

Original article

Efficacy of Flavocide™ against *Ixodes scapularis* (Acari: Ixodidae) nymphs in an adaptable phenocosm test arenaMaria V. Murgia¹, Phurchhoki Sherpa¹, Brianna Gast, Kayla Bliven[✉], Catherine A. Hill^{*}

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ABSTRACT

Ticks are agents causing tick-borne diseases (TBDs) in the United States, with the blacklegged tick (*Ixodes scapularis* Say) posing particular concern. Synthetic acaricides remain an important approach for tick control and TBDs prevention. However, increasing public concern and emerging acaricide resistance highlight the need for alternative products. In this study, we evaluated the efficacy of an undiluted oil-in-water emulsion of Flavocide™ (FLC-EW; 50% AI), a nature-identical active with a novel mode of action, in comparison with two commercial standards: Talstar-P, a pyrethroid product, and Outdoor Pest Control (OPC), a natural product, against *I. scapularis* nymphs under laboratory conditions. To assess both product efficacy and tick behavior, we developed a portable test arena ("Phenocosm") capable of incorporating substrates (leaf litter, mulch, and turfgrass) simulating natural tick habitats. In contact assays, preliminary assessment of FLC-EW indicated significantly higher mortality than Talstar-P at 24 and 48 hrs post-exposure (PE), despite lower knockdown (KD) at 1 hr PE. FLC-EW exhibited residual activity through four weeks. OPC exhibited only repellency against *I. scapularis* nymphs. Tick viability assays without acaricide indicated tick survival above 80% at week four in Phenocosms with leaf litter and mulch, and 78% in turfgrass at week three. Overall, >55% of ticks were consistently found sheltering on or beneath substrates with implications for acaricide applications. These findings suggest the acaricidal potential of FLC-EW and support further studies to assess its efficacy at lower AI concentrations. Additionally, they highlight the Phenocosm as a versatile test arena for assessing acaricide efficacy and tick behavior.

1. Introduction

Ticks are an increasing global public health threat. In the United States (US), the number of individuals diagnosed with and/or treated for tick-borne diseases (TBDs) has steadily increased (Rosenberg et al., 2018). In 2022, over 71,346 TBD cases were reported to the US Centers for Disease Control and Prevention (CDC, 2024), although actual numbers may be significantly higher (Kugeler et al., 2021). The blacklegged tick, *Ixodes scapularis* Say (Acari: Ixodidae), is widely distributed across the eastern and midwestern regions of North America. It transmits seven of the 19 tick-borne pathogens recognized in the US, namely *Borrelia burgdorferi* and *B. mayonii* (causative agents of Lyme disease), *B. miyamotoi* (hard tick relapsing fever), *Anaplasma phagocytophilum* (anaplasmosis), *Babesia microti* (babesiosis), *Ehrlichia muris eaulairensis* (ehrlichiosis), and Powassan virus (Powassan virus disease) (Eisen et al., 2017; Eisen and Eisen, 2018; Eisen and Paddock, 2021; Foster et al.,

2023). The nymphal stage of *I. scapularis* poses particular risk to human health since its small size makes detection and timely removal difficult (Falco et al., 1999; Ostfeld et al., 2006; Pepin et al., 2012; Eisen and Eisen, 2016). Data suggest pronounced expansion in the geographical distribution of *I. scapularis* (Eisen and Eisen, 2018), particularly since 1998, primarily in the northeastern and north-central states (Eisen and Eisen, 2023; Eisen et al., 2016). The increasing prevalence of *I. scapularis* highlights the increasing risk of TBDs in the US.

Controlling tick populations remains one of the most effective approaches to reducing TBDs. Area-wide application of acaricides has proven effective in suppressing tick populations (Jordan and Schulze, 2020; Eisen and Stafford, 2021). However, synthetic acaricides approved for vector control face challenges due to human and environmental health risks (Sagiv et al., 2023; EPA, 2023; 2024; 2025), and continued control is threatened by the emergence of acaricide resistance in tick populations (Eiden et al., 2015; Burtis et al., 2021). These issues

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underscore the need for the development of new and effective acaricides that also minimize environmental and non-target impacts.

Here, we performed a preliminary investigation of the potential of Flavocide™, based on a naturally occurring beta-triketone plant compound, prepared by Bio-Gene Technology Ltd., Melbourne, Australia, for development as a nature-identical active (NP) to control hard tick pests. Botanical acaricides are increasingly viewed as viable alternatives to synthetic products due to their perceived lower environmental and human health risks. We evaluated the efficacy of an undiluted oil-in-water emulsion of Flavocide™ (FLC-EW; 50% AI) against *I. scapularis* nymphs under controlled laboratory conditions, using a novel test arena, the Phenocosm, developed at Purdue University to assess tick phenotypic responses. Assessing acaricidal products requires a scalable and versatile platform suitable for both laboratory and field testing. To address these needs, we developed the Phenocosm, a portable test arena designed to simulate natural tick habitats, while enabling evaluation of acaricide efficacy and observation of tick biology and behavior. The Phenocosm consists of a transparent, durable plastic cylinder sealed at both ends, with a vented top lid for air circulation. In its basic configuration, the Phenocosm is lined with treated filter paper, allowing ticks to crawl on or beneath the filter paper, and vertically on the walls, providing multiple routes for ticks to contact or escape a product-treated surface (repellent and/or toxicant). The design also accommodates more complex configurations with the addition of environmental substrates such as leaf litter, mulch, or turfgrass to simulate the typical conditions that would be encountered in the natural habitat of the tick. Moreover, the enclosed and compact design facilitates efficient tick recovery and observation.

Two complementary laboratory experiments were performed to evaluate (1) the acaricidal efficacy of FLC-EW and (2) the viability of ticks and functional suitability of the Phenocosm configured with each of three separate substrates (leaf litter, mulch, and turfgrass). These substrates were selected to simulate typical *I. scapularis* habitats encountered in natural and managed environments, such as on landscaped public or private property. In the first experiment, we tested the contact efficacy of FLC-EW on the same day of product application and at two- and four-weeks post-application (residual activity) against *I. scapularis* nymphs under forced-exposure conditions using the basic Phenocosm configuration and test articles applied to filter paper. FLC-EW was compared against two commercially available products used as positive controls: Talstar-P, a widely used product containing the synthetic pyrethroid bifenthrin, and EcoTreat Outdoor Pest Control (OPC), a natural product alternative comprising cedarwood oil as the primary active ingredient (AI). These comparisons provided a baseline for the performance of FLC-EW under controlled, laboratory conditions. In addition, they suggested the acaricide potential of FLC-EW and supported future studies to assess its efficacy at lower AI concentrations. In the second experiment, we evaluated tick survival in Phenocosms containing substrates that mimic natural tick microhabitats. Leaf litter, mulch, or turfgrass were separately added to the Phenocosms to simulate field environments, and tick viability and behavior were monitored over four weeks in the absence of acaricides. These experiments demonstrated the versatility of the Phenocosm as a platform for acaricide efficacy testing and tick behavior studies.

2. Materials and methods

2.1. Tick source and maintenance

Twelve- to fifteen-week-old *I. scapularis* nymphs were obtained from the Tick Rearing Facility, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK, USA. Ticks were maintained at ~24 °C and >80% RH, under a 12:12 hrs (light: dark) photoperiod. For viability assays, ticks were marked dorsally with non-toxic, neon blacklight acrylic paint (Folkart, Norcross, GA, USA) three to four days prior to testing and kept in air-permeable 7 × 7 cm paper

biopsy bags (Cancer Diagnostics, Inc., Durham, NC, USA; Item code: BBG100) with 10 ticks per bag, placed inside a humidified bucket.

2.2. Phenocosm design and construction

Phenocosms were constructed from ultra-thin walled, clear polyethylene cylinders of 10 cm diameter (Reference#: PRT.020–4.050; Cleartec Packaging, Park Hills, MO, USA). The cylinders were 10 cm tall for acaricide efficacy testing (Fig. 1) and 15 cm tall for viability assays when environmental substrates were incorporated with above ground height of 10 cm (Fig. 2). Each end was sealed with fitted clear plastic lids (Reference#: PCC4.000; Cleartec Packaging, Park Hills, MO, USA). For acaricide efficacy testing, the upper lid contained a 5 cm diameter vent sealed with cotton enclosed in tulle to allow airflow and prevent tick escape. For the substrate-based viability assays, the Phenocosm was modified to include an 8.5 cm diameter vent on the top lid, four 2.5 cm diameter side windows, and 10 - 12 small drainage holes (~2 mm diameter) on the bottom lid. All openings were covered with a layer of sheer organza fabric (Item# 317,660,829,582,540,823; Michaels, Irving, TX, USA) affixed with hot glue.

3. Experiment 1: acaricide efficacy assays with the basic configuration of phenocosm

Test Products: FLC-EW is an oil-in-water emulsion formulation containing 50% flavesone, a beta-triketone compound typically found in plants from the Myrtaceae family, including *Eucalyptus* spp. native to Australia. FLC-EW was formulated and provided by Bio-Gene Technology Ltd. (Melbourne, Australia). Two commercially available products served as reference standards: Talstar-P (FMC Corporation, Philadelphia, PA, USA), a pyrethroid product containing 7.9% bifenthrin, and OPC (Wondercide LLC, Round Rock, TX, USA), a natural product containing 40.42% cedarwood oil, 8.74% sesame oil, and 3.5% sodium lauryl sulfate. Talstar-P and OPC were diluted in double-distilled water according to label instructions to a final concentration of active ingredients of 0.062%, and 0.16%, respectively. The commercial products were used at the dilution recommended by the manufacturer for field application. The FLC-EW formulation was tested undiluted (50% AI) at recommendation of the supplier and in order to gather baseline information on efficacy against ticks and justify further investment in development for tick control. Given the focus and scope of the current study, additional experiments to evaluate dose-response and minimum effective dose were not performed. However, these are under consideration for future work. Untreated filter paper served as the universal negative control with future studies possibly expanding to include multiple organic solvents as appropriate for each positive control included in study design. The diluent for all products and formulations tested was water, which was observed to evaporate from test filter papers under the ambient conditions of the assay. Therefore, an assumption was made that water-impregnated controls provided the functional equivalent of an untreated paper. While we did not empirically verify this, the use of an aqueous carrier rather than an organic solvent reduces the likelihood that solvent effects confounded our results.

Product Application: In a fume hood, 2 ml of each product was applied to Whatman filter paper (10 cm diameter) placed in clear plastic lids (10 cm diameter; Reference#: PCC4.000; Cleartec Packaging, Park Hills, MO, USA). Considering its high viscosity, FLC-EW was dispensed as small droplets across the lid surface before placing the filter paper on top for even saturation (Fig. 1; Panel 1). Lids were gently tilted and rotated to ensure even coverage, then allowed to soak for 10 min. Treated filter papers were then air-dried on wire racks in the fume hood for 2 hrs (FLC-EW) or 45 min (Talstar-P and OPC).

Contact Efficacy Assays: For each test product and its negative control, three biological replicates were performed, each consisting of three technical replicates with five ticks (i.e., 15 ticks per biological replicate). Dry treated or untreated filter paper was placed at the bottom of the

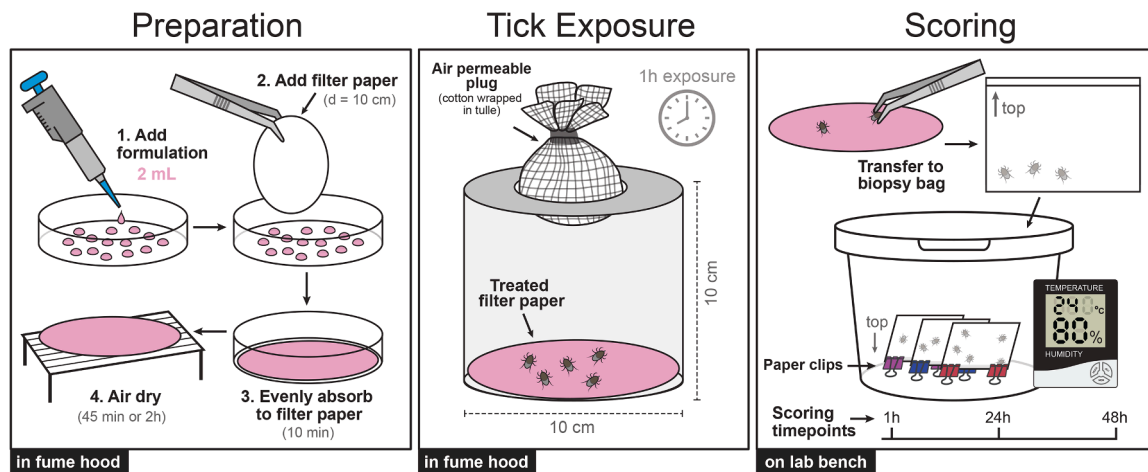


Fig. 1. Diagram of contact and residual assay setup used to evaluate acaricidal efficacy against *Ixodes scapularis* nymphs. Test product/formulation (2 ml) were applied onto 10 cm diameter filter papers inside a fume hood and allowed to air dry on wire racks (45 min for Talstar-P and OPC and 2 hrs for FLC-EW). Ticks were then exposed to treated papers in the Phenocosms for 1hr, during which their locations were recorded at 5 min interval. After that, ticks were transferred to paper biopsy bags and kept in humidified buckets for scoring at 1, 24, and 48 hrs post-exposure.

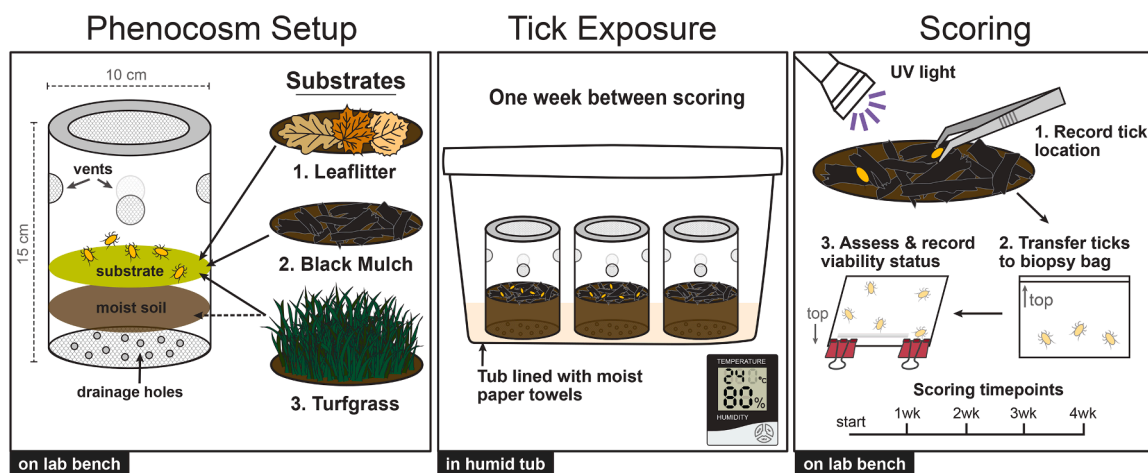


Fig. 2. Diagram of tick viability assay setup and scoring. Phenocosms contained one of three substrate types (leaf litter, black mulch, or turfgrass) and were placed in tubs lined with moist paper towels to maintain humidity. Ticks' viability was assessed weekly for three or four weeks depending on substrate. During each scoring, ticks were retrieved using UV light, their locations were recorded and their viability status was assessed in paper biopsy bags. Dead ticks were removed.

Phenocosm, and ticks were introduced to the center (Fig. 1; Panel 2). The Phenocosms were then placed in sealed Ziploc bags with moist paper towels to maintain humidity (~80%) during the 60 min exposure. During exposure, tick location ('top' or 'bottom' of filter paper, Phenocosm 'wall,' or 'not visible') was recorded at 5 min intervals.

After the 60 min exposure period, ticks were transferred to paper biopsy bags and held in clean, humidified buckets (24°C, >80% RH). Knockdown (KD) and mortality were scored at 1, 24, and 48 hrs post-exposure (Fig. 1; Panel 3). Ticks were considered *alive* if they moved normally in response to touch or breath; *knocked down* if they could move their limbs but were unable to crawl; and *dead* if they were unresponsive to stimulus and either had rigid or curled legs. Occasionally, *dead* ticks also exhibited exposed hypostomes and/or splayed palps.

Residual Activity Assays: Treated filter papers were stored individually in sealed Ziploc bags, placed inside separate plastic containers for each test product, and held in a fume hood at room temperature. At two or four weeks of storage after product application, residual activity was assessed following the same procedure described above for contact efficacy assays.

4. Experiment 2: evaluating tick viability in simulated tick habitats

The viability of *I. scapularis* nymphs was evaluated in Phenocosms containing three substrate types commonly associated with tick habitats: leaf litter (red and white oaks, poplar, and mulberry leaves collected locally), black mulch (Vigoro Corporation, Lake Forest, IL, USA), and tall fescue turfgrass sod (*Schedonorus arundinaceus*) grown in a greenhouse (Fig. 2; Panel 1). Tall fescue was selected because it is a cool season turfgrass commonly found in Midwestern yards. The base of each Phenocosm was packed with ~1–2 cm moist soil, and test substrates were placed on top. For turfgrass, a freshly cut sod plug (10 cm diameter) containing soil was used without the need for a soil layer. Five labeled ticks were introduced at the center of each substrate. Phenocosms were then placed in a humidified tub (Fig. 2; Panel 2). Each week, for up to 4 weeks, ticks were identified and retrieved using a UV flashlight and transferred to paper biopsy bags using fine-tipped tweezers (Fig. 2; Panel 3). The location of each tick was recorded as (i) on or beneath the *substrate*, (ii) on or in the *soil*, (iii) on the Phenocosm *wall*, or (iv) *not found*. Retrieved ticks were assessed for mortality and, if alive, placed back into the center of the substrate. Assays were terminated after four weeks for leaf litter and

mulch and after three weeks for turfgrass (due to a mold issue). For each substrate type, three biological replicates were conducted, each consisting of three technical replicates with five ticks per replicate.

4.1. Statistical analysis

All analyses were performed in R (v. 4.4.0; [R Core Team, 2024](#)) using generalized linear mixed models (GLMMs) with the glmmTMB package (v. 1.1.10; [Brooks et al., 2017](#)). Although Abbott's formula is often used to adjust for control mortality in efficacy studies, recent work ([Piepho et al., 2024](#)) highlights its statistical limitations and potential to introduce bias. Following their recommendation, we used GLMMs as a more robust alternative to assess product efficacy. GLMMs account for both fixed and random effects in hierarchical (nested) experimental designs and accommodate response variables that do not follow a normal distribution.

Efficacy Data: Contact, residual-week 2, and residual-week 4 data were analyzed separately using Bayesian GLMMs with a binomial distribution and a logit link. Weakly informative normal priors (mean = 0, SD = 3) were applied to the fixed effects (treatment and time) to regularize estimation and reduce the risk of overfitting ([Bolker, 2025](#)). Random effects were specified hierarchically (nested): tick batch within technical replicate within biological replicate.

Due to quasi-complete separation (e.g., no KD or mortality in controls or OPC), final models included only FLC-EW and Talstar-P data. Post hoc pairwise comparisons between FLC-EW and Talstar-P at each post-exposure time point were conducted using Tukey's Honestly Significant Difference (Tukey HSD), in the emmeans package (v. 1.10.5; [Lenth, 2025](#)). KD and mortality were modeled separately.

Tick Behavior: Tick location was categorized as "in-contact" (top or bottom of filter paper) or "not-in-contact" (on the walls of Phenocosms). An 'adjusted total' was used in all analyses [adjusted total = initially added total ticks (5 per Phenocosm) minus the number of 'not visible' ticks]. Although observations were recorded every 5 min, location analyses were conducted only at 0, 15, 30, 45, and 60 min, using the same GLMM framework as efficacy models. Unlike the efficacy analyses, OPC and control groups were included in the location analyses.

Viability Data: Weekly percent survival, mortality, and unrecovered rates were calculated for each substrate. Because the goal was to evaluate whether a substrate was suitable for use with the Phenocosm, no statistical comparisons were made between substrates. Substrates were considered unsuitable if weekly tick mortality exceeded 10%.

5. Results

The following section presents results from the two experiments evaluating the efficacy of acaricidal treatments and the viability of ticks in Phenocosms with substrates. Results are organized by experiment and treatment type with separate analyses of mortality, KD, tick behavior (location), and substrate-based viability.

6. Experiment 1: acaricide efficacy assays

Contact assays were conducted the same day of product application to evaluate the efficacy of FLC-EW, Talstar-P, and OPC. KD and mortality were scored at 1, 24, and 48 hrs post-exposure. As OPC caused no KD or mortality at any time points during contact assays, it was excluded from statistical comparison and residual assays. Because of ambiguity in interpreting KD at later timepoints (complicated by the possibility that non-knocked-down ticks could have either been alive or dead), only 1 hr post-exposure KD was analyzed. Mortality data were evaluated across all time-points. To assess residual activity, filter papers treated with FLC-EW and Talstar-P were stored at room temperature and tested at two and four weeks after product application. Tick locations were recorded over the 60 min exposure period to assess potential repellency behavior. Results for mortality, KD, and tick behavior (location) are presented in

separate subparagraphs below. For all assays, we observed <10% mortality in ticks assigned to the control group and no evidence of KD.

Contact Mortality: After 1 hr post-exposure, the odds of mortality were 1.68 times higher in the FLC-EW group compared to the Talstar-P group, but this difference was not statistically significant (p -value = 0.39). However, FLC-EW induced significantly higher mortality than Talstar-P at 24 hrs (odds ratio [OR] = 8.27, p -value < 0.01), and this effect persisted at 48 hrs post-exposure (OR = 11.40, p -value < 0.05) ([Fig. 3A](#)).

Contact KD (1 hr post-exposure): Talstar-P caused a significantly higher proportion of knockdown compared to FLC-EW at 1 hr post-exposure (OR = 6.99, p -value < 0.001; [Fig. 4A](#)).

Residual Week 2 - Mortality: At 1 and 24 hrs post-exposure, mortality did not differ significantly between FLC-EW and Talstar-P (1 hr: OR = 3.19, p -value = 0.23; 24 hrs: OR = 2.16, p -value = 0.14). However, at 48 hrs, FLC-EW showed significantly higher mortality than Talstar-P (OR = 6.25, p -value < 0.05) ([Fig. 3B](#)).

Residual Week 2 - KD (1 hr post-exposure): Talstar-P caused significantly greater KD than FLC-EW (OR = 10.24, p -value < 0.001; [Fig. 4B](#)).

Residual Week 4 - Mortality: FLC-EW consistently resulted in significantly higher mortality compared to Talstar-P across all post-exposure time points: 1 hr (OR = 8.01, p -value < 0.05); 24 hrs (OR = 64.20, p -value < 0.001); and 48 hrs (OR = 42.73, p -value < 0.001) ([Fig. 3C](#)).

Residual Week 4 - KD (1 hr post-exposure): Although Talstar-P resulted in a higher proportion of KD than FLC-EW, the difference was not statistically significant (OR = 1.72, p -value = 0.30; [Fig. 4C](#)).

Tick Location During Contact Assay: Ticks exposed to OPC were significantly less likely to be in contact with the treated filter paper compared to those in the corresponding control group at: 30 min (OR = 7.12, p -value < 0.01); 45 min (OR = 7.03, p -value < 0.01); and 60 min (OR = 7.92, p -value < 0.001). Similarly, the probability of ticks contacting OPC-treated filter paper was significantly lower than that of ticks contacting FLC-EW treated filter paper at: 30 min (OR = 5.95, p -value < 0.05); 45 min (OR = 19.51, p -value < 0.001); and 60 min (OR = 56.39, p -value < 0.001). While the probability of ticks being in contact with Talstar-P treated filter paper was higher than the ticks being in contact with OPC treated filter paper at all timepoints after 15 min, the difference reached statistical significance only at 60 min (OR = 11.80, p -value < 0.01). No significant differences were observed between FLC-EW and Talstar-P, or between either product and their respective controls ([Fig. 5](#)).

7. Experiment 2: tick viability in phenocosms with substrates

To evaluate the suitability of the Phenocosm for future semi-field or field-based experiments, tick viability assays were conducted using simulated tick habitats with three common substrates: leaf litter, mulch, and turfgrass, without chemical treatment and under controlled laboratory conditions.

Leaf litter: After one week in Phenocosms with leaf litter, an average of 80% of ticks remained alive, 4.4% were dead, and 15.5% were unrecovered. By week four, the percentage of unrecovered ticks decreased to 9% while survival and mortality increased to 84% and 7%, respectively ([Fig. 6](#)). The increase in the percentage of live ticks observed at week four reflected the deconstruction of the Phenocosm, which occurred at assay termination and enabled greater recovery of nymphs likely concealed within leaf litter and soil layers during prior weekly assessments. Most ticks were observed directly on leaf litter at both one week (77.8%) and four weeks (60.7%), with smaller proportions on the soil surface (2% at one week; 25.9% at four weeks) and on the Phenocosm wall (7% and 4% at one and four weeks, respectively) ([Fig. 7](#)).

Mulch: In Phenocosms with mulch, tick survival averaged 96% at week one and declined to 88% at week four. The percentage of mortality increased from 2% to 10%, while the percentage of unrecovered ticks remained at 2% at both weeks ([Fig. 6](#)). At week one, 97.8% of ticks were found on or beneath the mulch surface, which decreased to 69% by week

Model Predicted Probability of Mortality

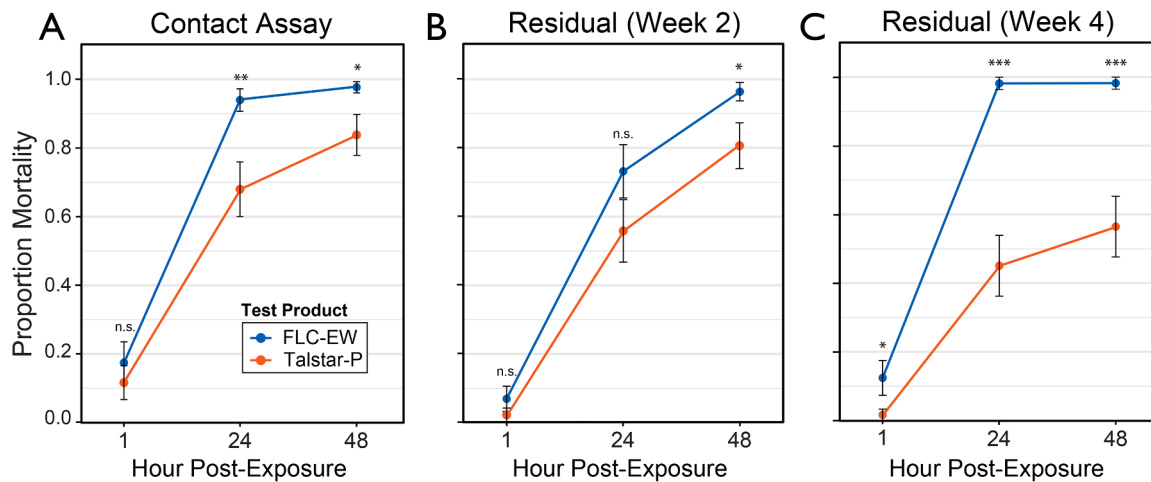


Fig. 3. GLMM model predicted tick mortality assessed at 1, 24, and 48 hrs post-exposure under three conditions: (A) contact assay following product application and drying; (B) residual assay at week 2 after product application; and (C) residual assay at week 4. Test products included Flavicide™ oil-in-water emulsion (FLC-EW; 50% AI, undiluted) and commercial pyrethroid product, Talstar-P (0.062% AI, diluted per label instructions). Dots represent model-predicted means $\pm$ SE. Significance of pairwise differences between treatments at each time point is indicated as: p -value < 0.05 (*), $p < 0.01$ (**), $p < 0.001$ (***), and n.s. = not significant.

Predicted Knockdown at 1-hr Post-Exposure

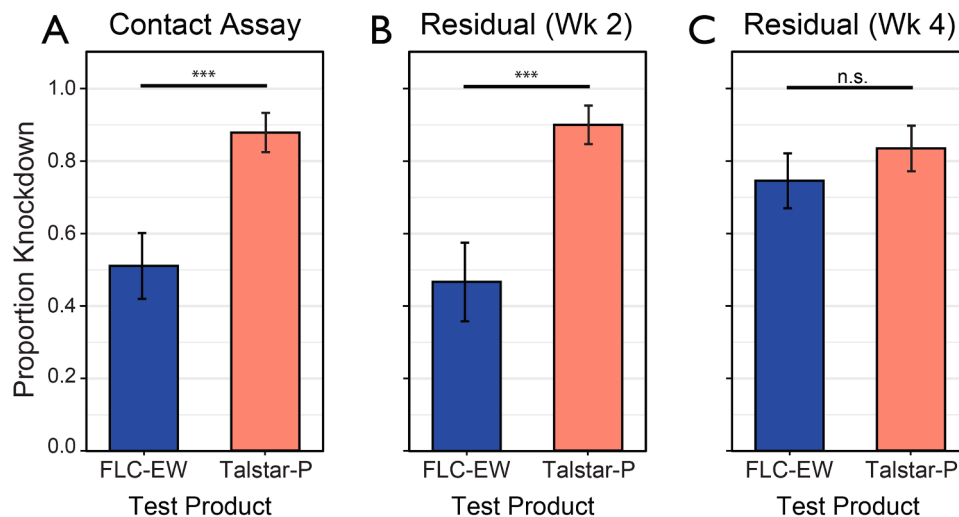


Fig. 4. Proportion of knockdown ticks at 1 hr post-exposure to FLC-EW (50% AI, undiluted) and Talstar-P (0.062% AI, diluted per label instructions) for: (A) contact assay; (B) residual assay at week 2; (C) residual assay at week 4. Bars represent GLMM model-predicted means $\pm$ SE. Asterisks denote statistical significance (*** p -value < 0.001 ; n.s. = not significant).

four, with corresponding increases in ticks found on the soil surface (24%) and Phenocosm wall (5%) (Fig. 7).

Turfgrass: At week one in Phenocosms with turfgrass, 86% of nymphs were alive, 4.4% were dead, and 9.4% could not be found. By week two, tick mortality reached 17.8%, with 80% alive, and 2.22% unrecovered. At week three, the survival rate fell to 78% and mortality increased to 22% (Fig. 6). Most ticks were recovered from grass blades at both week one (64%) and week three (65%), while others were found on the soil surface (26% at week one; 35% at week three) (Fig. 7).

8. Discussion

Synthetic acaricides remain a primary approach to area-wide tick control and TBDs prevention (Eisen, 2024). Their use, however, is

constrained by several challenges, including low public uptake and adoption (Cuaderna et al., 2023; Eisen and Stafford, 2021; Jordan and Schulze, 2020), the limited number of acaricide classes approved for vector control, concerns over human and environmental health, and the emergence of acaricide resistance among tick populations (Eiden et al., 2015; Burtis et al., 2021; Sagiv et al., 2023; EPA, 2024). These constraints underscore the need to evaluate novel compounds and formulations and develop new technologies that are affordable, potent, have minimal impacts on non-target organisms, and are easy to use. In this context, botanical products provide a promising path forward.

This study is a preliminary assessment of the contact efficacy and residual activity of a novel oil-in-water formulation of Flavicide™ (FLC-EW; 50% AI), developed by Bio-Gene Technology, Ltd., in comparison with two commercially available products: Talstar-P, a synthetic

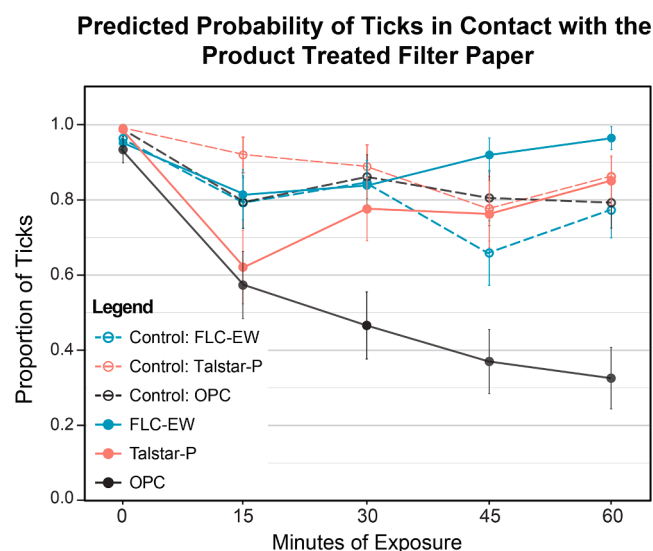


Fig. 5. Proportion of *I. scapularis* nymphs in contact with treated filter paper over the 60-min exposure period for each treatment group: FLC-EW (50% AI, undiluted), Talstar-P (0.062% AI, diluted per label instructions), OPC (0.16% AI, diluted per label instructions), and their respective controls (Control: FLC-EW, Control: Talstar-P, and Control: OPC). Tick locations were analyzed at 15-min intervals.

pyrethroid product, and OPC, a cedarwood oil-based natural product (tested only in contact assays). In contact assays, FLC-EW induced greater than 90% tick mortality at 24 hrs, which exceeded 95% by 48 hrs post-exposure, whereas Talstar-P achieved approximately 70% mortality at 24 hrs and slightly above 80% at 48 hrs. Talstar-P caused approximately 90% KD at 1 hr post-exposure, compared to approximately 50% KD of ticks in response to FLC-EW, suggesting limited repellency associated with the FLC-EW formulation and different molecular modes of action between bifenthrin and flavesone. This finding is consistent with a previous study, where Flavicide™ was reported to

exhibit delayed KD_{50} and KD_{90} against *Aedes aegypti* and *Musca domestica* in comparison to permethrin (Miller and Peters, 2017). Although the toxic mode of action (MoA) of the AI in FLC-EW is not fully known, evidence suggests that it is distinct from the established molecular targets and insecticide classes recognized by the Insecticide Resistance Action Committee (IRAC) (May 2016; Bio-Gene Technology Limited, 2025). The modest KD effect may reflect limited airborne exposure under the study conditions, suggesting that FLC-EW may have limited potential as a passive spatial product for tick control without additional formulation optimization or changes to product delivery and administration routes. The pattern of low KD at 1 hr and high mortality observed with FLC-EW at 24 and 48 hrs post-exposure is consistent with a MoA associated with disruption of metabolic processes. Recent studies suggest that beta-triketone compounds, including flavesone, may act via two distinct MoAs in arthropods- disruption of hydroxyphenylpyruvate dioxygenase (HPPD) leading to inhibition of tyrosine catabolism, as reported in *Sarcoptes scabiei* mites (Nammunige et al., 2025) and inhibition of carbonic anhydrase (leptospermone), as in *Aedes aegypti* mosquitoes (McComie et al., 2025). Biochemical and toxicological studies are needed to determine the involvement of HPPD and carbonic anhydrase targets in the MoA of flavesone against *I. scapularis* and other tick species.

Preliminary assessment of the residual activity of FLC-EW undiluted formulation indicated toxicity against *I. scapularis* nymphs for up to four weeks in laboratory assays, with >70% mortality observed at 24 hrs post-exposure. These findings indicate that FLC-EW retains activity for multiple weeks under laboratory conditions, suggesting potential for extended tick control in field applications. This justifies further studies to evaluate formulation performance at lower AI concentration under laboratory conditions first, then, if confirmed, in the field. Notably, ticks exposed to filter papers treated with FLC-EW and stored for four weeks exhibited higher mortality than those exposed to filter papers stored for two weeks. The four-week-old filter papers appeared to retain a higher oil content, which may have facilitated persistence of the AI in the organic fraction and enhanced uptake by ticks through tarsal contact. Alternatively, other unmeasured changes in the formulation or exposure conditions during storage may have contributed to the observed

Weekly Tick Viability

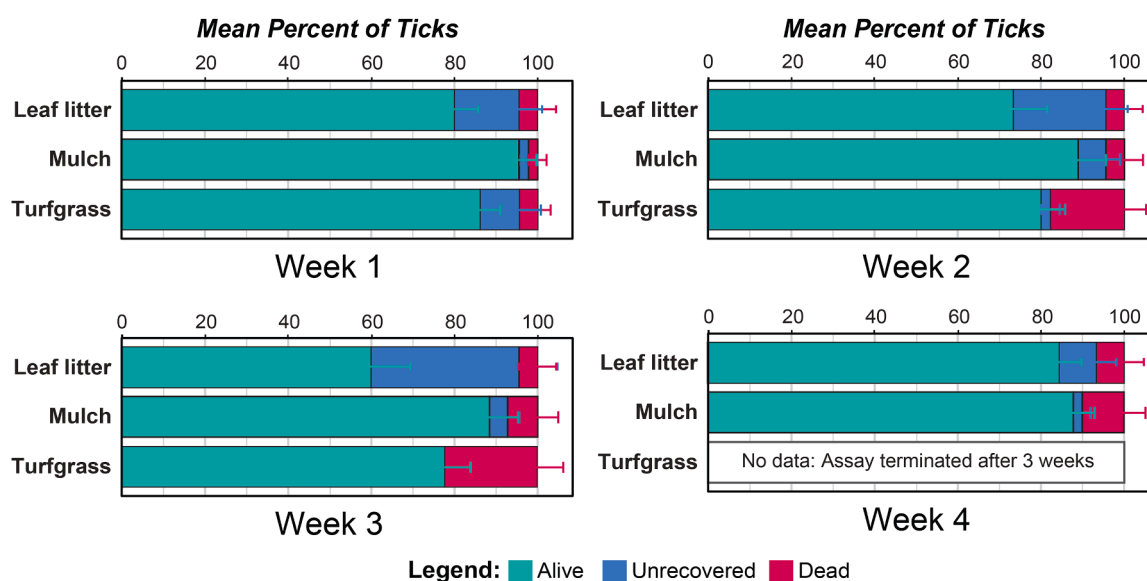


Fig. 6. Viability of *I. scapularis* nymphs over time in Phenocosms with three different substrates (leaf litter, mulch, and turfgrass). Mean percentages of ticks alive, dead, or unrecovered were recorded weekly for up to four weeks. The turfgrass assay was terminated at week 3 due to excessive mold. Bars represent weekly mean percent of tick status across substrates $\pm$ SE. The apparent increase in the percentage of live ticks at week 4 reflects an improved recovery of previously undetected nymphs upon Phenocosm deconstruction at assay termination, rather than an actual increase in survival.

Location of ticks in the phenocosms with substrate

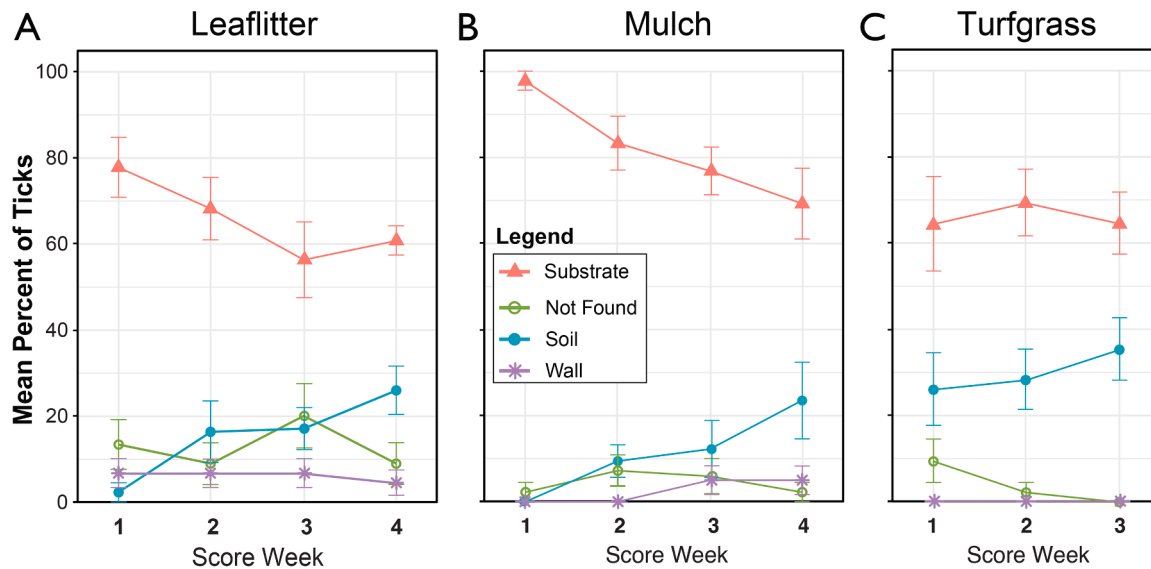


Fig. 7. Mean percent of ticks found on or within each substrate, on the soil surface, on the Phenocosm wall, or not found, at weekly scoring intervals. Data are shown for leaf litter and mulch through week 4, and for turfgrass through week 3 (assay terminated due to mold).

difference.

The FLC-EW represents a first-generation formulation and was used undiluted (50% AI) at recommendation of the supplier to determine its baseline efficacy against ticks. Study design necessarily incorporated two commercial products, Talstar-P and OPC tested at their labeled field application rates (0.062% and 0.16% AI, respectively), representing a synthetic pyrethroid and natural product as positive controls. Therefore, the efficacy results for FLC-EW and the commercial standards should be interpreted with test concentration in mind. The purpose of this study was to benchmark the acaricidal activity of a novel nature-identical AI and preliminary formulation in comparison to commercial formulations under standardized laboratory conditions, rather than to evaluate relative potency at equivalent concentrations. Identifying the lowest effective concentration of FLC-EW would be a necessary next step for commercialization. This work may be performed with second-generation formulations of FLC specifically developed for the application and control of ticks. Laboratory and semi-field assays to evaluate minimum effective dose and assess residual activity are anticipated as part of future studies. Should such studies prove successful, randomized field trials in natural habitats with wild tick populations will be conducted to further evaluate the potential of FLC for tick control and support product registration. Phenocosm studies could also be expanded to include other substrates and other tick life stages and species.

OPC, the commercial natural product containing cedarwood oil, used as a positive control, is labeled to both kill and repel ticks. In this study, ticks did not exhibit KD or mortality following exposure to OPC. Significantly fewer ticks were observed in contact with OPC-treated filter paper compared to untreated controls suggestive of a repellency effect. While the present study did not directly assess repellency, various *in vitro* and *in vivo* repellency tests have been developed (Bissinger and Roe, 2010; Adenubi et al., 2018; Burtis et al., 2023) and could be employed to further investigate this phenomenon. Previous studies have reported limited efficacy of cedarwood oil-based products such as Cedarcide PCO Choice and Tick Killz over a two-week period (Dyer et al., 2021) and observed repellency of cedarwood oil to *I. scapularis* and *Amblyomma americanum* Linnaeus nymphs (Carroll et al., 2011). Sesquiterpene derivatives of cedarwood oil, such as nootkatone and cedrol, have demonstrated toxicity against *I. scapularis* nymphs in previous studies (Panella et al., 1997, 2005; Dolan et al., 2009; Eller et al.,

2014). Our assays were designed to evaluate efficacy via tarsal contact and absorption. Consequently, potential efficacy resulting from direct cuticular application to the tick scutum was not investigated, and this alternative delivery route may yield different results.

The Phenocosm proved a versatile platform for (1) assessment of efficacy and (2) observations of tick behavior in multiple simulated tick microhabitats. Unlike some other test arenas described in the literature, the Phenocosm allows ticks to move freely in both vertical and horizontal directions and facilitates near-complete recovery of ticks at the end of each trial, improving quantification of treatment and substrate effects. Viability assays confirmed that the Phenocosm sustained *I. scapularis* nymphs for four weeks in leaf litter and mulch, with survival exceeding 80%. Tick survival rate in turfgrass was >75% through week three, although mortality exceeded 10% at week two, which increased to >20% at week three, presumably due to mold growth on the grass, leading to early termination of the assays. These results support the utility of the Phenocosm for longitudinal efficacy and behavioral studies using leaf litter and mulch substrates. Design modifications to increase air flow and reduce humidity could extend the utility of the Phenocosm turfgrass-based studies beyond the current two week maximum, although challenges around growth of turfgrass will also need to be overcome.

Across weeks one through four, 56–98% of ticks were located on or beneath the substrate surface, with >20% migrating to the underlying soil layer by weeks three and four, regardless of substrate. This behavior likely reflects avoidance of environmental stressors such as desiccation or light, both of which influence questing and survival in *Ixodes* nymphs (Perret et al., 2003). The consistent tendency of ticks to shelter beneath substrates, particularly in leaf litter and mulch, has important implications for acaricide applications in environments where such cover is common and human-tick encounter risk is elevated (Falco and Fish, 1988; Maupin et al., 1991; Feldman et al., 2015; Robert et al., 2025). The finding highlights the importance of considering habitat structure both when predicting tick encounter risk and when designing integrated tick control strategies that combine chemical, biological, and habitat-based interventions.

Effective acaricide treatments must also account for substrate penetration to reach microhabitats where ticks persist. Standard spray applications for tick control primarily deposit acaricides on the surface

of substrates commonly encountered on public and private property such as leaf litter, mulch, or turfgrass while ticks remain concealed beneath these layers or within the upper layer of soil. The extent to which lethal concentrations of acaricides reach these refugia is influenced by formulation type, spray volume, and substrate structure. If coverage or penetration is inadequate, ticks in sheltered microhabitats may survive and recolonize, thus reducing treatment efficacy and potentially contributing to the development of acaricide resistance through repeated sublethal exposure. Further, the persistence of resistant populations could increase the risk of tick bites and disease transmission.

9. Conclusion

In our preliminary assessment, FLC-EW undiluted formulation (50% AI) demonstrated potential for tick control, resulting in >90% mortality of *I. scapularis* nymphs at 24 hrs post-exposure in contact assays and robust residual efficacy (greater than 70% mortality up to four weeks) under laboratory conditions. In contact assays, FLC-EW was associated with modest KD (~50% KD at 1 hr) and robust mortality of ticks at later time points (>90% at 24 and 48 hrs). Talstar-P exposure was associated with 90% KD at 1hr, and 70 and above 80% mortality at 24 and 48 hrs, respectively, while OPC exposure was associated with repellency but no toxicity. Further studies to assess the efficacy of FLC-EW dilutions and formulations will enable a better understanding of the potency of this novel AI compared to commercial products currently in use for tick control.

In its basic configuration, the Phenocosm provided a controlled and repeatable arena design for evaluating acaricidal efficacy. When combined with different substrates, it provided a flexible platform enabling near-complete tick recovery and facilitated observation of tick behavior. Tick recovery was improved following deconstruction of the Phenocosms at assay termination, presumably as this step enabled a more comprehensive examination of substrate and soil. During weekly assessments, complete recovery of the nymphs was constrained by the complexity of substrate and the basal soil layer. The apparent increase in live tick percentages observed at week three (turfgrass) should be interpreted accordingly. Substrate-specific viability assays demonstrated that Phenocosms containing leaf litter and mulch supported survival of nymphs above the 80% threshold for up to four weeks, whereas turfgrass resulted in >10% mortality of nymphs starting at week two and >20% mortality at week three, thus necessitating assay termination and indicating the need for design modifications to improve airflow and humidity control. Collectively, this study establishes a methodological foundation for evaluating the interaction between substrate type and the efficacy of actives and formulated products, and for extending Phenocosm applications to large-scale field studies and product efficacy trials. Observations of tick behavior across leaf litter, mulch, and turfgrass substrates showed that most ticks remained on or beneath surface layers, underscoring the importance of integrated pest management strategies that ensure acaricide penetration into these microhabitats.

CRediT authorship contribution statement

Maria V. Murgia: Writing – review & editing, Methodology, Investigation, Conceptualization. **Phurchhoki Sherpa:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Brianna Gast:** Investigation. **Kayla Bliven:** Investigation. **Catherine A. Hill:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

M.V.M.: I have nothing to declare.

P.S.: I have nothing to declare.

B.G.: I have nothing to declare.

K.B.: I have nothing to declare.

C.A.H.: She is a consultant and member of the Bio-Gene Scientific Advisory Board.

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Data availability

Data will be made available on request.

References

- Adenubi, O.T., McGaw, L.J., Eloff, J.N., Naidoo, V., 2018. In vitro bioassays used in evaluating plant extracts for tick repellent and acaricidal properties: a critical review. *Vet. Parasitol.* 254, 160–171. <https://doi.org/10.1016/j.vetpar.2018.03.008>.
- Bio-Gene Technology Limited. Flavocide™. Available from: <https://bio-gene.com.au/products/flavocide/>, (Accessed 7 July 2025).
- Bissinger, B.W., Roe, R.M., 2010. Tick repellents: past, present, and future. *Pestic Biochem. Physiol.* 96, 63–79. <https://doi.org/10.1016/j.pestbp.2009.09.010>.
- Bolker, B., 2025. Priors in *glmmTMB*. <https://cran.r-project.org/web/packages/glmmTMB/vignettes/priors.html> (Accessed 15 May 2025).
- Brooks, M., Kristensen, E., Benthem, K., van, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Mächler, M., Bolker, B.M., 2017. *glmmTMB* balances speed and flexibility among packages for zero-inflated generalized Linear Mixed modeling. *R J.* 9, 378. <https://doi.org/10.32614/RJ-2017-066>.
- Burtis, J.C., Poggi, J.D., Payne, B., Campbell, S.R., Harrington, L.C., 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. <https://doi.org/10.1093/jme/tjab054>.
- Burtis, J.C., Ford, S.L., Parise, C.M., Foster, E., Eisen, R.J., Eisen, L., 2023. Comparison of in vitro and in vivo repellency bioassay methods for *Ixodes scapularis* nymphs. *Parasites Vectors* 16. <https://doi.org/10.1186/s13071-023-05845-7>.
- Carroll, J.F., Tabanca, N., Kramer, M., Elejalde, N.M., Wedge, D.E., Bernier, U.R., Coy, M., Becnel, J.J., Demirci, B., Bager, K.H.C., Zhang, J., Zhang, S., 2011. Essential oils of *Cupressus funebris*, *Juniperus communis*, and *J. chinensis* (Cupressaceae) as repellents against ticks (Acari: Ixodidae) and mosquitoes (Diptera: Culicidae) and as toxicants against mosquitoes. *J. Vector Ecol.* 36, 258–268. <https://doi.org/10.1111/j.1948-7134.2011.00166.x>.
- Centers for Disease Control and Prevention (CDC), 2024. Tickborne Disease Surveillance Data Summary. Available from: <https://www.cdc.gov/ticks/data-research/facts-sheets/tickborne-disease-surveillance-data-summary.html> (Accessed 9 October 2024).
- Cuaderna, M.K.Q., Mader, E.M., Safi, A.G., Harrington, L.C., 2023. Knowledge, attitudes, and practices for tick bite prevention and tick control among residents of Long Island, New York, USA. *Ticks Tick Borne Dis.* 14, 102124. <https://doi.org/10.1016/j.ttbdis.2023.102124>.
- Dolan, M.C., Jordan, R.A., Schulze, T.L., Schulze, C.J., Manning, M.C., Ruffolo, D., Schmidt, J.P., Piesman, J., Karchesky, J.J., 2009. Ability of two natural products, Nootkatone and Carvacrol, to suppress *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in a Lyme disease endemic area of New Jersey. *J. Econ. Entomol.* 102, 2316–2324. <https://doi.org/10.1603/029.102.0638>.
- Dyer, M.C., Requistina, M.D., Berger, K.A., Puggioni, G., Mather, T.N., 2021. Evaluating the effects of minimal risk natural products for control of the tick, *Ixodes scapularis* (Acari: Ixodidae). *J. Med. Entomol.* 58, 390–397. <https://doi.org/10.1093/jme/tjaal88>.

- Eiden, A.L., Kaufman, P.E., Oi, F.M., Allan, S.A., Miller, R.J., 2015. Detection of permethrin resistance and fipronil tolerance in *Rhipicephalus sanguineus* (Acari: Ixodidae) in the United States. *J. Med. Entomol.* 52, 429–436. <https://doi.org/10.1093/jme/tjv005>.
- Eisen, L., 2024. Efficacy of unregulated minimum risk products to kill and repel ticks. *Emerg. Infect. Dis.* 30. <https://doi.org/10.3201/eid3001.230813>.
- Eisen, L., Eisen, R.J., 2023. Changes in the geographic distribution of the blacklegged tick, *Ixodes scapularis*, in the United States. *Ticks Tick Borne Dis.* 14, 102233. <https://doi.org/10.1016/j.ttbdis.2023.102233>.
- Eisen, L., 2016. Critical evaluation of the linkage between tick-based risk measures and the occurrence of Lyme disease cases: Table 1. *J. Med. Entomol.* 53, 1050–1062. <https://doi.org/10.1093/jme/tjw092>.
- Eisen, R.J., Paddock, C.D., 2021. Tick and tickborne pathogen surveillance as a public health tool in the United States. *J. Med. Entomol.* 58, 1490–1502. <https://doi.org/10.1093/jme/tjaa087>.
- Eisen, R.J., Stafford, K.C., 2021. Barriers to effective tick management and tick-bite prevention in the United States (Acari: Ixodidae). *J. Med. Entomol.* 58, 1588–1600. <https://doi.org/10.1093/jme/tjaa079>.
- Eisen, R.J., Eisen, L., 2018. The Blacklegged tick, *Ixodes scapularis*: an increasing public health concern. *Trends Parasitol.* 34, 295–309. <https://doi.org/10.1016/j.pt.2017.12.006>.
- Eisen, R.J., Eisen, L., Beard, C.B., 2016. County-scale distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in the continental United States. *J. Med. Entomol.* 53, 349–386. <https://doi.org/10.1093/jme/tjv237>.
- Eisen, R.J., Kugeler, K.J., Eisen, L., Beard, C.B., Paddock, C.D., 2017. Tick-borne zoonoses in the United States: persistent and emerging threats to Human health. *ILAR J.* 58, 319–335. <https://doi.org/10.1093/ilar/ilx005>.
- Eller, F.J., Vander Meer, R.K., Behle, R.W., Flor-Weiler, L.B., Palmquist, D.E., 2014. Bioactivity of Cedarwood oil and Cedrol against arthropod pests. *Environ. Entomol.* 43, 762–766. <https://doi.org/10.1603/EN13270>.
- Environmental Protection Agency (EPA), 2023. EPA Announces Accelerated Action on Four Organophosphate Pesticides Based on Updated Exposure Assessments. Available from: <https://www.epa.gov/newsreleases/epa-announces-accelerated-action-four-organophosphate-pesticides-based-updated-0> (Accessed 10 October 2024).
- Environmental Protection Agency (EPA), 2024. EPA Issues Emergency Order to Stop Use of Pesticide Dacthal to Address Serious Health Risk. Available from: <https://www.epa.gov/newsreleases/epa-issues-emergency-order-stop-use-pesticide-dacthal-address-serious-health-risk-4> (Accessed 7 November 2024).
- Environmental Protection Agency (EPA), 2025. Chlorpyrifos. Available from: <https://www.epa.gov/ingredients-used-pesticide-products/chlorpyrifos> (Accessed 22 May 2025).
- Falco, R.C., Fish, D., 1988. Prevalence of *Ixodes dammini* near the homes of Lyme disease patients in Westchester County, New York. *Am. J. Epidemiol.* 127, 826–830. <https://doi.org/10.1093/oxfordjournals.aje.a114865>.
- Falco, R.C., McKenna, D.F., Daniels, T.J., Nadelman, R.B., Nowakowski, J., Fish, D., Wormser, G.P., 1999. Temporal relation between *Ixodes scapularis* abundance and risk for Lyme disease associated with Erythema Migrans. *Am. J. Epidemiol.* 149, 771–776. <https://doi.org/10.1093/oxfordjournals.aje.a009886>.
- Feldman, K.A., Connally, N.P., Hojgaard, A., Jones, E.H., White, J.L., Hinckley, A.F., 2015. Abundance and infection rates of *Ixodes scapularis* nymphs collected from residential properties in Lyme disease-endemic areas of Connecticut, Maryland, and New York. *J. Vector Ecol.* 40, 198–201. <https://doi.org/10.1111/jvec.12153>.
- Foster, E., Maes, S.A., Holcomb, K.M., Eisen, R.J., 2023. Prevalence of five human pathogens in host-seeking *Ixodes scapularis* and *Ixodes pacificus* by region, state, and county in the contiguous United States generated through national tick surveillance. *Ticks Tick Borne Dis.* 14, 102250. <https://doi.org/10.1016/j.ttbdis.2023.102250>.
- Jordan, R.A., Schulze, T.L., 2020. Availability and nature of commercial tick control services in three Lyme disease endemic states. *J. Med. Entomol.* 57, 807–814. <https://doi.org/10.1093/jme/tjz215>.
- Kugeler, K.J., Schwartz, A.M., Delorey, M.J., Mead, P.S., Hinckley, A.F., 2021. Estimating the frequency of Lyme disease diagnoses, United States, 2010–2018. *Emerg. Infect. Dis.* 27, 616–619. <https://doi.org/10.3201/eid2702.202731>.
- Lenth, R., 2025. emmeans: Estimated Marginal Means, Aka Least-Squares Means. R package version 1.11.1-00001. <https://rvinth.github.io/emmeans/>.
- Maupin, G.O., Fish, D., Zultowsky, J., Campos, E.G., Piesman, J., 1991. Landscape ecology of Lyme disease in a residential area of Westchester County, New York. *Am. J. Epidemiol.* 133, 1105–1113. <https://doi.org/10.1093/oxfordjournals.aje.a115823>.
- May, P., 2016. New β -triketone insecticides offer novel mode of action to control resistant insects. *Int. Pest Control* 310–311.
- McComic, S.E., Chen, R., Mishra, S., Geldenhuys, W.J., Cantrell, C.L., Burgess, E.R., Anderson, T.D., Swale, D.R., 2025. Mode of toxicity of the β -triketone leptospermone to *Aedes aegypti* mosquitoes. *Pestic. Biochem. Physiol.* 210, 106401. <https://doi.org/10.1016/j.pestbp.2025.106401>.
- Miller, P., Peters, B., 2017. Flavesone: a novel insecticide for the control of urban pests. In: Davies, P.M., Pfeiffer, C., Robinson, W.H. (Eds.), *Proceedings of the Ninth International Conference on Urban Pests. Presented at the Ninth International Conference on Urban Pests. Pureprint Group, Uckfield, UK*, pp. 245–251.
- Nammunige, N.A., Agnew-Francis, K.A., Fernando, D.D., Taylor, S., Lu, H., Chow, S., Hartel, G., Okano, S., Williams, C.M., Fischer, K., 2025. β -triketones from *leptospermum scoparium* (mānuka) oil show potential as scabicides. *Phytomedicine* 136, 156321. <https://doi.org/10.1016/j.phymed.2024.156321>.
- Ostfeld, R.S., Price, A., Hornbostel, V.L., Benjamin, M.A., Keesing, F., 2006. Controlling ticks and tick-borne zoonoses with biological and chemical agents. *BioScience* 56, 383. [https://doi.org/10.1641/0006-3568\(2006\)056%255B0383:CTATZW%255D2.0.CO;2](https://doi.org/10.1641/0006-3568(2006)056%255B0383:CTATZW%255D2.0.CO;2).
- Panella, N.A., Dolan, M.C., Karchesy, J.J., Xiong, Y., Peralta-Cruz, J., Khasawneh, M., Montenieri, J.A., Maupin, G.O., 2005. Use of novel compounds for pest control: insecticidal and acaricidal activity of essential oil components from Heartwood of Alaska Yellow Cedar. *J. Med. Entomol.* 42.
- Panella, N.A., Karchesy, J., Maupin, G.O., Malan, J.C.S., Piesman, J., 1997. Susceptibility of immature *Ixodes scapularis* (Acari: ixodidae) to plant-derived acaricides. *J. Med. Entomol.* 34, 340–345. <https://doi.org/10.1093/jmedent/34.3.340>.
- Pepin, K.M., Eisen, R.J., Mead, P.S., Piesman, J., Fish, D., Hoen, A.G., Barbour, A.G., Hamer, S., Diuk-Wasser, M.A., 2012. Geographic variation in the relationship between Human Lyme disease incidence and density of infected host-seeking *Ixodes scapularis* nymphs in the Eastern United States. *Am. J. Trop. Med. Hyg.* 86, 1062–1071. <https://doi.org/10.4269/ajtmh.2012.11-0630>.
- Perret, J.-L., Guerin, P.M., Diehl, P.A., Vilmant, M., Gern, L., 2003. Darkness induces mobility, and saturation deficit limits questing duration, in the tick *Ixodes ricinus*. *J. Exp. Biol.* 206, 1809–1815. <https://doi.org/10.1242/jeb.00345>.
- Piepho, H.-P., Malik, W.A., Bischoff, R., El-Hasan, A., Scheer, C., Sedlmeier, J.E., Gerhards, R., Petschenka, G., Voegele, R.T., 2024. Efficacy assessment in crop protection: a tutorial on the use of Abbott's formula. *J. Plant Dis. Prot.* 131, 2139–2160. <https://doi.org/10.1007/s41348-024-00968-0>.
- R Core Team, 2024. R: A language and Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>.
- Robert, K., Van Gestel, M., Lathouwers, M., Fonville, M., Sprong, H., Matthysen, E., Heylen, D., 2025. Abundance of ticks and tick-borne pathogens in domestic gardens in Belgium, 2020–2022: a citizen science approach. *BMC Public Health* 25. <https://doi.org/10.1186/s12889-025-23221-1>.
- Rosenberg, R., Lindsey, N.P., Fischer, M., Gregory, C.J., Hinckley, A.F., Mead, P.S., Paz-Bailey, G., Waterman, S.H., Drexler, N.A., Kersh, G.J., Hooks, H., Partridge, S.K., Visser, S.N., Beard, C.B., Petersen, L.R., 2018. Vital signs: trends in reported VectorVital signs: trends in reported vector borne disease cases — United States and Territories, 2004–2016. *MMWR Morb. Mortal. Wkly. Rep.* 67, 496–501. <https://doi.org/10.15585/mmwr.mm6717e1>.
- Sagiv, S.K., Mora, A.M., Rauch, S., Kogut, K.R., Hyland, C., Gunier, R.B., Bradman, A., Deardorff, J., Eskenazi, B., 2023. Prenatal and childhood exposure to organophosphate pesticides and behavior problems in adolescents and young adults in the CHAMACOS study. *Environ. Health Perspect.* 131, 067008. <https://doi.org/10.1289/EHP11380>.