upper surface of the soil core (1.2 cm) was thoroughly examined with a UV LED flashlight and fine-pointed tweezers for 0.5 h. Bagged leaf litter and the soil core were re-examined for 0.5 h each day for three days post recovery from the field. Recovered ticks were tallied on a per day basis for each treatment and subjected to one way analysis of variance (ANOVA), and Fisher's post hoc test if the F value proved significant. These analyses were performed for tick type and stage versus products and for product versus tick type and stage for each sampling day. The statistical analysis was performed in Microsoft Excel. A P value of 0.05 or lower was considered a significant result. A total of 88 analyses were performed, including tick to product and product to life stage and type of tick, for the 2020-21 season and the 2022 season, which also compared product longevity. Out of these 88 comparisons, 68 were statistically significant. The reference treatment produced the greatest survivorship in all types and life stages of ticks trialed. Deer tick nymphs had consistently lowest survivorship, even in the reference treatments. Lone star adults consistently had highest survivorship for the most part. The synthetic pyrethroid treatments had the least survivorship and the natural oils had more survivorship for the most part compared to the synthetic pyrethroids. ES natural oil had consistently higher survivorship than the other natural oil, NC, or the pyrethroids, again for the majority of the analyses. Data analysis is underway and a manuscript reporting these results is in preparation.

1.d. Determine the effectiveness of a pheromone-based acaricide product (SPLAT TK: ISCA Technologies). In addition to delays as a result of the Covid-19 pandemic, issues with obtaining SPLAT-TK from the manufacturer, ISCA Technologies, there was no progress on this project. The product currently remains unavailable from the manufacturer. Attempts to develop a more efficient method of dispersing the SPLAT-TK in the field were not successful. Previous field trials with SPLAT-TK required manual application of the product using toilet bowl brushes. Although a significant reduction in the number of host-seeking *I. scapularis* nymphs was observed on treated plots, the application method precluded knowing how much SPLAT-TK was dispersed.

Objective 2. Evaluate the repellent effects of existing and new compounds, using *in vitro* vertical bioassays and trials with human hosts wearing treated clothing (with IRB approval and consent) using laboratory reared ticks. We did not complete the human host trials for this study as we could not get *H. longicornis* to stay on the human sock, demonstrating their dislike for attaching to humans. We investigated the repellent efficacy of botanical compounds – including carvacrol, eugenol, nerolidol, eucalyptol, and thymol – against *A. americanum* and *H. longicornis* nymphs.

Repellents. Active ingredients (AIs) including carvacrol, eucalyptol, eugenol, nerolidol, and thymol were obtained from Sigma Aldrich (St. Louis, MO) and tested for repellency against *A. americanum* or *H. longicornis* nymphal ticks. Compounds were diluted in acetone to create a range of concentrations between 0.5-10%. A commercial 25% DEET product (OFF Deep Woods, Johnson & Johnson; New Brunswick, NJ) diluted in acetone was prepared and included in all assays as a standard treatment.

Ticks. *A. americanum* and *H. longicornis* nymphs were obtained from BEI Resources Repository (<https://www.beiresources.org>). Nymphs were stored at 22°C and RH ≥75%. Only actively questing nymphs were used in the assays. Temperature and humidity of the room were recorded with a data logger for each assay below and ranged from 22-23 °C and 35-40% RH. Preliminary experiments were conducted with different assay designs before selecting the two most effective assays below. In addition, range finding assays and comparisons of drying time were conducted to determine optimal conditions for testing.

Horizontal repellency assay. General methods for horizontal tick repellencies were followed as previously described by Bissinger (2009) with minor modifications. Briefly, after dilution of each compound and control in acetone to obtain a range of concentrations, 300 µl of solution was applied to one half of a circular 9 cm diameter Whatman no. 1 filter paper (Marlborough, MA). Another half of filter paper was saturated in 300 µl of acetone alone to serve as a negative control. Filter papers were treated 30 mins before the start of the experiment. Petri dish lids were lined with one treated and one control filter paper half using double-sided tape.

Five *A. americanum* or *H. longicornis* nymphs were introduced to each petri dish arena using a small paintbrush, and dishes were secured with a rubber O-ring from Grainger (Lake Forest, IL) sandwiched between the petri dish top and bottom. Tick activity was observed and

recorded at 5, 10, 15, 20, 25, 30, 45, 60, 120 and 180 mins. Tick position on the treated or untreated filter paper half was recorded at each time point. Petri dishes were oriented on the work surface such that the line dividing the treated and untreated sides was perpendicular to and equidistant from the observer to reduce the influence of their host cues on the ticks' movement. Mean repellency over the course of the assay was calculated as follows:

$$\text{Repellency} = \frac{\text{mean \# of nymphs on treated paper} - \text{mean \# of nymphs on untreated paper}}{\text{mean \# of nymphs on untreated paper}} \times 100$$

Vertical repellency assay. Assays were conducted following the methods of Carroll (et al. 2004) with modifications. Briefly, filter papers were cut into 4x8 cm rectangles. On each rectangle, a pencil was used to draw horizontal lines 2 cm from the top and bottom edges dividing the paper into three zones. Each compound or control was diluted as described above, then 150 µl of each concentration was applied to the middle 4x4 cm zone of a filter paper rectangle. One filter paper rectangle was treated with 150 µl of acetone only to serve as a negative control. Filter papers were treated 30 mins before the start of the experiment.

Two ring stands were placed 50 cm apart, with ring heights adjusted to 16 cm above the work surface and a wooden dowel suspended between them. Five 8 cm long wire hangers were attached to the dowel using binder clips, and another binder clip was secured at the bottom of the wire. The top edge of one treated filter paper rectangle was secured to each hanging clip. Below each hanging filter paper, a 50-mm glass petri dish was secured inside a 90-mm glass petri dish using double-sided tape. The filter papers were positioned so that their bottom edges just barely touched the bottoms of the 50-mm petri dishes. The 90-mm petri dishes were filled with water creating moats to prevent nymphs from escaping. Filter paper hangers were oriented on the work surface such that each filter paper was perpendicular to and equidistant from the observer to reduce the influence of host cues on the ticks' positions.

Five *A. americanum* nymphs were placed into the inner petri dish using a fine paint brush. Tick locations were recorded at 2, 4, 7, 10, 15, 20, and 30 minutes after placement into the dishes. The number of ticks repelled for each treatment was considered the number that stayed in untreated zones or immediately fell off. Any ticks that crossed the treated zones were considered not repelled and were immediately removed from the assay to prevent them from falling and being recounted. The percentage of ticks that were repelled in each treatment and replicate was calculated.

Data analysis. Horizontal and vertical repellency was determined for each assay as percent repellency (described above). Results were analyzed using Kruskal-Wallis one-way analysis of variance followed by *post hoc* Student–Newman–Keuls test. Analyses were performed in R version 3.6.1.

Results

H. longicornis. Mean repellency from three horizontal assays was comparable to DEET for carvacrol, eugenol, and nerolidol at all tested concentrations $\leq 10\%$ (Table 1. Kruskal-Wallis chi-squared = 17.3, d.f. = 8, p-value = 0.027).

Table 1. Mean repellency of active ingredients against H. longicornis in 3-hour horizontal assays.

AI	Conc.	Mean percent repelled		
		Trial 1	Trial 2	Trial 3
DEET	0.5%	100	100	100
	5%	100	100	100
Carvacrol	0.5%	96	100	96
	5%	100	100	100
Eugenol	0.5%	100	100	96
	1%	100	96	92
Nerolidol	1%	100	100	100
	10%	96	100	100

A. americanum. Carvacrol, eugenol, nerolidol, and thymol showed comparable repellent activity to DEET in two horizontal assays (Table 2. Kruskal-Wallis chi-squared = 23.2, d.f. = 13, p-value = 0.039). All AI concentrations tested except for 1% thymol and 1% eucalyptol showed comparable repellent activity to DEET in two vertical assays (Table 3. Kruskal-Wallis chi-squared = 21.8, d.f. = 12, p-value = 0.040).

Table 2. Mean repellency of active ingredients against A. americanum in 3-hour horizontal assays.

AI	Conc.	Mean percent repelled	
		Trial 1	Trial 2
Carvacrol	1%	56	80
	10%	80	96
DEET	0.5%	76	84
	5%	64	88
	25%	84	92
Eucalyptol	1%	11	4
	10%	35	44
Eugenol	1%	80	96
	10%	96	100
Nerolidol	1%	72	60
	10%	88	96
Thymol	1%	77	80
	10%	92	100

Table 3. Percent of A. americanum nymphs repelled by each active ingredient in 30-min vertical assays.

AI	Conc.	Percent repelled	
		Trial 1	Trial 2
Carvacrol	1%	100	60
	10%	100	100
DEET	0.5%	60	60
	25%	100	100
Eucalyptol	1%	0	20
	10%	40	40
Eugenol	1%	80	40
	10%	80	100
Nerolidol	1%	20	60
	10%	60	100
Thymol	1%	0	40
	10%	100	100

Objective 3. Determine the baseline susceptibility and resistance to acaricides for populations of *I. scapularis*, *A. americanum*, and *H. longicornis* in the United States.

Pesticide resistance in medically significant disease vectors can negatively impact the efficacy of control efforts. Research on ticks has focused primarily on resistance in species of veterinary significance which experience relatively high degrees of control pressure. Resistance in tick vectors of medical significance has received little attention, in part because area-wide broadcast of pesticides is not used to control these generalist tick species. One of the few efficacious methods currently in use for area-wide control of medically important ticks, including *Ixodes scapularis*, is the deployment of 4-poster devices. Deer self-apply a topical acaricide (permethrin) while feeding on corn from the devices. A 4-poster program using permethrin has been deployed on Shelter Island, NY to control *I. scapularis* populations since 2008. We collected engorged female ticks from deer in this management area and a location in the Mid-Hudson River Valley, NY without area-wide tick control. Larvae were reared from egg masses and their susceptibility to permethrin was tested. Larvae originating from a long-term laboratory colony were used as a susceptible baseline for comparison. Compared against the laboratory colony, resistance ratios (RR) at LC-50 for Shelter Island and Hudson Valley *I. scapularis* were 1.87 and 1.51, respectively. The susceptibilities of the field populations to permethrin were significantly lower than that of the colony ticks. We provide the first data using the larval packet test to establish baseline susceptibility for *I. scapularis* to permethrin along with information relevant to understanding resistance emergence in tick populations under sustained control pressure from 4-poster devices. A manuscript reporting this work was published (Burtis et al. 2021).

Objective 4. Employ simulation modeling as a powerful tool to identify optimal deployment of individual and integrated tick management approaches under variable conditions, which will inform the development of updated tick management guidelines.

4.a. Updated Tick Management Guidelines. Unfortunately, we were not able to complete the updates for the Tick management guidelines proposed in our application. Dr. Stafford is now retired and is currently working on those updates.

4.b. Computer simulation model to identify optimal deployment of individual and integrated tick management approaches in different settings.

We have completed the development of a modeling platform to assess the effectiveness of different *Ixodes scapularis* tick management approaches. The platform includes a climate-driven mechanistic model of tick and pathogen population dynamics and has been adapted to simulate the impact of different control treatments, which are either conducted individually or in combination, with different application timing and duration. We piloted the platform by assessing three safe and low-toxicity measures, namely reductions in white-tailed deer population, broadcast area-application of the entomopathogenic fungus *Metarhizium anisopliae* (Met52), and fipronil-based small rodent bait boxes. To assess the impact of these control efforts, we estimated the percent reduction of the nymphal peak, the percent control, and the percentage change in the area under the phenology curve associated with each single treatment and combined efforts. We initially calibrated the model using published data from an integrated tick management program to reduce *Ixodes scapularis* nymphs implemented in the town of Redding, southwestern Connecticut, conducted from January 2013 through September 2016. We then simulated multiple timing and intensities of intervention. In simulations, the three-treatment combined effort had the overall highest impact on suppressing *Ixodes scapularis* nymphs. Under intermediate intervention intensities, the average reduction in the area under the nymphal phenology curve was 74.0% (Figure 1). Simulation outputs show that a two-week earlier broadcast area-application of Met52 would result in a 12.1% reduction of the area under the phenology curve. The platform can be expanded to include other sets of interventions, including habitat-targeted acaricide spraying, hosts-targeted acaricides and vaccines, and reductions in tick habitat suitability through landscape and vegetation management. Future applications can be implemented in military bases where enough tick data has been collected to initially calibrate the model. Computer simulation is a powerful tool to identify the optimal deployment of individual and combined tick management approaches, which can synergistically contribute to short- to long-term, cost-effective and sustainable control of tick-borne diseases in integrated vector management guidelines. A manuscript is in preparation.

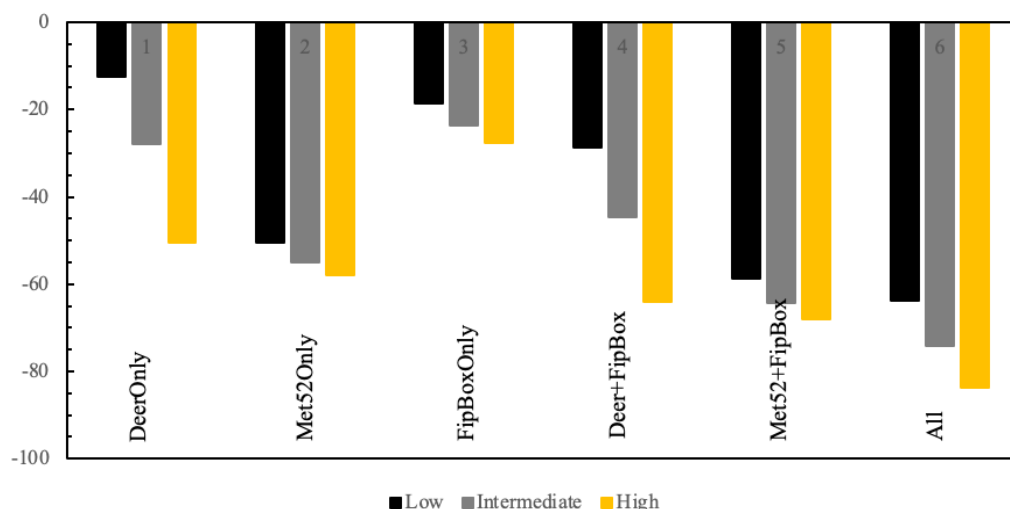


Figure 1. Average percent change in the area under the phenology curve resulting from simulated reductions of 25, 50 and 75% in deer populations ('Deer', low, intermediate and high, respectively); 40, 60 and 80% increased mortality of host seeking nymphs and adults resulting from Met52 applications ('Met52' low, intermediate, high, respectively); and increased mortality in larvae and nymphs on hosts from fipronil

KEY RESEARCH ACCOMPLISHMENTS:

- Demonstrated the high efficacy of 10% permethrin topical treatment of white-tailed deer via 4-poster devices in reducing host seeking tick densities and *Ehrlichia* infection rates of *A. americanum*. The high-density deployment of one 4-poster device/12.7 ha reduced densities of host-seeking adults (93% reduction), nymphs (92%), and larvae (96%) over the course of the study from 2018 to 2020.
- Fipronil-laced bait was readily accepted by white footed mice and other rodent tick borne pathogen reservoirs and reduced juvenile *I. scapularis* burdens on rodents.
- Evaluated for the first time, susceptibility of *I. scapularis* to permethrin via larval packet assays in an area of long term 4-poster device deployment. Resistance ratios (RR) at LC-50 for Shelter Island and Hudson Valley (no 4 poster use) *I. scapularis* were 1.87 and 1.51, respectively. The susceptibilities of the field populations to permethrin were significantly lower than that of the colony ticks. This baseline susceptibility data is relevant to understanding resistance emergence in tick populations under sustained control pressure from 4-poster devices.
- Over 80 outdoor contained trials (ARENA) were conducted to evaluate efficacy of synthetic pyrethroids and 25 b exempt products for *A. americanum* and *I. scapularis* control with analysis underway.
- Horizontal and vertical repellent assays of botanical compounds – including carvacrol, eugenol, nerolidol, eucalyptol, and thymol – against *A. americanum* and *H. longicornis* nymphs demonstrated high repellency of botanicals, comparable to 25% DEET, over 3hrs for *H. longicornis*. All compounds at a range of concentrations tested except for 1%

thymol and 1% eucalyptol showed comparable repellent activity to DEET over 3hrs for *A. americanum*.

- Simulation studies of integrated tick management tools such as Met52 highlight optimal deployment methods for new and conventional tick control strategies. The platform can be expanded to include other sets of interventions, including habitat-targeted acaricide spraying, hosts-targeted acaricides and vaccines, and reductions in tick habitat suitability through landscape and vegetation management.

REPORTABLE OUTCOMES:

Publications citing this grant award are included in the appendices.

Manuscripts. In preparation:

Modeling platform to assess the effectiveness of single and integrated *Ixodes scapularis* tick control methods. Daniel Ruiz-Carrascal, Scott C. Williams, Jonathan Bastard and Maria Diuk-Wasser

Repellent Assays to Assess Efficacy of Botanical Compounds Against *A. americanum* and *H. longicornis* Nymphal Ticks. Elisabeth Martin and L.C. Harrington (formatted for Arthropod Tests)

ARENA-based assessment of tick control products. M. Cucura et al.

Published:

Burtis, J. C., J. D. Poggi, B. Payne, S. R. Campbell, and L. C. Harrington. 2021. Susceptibility of *Ixodes scapularis* (Acari: Ixodidae) to Permethrin Under a Long-Term 4-Poster Deer Treatment Area on Shelter Island, NY. J Med Entomol 58: 1966-1969.

Williams, S. C., K. C. Stafford, 3rd, M. A. Linske, D. E. Brackney, A. M. LaBonte, H. R. Stuber, and D. W. Cozens. 2021. Effective control of the motile stages of *Amblyomma americanum* and reduced *Ehrlichia* spp. prevalence in adults via permethrin treatment of white-tailed deer in coastal Connecticut, USA. Ticks Tick Borne Dis 12: 101675.

Williams, S. C., M. A. Linske, and K. C. Stafford, 3rd. 2023. Orally delivered fipronil-laced bait reduces juvenile blacklegged tick (*Ixodes scapularis*) burdens on wild white-footed mice (*Peromyscus leucopus*). Ticks Tick Borne Dis 14: 102189.

No other reportable outcomes including patents or licenses have been applied for or issued, degrees obtained, development of cell lines/reagents, informatics, funding applied for, employment opportunities have resulted from this funding.

The following personnel received pay from the research effort:

Elisabeth Martin, temporary technician 50% FTE (Cornell)
Daniel Ruiz-Carrascal, Postdoctoral student (50% FTE; Columbia University
Research Technician I (50% FTE; CAES)
Seasonal research assistant I (summer seasonal; CAES)
Seasonal summer assistants 2 (Fordham)
Tamson Yeh (0.03% FTE) (Suffolk County)
Dan Gilrein (0.03% FTE) (Suffolk County)

CONCLUSION: In this study, we have evaluated a number of new tools and approaches for tick and tick borne disease management that can be adopted by military and civilians to reduce the burden of tick borne infections.

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- Williams, S. C., M. A. Linske, and K. C. Stafford, 3rd. 2023. Orally delivered fipronil-laced bait reduces juvenile blacklegged tick (*Ixodes scapularis*) burdens on wild white-footed mice (*Peromyscus leucopus*). *Ticks Tick Borne Dis* 14: 102189.

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Appendix II. Williams, S. C., K. C. Stafford, 3rd, M. A. Linske, D. E. Brackney, A. M. LaBonte, H. R. Stuber, and D. W. Cozens. 2021. Effective control of the motile stages of *Amblyomma americanum* and reduced Ehrlichia spp. prevalence in adults via permethrin treatment of white-tailed deer in coastal Connecticut, USA. Ticks Tick Borne Dis 12: 101675.

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Short Communication

Susceptibility of *Ixodes scapularis* (Acari: Ixodidae) to Permethrin Under a Long-Term 4-Poster Deer Treatment Area on Shelter Island, NY

James C. Burtis,^{1,2,5} Joseph D. Poggi,¹ Beau Payne,³ Scott R. Campbell,⁴ and Laura C. Harrington¹

¹Department of Entomology, Cornell University, Ithaca, NY 14853, USA, ²Division of Vector-Borne Disease, Centers for Disease Control and Prevention, Fort Collins, CO 80521, USA, ³Shelter Island Police Department, Shelter Island, NY 11964, USA, ⁴Suffolk County Department of Health Services, Yaphank, NY 11980, USA, and ⁵Corresponding author, e-mail: burtis.james@gmail.com

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Abstract

Pesticide resistance in medically significant disease vectors can negatively impact the efficacy of control efforts. Resistance research on ticks has focused primarily on species of veterinary significance that experience relatively high degrees of control pressure. Resistance in tick vectors of medical significance has received little attention, in part because area-wide pesticide applications are not used to control these generalist tick species. One of the few effective methods currently used for area-wide control of medically important ticks, including *Ixodes scapularis* Say (Acari: Ixodidae), is deployment of 4-poster devices. Deer self-apply a topical acaricide (permethrin) while feeding on corn from the devices. A 4-poster program using permethrin has been deployed on Shelter Island, NY to control *I. scapularis* populations since 2008. We collected engorged female ticks from deer in this management area and a location in the Mid-Hudson River Valley, NY without area-wide tick control. Larvae were reared from egg masses and their susceptibility to permethrin was tested. Larvae originating from a long-term laboratory colony were used as a susceptible baseline for comparison. Compared against the laboratory colony, resistance ratios at LC-50 for Shelter Island and Hudson Valley *I. scapularis* were 1.87 and 1.51, respectively. The susceptibilities of the field populations to permethrin were significantly lower than that of the colony ticks. We provide the first data using the larval packet test to establish baseline susceptibility for *I. scapularis* to permethrin along with information relevant to understanding resistance emergence in tick populations under sustained control pressure from 4-poster devices.

Key words: pesticide resistance, larval packet test, acaricide, *Ixodes scapularis*, 4-poster device

Pesticide resistance is a common issue in arthropod pathogen vector populations. Considerable research has focused on pesticide resistance in medically significant mosquito species, with well-developed resistance testing guidelines from the World Health Organization (WHO 2016a,b) and the Centers for Disease Control and Prevention (CDC; McAllister and Scott 2019). Pesticide resistance in mosquito populations has been implicated in management failures (Brouqui et al. 2012, Estep et al. 2018) and monitoring is considered an essential component of mosquito control programs (National Association of County and City Health Officials [NACCHO] 2017). This is not the case for ticks, where most management and pesticide resistance guidelines and research have focused on species that impact domestic animals, particularly cattle ticks such as *Rhipicephalus microplus*

(Canestrini) (Acari: Ixodidae) and *Rhipicephalus annulatus* (Say) (Food and Agriculture Organization of the United Nations [FAO] 2004). These ticks are one-host species, spending most of their life cycle on cattle, and causing enormous economic damage globally (Jonsson et al. 2001, Kivaria 2006). Cattle tick management often involves applying acaricides directly to cattle and resistance has been reported for multiple pesticide classes (George et al. 2004, Reck et al. 2014). Recently, permethrin resistance also has been detected in an endophilic three-host tick, *Rhipicephalus sanguineus* (Latreille), from multiple populations in Florida and Texas (Eiden et al. 2015).

Most ticks that transmit human disease agents are three-host species, which feed on different vertebrate hosts at each life stage and spend large portions of time off-host in the environment. Consequently,

host-targeted methods are challenging for medically important tick species. One exception is deployment of 4-poster devices that can effectively control *Ixodes scapularis* Say and *Amblyomma americanum* (L.) by targeting deer, their primary reproductive host (Stafford and Williams 2017). *Ixodes scapularis* is the most medically significant vector in the eastern United States, transmitting pathogens responsible for Lyme disease, anaplasmosis, babesiosis, and Powassan virus disease. The permethrin-treated application rollers of 4-poster devices treat deer at attached feeding stations offering corn (Pound et al. 2000). This method has effectively reduced questing tick densities, but whether the resulting selection pressure is strong enough for resistance to emerge in tick populations is unclear.

In an effort to reduce tick populations on Shelter Island, NY, a program deploying 4-poster devices was maintained from 2008 to 2019. A study conducted on the island between 2008 and 2010 tested the efficacy of 60 devices (1.9/km²) dispersed over the town of Shelter Island and the associated Mashomack Preserve. They observed 85% fewer questing *I. scapularis* nymphs by the final year (Curtis et al. 2011). After study completion, the management program continued with 36 devices. This long running program targeting a relatively isolated island population of *I. scapularis* provides an excellent opportunity to test permethrin resistance emergence. We, therefore, collected engorged female *I. scapularis* from deer on Shelter Island. We similarly collected engorged female *I. scapularis* from deer at the Cary Institute of Ecosystem Studies (CIES) in Millbrook, NY. This site represents a tick population under lower control pressure; no 4-poster devices are in operation and pesticides are not widely applied on the property. Permethrin susceptibility of larvae reared from these specimens was compared with larvae from a long-term laboratory-reared colony maintained by CDC.

Methods

Tick Collection and Rearing

Engorged female *I. scapularis* were collected in November 2019 directly from hunter-killed white-tailed deer [*Odocoileus virginianus* (Zimmermann)] on Shelter Island, NY (N 41° 4' 4.17"; W 72° 20' 19.54") and grounds of CIES in Millbrook, NY (N 41° 47' 6.04"; W 73° 44' 1.76") in coordination with ongoing deer management programs. Engorged females were removed with tweezers, placed in 50 ml falcon tubes, and transported to the laboratory. Engorged females, egg masses, and larvae were stored in humidified vials inside an incubator set to 24°C, 70% RH, with a 16:8 (L:D) light cycle. The laboratory strain of *I. scapularis* was provided by CDC from a long-term colony and reared as described previously (Troughton and Levin 2007). Original colony material was collected from Rhode Island and has been maintained in colony since 2003. It is periodically refreshed with males from the field. Females were monitored weekly, until egg masses were laid at which point, they were monitored every 24 h. Once larvae hatched, they were aged to 14–18 d and used in bioassays.

Larval Packet Test Bioassay

The larval packet test (FAO 1984) was used to determine permethrin susceptibility of the three *I. scapularis* strains (CDC/CIES/Shelter Island). Technical grade permethrin (99%, Chem Service Inc., West Chester, PA) was dissolved in a 2:1 solution of trichloroethylene (TCE) and olive oil. Six permethrin concentrations between 1.33 and 0.04 mg/ml were prepared for the bioassay. A control, containing only TCE and olive oil mixture with no permethrin, was included for each set of replicates. Control mortality was adjusted using Abbott's

correction (Abbott 1925). Four to eight replicates were conducted at each concentration and tick strain, depending upon availability of larvae.

For each replicate, seven filter papers (Whatman No. 1, Maidstone, England) were cut into uniform packets (7.6 × 8.9 cm). Each paper was inoculated with 1 ml of the target concentration of permethrin solution. The control papers were inoculated with 1 ml of the control solution described above. Papers were suspended in a fume hood for 2 h to evaporate the TCE. Once dry, the filter paper was folded in half and two #2 bulldog clips were used to enclose the sides of the packet. The top was left open and approximately 100 *I. scapularis* larvae were added to each packet using a fine brush. Once the larvae were added, a third bulldog clip was used to seal the packet. Packets were suspended inside an aquarium in an incubator with a 16:8 light cycle at 24°C and 70% RH. After 24 h, packets were opened, and mortality was quantified by counting viable and non-viable ticks. Ticks were categorized as non-viable if they did not respond to being breathed upon.

Data were analyzed using R (version 4.0.2). Three probit analyses (CDC/CIES/Shelter Island) were conducted using the glm() command in the MASS package. Lethal concentration values (LC-50/LC-95/LC-99) and their associated 95% confidence intervals were used to determine whether susceptibility differed significantly between the three tick strains. Resistance ratios (RRs) at the LC-50 value were calculated, using the CDC colony as a baseline (Yu 2014). Significant differences in susceptibility between *I. scapularis* strains were determined by comparing the LC-50s and their 95% CIs.

Results

Nine fully engorged *I. scapularis* females were collected from deer on Shelter Island, seven of which laid viable egg masses. At CIES, six fully engorged females were collected, four of which laid viable egg masses. All five engorged females supplied by CDC produced egg

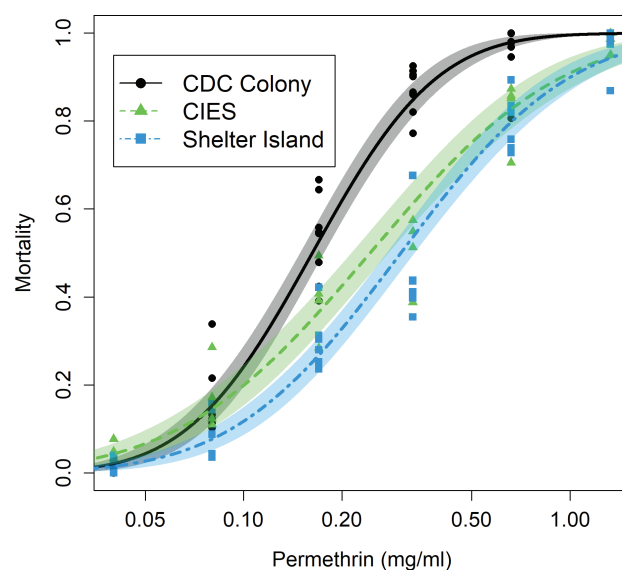


Fig. 1. Scatterplots displaying permethrin susceptibility curves for *Ixodes scapularis* larvae from the two field populations (CIES/Shelter Island) and the CDC laboratory colony. Lines represent the predictions resulting from probit analyses and the shaded areas are 95% confidence intervals. A jitter was used to add a small amount of noise to these data to reveal overlapping points on this figure.

Table 1. Results of the probit analyses for *Ixodes scapularis* larvae from the two field populations for which susceptibility to permethrin was tested (CIES/Shelter Island), and the CDC laboratory colony serving as the susceptible baseline

	No. egg masses included	No. replicates tested	df	Slope (SE)	LC-50 ($\pm$ 95% CI)	LC-95 ($\pm$ 95% CI)	LC-99 ($\pm$ 95% CI)	RR (LC-50)	χ^2
CDC colony	5	8	46	3.34 (0.19)	0.162 ($\pm$ 0.01)	0.505 ($\pm$ 0.08)	0.808 ($\pm$ 0.18)	1	303
CIES	4	4	22	2.47 (0.13)	0.244 ($\pm$ 0.03)	1.39 ($\pm$ 0.42)	2.84 ($\pm$ 1.19)	1.51	220
Shelter Island	7	6	34	2.18 (0.14)	0.303 ($\pm$ 0.03)	1.41 ($\pm$ 0.30)	2.66 ($\pm$ 0.78)	1.87	337

LC values are presented in milligrams per milliliter. 'No. replicates tested' represents the number of replicate packets (each with ~100 larvae) included for the six tested permethrin concentrations for the field populations and susceptible control colony. RR is the resistance ratio at LC-50 (determined by dividing the LC-50 of the field colonies by that of the CDC colony). χ^2 values represent the goodness-of-fit for the probit analyses.

masses and larvae. Bioassays included totals of 3,569 larvae from seven egg masses from Shelter Island, 2,347 larvae from four egg masses from CIES, and 5,324 larvae from five egg masses from the CDC colony (Fig. 1). Results for probit analyses for Shelter Island ($t = 18.3$; $df = 34$; $P < 0.001$), CIES ($t = 17.4$; $df = 46$; $P < 0.001$), and the CDC colony ($t = 14.8$; $df = 22$; $P < 0.001$) are shown in Table 1, along with the LC values and LC-50 RRs.

Discussion

Ixodes scapularis collected from Shelter Island and CIES were less susceptible to permethrin than the CDC colony, with RRs of 1.87 and 1.51, respectively. Furthermore, specimens from Shelter Island were significantly less susceptible than those from CIES, but the difference in RRs was relatively small. It appears that field populations of *I. scapularis* may be less susceptible than the CDC laboratory colony, regardless of selective pressure from 4-poster devices. No other studies reporting *I. scapularis* RRs have been published. For other species, the RR threshold values for a population to be considered resistant have been set between 2 and 10 (Castro-Janer et al. 2011, Coles and Dryden 2014, Eiden et al. 2015). When connected to field efficacy, RR thresholds can vary by species, active ingredient, and bioassay method (Barré et al. 2008, Lovis et al. 2011). Field populations with RR values < 2 are rarely considered resistant, but further evaluation is needed to connect these thresholds to *I. scapularis* control efficacy. The absence of established susceptible colonies for *I. scapularis* is another limitation. The detection of pesticide resistance in mosquitoes generally employs the use of well-established susceptible lines that have been maintained under laboratory conditions, often for decades (Vontas et al. 2012). Unfortunately, no such established susceptible colonies exist for *I. scapularis* or other medically significant tick species.

Although, we found no permethrin resistance in the Shelter Island *I. scapularis*, it is possible that resistance might emerge under more intensive management regimes using 4-poster devices. Recent 4-posters densities on Shelter Island are approximately 1.2 devices/km², down from the 1.9 devices/km² in the original study, when a significant reduction in questing *I. scapularis* was observed (Curtis et al. 2011). Remaining devices were placed so that at least one device was within the majority of the home ranges of deer on the island. Tick sampling has continued on Shelter Island and tick densities do not appear to have rebounded, but data collection was less rigorous after the initial study (B. Payne, personal communication). There is evidence that both deer and 4-poster density influence the efficacy of 4-poster devices (Stafford and Williams 2017). A large-scale study observed significant reductions in questing tick densities with 4–5 devices/km² (Pound et al. 2009). Other studies using lower device densities (1.65 devices/km²) have shown limited efficacy (Grear et al. 2014). Permethrin susceptibility of tick populations should be

monitored from other 4-poster control areas so that guidelines for managing pesticide resistance in the field can be developed.

The emergence of resistance in *I. scapularis* populations is likely to differ from that in mosquitoes, which may ultimately make resistance in ticks easier to track in the field. Hard ticks have significantly longer life cycles and therefore longer generation times than mosquitoes. This should reduce the amount of temporal variability in resistance status. The 11-yr deployment of the 4-poster program on Shelter Island only represents approximately six generations, so the emergence of resistance would require a high degree of control pressure. It is common for resistance to emerge rapidly in mosquito populations in response to targeted control efforts (Barbosa et al. 2011, Matowo et al. 2019), but for ticks this will occur on a longer timescale. This may reduce the frequency of sampling needed to monitor resistance in *I. scapularis* populations. Furthermore, ticks can move long distances attached to their hosts (Madhav et al. 2004, Diuk-Wasser et al. 2020), potentially increasing population mixing and reducing the probability that resistance will emerge due to localized control efforts.

Our sample sizes were limited due to the cessation of the Shelter Island 4-poster program, but results presented here provide the first field-based pesticide susceptibility assessment for *I. scapularis* collected from an area with potential long-term pressure. Future efforts to establish susceptible reference colonies, monitor field susceptibility, and develop operational resistance thresholds are critical to ensure long-term efficacy of existing and new pesticide-based tick control strategies.

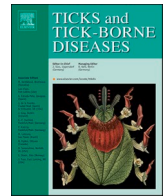
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Effective control of the motile stages of *Amblyomma americanum* and reduced *Ehrlichia* spp. prevalence in adults via permethrin treatment of white-tailed deer in coastal Connecticut, USA

Scott C. Williams^{a,b,*}, Kirby C. Stafford III^{a,c}, Megan A. Linske^{a,c}, Douglas E. Brackney^{a,d}, Andrew M. LaBonte^e, Heidi R. Stuber^{a,c}, Duncan W. Cozens^{a,d}

^a Center for Vector Biology & Zoonotic Diseases, The Connecticut Agricultural Experiment Station, P.O. Box 1106, 123 Huntington Street, New Haven, CT, 06504, United States

^b Department of Forestry and Horticulture, The Connecticut Agricultural Experiment Station, P.O. Box 1106, 123 Huntington Street, New Haven, CT, 06504, United States

^c Department of Entomology, The Connecticut Agricultural Experiment Station, P.O. Box 1106, 123 Huntington Street, New Haven, CT, 06504, United States

^d Department of Environmental Sciences, The Connecticut Agricultural Experiment Station, P.O. Box 1106, 123 Huntington Street, New Haven, CT, 06504, United States

^e Connecticut Department of Energy and Environmental Protection, Wildlife Division, 391 Route 32, North Franklin, CT, 06254, United States

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ABSTRACT

The lone star tick, *Amblyomma americanum*, is a common human-biting species whose range has been largely restricted to the southeastern United States, until recent detections of established populations on Long Island, New York and throughout coastal southern New England. We evaluated the effectiveness of topical treatment of 10 % permethrin delivered via 4-poster devices to white-tailed deer, *Odocoileus virginianus*, in the management of a newly discovered *A. americanum* population in Norwalk, Connecticut. Using a high-density deployment of one 4-poster device/12.7 ha, we were successful in significantly reducing densities of host-seeking adults (93 % reduction), nymphs (92 %), and larvae (96 %) from 2018 to 2020. We also documented a significant reduction (87 %) in parasitizing adults and nymphs on white-tailed deer from 2018 to 2019. The prevalence of *Ehrlichia chaffeensis* and *Ehrlichia ewingii* combined in host-seeking adults declined significantly from 47 % at the time the *A. americanum* population was discovered in 2017 to 7% in 2020. However, the prevalence in nymphs remained static (~9%) throughout the study period. These data demonstrate that, when properly deployed in a density-dependent manner in terms of deer abundance, 4-poster devices can effectively manage parasitizing and host-seeking *A. americanum* populations and reduce the prevalence of two ehrlichial species of public health importance.

1. Introduction

The lone star tick, *Amblyomma americanum*, is a common and aggressive three-host tick typically found throughout the southeastern and midwestern United States (Raghavan et al., 2019). However, in recent years, its range has been expanding northward along coastal areas where milder climates and chronically overabundant white-tailed deer, *Odocoileus virginianus*, herds persist (Molaei et al., 2019). *Amblyomma americanum* is of public health concern as they are known vectors of *Ehrlichia chaffeensis* and *Ehrlichia ewingii*, for both of which

white-tailed deer are reservoir competent (Goddard and Varela-Stokes, 2009). Additionally, *A. americanum* has been implicated in vectoring *Francisella tularensis* (tularemia), Bourbon virus (Bourbon virus disease), as well as Heartland virus (Heartland virus disease) and bites by this tick have been associated with alpha gal syndrome (red meat allergy) (Stafford et al., 2018; Molaei et al., 2019; Godsey et al., 2020). Historically, New Jersey was regarded as the northern extent of the range of *A. americanum* along the Atlantic Coast, but in recent decades, breeding populations have been reported in the milder climates of Suffolk County, New York, Newport County, Rhode Island, and Barnstable, Nantucket,

* Corresponding author at: Department of Forestry and Horticulture, The Connecticut Agricultural Experiment Station, 123 Huntington Street, P.O. Box 1106, New Haven, CT, 6504, United States.

E-mail addresses: scott.williams@ct.gov (S.C. Williams), kirby.stafford@ct.gov (K.C. Stafford), megan.linske@ct.gov (M.A. Linske), doug.brackney@ct.gov (D.E. Brackney), andrew.labonte@ct.gov (A.M. LaBonte), heidi.stuber@ct.gov (H.R. Stuber), duncan.cozens@ct.gov (D.W. Cozens).

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and Dukes Counties, Massachusetts (Jordan and Egizi, 2019; Molaei et al., 2019). A recent study documented that adult female *A. americanum* overwintering survival was higher than that of males with a maximum of 78 % survival under winter conditions in Connecticut and 73 % as far north as coastal Maine (Linske et al., 2020).

In 2017, Connecticut's first known established breeding population of *A. americanum* was documented in the City of Norwalk in Fairfield County (Stafford et al., 2018); a second population has since been documented in New Haven County (Molaei et al., 2019). The original location, Manresa Island, is a peninsula surrounded by Long Island Sound and is the site of the decommissioned coal/oil-fired Norwalk Harbor Power Station owned by NRG Energy, Inc. (Houston, TX, USA). After discovery of the population, we approached NRG and reached an arrangement to permit us access to the site that is otherwise closed to the public to monitor the *A. americanum* infestation and attempt its management.

Amblyomma americanum abundances have been closely associated with white-tailed deer abundances (Patrick and Hair, 1978; Bloemer et al., 1986, 1988, 1990). As was the case with the discovery of this population, *A. americanum* can be so abundant they occasionally overwhelm and kill their ungulate hosts (Bolte et al., 1970; Stafford et al., 2018). As a result of this association and the abundance of deer on Manresa Island, we concluded that quantifying deer density and deploying 4-poster devices would be an excellent strategy in the management of this somewhat isolated *A. americanum* population. The 4-poster device is a passive treatment station developed by scientists with the United States Department of Agriculture-Agricultural Research Service in Kerrville, Texas, USA. Deer are lured to the device and self-apply a topical acaricide to their head, neck, and ears as they contact treated rollers while feeding on corn within the device (Pound et al., 2000a, 2000b). The 4-poster device has had historic successes in managing local populations of blacklegged ticks, *Ixodes scapularis*, on white-tailed deer using 10 % permethrin in Maryland (Solberg et al., 2003) and on both *I. scapularis* and *A. americanum* on Shelter and Fire Islands, New York (Curtis et al., 2011). The devices were also tested using 2% amitraz in New Jersey as part of an integrated approach (Schulze et al., 2007) and in Rhode Island, Connecticut, New York, New Jersey, and Maryland as part of the multistate Northeast Areawide Tick Control Project (NATCP; Pound et al., 2009a, 2009b). While some limitations exist in their use, if properly distributed across the landscape relative to deer density, 4-poster devices are effective at reducing tick abundances (Stafford and Williams, 2017).

Our objectives were to use trail camera imagery to estimate local deer density and test the effectiveness of 10 % permethrin topically delivered to deer on Manresa Island via 4-poster devices in the management of *A. americanum* and associated *Ehrlichia* spp. over a three-year period (2018–2020). We routinely drag sampled permanent transects of known length from early May to September to determine the density of host-seeking ticks, used trail cameras to estimate the size of the deer herd, captured white-tailed deer to quantify burdens of parasitizing *A. americanum*, and tested host-seeking *A. americanum* for the presence of *E. chaffeensis* and *E. ewingii*. Permethrin treatment of adult and nymphal *A. americanum* parasitizing white-tailed deer in 2018 would have had no impact on host-seeking tick abundances in that year and we expected would result in reductions in parasitizing tick burdens of both stages in 2019 and again in 2020. We also expected lower densities of host-seeking larvae, nymphs, and adults in 2019 than in 2018 and fewer still in 2020. Because we were targeting parasitizing nymphs and adults on the major *Ehrlichia* spp. reservoir, we suspected this would result in a corresponding decrease in infection prevalence with *E. chaffeensis* and *E. ewingii* in both stages over time.

2. Materials and methods

2.1. Study area

No deer management occurs on the 50.6 ha Manresa Island property. Additionally, it is closed to the public and is under 24-h security detail with dense residential housing bordering it to the north and on the mainland to the west and northwest. A perimeter fence separates it from these residential areas. Within the property, the majority of the forested area (37.2 ha) is enclosed by an interior fence while the remaining 13.4 ha containing the power station infrastructure is not (Fig. 1). Marginal tick habitat exists outside the fence as this area consists of mostly pavement, buildings, and maintained lawn. The fenced interior property is ideal tick habitat as it has revegetated over time and is now dominated by grey birch, *Betula populifolia*, with some pitch pine, *Pinus rigida*, eastern white pine, *Pinus strobus*, eastern redcedar, *Juniperus virginiana*, black locust, *Robinia pseudoacacia*, and pin oak, *Quercus palustris*. Native grasses and sedges along with northern bayberry, *Morella pensylvanica*, dominate the upland understory, saltmeadow cordgrass, *Spartina patens*, dominates saltwater marshes, and brackish and freshwater marshes are dominated by common reed, *Phragmites australis*.

Manresa Island's coastal proximity, wetlands systems, and limited human presence have allowed wildlife to flourish. Several dozen osprey, *Pandion haliaetus*, nests were active on site, eastern coyotes, *Canis latrans* var., and multiple species of waterfowl and wading shorebirds readily utilize the property and eastern wild turkey, *Meleagris gallopavo silvestris*, diamondback terrapin, *Malaclemys terrapin*, and white-tailed deer are found in high abundance. The fencing on the property was not deer-proof; we routinely witnessed and photographed deer jumping over fencing. Further site description and location within Connecticut can be found in Stafford et al. (2018). *Ixodes scapularis* and American dog tick, *Dermacentor variabilis*, were also present on site, but because 98–99% of sampled ticks were *A. americanum*, our analyses focused on their abundances specifically.

2.2. Permethrin treatment

Four 4-poster devices (C. R. Daniels, Inc., Ellicott City, MD, USA) were placed across the property where we had visually observed deer utilizing the various habitats. Two were positioned in the wooded area within the confines of the interior fence and two were sited outside the interior fence, generally in the northern and southern extremes of the property (Fig. 1). This resulted in a density of one active 4-poster per 12.7 ha. Devices were activated ahead of the nymphal and adult *A. americanum* peaks in late April to early May and were maintained weekly through August 2018, 2019, and 2020. To avoid deer dominating and excluding other deer from accessing 4-poster devices, one 22.7 kg bag of whole corn was placed in each of the four devices weekly. After an initial soaking in 40 mL, each of the four paint rollers per device received a weekly application of 10 mL of 10 % permethrin ('4-poster' tickicide, Y-Tex Corporation, Cody, WY, USA) (40 mL total/device/week).

2.3. Host-seeking *A. americanum* sampling

Host-seeking *A. americanum* were sampled primarily during periods of peak activity from 2018 to 2020. Adults and nymphs were sampled weekly throughout May and June and larvae weekly throughout their peak in August and September. A 1.0 m² piece of white duck cloth attached to a wooden dowel was dragged over vegetation along permanent transects of known distance (100 m) and checked for ticks every 10–15 m. Because ~75 % of the property was fenced and ~25 % was not, three permanent transects were established within the interior fence and one outside. Transects were established within the property interior ranging in distance from 70 to ~300 m to the closest 4-poster device (Fig. 1). All collected ticks were removed from drags, placed in

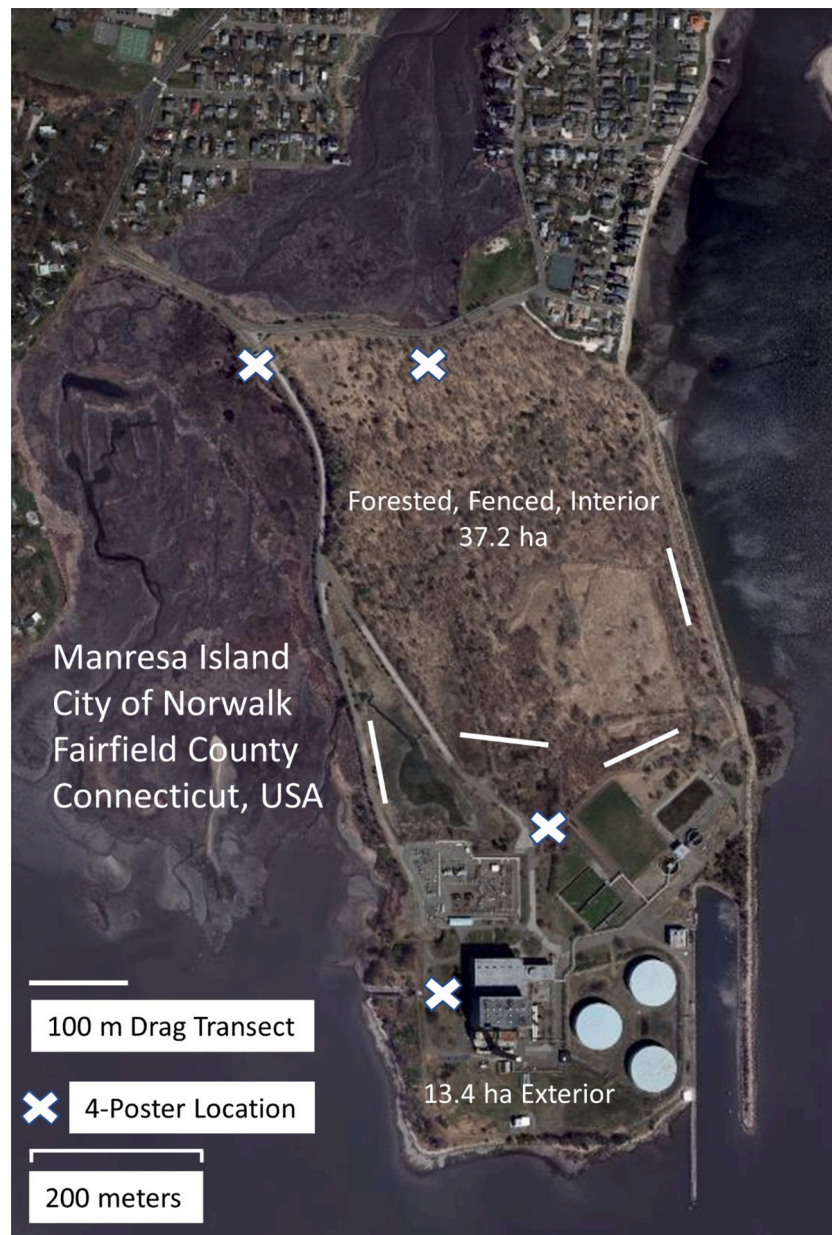


Fig. 1. Study site with 4-poster locations and permanent tick dragging transects indicated.

containers, labeled, and brought back to the laboratory for counting and species identification. Retained live adult and nymphal *A. americanum* were then placed in vials in an incubator at 4 °C until they could be processed for *Ehrlichia* infection.

For adult and nymphal *A. americanum*, we determined a mean daily density/100 m² using data from all four transects and total drag distance for each day of sampling during their peak activity periods in May and June 2018–2020. We also derived a single mean daily density/sampling day for larval *A. americanum* during their activity peak in August and September 2018–2020 as there was high variability in drag sampling success based on transect location within the site, month, and daily weather conditions. Mean daily densities for host-seeking larval and adult *A. americanum* were subjected to one-way analysis of variance (ANOVA) with the Holm-Sidak method used for multiple comparisons between years. Host-seeking nymphal data were analyzed with Welch's *t*-test. Square root and natural log transformations were required to normalize data.

2.4. *Ehrlichia* spp. screening in *A. americanum*

Male, female, and nymphal *A. americanum* were screened for baseline *E. chaffeensis* and *E. ewingii* presence by the Centers for Disease Control and Prevention (CDC) Rickettsial Zoonoses Branch in Atlanta, GA in 2017 after initial discovery of the established population, but before we began 4-poster device intervention in 2018. These data were reported previously in [Stafford et al. \(2018\)](#). Unfortunately, retained samples from our first intervention year expired in incubators before we had the capacity to test them. However, samples from 2019 and 2020 were tested for *E. chaffeensis* and *E. ewingii* infection in-house at The Connecticut Agricultural Experiment Station, duplicating the 2017 CDC methodology found in [Stafford et al. \(2018\)](#), aside from use of a different extraction kit (MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, Thermo Fisher Scientific, Waltham, MA, USA). We used Pearson's chi-square test with Yate's correction for continuity for small sample sizes to determine multiple pairwise differences in percent *Ehrlichia* spp. infection between years in both the adult and nymphal cohorts. Values

with $P < 0.05$ were deemed significantly different.

2.5. Deer capture and parasitizing tick burdens

In May and June 2018 and 2019, deer were captured opportunistically from a vehicle or ground blind over established bait sites using an explosive-injection dart rifle system (Pneu-Dart Inc., Williamsport, PA, USA) equipped with a 4-power scope. State of Connecticut Covid-19 travel restrictions precluded us from capturing animals in 2020. Darts contained a combination of 367 mg zolazepam hydrochloride and tiletamine hydrochloride (Telazol®, Fort Dodge Animal Health, Fort Dodge, IA, USA) and 220 mg xylazine hydrochloride (Rompun®, Bayer Corp., Pittsburgh, PA, USA). Darts also contained a transmitter for aid in locating sedated animals (Kilpatrick et al., 1996). Sedated deer were first blindfolded and Puralube® (Fougera, Melville, NY, USA) was applied to the eyes to prevent desiccation of the cornea.

Prior to ear-tagging animals, the tag was positioned adjacent to the back of each ear of each animal and photographed for scale. Thereafter, animals were ear-tagged and the effects of Rompun reversed with a 200-mg (≈ 3.0 mg/kg) injection of tolazoline hydrochloride (Tolazine®, Lloyd, Inc., Shenandoah, IA, USA). Contact names and phone numbers were clearly placed on ear tags to identify captured deer and advise against consumption by humans within the 60-day withdrawal period for immobilizing agents to be fully metabolized. Capture and handling efforts were carried out by staff of the Connecticut Department of Energy and Environmental Protection, Wildlife Division following guidelines set forth by Sikes (2016) and were previously approved by the Connecticut Agricultural Experiment Station's Institutional Animal Care and Use Committee.

Photographs of tick burdens and ear tags were downloaded onto a computer. For each image, the outline of the photographed ear tag was digitally traced and overlain on the image of the parasitizing ticks on the back of the deer's ears. Ticks found within the outline were counted and the outline repositioned as needed until all ticks were accounted for. We calculated the surface area of the physical ear tags and were able to determine the density of parasitizing nymphal and adult *A. americanum* /100 cm² combined to compare burdens between deer in both years. There were a few nymphal *I. scapularis* documented parasitizing deer during this time, but the vast majority ($> 99\%$) were adult and nymphal *A. americanum*. There were other deer captured and ear tagged as part of a separate deer movement study that were not included in the parasitizing *A. americanum* analysis, but were available to be photographed for deer density estimation.

2.6. Deer density estimation

Motion activated trail cameras (M-880 (Gen 2), Moultrie, Birmingham, AL, USA) were placed over each 4-poster device from 10 July – 17 August 2018 in an attempt to quantify the number of deer that utilized both the property and 4-poster devices. We used a modified version of the Jacobson et al. (1997) method for censusing white-tailed deer using trail cameras. Jacobson et al. (1997) called for using antler configuration to identify individual males in order to establish a population cohort of known abundance with which to compare frequency of photographs with the doe and fawn cohorts. The paucity of branch-antlered bucks on Manresa Island prevented us from using this technique, but we had a cohort of easily identifiable uniquely ear-tagged does. Within each photograph taken, we counted the number of bucks, tagged does, untagged does, and fawns and determined which individually ear-tagged does were photographed. We used the ratio of untagged:tagged does in photographs and the number of individually tagged does photographed to estimate the total doe population.

$$N_d = D + (D * (P_u / P_t))$$

N_d = estimated doe population

D = number of individually tagged does photographed

P_u = total number of untagged does in photographs

P_t = total number of tagged does in photographs

We then used the ratio of bucks:does photographed to determine the estimated number of bucks.

$$N_b = (P_b / (P_u + P_t)) * N_d$$

N_b = estimated buck population

P_b = total number of bucks in photographs

We then used the ratio of fawns:bucks photographed to estimate the number of fawns.

$$N_f = (P_f / P_b) * N_b$$

N_f = estimated fawn population

P_f = total number of fawns in photographs

3. Results

3.1. Permethrin treatment

In 2018, the combination of excessive precipitation, feeding by local residents, and the novelty of the 4-poster devices somewhat limited consumption by deer. Corn within one device grew mold, preventing deer from being able to feed from it for several weeks. Despite this initial setback, an average of nearly 50 kg of corn was consumed weekly site-wide in 2018. Throughout 2019 and 2020, conditions were drier, animals were accustomed to 4-poster presence, and devices performed well. On each weekly visit in the latter two years, the 22.7 kg of corn placed in each 4-poster device the previous week had been consumed, and on occasion, empty 4-posters were laying on their sides, due to frustrated animals knocking them over in search of more corn. For each year, devices were activated from May through the end of August. In both 2019 and 2020, 64 bags of corn (one bag/week/device for 16 weeks) were distributed and just over 3.0 L of tickicide used.

3.2. Host-seeking *A. americanum* densities

Mean daily adult *A. americanum* densities were square root transformed before being subjected to one-way ANOVA with the Holm-Sidak method used for multiple comparisons between years. Transformed data passed the Shapiro-Wilk normality test ($P = 0.814$) and Brown-Forsythe test for equal variance ($P = 0.065$). Significant differences existed in mean adult *A. americanum* densities by year ($F_{2,13} = 32.276$, $P < 0.001$); as suspected, mean density decreased significantly from 2018 ($\bar{X} = 23.8 \pm 3.1$ adults/100 m²) to 2019 ($\bar{X} = 6.4 \pm 1.7$ adults/100 m²; $P < 0.001$) and again from 2019–2020 ($\bar{X} = 1.7 \pm 0.4$ adults/100 m²; $P = 0.010$) (Fig. 2A).

Mean daily nymphal density data were not normally distributed; square root transformation was successful in normalizing data (Shapiro-Wilk, $P = 0.691$) but failed the Brown-Forsythe test for equal variance ($P < 0.05$). As a result, we used a one-tailed Welch's *t*-test (which does not assume equal variance) to compare mean daily host-seeking nymphal densities from 2018 to 2019 and 2019–2020 on square root transformed data. Host-seeking nymphal densities declined significantly from 2018 ($\bar{X} = 205.2 \pm 75.1$ nymphs/100 m²) to 2019 ($\bar{X} = 24.3 \pm 8.8$ nymphs/100 m²; $t_{4,6} = 2.271$, $P = 0.038$). However, densities from 2019 did not differ from 2020 densities ($\bar{X} = 16.7 \pm 5.3$ nymphs/100 m²; $t_{7,9} = 0.929$, $P = 0.190$) (Fig. 2B).

Mean daily larval *A. americanum* densities were natural log transformed before being subjected to a one-way ANOVA with the Holm-Sidak method used for multiple comparisons between years. Transformed data passed the Shapiro-Wilk normality test ($P = 0.447$) and Brown-Forsythe test for equal variance ($P = 0.802$). Significant differences existed in mean larval *A. americanum* densities by year ($F_{2,10} = 12.493$, $P = 0.002$); mean densities declined significantly from 2018 ($\bar{X}$

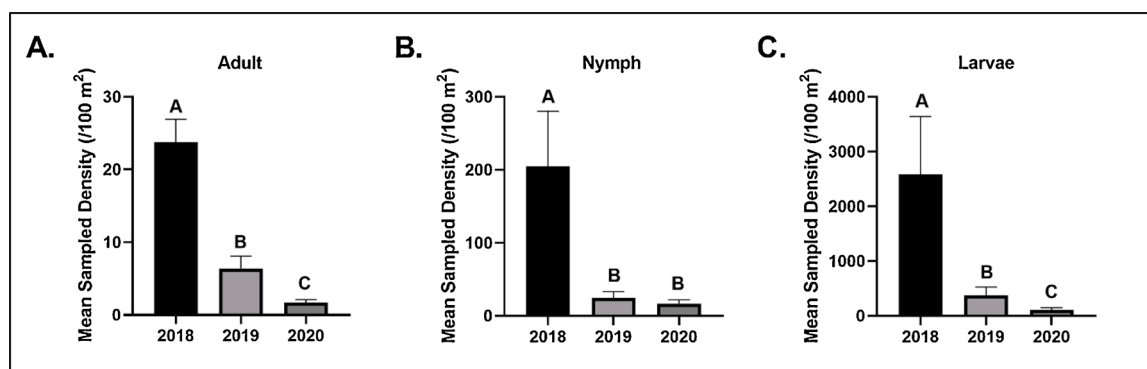


Fig. 2. Densities of host-seeking adult (A), nymphal (B), and larval (C) *Amblyomma americanum* on Manresa Island, Norwalk, Connecticut from 2018 to 2020. Means with differing letter assignments are significantly different within each life stage.

= 2583.9 ± 1056.7 larvae/100 m²) to 2019 ($\bar{X} = 368.7 \pm 154.8$ larvae/100 m²; $P = 0.016$) and again from 2019–2020 ($\bar{X} = 104.8 \pm 42.7$ larvae/100 m²; $P = 0.044$) (Fig. 2C).

3.3. *Ehrlichia* spp. screening in *A. americanum*

We detected *E. ewingii* in 19 of 629 individually screened *A. americanum* (3.0 %) and *E. chaffeensis* in 62 (9.9 %; Table 1). The cohort with the highest percent infection with both *Ehrlichia* spp. was in males from 2017 (55.1 %) and the lowest was in males in 2019 (0.0 %), the second year of 4-poster deployment (Table 1). The 2017 combined adult percent infection with *Ehrlichia* spp. (47.0 %) was significantly higher than both 2019 (2.3 %; $\chi^2 = 83.4$, $P < 0.0001$) and 2020 (7.1 %; $\chi^2 = 25.4$, $P < 0.0001$) though, 2019 did not differ significantly from 2020 ($\chi^2 = 2.3$, $P = 0.08$; Fig. 3A). Despite significant decreases in abundance, nymphal infection prevalence remained static and did not differ from 2017 (8.7 %) to 2019 (8.3 %; $\chi^2 = 0.008$, $P = 1.0$) or from 2019–2020 (9.4 %; $\chi^2 = 0.063$, $P = 1.0$) (Fig. 3B).

3.4. Deer capture and parasitizing tick burdens

In 2018, 11 deer (8 does, 3 bucks) were included in the parasitizing *A. americanum* analysis and 9 does in 2019. In 2018, parasitizing, engorged adult and nymphal *A. americanum* burdens were so severe on some animals, that their weight caused ears to droop forward and down instead of their usual erect posture (Fig. 4). Mean parasitizing

A. americanum density (/100 cm² of deer ear) data were not normal and were not able to be successfully transformed. As a result, we ran a Mann-Whitney sum test on ranks with Yates continuity correction applied to determine differences between the two years. Combined parasitizing adult and nymphal *A. americanum* densities were significantly reduced from 2018 ($\bar{X} = 708.5 \pm 219.5$ ticks/100 cm²) to 2019 ($\bar{X} = 95.5 \pm 19.2$ ticks/100 cm²; Mann-Whitney $U = 80.5$, $P = 0.002$) (Fig. 5).

3.5. Deer density estimation

Over 4000 camera images were analyzed and 9 unique ear-tagged does (D) were positively identified in photographs. Tagged bucks ($n = 3$) were photographed infrequently and were such an insignificant contribution to this analysis, we proceeded as if all bucks were not tagged. A total of 142 instances of untagged does (P_u) appeared in photographs and 189 tagged does (P_t), resulting in an estimated doe population (N_d) of 15.8 ($9 + 9(142/189) = 15.8$). There were also a total of 142 instances in which bucks (P_b) were photographed which resulted in an estimated buck population (N_b) of 6.8 ($142/(142 + 189) * 15.8 = 6.8$). There were a total of 54 instances in which fawns (P_f) were photographed, resulting in an estimated fawn population (N_f) of 2.6 ($(54/142) * 6.8 = 2.6$). As a result, we rounded decimals up to the nearest whole number and estimated there were ~ 26 deer (16 does, 7 bucks, 3 fawns) utilizing 4-posters. There were seven additional individuals (6 does, 1 buck) that had been previously captured and ear tagged on site that were never photographed during this interval. We then determined there were minimally 33 deer utilizing the 50.6 ha site in 2018. While our 2018 imagery data revealed a poor fawn:doe ratio (1:6), in our estimation, the deer population remained stable in 2019 and again in 2020.

4. Discussion

We documented dramatic and significant declines in all motile stages of host-seeking *A. americanum* as well as parasitizing nymphs and adults after three years of 4-poster deployment. Similar dramatic declines in all stages of *A. americanum* using 4-poster devices were reported using 2% Amitraz in Maryland (Carroll et al., 2009) and New Jersey (Schulze et al., 2009) as part of the larger NATCP (Pound et al., 2009b) as well as using 10 % permethrin in New York (Curtis et al., 2011). Both Carroll et al. (2009) and Pound et al. (2009b) suggested that deer pelage is thinner in warm summer months when *A. americanum* are active, allowing for greater coverage of and dermal exposure to topical acaricides, hereby increasing effectiveness as compared to colder months when *I. scapularis* are feeding on animals with heavy winter coats. While this may be true, we think there is also an ecological explanation regarding the increased effectiveness of 4-posters on *A. americanum* at the northern reaches of their range.

Table 1

Number of female, male, and nymphal host-seeking *Amblyomma americanum* tested (# Tested), positive for *Ehrlichia chaffeensis*, positive for *Ehrlichia ewingii*, percent positive for each and combined (Total %) at time of discovery of the tick population (2017*) and during the second (2019) and third year (2020) of 4-poster deployment.

Year	Stage	# Tested	# Infected (%)		Total %
			<i>E. chaffeensis</i>	<i>E. ewingii</i>	
2017*	Nymphs	104	1 (1.0)	8 (7.7)	8.7 %
	Adult	100	44 (44.0)	3 (3.0)	47.0 %
	Female	51	18 (35.3)	2 (3.9)	39.2 %
	Male	49	26 (53.1)	1 (2.0)	55.1 %
	Nymphs	96	7 (7.3)	1 (1.0)	8.3 %
2019	Adults	177	1 (0.6)	3 (1.7)	2.3 %
	Female	96	1 (1.0)	3 (3.1)	4.2 %
	Male	81	0 (0.0)	0 (0.0)	0.0 %
	Nymphs	96	6 (6.3)	3 (3.1)	9.4 %
	Adults	56	3 (5.4)	1 (1.8)	7.1 %
2020	Female	28	1 (3.6)	0 (0.0)	3.6 %
	Male	28	2 (7.1)	1 (3.6)	10.7 %
	Totals	629	62	19	12.9 %

* 2017 *Ehrlichia* spp. infection results were previously reported in Stafford et al. (2018).

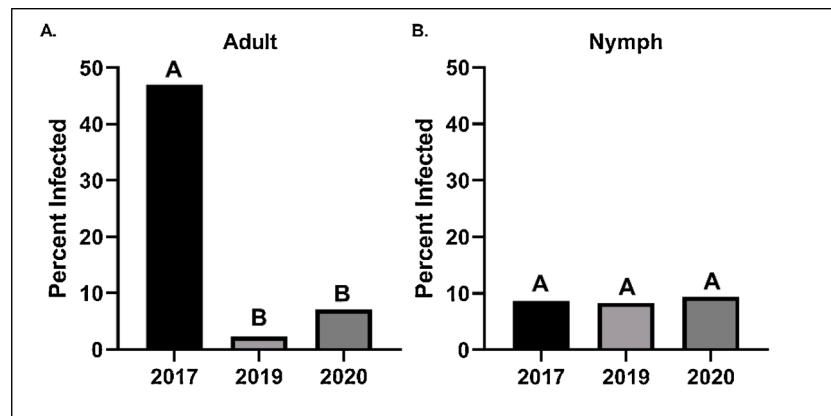


Fig. 3. Combined percent infection with both *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in host-seeking adult (A) and nymphal (B) *Amblyomma americanum* on Manresa Island, Norwalk, Connecticut at time of discovery of the tick population (2017) and in the second (2019) and third year (2020) of 4-poster deployment. Means with differing letter assignments are significantly different within each life stage.



Fig. 4. Varying parasitizing adult and nymphal *Amblyomma americanum* densities on the ears of captured white-tailed deer on Manresa Island, Norwalk, Connecticut when 4-posters were novel and had been only recently activated in May – June 2018.

While *I. scapularis* are cold-hardy and research has shown they can withstand winter temperatures with proper insulative barriers (Linske et al., 2019), *A. americanum* have been shown to be cold intolerant with prolonged exposure to subfreezing temperatures resulting in increased mortality, regardless of insulation (Needham et al., 1996; Linske et al., 2020). In their southeastern range where cold temperatures are not as limiting a factor, a single year or less is required for *A. americanum* to complete their life cycle, but two or three years may be needed in Maryland and further north where subfreezing temperatures are not uncommon (Pound et al., 2009b). In Georgia, adult *A. americanum* are active April to June and nymphs have a bimodal activity distribution in May through July and August to September (Semtner and Hair, 1973). In southern New England, to accommodate periods of dormancy in cold weather, adult and nymphal *A. americanum* are most active contemporaneously (May – June; Fig. 6) and synchronously parasitize their single most abundant and largest host, white-tailed deer. The fact that latter life stages of *A. americanum* prefer to parasitize white-tailed deer (Allan

et al., 2010) and that more than half of adult females and 97 % of nymphs can be found attached to the inner and outer ear (Bloemer et al., 1988) makes them particularly vulnerable to topical acaricide treatment via 4-poster, even in areas of extreme infestation.

Pound et al. (2009b) and Carroll et al. (2009) reported dramatic reductions in nymphs in the first year of treatment just as we documented a similar dramatic reduction in host-seeking nymphs after the first treatment year on Manresa Island (Fig. 2B). Because the host (deer) and feeding site (ears) are so specific in nymphal *A. americanum*, 4-posters can apply acaricide directly to the ticks themselves instead of relying on deer to groom product throughout the rest of their body as is the case in topical treatment of *I. scapularis*. This coupled with the fact that two stages of this tick are being targeted simultaneously by 4-posters lends itself to rapid reduction in parasitizing and as a result, host-seeking *A. americanum* abundances. Despite an 87 % reduction in density of parasitizing *A. americanum* from 2018 to 2019, mean parasitizing tick burdens were still fairly high. Unfortunately, we were not able to

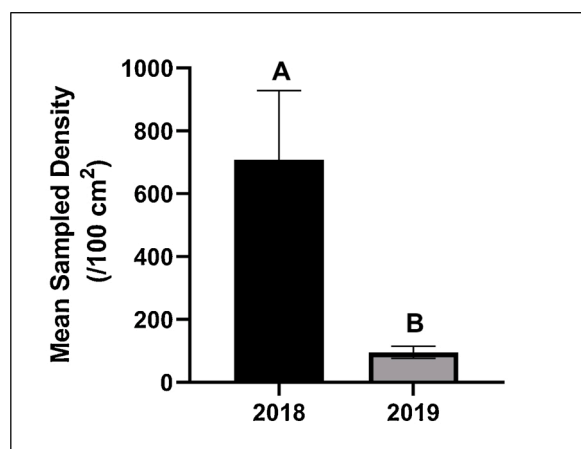


Fig. 5. Mean densities of adult and nymphal *Amblyomma americanum* parasitizing the ears of white-tailed deer captured on Manresa Island, Norwalk, Connecticut May – June 2018 and 2019. Means with differing letter assignments are significantly different.

capture deer in spring 2020 due to restrictions on travel because of Covid-19, but we would have expected to see a comparable reduction in mean parasitizing *A. americanum* density in the third year of 4-poster deployment, consistent with reductions witnessed in all stages of host-seeking ticks.

Because of the relatively small area of the site, placing only four devices resulted in the high-density distribution of one device/12.7 ha. The 4-poster manufacturer (CR Daniels, Inc.) recommend minimally one device/16 ha and as few as one/28–40 ha in some situations. Williams and DeNicola (2000) suggested one device/50–60 ha was sufficient in a study in Old Lyme, CT though Solberg et al. (2003) and Schulze et al. (2007) recommended one device/20 ha. Obviously, 4-poster distribution is not directly tied to land area, but rather to the number of deer within the proposed treatment area; 4-poster device deployment density needs to account for overall deer density in order to effectively treat a significant portion of the population. In our case, using cameras placed over 4-poster devices, we estimated there were 33 deer on site resulting in a deployment density of minimally one device/8.25 deer. By comparison, in a four-year study in Fairfax County, VA, 20 4-posters were distributed over 1780 ha of parkland (one device/89 ha) with an estimated 821 deer (one device/41 deer; Fairfax County, VA Publication 2016). Not surprisingly, there were no differences in burdens of parasitizing *I. scapularis* or *A. americanum* on harvested white-tailed deer between treated and control sites and no difference in host-seeking

A. americanum adult and nymphal abundances (Fairfax County, VA Publication 2016). We feel that our 4-poster density coupled with our purposeful limited feeding strategy resulted in the repeated treatment of the majority, if not all, deer utilizing the property.

The high-density deployment of 4-poster devices was likely a major key to our success. But to avoid bait site dominance issues that have been reported in the past and to entice curious subordinate deer to be treated in an attempt to deliver permethrin to the majority of the population, we purposefully limited whole corn volume in devices. By adding only 22.7 kg at a time, we surmised that dominant animals would consume the majority of the corn and be treated with permethrin while educating subordinate animals of corn presence. Once corn was largely evacuated after a day or two, obsession with the feeder by dominant animals would wane and we suspect curious subordinate (and dominant) animals would periodically investigate the devices for the presence of corn and get dosed with permethrin in doing so. This behavior cycle would repeat throughout the duration of the peak adult and nymphal *A. americanum* activity as corn was added weekly. Corn within devices was meant as an attractant to entice deer to be treated with permethrin periodically, not to provide them a daily food source with daily acaricide treatment. We did not quantify this feeding strategy but suspect this is in part why we had such success.

Despite the lack of a control site for comparison, we feel it is clear that our 4-poster management strategy had a devastating impact on both parasitizing and host-seeking *A. americanum* adults and nymphs beyond naturally occurring annual fluctuations. The void we created in these two cohorts is what likely resulted in a significant decrease in questing larvae over time. Additionally, we documented significant and dramatic decreases in *Ehrlichia* spp. infection in adult *A. americanum* from 2017 to the third year of 4-poster deployment. This is in stark contrast to Harmon et al. (2011) who deployed 4-posters with 10 % permethrin in a community in Tennessee for the specific purpose of controlling *A. americanum* in the wake of an ehrlichiosis outbreak in residents. While they documented some control of *A. americanum* in the vicinity of devices, they concluded that low density 4-poster distribution had “... little large-scale effect on the health risk posed by ticks in this community” (Harmon et al., 2011). It should be noted that Harmon et al. (2011) deployed eight 4-poster devices on a 52.6 km² property, resulting in the equivalent density of one for every 657.5 ha. As a result, Harmon et al. (2015) also concluded that low density 4-poster deployment was ineffective in controlling *Ehrlichia* infection in adult and nymphal *A. americanum*. While deer density was not reported, it is very likely that this low-density deployment resulted in one 4-poster device/100 or even several hundred deer. For an effective tick management program to be successful, managers will need to have a relative estimate of deer densities prior to distributing 4-posters. Thereafter, utilizing trail cameras at

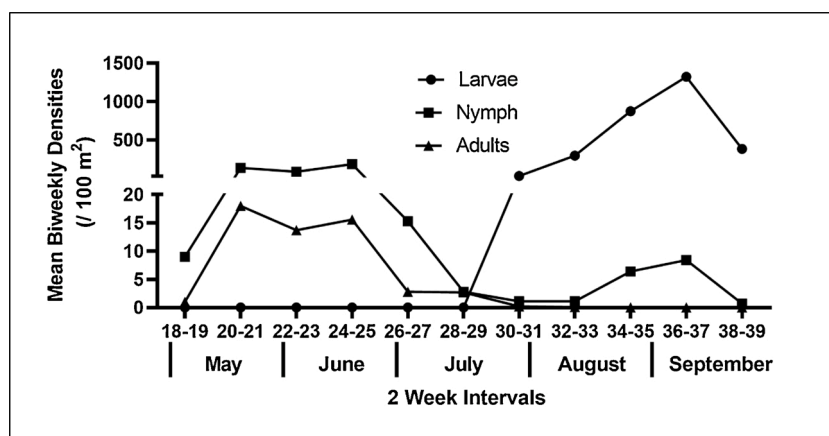


Fig. 6. Mean biweekly (to reduce variability) sampled *Amblyomma americanum* densities (/100 m²) by stage for 2018 – 2020 on Manresa Island, Norwalk, Connecticut.

active 4-posters could further evaluate deer density estimates to determine if deployment density is adequate.

While we had success in reducing *Ehrlichia* spp. infection in adult *A. americanum*, infection in nymphs remained remarkably consistent and unchanged from population discovery through the latter two years of 4-poster deployment (Fig. 3B). We deduced that it was unlikely these nymphs obtained their initial larval bloodmeals from permethrin-treated deer as they were still alive and actively host-seeking. As a result, we suspected larvae were feeding on and obtaining *Ehrlichia* spp. from alternate hosts. In Brazil, blood samples were taken from various species of birds with *E. chaffeensis* detected using molecular techniques, leading researchers to conclude that wild birds are likely competent reservoirs (Machado et al., 2012). Additionally, in bloodmeal analysis of 19 *Ehrlichia*-infected host-seeking *A. americanum*, Harmon et al. (2015) determined that four had fed upon wild turkey and four on other bird species. In addition, through DNA analysis of adult and nymphal *A. americanum*, Harmon et al. (2015) reported that wild turkey provided more than double the number bloodmeals than did white-tailed deer. Wild turkey are frequently reported hosts for immature stages of *A. americanum* (Allan et al., 2010; Scott et al., 2014; Hertz et al., 2017) and were the other, dominant, relatively large-bodied reservoir host on Manresa Island. This led us to conclude that larvae likely consistently parasitized wild turkey and had the opportunity to obtain both species of *Ehrlichia* during their first bloodmeal which remained unchanged over the three years, despite 4-poster activation. While we routinely documented wild turkey feeding from 4-poster devices, their heads and necks were slender enough to access corn within the troughs without contacting permethrin-soaked rollers.

5. Conclusions

The overall reduction in all motile stages of *A. americanum* in conjunction with the reduction in *Ehrlichia* spp. presence in adults are indications of the successful management of this recently established population. We determined that a 4-poster distribution regime that accommodates not only land area but also density of white-tailed deer can effectively reduce tick abundances and associated *Ehrlichia* spp. infection. Additionally, the purposeful limiting of bait in order to accommodate herd feeding behavior likely treated the majority of animals that play a key role in the *A. americanum* life cycle. However, in future efforts to further reduce the presence of *A. americanum* and infection with *Ehrlichia* spp., a multi-species approach that targets white-tailed deer as well as the wild turkey/avian reservoir would be required. This case study on the reduction of a recently discovered *A. americanum* infestation with 4-poster devices was successful and can be used as a reference for future management strategies. Managers should understand that scaled-up attempts to manage large herds of white-tailed deer with 4-posters would require one device for approximately every 8–12 animals, regardless of land area, and we strongly suspect that the purposeful limiting of corn would dose a higher proportion of deer with permethrin.

Declaration of Competing Interest

The authors report no declarations competing of interest.

Acknowledgments

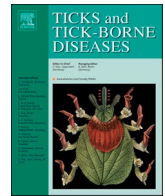
We thank NRG Energy, Inc. for permitting us access to their property and P. Bukowsky and J. Ferrara for facilitating that access. Thanks to B. Embacher and J. Kilburn for deer capture, camera maintenance, imagery analysis, and 4-poster maintenance, M. Short for 4-poster transport and maintenance, tick sampling, and imagery analysis, and S. Karpathy and C. Paddock of the Centers for Disease Control and Prevention for *Ehrlichia* detection in the 2017 samples. We also thank J. Cantoni and seasonal assistants B. Willett, M. DeNicola, and H. Badey for tick sampling.

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Original article

Orally delivered fipronil-laced bait reduces juvenile blacklegged tick (*Ixodes scapularis*) burdens on wild white-footed mice (*Peromyscus leucopus*)Scott C. Williams^{a,b,*}, Megan A. Linske^{a,c}, Kirby C. Stafford III^{a,c}^a Center for Vector Biology & Zoonotic Diseases, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06511, USA^b Department of Environmental Science and Forestry, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06511, USA^c Department of Entomology, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06511, USA

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ABSTRACT

While the topical acaricidal treatment of rodent pathogen reservoirs has been readily explored over the past several decades, oral systemic acaricidal treatment is only recently gaining traction as an alternative approach to the management of ticks and tick-borne pathogens. Recent laboratory tests have shown promise in the effectiveness of this systemic strategy against the blacklegged tick (*Ixodes scapularis*) and a Canadian field evaluation was recently published, but no recent field data from the United States yet exist. With this research, we sought to field deploy a commercially available fipronil-laced bait (Kapat® Flea Control Bait, Scimetrics LLC., Wellington, CO, USA; 0.005% fipronil; Environmental Protection Agency Reg. No. 72500-28), in an alternate use targeting white-footed mice (*Peromyscus leucopus*) to determine bait acceptance and potential impacts to juvenile *I. scapularis* burdens. Bait was readily accepted by wild *P. leucopus* and other rodent reservoirs. An *ad libitum* distribution strategy as well as placing smaller volumes of fipronil-laced bait within individual Sherman traps both resulted in significant reductions (57–94%) in juvenile *I. scapularis* burdens as compared to control over two years. The oral delivery of systemic acaricides shows promise in reduction of *I. scapularis* burdens on *P. leucopus* and should be further explored to determine effectiveness on host-seeking tick abundances, associated pathogen infection, and potentially incorporated into integrated tick management programs.

1. Introduction

The causative agents of Lyme disease and other tick-borne diseases persist in our environment and reported human cases are only increasing in frequency. Due in part to a warming climate, tick species are not only expanding their ranges, but are increasing in abundance within current ranges which combined, will result in continued increases in human illness (Sonenshine, 2018). Because of these factors and risk to the health of the public, it is clear that human intervention is needed to break pathogen transmission cycles and decrease overall tick abundances (Eisen and Stafford, 2021). Broadcast applications of biopesticides and synthetic pyrethroids can be effective in reducing *Ixodes scapularis* densities, but challenges exist regarding the real or perceived concerns with overapplication of product and potential deleterious impacts to the environment and/or beneficial insects (Maund et al., 2012; Elias et al., 2013; Fishchhoff et al., 2017; Schulze and Jordan, 2021). In contrast, killing ticks on hosts (host-targeted) approaches use reduced

volumes of acaricide with decreased likelihood of negatively impacting non-target species. Such host-targeted strategies are a more directed approach which require less product due to the feeding biology of *I. scapularis*; various life stages are concentrated on known host species during known times of year (Dolan et al., 2004). It is during these known times that parasitizing tick species can be effectively targeted.

However, host-targeted treatments are best utilized in an integrated strategy which is the preferred method of tick management that targets multiple tick stages, behaviors, and hosts simultaneously (Stafford et al., 2017; Little et al., 2020; Mandli et al., 2021; Linske et al., 2022). Individual methods within an integrated tick management (ITM) strategy can include spraying of synthetic acaricides (Hinckley et al., 2016; Bron et al., 2021; Bickerton et al., 2022), biopesticides (Williams et al., 2018a, 2018b; Dyer et al., 2021; Schulze and Jordan, 2021), or natural products (Bissinger et al., 2014; Machtinger and Li, 2017; Klafke et al., 2021) to combat various species and stages of host-seeking ticks. Vegetation and/or host management can be used within an ITM framework as well

* Corresponding author at: Department of Environmental Science and Forestry, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06511, USA.

E-mail addresses: scott.williams@ct.gov (S.C. Williams), megan.linske@ct.gov (M.A. Linske), kirby.stafford@ct.gov (K.C. Stafford).

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to reduce ideal habitats and bloodmeal acquisition success (Telford, 2017; Williams et al., 2017; Williams et al., 2018a, 2018b). Partially due to politics that can often accompany and limit effective host reduction (specifically white-tailed deer, *Odocoileus virginianus*), host-targeted strategies have become a feasible and accepted strategy for tick management (Williams et al., 2018b). Because of the familiarity with feeding ecologies of various tick species, it is known when many will be parasitizing reproductive hosts to be effectively targeted. For instance, in New England, we know a significant proportion of female *I. scapularis* can be found feeding on *O. virginianus* in the autumn months (Stafford, 2007). In the mid-late summer months, white-footed mice (*Peromyscus leucopus*) are infested with numerous larval *I. scapularis* (Stafford, 2007). Because of this knowledge, these feeding ticks are particularly vulnerable to host-targeted abatement strategies.

To date, the majority of approved host-targeted work has included topical application of acaricides to *O. virginianus* (Stafford and Williams, 2017) and small rodents (Hinckley et al., 2021; Keesing et al., 2022). The 4-poster (C. R. Daniels, Inc., Ellicott City, MD, USA) is a device that uses whole corn to entice *O. virginianus* to self-apply the topical acaricide permethrin to head and neck via saturated paint rollers that are proximate to a feeding trough. The 4-poster has been used successfully to manage ticks for decades (Pound et al., 2000; Schulze et al., 2007; Williams et al., 2021), but positive results are labor intensive, expensive, and can introduce hundreds of thousands of calories into the host population, potentially increasing fitness and fecundity (Edwards et al., 2016; Burtis et al., 2021). A similar strategy on a smaller scale, is the use of fipronil-based rodent-targeted bait boxes (Select TCS Tick Control System, Connecticut Tick Control, LLC., Norwalk, CT, USA) (Schulze et al., 2007; Williams et al., 2018a, 2018b; Linske et al., 2022). These boxes utilize a non-toxic bait to attract small rodents and when they access bait within, are forced to contact a fipronil-saturated wick. Similar successes have been witnessed using fipronil-based rodent-targeted bait boxes, but they too are expensive and can be labor intensive depending on scale (Dolan et al., 2004; Schulze et al., 2017; Jordan and Schulze, 2019). Both strategies are somewhat cumbersome in that they utilize an attractant to ensure the animal makes contact with topical acaricides. A more pragmatic approach would be to lace bait with acaricide and directly treat hosts systemically with its consumption.

The bacterium *Yersinia pestis* (the causal agent of plague) is vectored by fleas from its small rodent reservoir and is transmissible to humans and other mammal species. Human cases of plague still do occur annually in the United States, but incidents pale in comparison to mortalities from previous centuries (Perry and Fetherston, 1997). However, plague can be devastating to populations of black-tailed prairie dogs (*Cynomys ludovicianus*; Stapp et al., 2004; Pauli et al., 2006) which are a crucial prey item for the critically endangered black-footed ferret (*Mustela nigripes*; Matchett et al., 2010; Eads and Biggins, 2019). As a result, there has been experimental research investigating the orally delivered systemic acaricide treatment of *C. ludovicianus* against fleas to reduce incidence of plague in their populations to benefit *M. nigripes* survival (Borchert et al., 2009; Biggins et al., 2010; Poché et al., 2017, 2020). While this line of research has been ongoing for decades in the western United States, only recently has the same systemic acaricidal strategy been investigated for management of *I. scapularis* on their small rodent hosts in eastern North America.

Under laboratory conditions, Pelletier et al. (2020) systemically dosed lab-reared deer mice (*Peromyscus maniculatus*) orally with 50 mg/kg and 12.5 mg/kg fluralaner resulting in 97% and 94% mortality of parasitizing larval *I. scapularis*, respectively, two days post treatment. Also under laboratory conditions, Poché et al. (2020) were able to successfully deliver an oral bait containing 0.005% fipronil to lab-reared *P. leucopus* resulting in 100% mortality of parasitizing larval *I. scapularis* 15 days after 48-hours exposure to treated bait. Also, in the laboratory under simulated field conditions, Poché et al. (2021) documented excellent control of parasitizing larval *I. scapularis* on lab-reared *P. leucopus* systemically treated with 0.005% fipronil, with up to 89%

mortality within 15 days of initial exposure to treated bait. Then recently, Pelletier et al. (2022) fed an experimental fluralaner-laced bait to wild *Peromyscus* mice in southern Quebec, Canada resulting in an 86% reduction in parasitizing larval and 72% reduction in parasitizing nymphal *I. scapularis* at their highest bait deployment density.

The purpose of our study was to determine if we could successfully deliver a commercially available fipronil-laced bait to wild *P. leucopus* in a Lyme disease endemic area of southern Connecticut and determine impacts of systemic acaricidal treatment to parasitizing juvenile *I. scapularis* burdens on treated animals. While we experimented with two different delivery strategies (*ad libitum* in bait boxes and smaller quantities in individual Sherman traps), we suspected that *P. leucopus* in the *ad libitum* feeding strategy locations would present significantly fewer parasitizing juvenile *I. scapularis* as compared to untreated, control areas.

2. Materials and methods

2.1. Study site

The study site was a privately-owned forested site in the Town of North Branford, New Haven County, Connecticut, USA. The property is heavily forested and is closed to the public. Overstory mature trees consist of oak (*Quercus* spp.), hickory (*Carya* spp.), sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), tulip poplar (*Liriodendron tulipifera*), declining white ash (*Fraxinus americana*) and eastern hemlock (*Tsuga canadensis*). Understory consists largely of invasives such as Japanese barberry (*Berberis thunbergii*), wine raspberry (*Rubus phoenicolasius*), multiflora rose (*Rosa multiflora*), and Japanese honeysuckle (*Lonicera japonica*).

2.2. Systemic fipronil treatment delivery

We obtained Kaput® Flea Control Bait (Scimetrics LLC., Wellington, CO, USA; 0.005% fipronil; Environmental Protection Agency Reg. No. 72500-28), which is commercially available and originally formulated to treat *C. ludovicianus* for fleas as described previously. The product consists of a grain treated with fipronil and also coated with a reddish dye to make it more visible to applicators. We established five (~0.25 ha) locations on the property (Dam, Icehouse, Rose's Brook, Sea Hill, Tommy Top) each > 1.5 km apart. At Icehouse and Sea Hill, we placed out four plastic bait boxes (Protecta Sidekick, Bell Laboratories Inc. Madison, WI, USA) that were accessible to small rodents such as *P. leucopus* and eastern chipmunk (*Tamias striatus*). The four bait boxes (bait box) were placed in a square configuration at approximately 30.0 m spacing and staked to the ground or affixed to tree saplings to prevent relocation by non-target wildlife. In late July 2021 and 2022 (corresponding with the beginning of larval *I. scapularis* activity in the region), we placed 200.0 g Kaput, which contained 10.0 mg fipronil, in each bait box and refreshed weekly through early/mid-September. We placed out Kaput at Icehouse and Sea Hill one week prior to the first trapping event in both years to provide animals time to learn bait site locations. At the Dam and Tommy Top treatment locations, we placed ~ 5.0 g of Kaput, which contained ~ 0.25 mg fipronil, in each Sherman live trap during rodent capture events (in trap) only, similar in strategy to Richer et al. (2014) and to be described below. Rose's Brook served as our control location where small rodents were trapped and sampled only with no treatment.

2.3. Rodent capture and tick sampling

At each of the five locations we established 6 × 6 trapping grids on 10.0-m spacing (50.0 × 50.0 m) which completely enveloped the square bait station configurations at Icehouse and Sea Hill. We sought to capture and sample small rodents weekly throughout the larval *I. scapularis* activity season both in 2021 and 2022, during which time, all five

locations were sampled contemporaneously. We placed a small volume of peanut butter at the rear of each Sherman trap prior to their deployment in the afternoon and gathered and processed captured *P. leucopus* the following morning. At the Dam and Tommy Top, we placed ~ 5.0 g Kaput in each Sherman trap as well and rodents were first exposed to fipronil at first capture. All captured *P. leucopus* were anesthetized using isoflurane in the open drop method in a plastic bag. Sedated *P. leucopus* were removed from the bag after which time they were sexed, mass determined, ear tagged (Style 1005-1; National Band and Tag Co., Newport, KY, USA), and the number of *I. scapularis* parasitizing the ears, nose, face, and chin were quantified.

Due to the fact that parasitizing ticks needed to remain on hosts to quantify treatment effectiveness over time, and based on decades of experience on the site known to be dominated by *I. scapularis*, and having previously handled upwards of 10,000 infested *P. leucopus* throughout Connecticut, it was assumed all parasitizing ticks were in fact, *I. scapularis*. Given that August is peak larval *I. scapularis* activity season in our region, it was also assumed that the vast majority of parasitizing *I. scapularis* were larvae, but we could not rule out the possibility of a parasitizing nymph or two being present, so refer to them collectively throughout the document as “juvenile *I. scapularis*.” Additionally, no anomalous parasitizing larval specimens, which would suggest other species were present aside from *I. scapularis*, were documented during the two-year study.

Partially sedated *P. leucopus* were placed back into individual Sherman traps and allowed to fully recover from the effects of isoflurane before being released to their original capture location. This trapping and handling protocol was permitted by the Connecticut Department of Energy and Environmental Protection, Wildlife Division (#2124002a) and was approved by the Connecticut Agricultural Experiment Station's Institutional Animal Care and Use Committee (P35-21) in adherence with guidelines set forth in Sikes (2016).

2.4. Data analysis

Parasitizing juvenile *I. scapularis* burdens for all individual *P. leucopus* captures (including recaptures) were analyzed using Kruskal-Wallis one way analysis of variance on ranks for both 2021 and 2022 separately due to the fact both data sets failed tests for normality and were not able to be successfully transformed. Dunn's method was used for multiple comparisons of means between treatments.

3. Results

3.1. Kaput distribution

After the initial distribution in 2021, on each occasion when we refreshed the 200.0 g of Kaput in each of the four bait box locations at both Icehouse and Sea Hill, the previous week's distribution had been fully consumed. We refilled bait stations on seven occasions in each year through the larval *I. scapularis* season, resulting in 1,400.0 g Kaput/bait box, or 0.07 g fipronil/box/year. Due to the presence of droppings, it was evident that boxes were being utilized by small rodents, but by the presence of tracks and prints left on boxes, it was obvious product was also being removed and likely consumed by raccoons (*Procyon lotor*) as well. It was clear that individual *P. leucopus* captured in Sherman traps containing Kaput had consumed at least some of the product contained within.

3.2. Rodent capture

Each of the five treatment locations were trapped on six occasions in 2021 (n = 1,080 trap nights) and on four occasions in 2022 (n = 720 trap nights). The 2021 effort resulted in the capture of 46 individual *P. leucopus* (14 females and 32 males) on 135 occasions (12% trap success); 19 individuals were captured once, six captured twice, three

captured thrice, five captured four times, four captured five times, and nine captured on all six occasions. One individual was recaptured and it was clear it had been tagged previously, but the tag had been dislodged. There were no other non-target species of any kind captured in 2021; 100% of captures were *P. leucopus*. In 2022, we had 239 *P. leucopus* captures resulting in 33% capture success. Due to time constraints and an overabundance of captures, we processed no more than 10 randomly selected captured *P. leucopus*/treatment location/capture event, resulting in the release of 53 captures unprocessed. As a result, we processed a subsample of 83 unique *P. leucopus* (41 females and 42 males) on 186 different occasions; 29 were captured once, 24 captured twice, 14 captured thrice, and 16 captured on all four occasions (Table 1). Two unique *P. leucopus* were recaptured and it was clear their ear tags had come out and third's tag was unreadable. In addition to the 239 *P. leucopus* captures, we captured and released three northern short-tailed shrews (*Blarina brevicauda*) in 2022.

3.3. Parasitizing juvenile *Ixodes scapularis*

We witnessed higher overall tick burdens on captured *P. leucopus* in 2021 ($\bar{X} = 5.0 \pm 0.66$ (standard error of the mean)) than in 2022 ($\bar{X} = 1.9 \pm 0.26$); treatment of parasitizing larvae in 2021 would have had no effect on 2022 larval burdens. In 2021, we documented a total of 671 parasitizing juvenile *I. scapularis* on the 135 captures, which resulted in a mean of 12.3 (± 2.0)/capture in the control site, 4.7 (± 1.3)/capture where Kaput was placed in the traps, and 2.5 (± 0.5)/capture in the bait box sites. Kruskal-Wallis ANOVA on Ranks determined that there were significant differences between treatments ($H_2 = 36.15$, $P < 0.001$). Dunn's Method for multiple comparisons between means determined mean parasitizing juvenile *I. scapularis* burdens were significantly lower (62% reduction) in the in-trap sites as compared to the control site ($Q = 2.89$, $P = 0.011$) and also lower (80% reduction) in the bait box sites ($Q = 5.69$, $P < 0.001$) as compared to control. No significant differences were detected between in-trap and bait box sites ($Q = 1.78$, $P = 0.223$), though, the in-trap tick burdens declined by more than 80% after the second trapping round as opposed to the bait box plots which declined by 97% after the initial trapping round (Fig. 1).

In 2022, we documented a total of 346 parasitizing juvenile *I. scapularis* on the 186 captures, which resulted in a mean of 4.7 (± 0.71)/capture in the control site, 2.0 (± 0.45)/capture where Kaput was placed in the traps (57% reduction), and 0.3 (± 0.10)/capture in the bait box sites (94% reduction). Kruskal-Wallis ANOVA on Ranks determined that there were significant differences between treatments ($H_2 = 56.09$, $P < 0.001$). Dunn's Method for multiple comparisons between means determined mean juvenile *I. scapularis* burdens were significantly lower on *P. leucopus* in the in-trap sites as compared to control ($Q = 3.70$, $P < 0.001$) only and bait box sites were significantly lower than both the control site ($Q = 6.64$, $P < 0.001$) and the in-trap sites ($Q = 3.34$, $P = 0.003$). Mean juvenile *I. scapularis* burdens at bait box sites started out very low and stayed at or below 0.37 ticks/capture throughout the season (Fig. 2). Mean juvenile *I. scapularis* burdens on *P. leucopus* in the in-trap sites started out comparable to the control site, then again decreased after the second trapping round in 2022 (Fig. 2).

4. Discussion

This was an exploratory study that repurposed an existing commercially available, orally delivered systemic acaricide formulation for use in combating *I. scapularis* parasitizing *P. leucopus*. Specifically, we sought to determine if *P. leucopus* would consume the fipronil-based product and what impact, if any, its consumption would have on *I. scapularis* burdens. While the topical treatment of rodents using fipronil-based rodent-targeted bait boxes has received much scrutiny with varying degrees of success in reducing *I. scapularis* abundances over the past several decades (Dolan et al., 2004; Schulze et al., 2007; Schulze et al.,

Table 1

Treatment assignments, location, number of trap nights, unique number of female and male white-footed mice (*Peromyscus leucopus*) captured, and total number of mouse captures (percent infested with at least one juvenile *Ixodes scapularis*) processed for 2021 and 2022.

Treatment	Location	2021				2022			
		Trap Nights	Female	Male	Captures Processed (% Infested)	Trap Nights	Female	Male	Captures Processed (% Infested)
Bait Box	Icehouse	216	3	8	29 (34)	144	10	9	38 (3)
Bait Box	Sea Hill	216	6	8	54 (41)	144	10	10	40 (28)
Control	Rose's Brook	216	3	10	29 (93)	144	8	7	40 (80)
In Trap	Dam	216	1	3	14 (57)	144	8	9	34 (62)
In Trap	Tommy Top	216	1	3	9 (67)	144	5	7	34 (32)
Totals		1,080	14	32	135 (54)	720	41	42	186 (41)

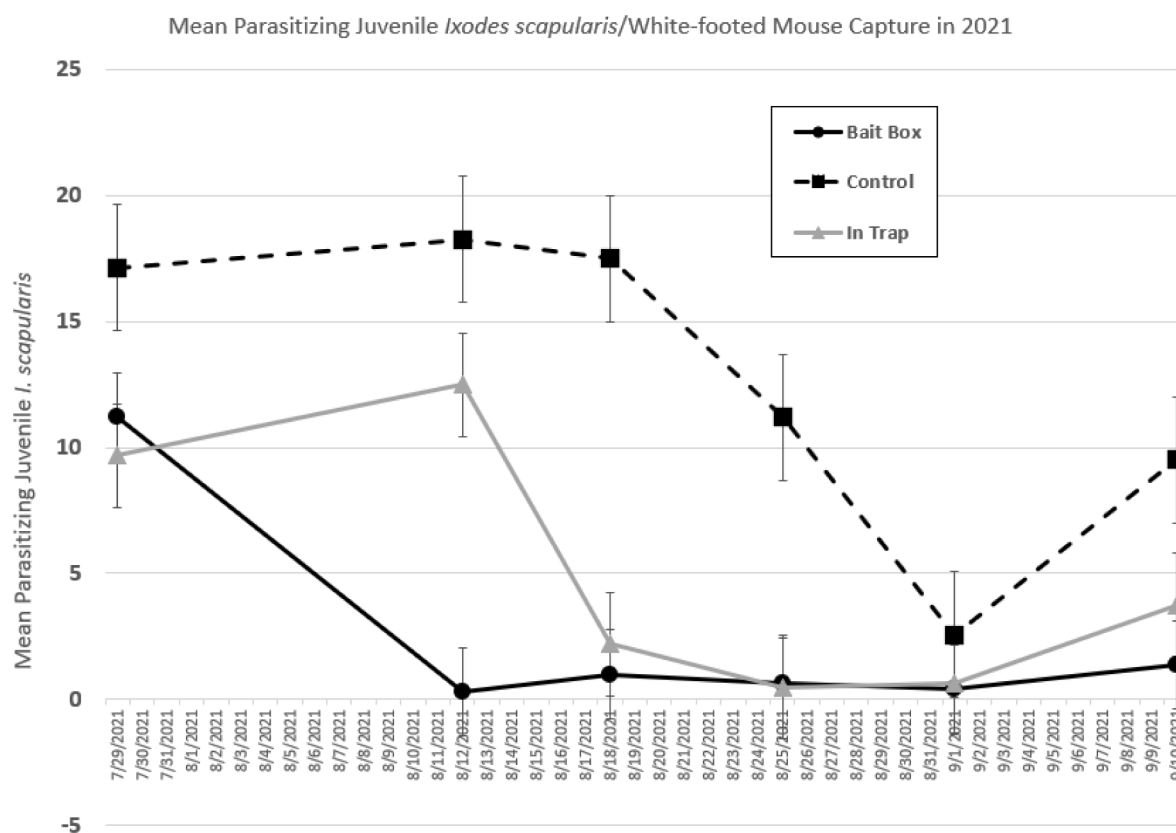


Fig. 1. Mean number of parasitizing juvenile *Ixodes scapularis* per white-footed mouse (*Peromyscus leucopus*) capture event in 2021. Error bars represent standard error of the mean.

2017; Williams et al., 2018a, 2018b; Jordan and Schulze, 2019; Eisen, 2021; Hinckley et al., 2021; Keesing et al., 2022; Linske et al., 2022), the oral systemic acaricidal treatment of rodent pathogen reservoirs is just recently being explored in the laboratory, and under field conditions in Canada (Pelletier et al., 2020, 2022; Poché et al., 2020, 2021).

Because we were not certain if *P. leucopus* would consume or get outcompeted for treated product, we tried two different deployment strategies. The first was the *ad libitum* bait box strategy (Icehouse and Sea Hill) where a relatively large volume of product (800.0 g) was available and refreshed weekly for seven weeks to wildlife reservoirs during the larval *I. scapularis* season. But with this strategy, there was no way of determining if a captured *P. leucopus* had contacted and consumed the product. Therefore, we placed a far lesser volume of product in individual Sherman traps at the Dam and Tommy Top sites such that we could document, by unique ear tag, which *P. leucopus* had likely consumed product at the time of capture.

Our differing deployment strategies were rendered moot as product was very readily consumed by small rodents on the landscape in both presentations. Similar to our previous work (Williams et al., 2020), the dye used on Kaput to increase visibility for applicators was readily

apparent on numerous captured individual *P. leucopus* but was not nearly as a reliable an indicator of contact as was Rhodamine B staining. However, captured *P. leucopus* within the bait box treatment sites started with mean burdens of over 11.0 juvenile *I. scapularis* at first capture in 2021, and thereafter, averaged 1.4/capture or below through 2021 and 2022 (Figs. 1 and 2). We suspect that the significant reduction in documented juvenile *I. scapularis* burdens on captured *P. leucopus* in bait box treatment sites at first capture and throughout 2022 was the result of learned behavior as to the location of the *ad libitum* bait boxes by small and medium-sized mammals on the landscape.

Boxes full of Kaput were placed out one week prior to the first capture event in both 2021 and 2022, but in 2021, boxes and bait within were new and animals were naïve to their presence. As a result, it took a few weeks for animals to learn the location of the bait source and we were able to document presumably relatively unaffected *I. scapularis* burdens after one week's exposure in 2021, which then declined precipitously thereafter (Fig. 1). However, when bait was placed out again at the same time of year in 2022, small and medium-sized mammals that had been exposed the previous year, were conditioned to the locations of bait boxes and had a much-reduced learning curve. As a result, the

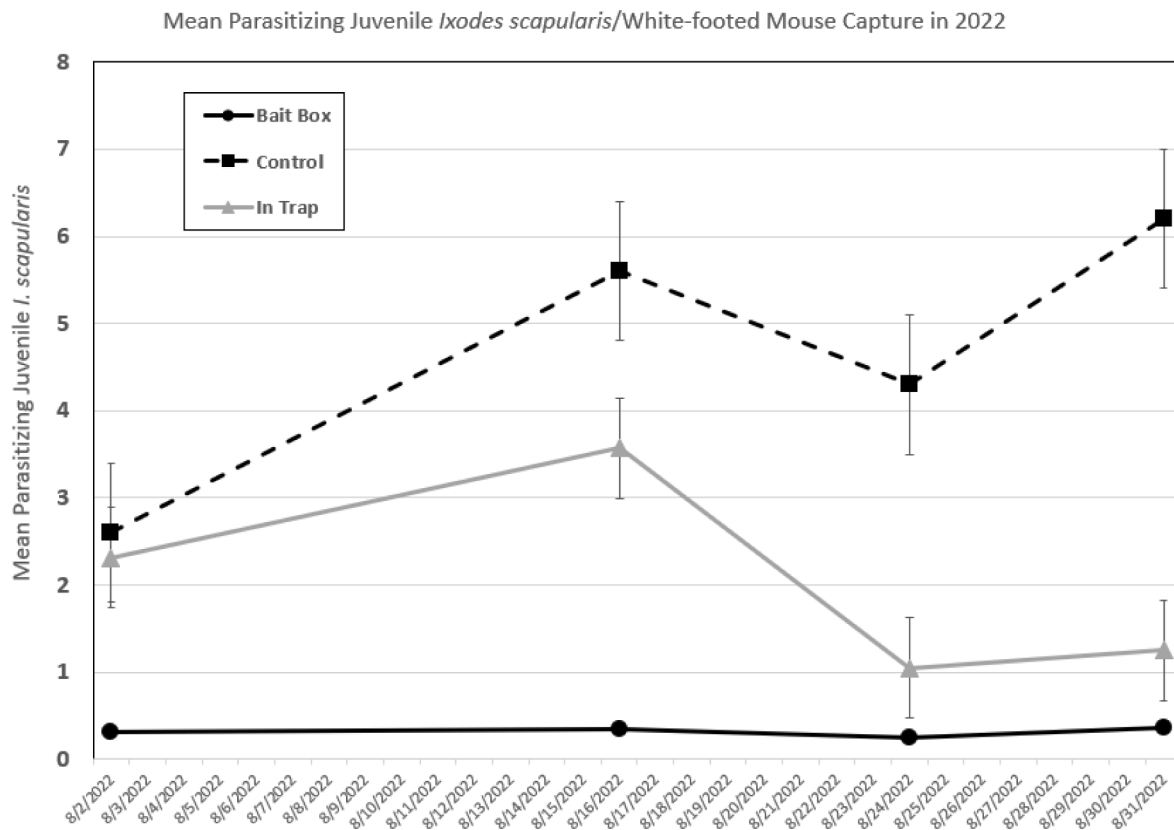


Fig. 2. Mean number of parasitizing juvenile *Ixodes scapularis* per white-footed mouse (*Peromyscus leucopus*) capture event in 2022. Error bars represent standard error of the mean.

presence of active bait boxes was made known through increased animal activity, bait was consumed nearly immediately, and the positive impacts of systemic fipronil treatment were witnessed in the *P. leucopus* reservoir at the first trapping event in 2022 without a similar delayed response as witnessed in 2021 (Fig. 2). Learned behavior of artificial bait site presence is very common in animals and has been documented previously in *O. virginianus* (Williams and DeNicola, 2000; Kilpatrick and Stober, 2002), wild pig (*Sus scrofa*) (Gielsing et al., 2011; Lavelle et al., 2018), gray wolves (*Canis lupus*) (Rossler et al., 2012), grey mouse lemurs (*Microcebus murinus*) (Lühns et al., 2009), and common carp (*Cyprinus carpio*) (Bajer et al., 2010) to name a few.

In contrast, at the in-trap treatment sites (Dam and Tommy Top), a much lesser volume of product was introduced to each Sherman trap such that the first *P. leucopus* exposure to fipronil was at the first capture event as opposed to one week prior at bait box sites. This difference can be seen in the delayed response in *I. scapularis* burden reduction as compared to bait boxes sites; reduction in *I. scapularis* burdens was witnessed at in-trap treatment sites during the third capture event in both years (Figs. 1 and 2), after captured *P. leucopus* had had two previous opportunities to consume treated product to be effective. Despite this difference in treatment timing, there was no detected difference in treatment effectiveness in the two deployment strategies in 2021. However, likely due to the larger volume of Kaput deployed and the learned behavior of bait box locations from one year to the next, tick burdens at bait box sites were significantly lower than in-trap sites in 2022. Regardless, both strategies were shown to be effective at reducing *I. scapularis* burdens as compared to control in both years.

Fipronil-based rodent-targeted bait boxes topically treat the rodent reservoir and would be the delivery method most comparable to our bait boxes filled with Kaput. Our resulting decrease in juvenile *I. scapularis* burdens on systemically treated *P. leucopus* (as compared to control) of 80% in 2021 and 94% in 2022 in the bait box treatments are

comparable, if not more effective at far lower cost, than results published for topical treatment of rodents with fipronil. While sample sizes were somewhat limited, Schulze et al. (2017) reported a mean $1.0 (\pm 2.3)$ juvenile *I. scapularis*/captured *P. leucopus* ($n = 14$) exposed to fipronil-based rodent-targeted bait boxes in August 2012 in New Jersey and $5.4 (\pm 4.8)$ /captured untreated *P. leucopus* ($n = 21$), or 81% fewer on treated animals than untreated. Schulze et al. (2017) also reported a mean $1.2 (\pm 1.9)$ juvenile *I. scapularis*/captured *P. leucopus* ($n = 13$) exposed to fipronil-based rodent-targeted bait boxes in August 2013 and $7.5 (\pm 1.0)$ /captured untreated *P. leucopus* ($n = 10$), 84% fewer on treated animals than untreated. Again, while somewhat limited by sample sizes, Jordan and Schulze (2019) reported a mean $2.5 (\pm 1.3)$ juvenile *I. scapularis*/captured *P. leucopus* ($n = 18$) exposed to fipronil-based rodent-targeted bait boxes in August 2014 and $4.8 (\pm 2.0)$ /captured untreated *P. leucopus* ($n = 9$), or 48% fewer on treated animals than untreated. Jordan and Schulze (2019) also reported a mean $2.9 (\pm 2.0)$ juvenile *I. scapularis*/captured *P. leucopus* ($n = 15$) exposed to fipronil-based rodent-targeted bait boxes in August 2015 and $15.0 (\pm 3.2)$ /captured untreated *P. leucopus* ($n = 23$), 81% fewer on treated animals than untreated. In addition, Dolan et al. (2004) reported that topical treatment of *P. leucopus* with fipronil reduced nymphal *I. scapularis* burdens by 68% and larval by 84%.

Orally delivered systemic acaricide treatment of household pets against ticks and fleas is common practice (Stafford et al., 2017; Eisen, 2023). However, there are currently no orally delivered acaricides commercially available for use in killing ticks feeding on wild rodent reservoirs (Eisen, 2023). We feel the results from this preliminary exploratory study show promise in the effectiveness of systemic treatment of free-ranging rodents with fipronil against juvenile *I. scapularis*. Next steps would be to determine if such treatment could be effective not only in reducing *I. scapularis* burdens, but also determine if such a strategy could be effective at reducing host-seeking nymphal *I. scapularis*

abundances and pathogen infection prevalence. Also, this research should facilitate the field evaluation of other formulations to be delivered to rodent reservoirs in the systemic acaricidal management of ticks and tick-borne pathogens. As mentioned in Eisen (2023), rodent-targeted approaches such as this strategy remain promising components of larger integrated tick management strategies and need to be further explored.

5. Conclusion

Oral delivery of systemic acaricides has the potential to reduce burdens of juvenile *I. scapularis* and other species of ticks parasitizing the small rodent reservoir. The direct targeting of small rodents during peak larval and nymphal activity season also has the potential to significantly reduce host-seeking tick abundances as well as reduce infection prevalence with various pathogens. This strategy should be tested for efficacy both alone and in combination with other treatments in an integrated tick management strategy. Successful implementation of this strategy has potential to reduce density of pathogen-infected ticks and presumably then reduce tick-borne illnesses in members of the public as well as domesticated animals and livestock.

CRediT authorship contribution statement

Scott C. Williams: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Megan A. Linske:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing. **Kirby C. Stafford:** Resources, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

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