



Lemongrass essential oil and DEET inhibit attractant detection in infected and non-infected *Ixodes scapularis* ticks

Kayla Gaudet^{a,b}, Luis Adriano Anholetto^b, N. Kirk Hillier^a, Nicoletta Faraone^{b,*}

^a Department of Biology, Acadia University, 33 Westwood Ave., Wolfville, NS, Canada B4P 2R6

^b Department of Chemistry, Acadia University, 6 University Ave., Wolfville, NS, Canada B4P 2R6

ARTICLE INFO

Keywords:

N,N-diethyl-3-methyl benzamide
Fumigation
Chemosensory system
Repellent
Essential oil
Inhibition

ABSTRACT

Blacklegged tick, *Ixodes scapularis* Say (Arachnida: Ixodidae), is a growing health concern for humans as vectors the causative agent of Lyme disease, *Borrelia burgdorferi*, and many other pathogens. Given the potential health threat *I. scapularis* entails, and the need to find effective strategies to prevent tick bites, it is pivotal to understand the chemosensory system of ticks and their host-seeking behaviour when exposed to repellents. In this study, we investigated whether the exposure to synthetic and plant-derived repellents impairs the ability of *I. scapularis* to detect attractants and host volatiles (butyric acid), and ultimately how these repellents interfere with host-seeking behaviour in both wild and lab-reared ticks. Furthermore, we screened wild ticks used in electrophysiology and Y-tube behavioural assays for presence of pathogens (*Borrelia*, *Anaplasma*, and *Babesia*) to evaluate if the bacterial infection status would affect the detection of butyric acid under the exposure to repellents. We determined that the exposure to DEET, lemongrass essential oil, citral, and geraniol significantly inhibited the ability of both lab-reared and wild adult female *I. scapularis* to detect and respond to butyric acid. We found that tick infection status does not significantly impact host-seeking behaviour in adult female *I. scapularis*. The knowledge gained from our study contributes to advance our understanding of host-seeking behaviour in ticks and the impact that the exposure to repellent has on the tick chemosensory system. These findings will be important for elucidating the mechanism of repellence in ticks and for the development of effective tick repellent management tools.

Introduction

Blacklegged ticks, *Ixodes scapularis* Say (Arachnida: Ixodidae), are obligate blood-feeding ectoparasites, responsible for the transmission of many pathogens, including *Borrelia burgdorferi*, the causative agent of Lyme disease. Given the potential health threat *I. scapularis* presents, it is important to understand how the chemosensory system of ticks and their host-seeking behaviours (Faraone et al., 2020) are affected when ticks are exposed to repellents.

Ticks use chemosensory receptor neurons to detect and distinguish between a broad range of chemical cues from their environment (Faraone, 2022; Kaupp, 2010; Sokolinskaya et al., 2010). Through the questing process, ticks detect chemical stimuli associated with hosts, and olfactory information is converted into nerve impulses through receptors and signal transduction. This causes a neuronal depolarization, which leads to the initiation of an action potential, which then allows signals to be transmitted to the brain for sensory coding (Kaupp, 2010;

Sokolinskaya et al., 2010; Persaud, 2013). An important distinguishing feature of tick olfaction is the presence of the Haller's organ, which is located on both foretarsi and contains porous olfactory sensilla, non-porous thermoreceptor sensilla, and hygroreceptors (Leonovich, 2021). The Haller's organ allows ticks to detect and differentiate chemical cues, which then elicit behavioural responses (Carr et al., 2017). During questing, *Ixodes* ticks extend their front legs and search for a passing host by detecting chemosensory stimuli through the Haller's organ. The removal of the Haller's organ results in some impairment of the chemosensory abilities (Carr et al., 2017; Faraone et al., 2019) indicating its role in detecting volatile compounds. Ticks also have pedipalps and chelicerae that host additional chemosensory sensilla. Although pedipalps have been shown to house sensory hairs with multiple roles (Faraone et al., 2019), their chemosensory function is not well characterized (Carr and Roe, 2016).

By combining electrophysiology with behavioural assays, the neural responses of the tick olfactory system can be linked to the tick

* Corresponding author.

E-mail address: nicoletta.faraone@acadiau.ca (N. Faraone).

<https://doi.org/10.1016/j.cris.2024.100096>

Received 12 September 2023; Received in revised form 12 September 2024; Accepted 16 September 2024

Available online 18 September 2024

2666-5158/© 2024 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

behaviour. This integration of data provides a more comprehensive understanding of how ticks perceive and respond to different odours, which can be critical for various applications, including developing strategies for tick control or understanding their role as disease vectors. Previous studies investigated the olfaction of ticks and other acarines through exposure to repellents (Faraone et al., 2020; Light et al., 2021; Romashchenko et al., 2012; Josek et al., 2021); however, further research is needed to understand the physiological effects of repellents, and the potential latency of effects after exposure.

Our lab has developed a novel electrophysiological method to investigate the chemosensory response of ticks to repellents (i.e., volatile organic compounds) and attractants (i.e., butyric acid) (Faraone et al., 2020). By using this novel electrophysiological approach termed “electroscutumography” (ESG), we investigated exposure to a known concentration of attractant, butyric acid, delivered simultaneously with a known repellent, such as geraniol or citral. After exposure to a combination of a repellent volatile and an attractant, ticks experienced a reduction in the overall electrophysiological response and action potential.

To identify compounds that can be used in tick management and to protect the public from tick bites, it is pivotal to have a deep understanding of the mechanism of chemoreception (e.g., how ticks detect and respond to chemical cues), and the related behavioural responses elicited (Josek et al., 2021; Faraone et al., 2020). Ticks rely on their chemosensory system to detect and respond to various chemical cues, such as host odours, pheromones, and other environmental cues. By understanding the mechanisms behind chemoreception, we can pinpoint the specific chemical compounds that attract or repel ticks. Behavioural studies are a key component for the identification of attractants and/or repellents, ultimately to develop effective pest control agents (Carr and Roe, 2016). *N,N*-diethyl-3-methyl benzamide (DEET) is considered as the gold standard of synthetic compounds commonly used to repel pest species such as mosquitoes (Leal, 2014). DEET is believed to interfere with receptors in mosquito (and other biting insects) antennae that detect L-lactic acid and carbon dioxide, the primary attractants emitted by humans and other animals (Koloski et al., 2020). Environmental and human health concerns have been raised with the use of DEET since it has been found to bioaccumulate in water and surface soil, posing a potential health threat to both aquatic and terrestrial organisms (Weeks et al., 2012; Zhu et al., 2020). Under specific conditions, potential human health concerns for the use of DEET are skin inflammation, tremors, seizures, hypotension, and bradycardia (Swale and Bloomquist, 2019). Therefore, the use of plant-derived repellents, such as lemongrass essential oil, have been considered as promising alternatives to synthetic repellents (Luker et al., 2023). Two main components present in the lemongrass essential oil composition are citral and geraniol, which have both exhibited repellent activity against ticks (Faraone et al., 2019) being linked to the overall observed repellent action exerted by the essential oil.

Previous studies suggest that bacterial infection in ticks may impact host-seeking behaviour, such as questing behaviour (Faraone, 2022) and *Borrelia* spp. can promote behavioural modifications in tick vectors manipulating the response to some olfactory stimuli (Romashchenko et al., 2012). *Borrelia burgdorferi* is the causative agent of Lyme disease transmitted to vertebrate hosts by *Ixodes* spp. ticks (Kurokawa et al., 2020). This bacterium has developed complex interactions with ticks to successfully colonize, multiply and, at the optimal time, exit the tick to invade another host. The spirochaete interacts with the tick gut and salivary gland proteins important for establishing infection and transmission to the vertebrate host (Kurokawa et al., 2020). Few studies have investigated whether the infection of *B. burgdorferi* in ticks could impact questing behaviour (Javed et al., 2021). A study done by Faulde and Robbins (2008) compared the number of infected *Ixodes ricinus* collected from dragging methods versus those collected on humans and found that a significantly higher number of infected ticks were found on humans rather than on the dragging tools. This suggests that infection of

B. burgdorferi may induce behavioural changes in adult ticks, possibly leading to an enhancement in host-finding ability. Overall, *Borrelia* infection might enhance ticks' strength, aggressiveness, and resistance to temperature fluctuations (Adamo et al., 2022; Benelli, 2020).

In this study, we explored whether the prolonged exposure to synthetic and plant-derived repellents impair the chemosensory ability of *I. scapularis* to detect butyric acid (a known attractant), and ultimately how these repellents interfere with the host-seeking behaviour in both wild and lab-reared ticks. We investigated how the prolonged exposure to known repellents such as DEET and lemongrass essential oil would interfere with the detection of butyric acid in both electrophysiological and behavioural studies, and if bacterial infection status would impact the ability of ticks to respond to butyric acid under the exposure to repellents. The knowledge gained from our study will advance our understanding of host-seeking behaviour in ticks and how it might be impacted by the presence of a repellent adding valuable information to unveil the mechanism behind repellents, and to develop effective tick management strategies.

Methods and materials

Chemicals. HPLC-grade hexane and histological-grade reagent alcohol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Geraniol (98 %), citral (95 %), and *N,N*-diethyl-3-methyl benzamide (DEET) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Butyric acid (≥ 98 %) was purchased from Bedoukian (Danbury, CT, USA). Lemongrass (*Cymbopogon citratus*) essential oil was purchased from New Direction Aromatics (Mississauga, ON, Canada).

Animal Care. Lab reared blacklegged ticks or deer ticks (*I. scapularis*) were purchased from the tick rearing facility at Oklahoma State University (Stillwater, OK, USA). Wild *I. scapularis* were collected from Kings, Annapolis, Lunenburg, Colchester, and Cumberland counties of Nova Scotia (Canada) between May 2022 and June 2023. All *I. scapularis* used in this study were adult females. Tick colonies were kept at Acadia University, Wolfville (NS, Canada) in plastic containers (21cm x 15cm x 7cm) with moist Kimwipes (Kimberly-Clark, Texas, USA). Ticks were kept at 4°C prior to starting experiments. One-week prior to experiments ticks were moved from the fridge to room temperature (20 ± 2°C) and kept on a 12:12 light/dark photoperiod. Ticks collected from the field were washed to prevent fungal growth (Binetruy et al., 2019). Wild ticks were transferred to a falcon tube (Fisherbrand, Fisher Scientific, ON, Canada) containing 10 % v/v bleach solution (Great Value™ bleach, Walmart Canada, Mississauga, ON, Canada) and a drop of dish soap (Down East, Dartmouth, NS, Canada). The solution containing the ticks was vortexed for 5 minutes and emptied into a sieve making sure no tick was left behind. The wash was repeated with 70 % v/v ethanol, where ticks were vortexed for 2 minutes. The last rinse was done with only distilled water, where ticks were vortexed for 2 minutes. After washing, all wild ticks were stored in plastic containers with wet Kimwipes. Each week tick colonies were checked for any potential fungal growth.

Tick Collection. Wild ticks were collected from Kings, Annapolis, Lunenburg, Colchester, and Cumberland counties of Nova Scotia (Canada) between May 2022 and June 2023. Ticks were collected by walking through the designated area to be sampled with a 1-m by 1-m² white cotton cloth dragging behind the collector. After dragging for approximately 8–10 m, the collector stopped to check the drag cloth and to remove any ticks (Salomon et al., 2020).

Tick Fumigation. Electrophysiological and behavioural studies to assess the response of ticks to butyric acid and hexane (control stimulus) were completed after exposing the ticks to hexane, butyric acid, lemongrass essential oil, DEET, citral, and geraniol by using a non-contact fumigation method adapted from a previous study by Sfara et al. (2009). During repellent exposure, ticks were exposed to the treatment in a 33 mL glass cylinder vial with a closed lid. A piece of filter paper (Fisherbrand, Fisher Scientific, ON, Canada), dampened with distilled water, was placed on the bottom of the container to create a

humid environment for the tick. An individual tick was placed inside the vial on the wet filter paper. A piece of round filter paper of the same diameter of the lid was placed inside the lid of the vial, and 10 μL of the designated treatment was applied to the filter paper at a concentration of 3.3 mg/mL to achieve in the fumigation chamber a final concentration of 1.0 mg of repellent / 1.0 mL of air. Then the lid was placed back on the glass container to allow a non-contact fumigation exposure of the treatment to the tick. The tick was exposed to the treatment by fumigation for 20 minutes. After the exposure to the treatment, ticks were readily used in electrophysiological and behavioural assays. We used 20 ticks for each treatment ($n=20$).

Electrophysiology. From previous electrophysiological studies (Faraone et al., 2020; Romashchenko et al., 2012), we developed a more efficient mounting technique that we called ‘electrotarsography’ based on the use of a fork-electrode set-up. Tarsi not bearing the Haller’s organs were surgically removed prior to mounting using surgical scissors and a dissecting microscope (AmScope SM-1BSX-64S, Irvine, CA, USA). High conductivity electrode gel (Signagel®, Parker Laboratories Inc, NJ, United States) was placed on the base electrode, and the tick was mounted dorsally between the base and the recording electrode of a Syntech EAG Probe (Syntech, Kirchzarten, Germany; Fig. 1). The tick’s electrophysiological response to stimuli was amplified via an Intelligent Data Acquisition Controller-2 (IDAC-2) (Syntech, Kirchzarten, Germany) and recorded through Data Acquisition for Gas Chromatograph with EAD (GcEad 2012 v1.2.4 (2012-06-24), Syntech, Kirchzarten, Germany). Electrotarsography (ETG) measured the stimulation of the tick chemosensory system to a given compound within a stimulus cartridge when a puff of air was directed through the cartridge to the mounted specimen. The recorded electrophysiological response may include the combined extracellular potentials of neurons from the synganglion indicating that the measured ETG may be an ensemble

response of all of the above. The voltage recorded in response to specific stimuli corresponded to the tick’s sensitivity to a particular compound.

We assessed whether ticks responded to different concentrations of tested compounds in a dose-response relationship presenting 0.1, 0.5, 1, 5, and 10 $\mu\text{g}/\mu\text{L}$. Stimulus cartridges contained a single piece of filter paper (Fisherbrand, Fisher Scientific, ON, Canada) that measured 7 mm x 35 mm were loaded with 10 μL of the compound of interest under a fume hood and let the solvent to evaporate for about 10 minutes. The loaded filter paper was placed into a disposable borosilicate glass pipette (Fisherbrand, Fisher Scientific, ON, Canada). Then the glass pipette was capped with 1-mL disposable pipette tip (Fisherbrand, Fisher Scientific, ON, Canada) and the tip of the pipette was sealed with dental wax (Electron Microscopy Sciences, Hatfield, PA, USA) to avoid any loss of volatile components. We tested the following compounds: hexane, butyric acid, lemongrass essential oil, DEET, citral, and geraniol. Only butyric acid elicited a detectable response showing a dose-response relationship with the magnitude of the response of the tick as a function of dose; therefore, we selected 100 μg as the concentration to use for all the compounds in further tests.

Compounds were diluted in HPLC-grade hexane and tested at 100 μg . All prepared stimulus cartridges were stored at -20°C until further use. After a maximum of 20 uses, stimulus cartridges were freshly prepared (as per standard protocol developed in our lab) (Gaudet et al., 2023). Compounds were presented to the tick in the following order: (1) air, (2) hexane, (3) butyric acid, (4) lemongrass essential oil, (5) DEET, (6) citral, and (7) geraniol. Given the short sequence of compounds, we did not deem it necessary to do a final hexane response for each replicate. A single odorant puff lasted 0.3 s controlled by a Syntech stimulus controller CS-55 V2.7 (Syntech, Kirchzarten, Germany). A constant humidified airflow (0.5 L/min) was delivered to the tick preparation. The flow rate of air for each puff is 3.54 L/min. A stimulus interval of 60

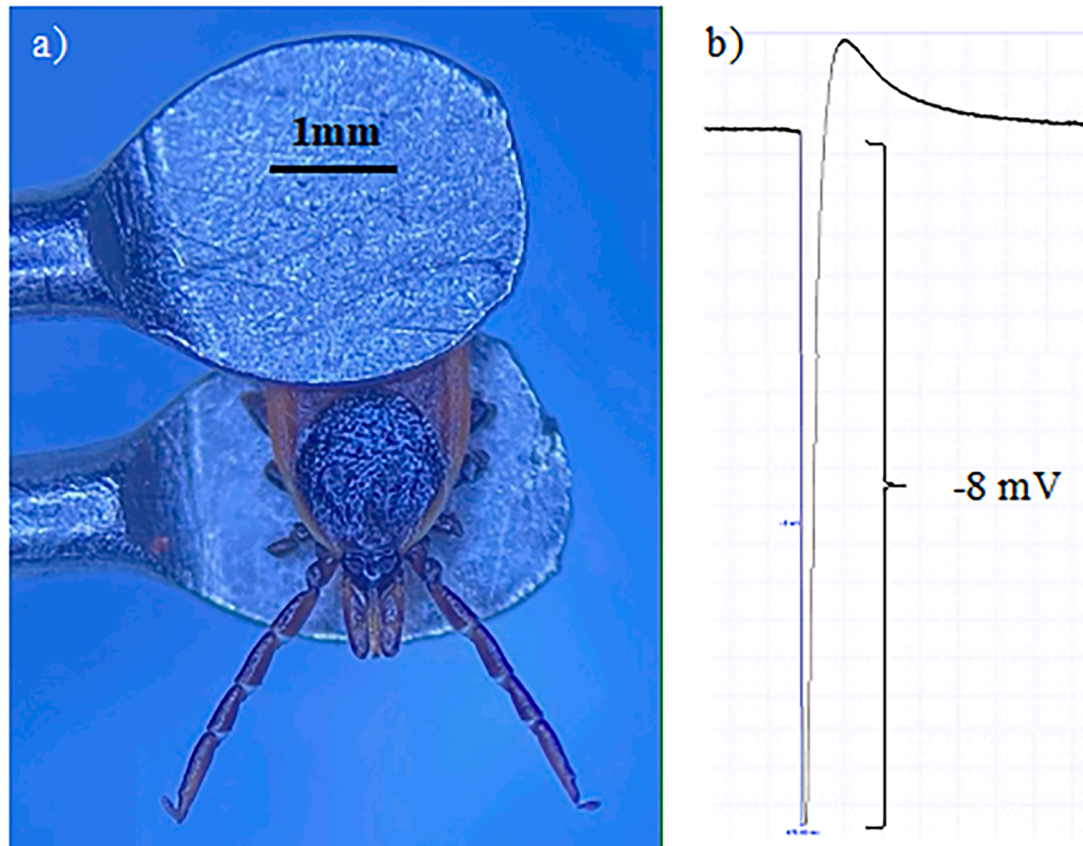


Fig. 1. a) Adult female *Ixodes scapularis* mounted dorsally on a fork-electrode probe using electrode gel for electrophysiology experiments. Legs not bearing the Haller’s organ were surgically removed prior mounting the tick on the fork electrode. b) Example of an electrophysiological response to 10 μg of butyric acid.

s was used to reduce any likelihood of adaptation or habituation due to stimulus presentation.

Y-Tube Behavioural Bioassay. Y-tube behavioural bioassays used in this study were performed according to [Josek et al. \(2021\)](#) and [Faraone et al. \(2020\)](#) (Fig. 2). A Y-tube olfactometer (Sigma Scientific, Micanopy, FL) diameter: 2 cm; entry arm: 10 cm; choice arms: 5 cm) placed at a 45° angle was used to examine behavioural responses of adult female *I. scapularis* (lab-reared and wild) ticks to butyric acid pre- and post-exposure to hexane, lemongrass essential oil, DEET, citral, geraniol, and butyric acid. The system was maintained at approximately 25°C with controlled humidity (65–70 %). Air was humidified by drawing air through a 150 mL Erlenmeyer flask containing water ([Josek et al., 2021](#)) and pushed through the arms of the Y-tube at a flow rate of 6.0 L/min. Ticks were carefully placed with a paintbrush in the glass Y-tube and were given a maximum of 7 minutes to make a choice between hexane or butyric acid loaded on filter paper strips (10 µg). Ticks were placed approximately 2 cm from the opening of the tube and the timer was started immediately. As test stimulus, 10 µL of butyric acid, was applied on a piece of filter paper (Fisherbrand, Fisher Scientific, ON, Canada) at a concentration of 1.0 µg/µL. A control stimulus, 10 µL of hexane, was applied on another piece of filter paper. Both test stimuli were allowed to dry for 5 minutes under a fume hood prior to be used in the bioassay. Filter papers were placed at the distal ends of each Y-tube arm. The position of the control and test stimuli (left or right arm of the Y-tube) was alternated after three trials, and the Y-tube bioassay was rinsed with 75 % v/v ethanol and dried between each tested compound ([Faraone et al., 2020](#)).

Bacterial Infection Analysis. Analysis of bacterial load in wild-collected ticks was performed at the Mount Allison University Lyme Disease Research Facility (Mount Allison University, Sackville, NB, Canada). We screened wild ticks for *Borrelia burgdorferi* s.l., *Borrelia burgdorferi* s.s., *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Babesia microti*, *Babesia odocoilei*, and *Babesia duncani* following a protocol developed by [Wills et al. \(2018\)](#). To test for *Borrelia*, DNA was extracted from each tick, and then an initial round of polymerase chain reaction (PCR) was performed to detect each of the *Borrelia*-specific loci. Subsequent PCR used the product of the first reaction as a new template to generate smaller, internal amplification fragments. The nested PCR approach improves upon both the specificity and sensitivity of conventional PCR. A tick was considered positive for the pathogen when inner amplicons from the amplified genes were detected by agarose gel electrophoresis. The same methodology was followed to test for *Anaplasma* and *Babesia* species ([Wills et al., 2018](#)).

Data Analyses. Electrophysiological responses were measured on GC-EAD software (GcEad 2012 v1.2.4, 2012-06-24, Syntech, Kirchzarten, Germany) and exported to Excel (Microsoft Excel for Mac, Version 16.54). To compare sensitivity of *I. scapularis* to butyric acid (100 µg) for

pre- and post-exposure to hexane, lemongrass essential oil, DEET, citral, and geraniol, a Kruskal-Wallis rank sum test was performed for both lab-reared and wild ticks ($\alpha = 0.05$), followed by Dunn multiple comparison with the Bonferroni method ($\alpha = 0.05$). These statistical tests were chosen given that data were not normally distributed - as determined from a Shapiro-Wilk normality test on the amplitude of response to butyric acid for each exposure compound. Additionally, a Kruskal-Wallis rank sum test was performed to compare the difference in butyric acid response between presence of any infection type versus lab (negative) ticks. To determine potential influence of pre-exposure treatment on choice in two-choice behavioural assays, *binomial tests* (performed using the R Stats Package) were performed for each exposure compound to determine whether the proportion of ticks that chooses the treatment side (butyric acid) over control side (hexane) was greater than 0.5 (one-tailed test with $\alpha = 0.05$). All statistical analyses were performed using R version 4.0.3 (R Core Team, 2020).

Results

Tick Collection. The number of wild *I. scapularis* ticks collected over the different counties of Nova Scotia, Canada, between May 2022 and June 2023 is reported in Table S1.

Electrophysiology. The response of *I. scapularis* wild and lab reared ticks to each of the stimuli (lemongrass essential oil, DEET, citral, and geraniol) was not significantly different from the solvent control (data reported in the Supplementary Materials, Fig. S1 and S2), indicating that no detectable response was induced by lemongrass essential oil, DEET, citral, and geraniol. In dose-response study, butyric acid elicited a detectable response showing a dose-response relationship with the magnitude of the response of the tick as a function of dose (Supplemental Materials, Fig. S3).

The response of *I. scapularis* wild ticks to butyric acid was significantly different between pre- and post-exposure to compounds, as determined from results of the *Kruskal-Wallis rank sum test* ($\chi^2 = 39.965$; $DF = 5$; $P < 0.0001$) in which ticks pre-exposure, and post-exposure to hexane, had a significantly greater response to butyric acid when compared to their response to butyric acid post-exposure to lemongrass essential oil, DEET, citral, and geraniol (Fig. 3).

For lab-reared ticks, the response of *I. scapularis* to butyric acid was significantly different between pre- and post-exposure compounds, as determined from results of the *Kruskal-Wallis rank sum test* ($\chi^2 = 12.984$; $DF = 5$; $P < 0.05$). Tick pre-exposure, and post-exposure to hexane, have a significantly larger response to butyric acid when compared to their response to butyric acid post-exposure to lemongrass essential oil, DEET, citral, and geraniol (Fig. 4).

There was no significant difference in the electrophysiological response of butyric acid when comparing presence of any infection type versus lab (negative) ticks, as determined from results of the *Kruskal-Wallis rank sum test* ($\chi^2 = 7.5633$; $DF = 6$; $P = 0.2719$).

Behavioural Studies. In pre-exposure experiments, wild ticks were significantly attracted to butyric acid ($P = 0.0004$), where 90 % of the ticks choose the treatment arm (Fig. 5). In post-exposure fumigation experiments to lemongrass essential oil and DEET, wild ticks were not significantly attracted or repelled to butyric acid.

In pre-exposure assays, lab-reared ticks were significantly attracted to butyric acid ($P = 0.0004$), where 90 % of the ticks choose the treatment arm (Fig. 6). In post-exposure experiments to hexane, the lab-reared ticks were significantly attracted to butyric acid ($P = 0.003$), where 85 % of the ticks choose the treatment arm (Fig. 6). In post-exposure assays to butyric acid, lab-reared ticks were significantly attracted to butyric acid ($P = 0.0004$), where 90 % of the ticks choose the treatment arm (Fig. 6). In post-exposure fumigation assays to citral and geraniol, ticks were not significantly attracted or repelled to butyric acid, where 55 % of the ticks choose the treatment arm (Fig. 6). In post-exposure fumigation assays to lemongrass essential oil, ticks were not significantly attracted or repelled to butyric acid, where 50 % of the ticks

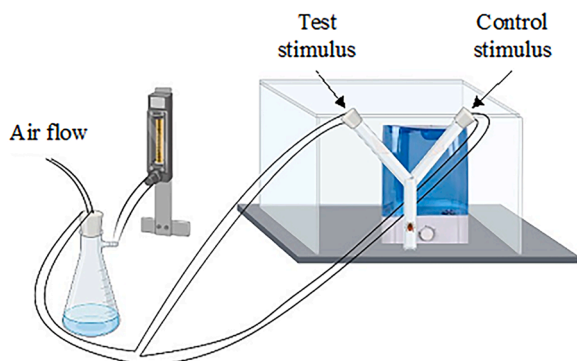


Fig. 2. Experimental set-up used to measure behavioural choice of adult female *Ixodes scapularis* ticks between butyric acid (i.e., test stimulus) or hexane (i.e., control stimulus), pre- and post- fumigation exposure to hexane, lemongrass essential oil, DEET, citral, geraniol, and butyric acid.

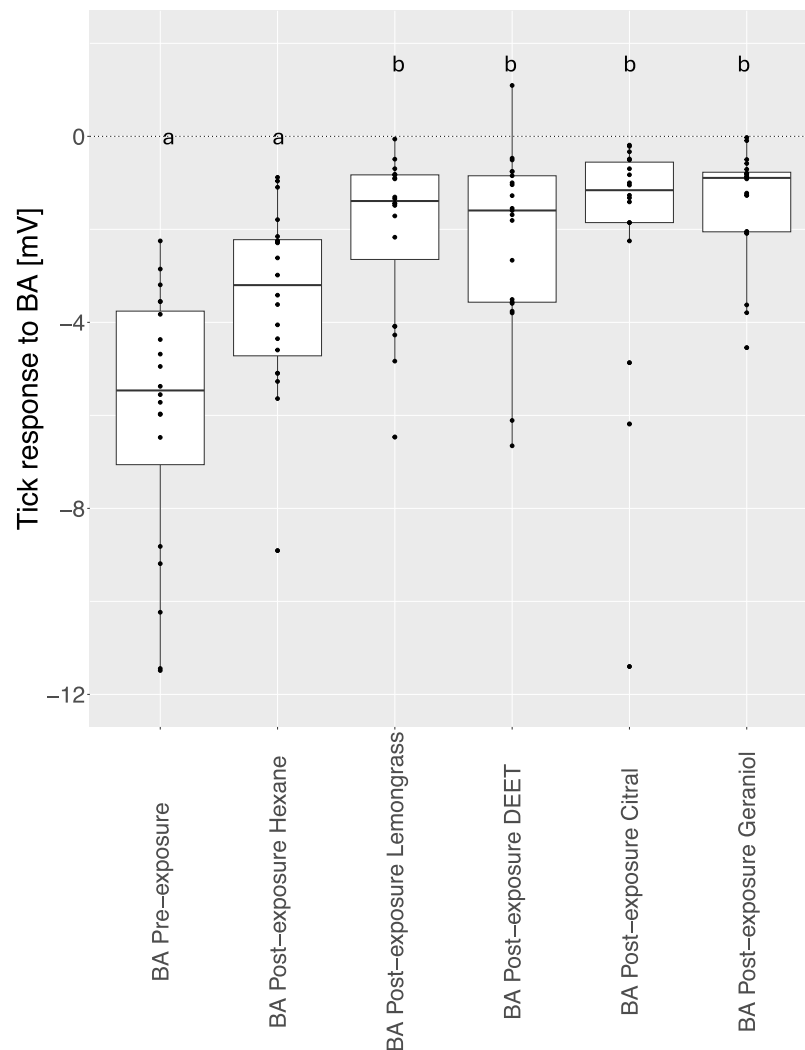


Fig. 3. Electrophysiological response of wild adult female *Ixodes scapularis* ticks to butyric acid (BA) (100µg) in pre- and post-fumigation exposure to hexane, lemongrass essential oil, DEET, citral, and geraniol (n = 20). In fumigation treatment, ticks were exposed to the designated compound at 1.0 mg of repellent / 1.0 mL of air for 20 minutes. The dashed line separates pre- and post-exposure experiments. Responses capped with different letters are significantly different ($P < 0.05$).

choose the treatment arm (Fig. 6). In post-exposure assays to DEET, ticks were not significantly attracted or repelled to butyric acid, where 45 % of the ticks choose the treatment arm (Fig. 6).

Additionally, infection status does not seem to impact attraction to butyric acid (Table 1). Any statistics is difficult to perform for individual infection groups given that certain combinations of infecting bacteria were not represented in behavioural studies.

Bacterial Infection Analysis. Several pathogens were detected in *I. scapularis* ticks including *B. burgdorferi*, *B. miyamotoi*, *A. phagocytophilum*, *B. odocoilei*, and *B. duncani* (Table S2; Fig. 7). All sample sites had some level of pathogen detection. *Borrelia burgdorferi* was the most detected pathogen. There was no detection of *Babesia microti* at any of the collection sites.

Discussion

In this project, we have investigated the effects of exposure to synthetic and plant-derived repellents on the chemosensory system of adult female *I. scapularis* ticks. The recorded tick responses (e.g., voltages) can be summarized as an overall response that combines the olfactory responses from Haller's organs and neuronal activities in the synganglion and neuromuscular activities triggered by olfactory stimulation. We found that both lab-reared and wild ticks had a decreased

electrophysiological response to butyric acid after being exposed to known repellents, such as lemongrass essential oil, DEET, citral, and geraniol. However, there was no detectable response to lemongrass, DEET, citral, and geraniol alone, being not significantly different from the solvent (data reported in the Supplementary Materials). Interestingly, we found no significant differences between the infected and non-infected groups.

A similar trend was previously observed (Faraone et al., 2020) where delivering butyric acid together with either geraniol or citral (main components with repellent activity present in lemongrass essential oil) decreases the overall electrophysiological response in ticks. The mode of action of blood-feeding arthropod repellents remains somewhat unclear, and several hypotheses have been put forward. For example, DEET, considered as the 'gold standard', has been widely investigated particularly in mosquitoes, and it has been proposed to function as inhibitor, interfering with the response of the olfactory system to a normally attractive chemical signal (i.e., lactic acid) (Dogan et al., 1999; Ditzgen et al., 2008). However, the inhibitory mechanism in mosquitoes has been challenged and described as a positive artifact due to the ability of DEET to trap the attractant when delivered together, as well as the ability of mosquitoes to smell and avoid DEET (Syed and Leal, 2008). Additionally, it has been suggested that DEET could act as a modulator of general olfactory receptor activity, disrupting the arthropod odour

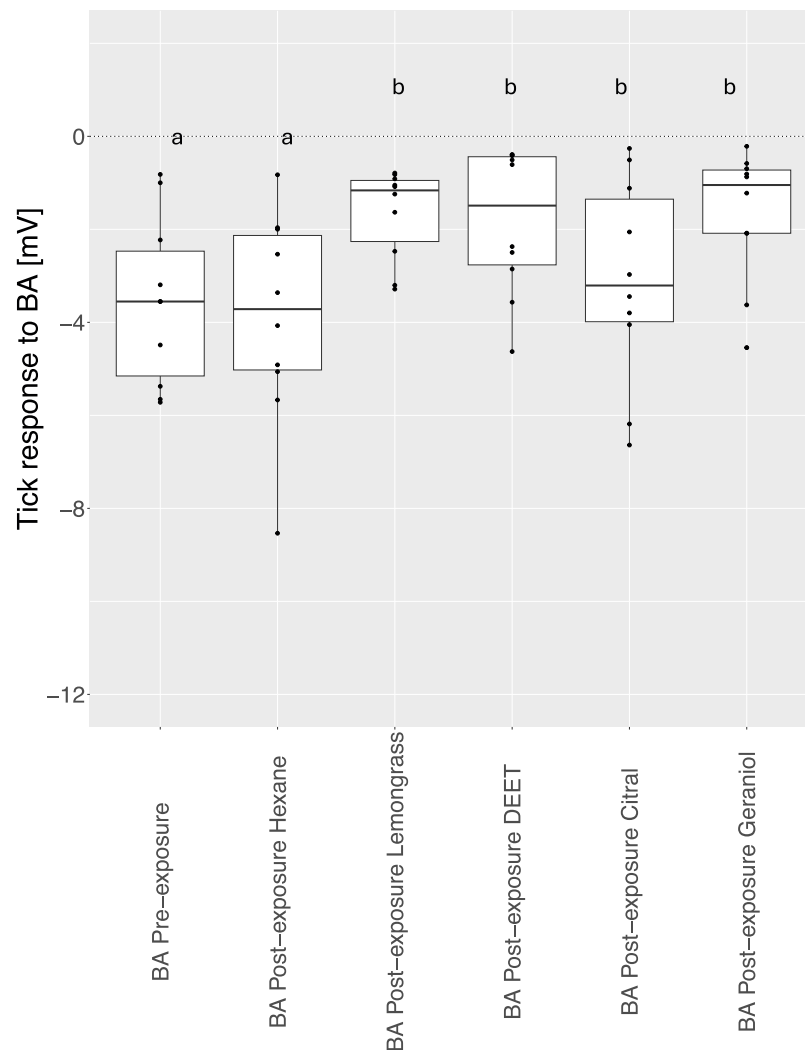


Fig. 4. Electrophysiological response of lab-reared adult female *Ixodes scapularis* ticks to butyric acid (BA) (100 μ g) in pre- and post- fumigation exposure to hexane, lemongrass essential oil, DEET, citral, and geraniol (n = 10). In fumigation treatment, ticks were exposed to the designated compound at 1.0 mg of repellent / 1.0 mL of air for 20 minutes. The dashed line separates pre- and post- exposure experiments. Responses capped with different letters are significantly different ($P < 0.05$).

code (Xu et al., 2019). In our results, the reduction in tick responsiveness by repellents to butyric acid suggests a mechanism involving a potential inhibitory effect on the tick chemosensory system, which results in a decrease response to an attractant compound post-exposure. We have observed this trend for both DEET and repellent natural compounds.

Many behavioural and electrophysiological studies indicate that olfactory receptors (ORs) in insect antennae respond to arthropod repellents and to carboxylic acids (Huff and Pitts, 2020). However, ORs and OR coreceptors (Orco) are absent in Acari (Gulia-Nuss et al., 2016; Eliash et al., 2017); on the other hand, ionotropic receptors (IRs) are known from Acari, and many such IRs are known to respond to acids from studies on insect species (Ray et al., 2023; Ni, 2021; Chen and Amrein, 2021), opening new questions on the mechanism by which ticks detect and process attractants and repellents.

Also, the role of odorant binding proteins (OBPs) has not been properly investigated in ticks. A protein family with similar structures of OBPs (therefore defined as 'OBP-like') have been previously identified in tick tarsi and palps (Renthal et al., 2017), and a more recent work reported the presence of only one member of the chemosensory protein family (CSP) (Gulia-Nuss et al., 2016). OBP-like proteins have been shown to be involved in chemosensory reception in acarines (Eliash et al., 2019; Renthal 2022), and they might be involved in the detection of repellents in ticks. Some of these proteins have a different

conformation when bound to an odorant, and repellents may disrupt the OBP-attractant interactions, challenging the transport of attractants to the olfactory receptors (Murphy et al., 2013; Xu et al., 2010), inducing a decreased in the attractant concentrations, and in the overall recorded response as shown in our results. It is still unclear why repellents did not induce a measurable response in ticks using electrophysiological analysis. Therefore, it would be important to investigate the molecular basis of this effect through comparative analyses (i.e., *Xenopus* oocytes expression) to target specific receptors and proteins.

Additionally, the impaired electrophysiological and behavioural response of ticks towards attractants might be the result of possible physical damage caused by the prolonged exposure to repellent volatiles through the fumigation assay. Previous studies have reported the fumigation effect inducing toxicity in arthropods exposed to essential oils, leading to physical damage of antennal receptor and antenna shedding (Xing et al., 2023). Citral can cause direct cell damage, precluding the arthropod's ability to successfully detect volatiles (Tak et al., 2017). To our knowledge, the repellent mode of action of citral in insects is through GABA receptors, or inhibition of acetylcholinesterase (Tak et al., 2017). The damaging effects of fumigation on the receptors may be associated to the subsequent decrease in sensitivity towards butyric acid observed after exposure to repellents.

Through behavioural studies, we found that the exposure to DEET,

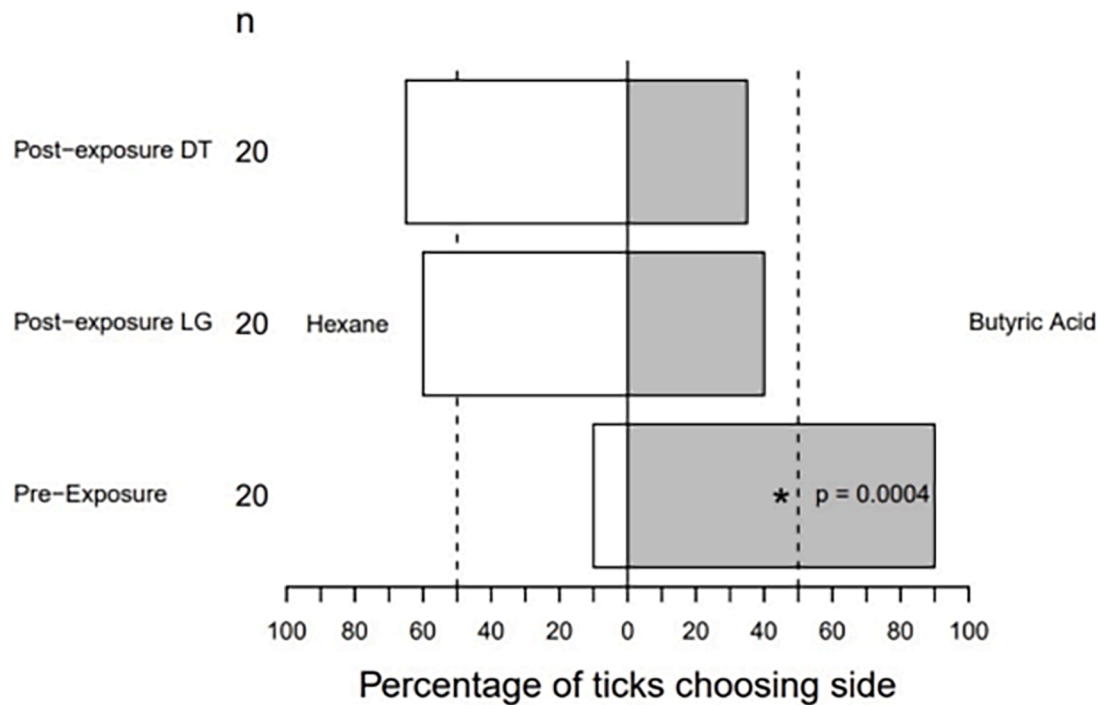


Fig. 5. Percentage of wild, adult, female *Ixodes scapularis* ticks choosing treatment (100 µg of butyric acid) or control (hexane) after 20 minutes in Y-tube two-choice behavioural assay for pre- and post-exposure to DEET (DT) and lemongrass (LG). The dashed line indicates the null expectation of 50 %. Results do not include non-responding ticks.

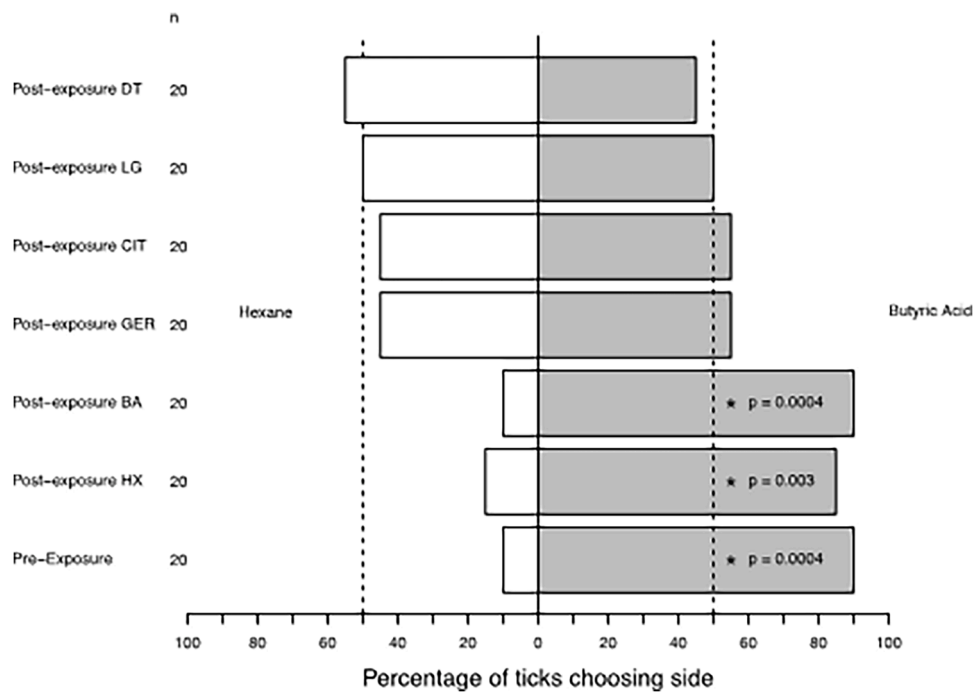


Fig. 6. Percentage of lab-reared, adult, female *Ixodes scapularis* ticks choosing treatment (100 µg of butyric acid, BA) or control (hexane, HX) in Y-tube two-choice behavioural assay for pre- and post-fumigation exposure to hexane (HX), butyric acid (BA), lemongrass essential oil (LG), DEET (DT), citral (CIT), and geraniol (GER). Dashed lines indicate the null expectation of 50 %. Results do not include non-responding ticks.

lemongrass essential oil, citral, and geraniol decreased the attraction to butyric acid in both wild and lab-reared adult females ticks. Similar results were found by Faraone et al. (2019), where *I. scapularis* nymphs were repelled by lemongrass essential oil constituents, including geraniol and citral, and DEET in a short-term repellency bioassay. In previous studies, lemongrass essential oil has shown to be an effective

repellent against mosquitoes and ticks (Oyedele et al., 2002; Luker et al., 2023). The fumigation repellent exposure inhibits the ability of ticks to detect the attractant possibly interfering with the transduction of neuronal information and decreasing the attractiveness of butyric acid by potential odour masking (Deletre et al., 2016).

The bacterial infection analysis revealed a high infection rate in tick

Table 1

Percentage of adult, female *Ixodes scapularis* ticks choosing treatment (100 µg of butyric acid) after 20 minutes in Y-tube two-choice behavioural assay for infected versus non-infected (lab-reared) ticks for pre- and post-exposure to repellents (DEET and lemongrass essential oil).

Infection Type	Sample size (n)	Percentage choosing butyric acid ± SE
<i>Borrelia burgdorferi</i> s.l.	15	60 % ± 51 %
<i>Borrelia burgdorferi</i> s.s.	14	57 % ± 51 %
<i>Borrelia miyamotoi</i>	1	100 %*
<i>Anaplasma phagocytophilum</i>	2	50 % ± 70 %
<i>Babesia odocoilei</i>	2	50 % ± 70 %
Negative (lab tick)	60	62 % ± 49 %

* SE not reported since the sample size is 1.

sampled in Nova Scotia. Among ticks sampled during this study, 67.7 % of *I. scapularis* female ticks were reported to carry *Borrelia* s.l. and 61.5 % of analyzed ticks were infected with *B. burgdorferi* s.s. *Ixodes scapularis* ticks, together with *Ixodes pacificus* ticks, are the principal vectors of the causative agent of Lyme disease and other tick-borne diseases in Canada. The high incidence of tick-borne pathogens found in our sampled ticks is in line with passive and active surveillance studies carried out in Canada in 2020 (Wilson et al., 2023), locating Nova Scotia as one of the provinces in Lyme disease risk areas. We determined that infection status does not significantly impact responsiveness to attractants (i.e., butyric acid) or effectiveness of repellents. Similar results were found by Berret et al. (2015), where infection of *B. burgdorferi* did not change the host-seeking behaviour of *Ixodes ricinus*. The study found that there was no significant difference between the *Borrelia* ecotype or *Borrelia* infection status on tick questing activity or attraction to rodent odor. Although infection status does not seem to affect host-seeking abilities in ticks, there are various studies suggesting that bacteria and viruses may

modify the traits of *I. ricinus*, *I. persulcatus*, *I. scapularis*, and *I. pacificus*. The presence of the pathogen might be associated with improving the overall tick survival (Herrmann and Gern, 2015). Multiple studies show that *Borrelia*-infected and *Anaplasma*-infected ticks are less sensitive to a dry environment than uninfected ticks, which decreases their risk to desiccation and allows them to survive in less favorable conditions (i.e., warmer and dryer climates) (Lefcort and Durden, 1996; Romashchenko et al., 2012; Herrmann and Gern, 2012). The increase in tick resilience translates in increasing questing time, following by better chances to finding a vertebrate host and transmitting the pathogen, even at higher elevations and under desiccating conditions before returning to a moist environment (i.e., leaf litter) to rehydrate (Herrmann and Gern, 2015). The lack of sensory and behavioural modulation of odor-induced responses by the pathogen may be influenced by the level of infection and spirochete load, as well as the combination of pathogen species (Herrmann and Gern, 2014). Further investigations considering pathogen infection level, environmental conditions of exposure, and recovery time post-exposure fumigation are required to better understand the impact of infection level on tick response to attractants and repellents.

The present study explored the effects of exposure of synthetic and plant-derived repellent compounds on tick response to butyric acid using electrophysiology and Y-tube behavioural assays. We found that ticks had a decreased behavioural response to butyric acid after being exposed to lemongrass essential oil, DEET, citral, and geraniol. The prolonged exposure induced a possible inhibitory effect by the repellent on the tick chemosensory system ability to detect an attractant. This experiment was performed using both wild and lab-reared ticks. We found that neither infection status nor wild-type versus lab-type had a significant impact on butyric acid responsiveness nor effectiveness of repellent compounds. Overall, we conclude that infection status does not seem to affect the response of tick chemosensory system to attractants and repellents, and on the host-seeking abilities as well. Additionally, we

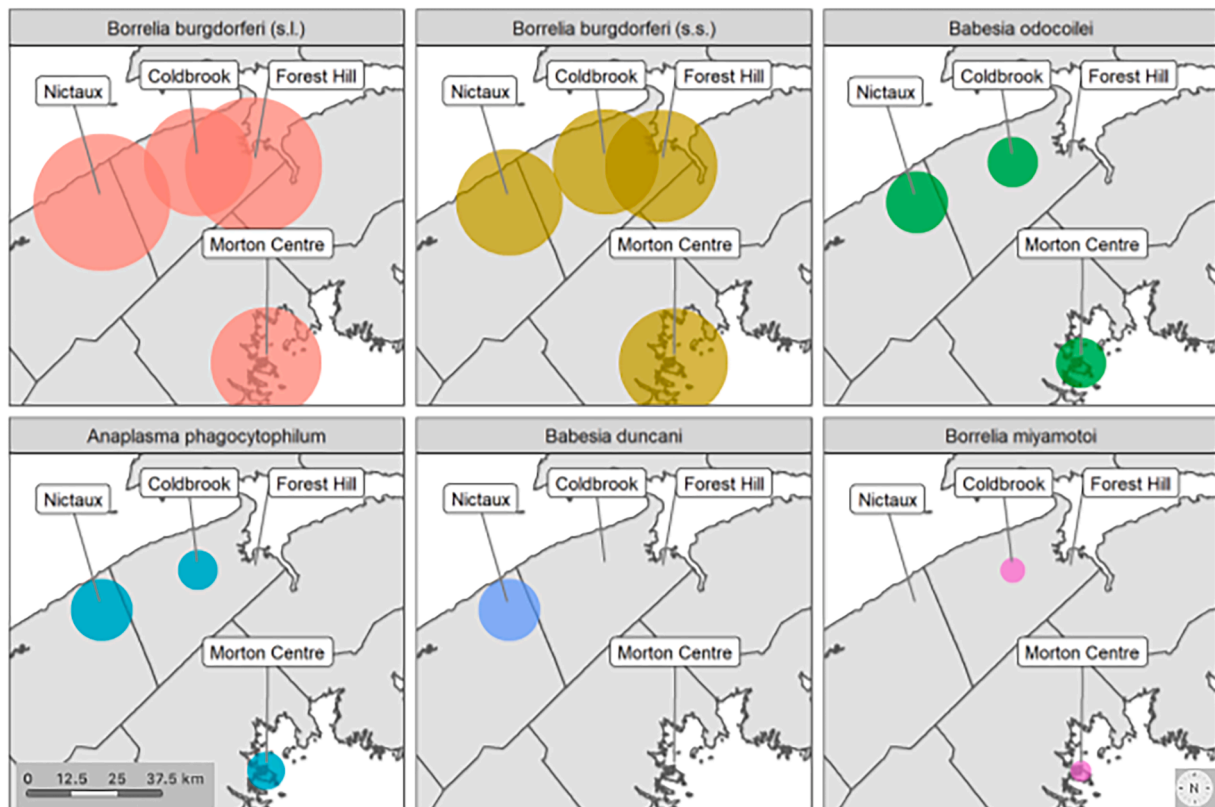


Fig. 7. Proportion of adult female *Ixodes scapularis* ticks infected with various pathogens for each of the sampled areas in Nova Scotia (Canada) from May to November 2022.

conclude that lemongrass essential oil and its main components, together with DEET, significantly impact the responsiveness of *I. scapularis* to butyric acid, therefore disrupting host-seeking abilities. On the other hand, the exposure to butyric acid or hexane did not impact the ability of ticks to detect the attractant in both electrophysiological and behavioural tests. Our results provide valuable insight into the olfactory system of ticks and their physiological response to repellent compounds contributing to uncovering new methods of interfering with tick-host seeking behaviour. More studies on the neuronal mechanisms that regulate odour sensing are required to verify the inhibitory mechanism resulting from the exposure of repellents compounds on the tick's chemosensory system, and whether ticks are able to recover the ability to detect an attractant afterward.

CRedit authorship contribution statement

Kayla Gaudet: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Luis Adriano Anholeto:** Investigation, Methodology, Writing – original draft. **N. Kirk Hillier:** Conceptualization, Writing – review & editing. **Nicoletta Faraone:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors have no competing interests to declare that are relevant to this article.

Data availability

The data that support the findings of this study are available in the supplementary material section.

Acknowledgements

We would like to acknowledge that this study was conducted in Mi'kma'ki, the ancestral and unceded territory of the Mi'kmaq. We thank Sarah Koerte, Vincent Wang, Miranda Amiro, Claire Hawboldt, Sophia Blanchard, Mia Lauzon, Laura Pickett, and Jacob Ouellette for their assistance with tick collection and behavioural studies.

Funding declaration

This research project was funded by NSERC Discovery Grant (RGPIN-2021-04126 to NF) and by Clean Foundation Nova Scotia (2022) to NF.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.cris.2024.100096](https://doi.org/10.1016/j.cris.2024.100096).

References

- Adamo, S.A., El Nabbout, A., Ferguson, L.V., Zbarsky, J.S., Faraone, N., 2022. Balsam fir (*Abies balsamea*) needles and their essential oil kill overwintering ticks (*Ixodes scapularis*) at cold temperatures. *Sci. Rep.* 12, 1–11. <https://doi.org/10.1038/s41598-022-15164-z>.
- Benelli, G., 2020. Pathogens manipulating tick behavior—through a glass, darkly. *Pathogens* 9, 664. <https://doi.org/10.3390/pathogens9080664>.
- Berret, J., Voordouw, M.J., 2015. Lyme disease bacterium does not affect attraction to rodent odour in the tick vector. *Parasit. Vect.* 8, 249. <https://doi.org/10.1186/s13071-015-0856-8>.
- Binetruy, F., Dupraz, M., Buysse, M., Duron, O., 2019. Surface sterilization methods impact measures of internal microbial diversity in ticks. *Parasit. Vect.* 12, 268. <https://doi.org/10.1186/s13071-019-351w7-5>.

- Carr, A.L., Mitchell III, R.D., Dhammi, A., Bissinger, B.W., Sonenshine, D.E., Roe, R.M., 2017. Tick Haller's organ, a new paradigm for arthropod olfaction: how ticks differ from insects. *Int. J. Mol. Sci.* 18, 1563. <https://doi.org/10.3390/ijms18071563>.
- Carr, A.L., Roe, M., 2016. Acarine attractants: chemoreception, bioassay, chemistry and control. *Pestic. Biochem. Phys.* 131, 60–79. <https://doi.org/10.1016/j.pestbp.2015.12.009>.
- Deletre, E., Schatz, B., Bourguet, D., Chandre, F., Williams, L., Ratnadass, A., Martin, T., 2016. Prospects for repellent in pest control: current developments and future challenges. *Chemoecology* 26, 127–142. <https://doi.org/10.1007/s00049-016-0214-0>.
- Ditzen, M., Pellegrino, M., Voshall, L.B., 2008. Insect odorant receptors are molecular targets of the insect repellent DEET. *Science* 319, 1838–1842. <https://doi.org/10.1126/science.1153121>.
- Dogan, E.B., Ayres, J.W., Rossignol, P.A., 1999. Behavioural mode of action of deet: inhibition of lactic acid attraction. *Med. Vet. Entomol.* 13, 97–100. <https://doi.org/10.1046/j.1365-2915.1999.00145.x>.
- Eliash, N., Singh, N.K., Thangarajan, S., Sela, N., Leshkowitz, D., Kamer, Y., Zaidman, I., Rafaeli, A., Soroker, V., 2017. Chemosensing of honeybee parasite, *Varroa destructor*: Transcriptomic analysis. *Sci. Rep.* 7. <https://doi.org/10.1038/s41598-017-13167-9>.
- Eliash, N., Thangarajan, S., Goldenberg, I., Sela, N., Kupervaser, M., Barlev, J., Altman, Y., Knyazer, A., Zaidman, I., Rafaeli, A., Soroker, V., 2019. *Varroa* chemosensory proteins: some are conserved across Arthropoda but others are arachnid specific. *Insect Mol. Biol.* 28, 321–341. <https://doi.org/10.1111/imb.12553>.
- Faraone, N., 2022. Host detection by ticks. Eds.: In: Ignell, R., Lazzari, C.R., Lorenzo, M. G., Hill, S.R. (Eds.), *Sensory ecology of disease vectors*, 1st ed. Wageningen Academic Publishers, Wageningen (NL), pp. 639–653. https://doi.org/10.3920/978-90-8686-932-9_24.
- Faraone, N., Light, M., Scott, C., MacPherson, S., Hillier, N.K., 2020. Chemosensory and behavioural responses of *Ixodes scapularis* to natural products: role of chemosensory organs in volatile detection. *Insects* 11, 502. <https://doi.org/10.3390/insects11080502>.
- Faraone, N., MacPherson, S., Hillier, N.K., 2019. Behavioral responses of *Ixodes scapularis* tick to natural products: development of novel repellents. *Exp. Appl. Acarol.* 79, 195–207. <https://doi.org/10.1007/s10493-019-00421-0>.
- Faulde, M.K., Robbins, R.G., 2008. Tick infestation risk and *Borrelia burgdorferi* s.l. infection-induced increase in host-finding efficacy of female *Ixodes ricinus* under natural conditions. *Exp. Appl. Acarol.* 44, 137–145. <https://doi.org/10.1007/s10493-008-9131-4>.
- Gaudet, K., Faraone, N., Hillier, N.K., 2023. Investigating chemoreception and behavioural responses of *Tetranychus urticae* (Trombidiformes: Tetranychidae) to organic acids, aldehydes and essential oil components. *Front. Agron.* 5, 1212705. <https://doi.org/10.3389/fagro.2023.1212705>.
- Gulia-Nuss, M., Nuss, A.B., Meyer, J.M., Sonenshine, D.E., Roe, R.M., Waterhouse, R.M., Sattelle, D.B., Fuente, Ribeiro, M., J., Megy, K., Thimmapuram, J., Miller, J.R., Walenz, B.P., Koren, S., Hostetler, J.B., Thiagarajan, M., Joardar, V.S., Hannick, L.L., Bidwell, S., Birren, B., 2016. Genomic insights into the *Ixodes scapularis* tick vector of Lyme disease. *Nature Comm.* 7, 10507. <https://doi.org/10.1038/ncomms10507>.
- Herrmann, C., Gern, L., 2012. Do the level of energy reserves, hydration status and *Borrelia* infection influence walking by *Ixodes ricinus* (Acari: Ixodidae) ticks? *Parasitol* 139, 330–337. <https://doi.org/10.1017/S0031182011002095>.
- Herrmann, C., Gern, L., 2014. Survival of *Ixodes ricinus* (Acari: Ixodidae) under challenging conditions of temperature and humidity is influenced by *Borrelia burgdorferi* sensu lato infection. *J. Med. Entomol.* 47, 1196–1204. <https://doi.org/10.1603/ME10111>.
- Herrmann, C., Gern, L., 2015. Search for blood or water is influenced by *Borrelia burgdorferi* in *Ixodes ricinus*. *Parasit. Vect.* 8, 6. <https://doi.org/10.1186/s13071-014-0526-2>.
- Huff, R.M., Pitts, R.J., 2020. Carboxylic acid responses by a conserved odorant receptor in culicine vector mosquitoes. *Insect Mol. Biol.* 29, 523–530. <https://doi.org/10.1111/imb.12661>.
- Javed, N., Bhatti, A., Paradkar, P.N., 2021. Advances in understanding vector Behavioural traits after infection. *Pathogens* 10, 1376. <https://doi.org/10.3390/pathogens10111376>.
- Josek, T., Sperrazza, J., Alleyne, M., Syed, Z., 2021. Neurophysiological and behavioral responses of blacklegged ticks to host odors. *J. Insect Phys.* 128, 104175. <https://doi.org/10.1016/j.jinsphys.2020.104175>.
- Kaupp, U.B., 2010. Olfactory signalling in vertebrates and insects: differences and commonalities. *Nat. Rev. Neurosci.* 11, 188–200. <https://doi.org/10.1038/nrn2789>.
- Koloski, C.W., Duncan, C.A., Rutherford, P.L., Cassone, B.J., 2020. Natural insensitivity and the effects of concentration on the repellency and survival of American dog ticks (*Dermacentor variabilis*) by DEET. *Exp. Appl. Acarol.* 82, 379–395. <https://doi.org/10.1007/s10493-020-00550-x>.
- Kurokawa, C., Lynn, G.E., Pedra, J.H.F., Pal, U., Fikrig, E., 2020. Interactions between *Borrelia burgdorferi* and ticks. *Nat. Rev. Microbiol.* 18, 587–600. <https://doi.org/10.1038/s41579-020-0400-5>.
- Leal, W.S., 2014. The enigmatic reception of DEET—the gold standard of insect repellents. *Curr. Opin. Insect Sci.* 6, 93–98. <https://doi.org/10.1016/j.cois.2014.10.007>.
- Lefcort, H., Durden, L.A., 1996. The effect of infection with Lyme disease spirochetes (*Borrelia burgdorferi*) on the phototaxis, activity, and questing height of the tick vector *Ixodes scapularis*. *Parasitol* 113, 97–103. <https://doi.org/10.1017/S0031182000066336>.
- Leonovich, S.A., 2021. Structure of Haller's organ and taxonomy of hard ticks of the subfamily Amblyomminae (Family Ixodidae). *Entomol. Rev.* 101, 709–724. <https://doi.org/10.1134/S0013873821050110>.

- Light, M., Faraone, N., Shutler, D., Cutler, G.C., Hillier, N.K., 2021. *Varroa destructor* (Mesostigmata: Varroidae) electrophysiological activity towards common yarrow (Asteraceae) essential oil and its components. *Can. Entomol.* 153, 211–221. <https://doi.org/10.4039/tce.2020.65>.
- Luker, H.A., Salas, K.R., Esmaili, D., Holguin, F.O., Bendzus-Mendoza, H., Hansen, I.A., 2023. Repellent efficacy of 20 essential oils on *Aedes aegypti* mosquitoes and *Ixodes scapularis* ticks in contact-repellency assays. *Sci. Rep.* 13, 1705. <https://doi.org/10.1038/s41598-023-28820-9>.
- Murphy, E.J., Booth, J.C., Davrazou, F., Port, A.M., Jones, D.N.M., 2013. Interactions of *Anopheles gambiae* odorant binding proteins with a human-derived repellent: implications for the mode of action of *N,N*-diethyl-3-methylbenzamide (DEET). *J. Biol. Chem.* 288, 4475–4485. <https://doi.org/10.1074/jbc.M112.436386>.
- Ni, L., 2021. The structure and function of ionotropic receptors in *Drosophila*. *Front. Mol. Neurosci.* 13, 638839.
- Oyedele, A.O., Gbolade, A.A., Sosan, M.B., Adewoyin, F.B., Soyelu, O.L., Orafidiya, O.O., 2020. Formulation of an effective mosquito-repellent topical product from lemongrass oil. *Phytomedicine* 9, 259–262. <https://doi.org/10.1078/0944-7113-00120>.
- Persaud, K.C., 2013. Engineering aspects of olfaction. In: Persaud, K.C., Marco, S., Gutiérrez-Gálvez, A. (Eds.), *Neuromorphic olfaction*, Eds. CRC Press/Taylor & Francis, Boca Raton (FL), pp. 1–58.
- Ray, G., Huff, R.M., Castillo, J.S., Bellantuono, A.J., DeGennaro, M., Pitts, R.J., 2023. Carboxylic acids that drive mosquito attraction to humans activate ionotropic receptors. *PLOS Neglect. Trop. Dis.* 17, e0011402. <https://doi.org/10.1371/journal.pntd.0011402>.
- Renthal, R., Manghnani, L., Bernal, S., Qu, Y., Griffith, W.P., Lohmeyer, K., Guerrero, F. D., Borges, L.M.F., Pérez de León, A., 2017. The chemosensory appendage proteome of *Amblyomma americanum* (Acari: Ixodidae) reveals putative odorant-binding and other chemoreception related proteins. *Insect Sci* 24, 730–742. <https://doi.org/10.1111/1744-7917.12368>.
- Renthal, R., 2022. Arthropod repellents and chemosensory reception. In: Corona, C., Debboun, M., Coats, J. (Eds.), *Advances in Arthropod Repellents*, Eds. Academic Press, Cambridge (MA, USA), pp. 141–162.
- Romashchenko, A.V., Ratushnyak, A.S., Zapara, T.A., Tkachev, S.E., Moshkin, M.P., 2012. The correlation between tick (*Ixodes persulcatus* Sch.) questing behaviour and synganglion neuronal responses to odours. *J. Insect Physiol.* 58, 903–910. <https://doi.org/10.1016/j.jinsphys.2012.04.004>.
- Salomon, J., Hamer, S.A., Swei, A., 2020. A beginner's guide to collecting questing hard ticks (Acari: Ixodidae): a standardized tick dragging protocol. *J. Insect Sci.* 20, 11. <https://doi.org/10.1093/jisesa/ieaa073>.
- Sfara, V., Zerba, E.N., Alzogaray, R.A., 2009. Fumigant insecticidal activity and repellent effect of five essential oils and seven monoterpenes on first-instar nymphs of *Rhodnius prolixus*. *J. Med. Entomol.* 46, 511–515. <https://doi.org/10.1603/033.046.0315>.
- Syed, Z., Leal, W.S., 2008. Mosquitoes smell and avoid the insect repellent DEET. *PNAS* 105, 13598–13603.
- Swale, D.R., Bloomquist, J.R., 2019. Is diethyltoluamide a dangerous neurotoxicant? *Pest. Manag. Sci.* 75, 2068–2070. <https://doi.org/10.1002/ps.5476>.
- Tak, J.H., Jovel, E., Isman, M.B., 2017. Synergistic interactions among the major constituents of lemongrass essential oil against larvae and an ovarian cell line of the cabbage looper, *Trichoplusia ni*. *J. Pest Sci.* 90, 735–744. <https://doi.org/10.1007/s10340-016-0827-7>.
- Wills, M.K.B., Kirby, A.M., Lloyd, V.K., 2018. Detecting the Lyme disease spirochete, *Borrelia burgdorferi*, in ticks using nested PCR. *Jove* 4, 56471. <https://doi.org/10.3791/56471>.
- Wilson, C., Gasmí, S., Bourgeois, A.C., Badcock, J., Carr, J., Chahil, N., Coatsworth, H., Dibernardo, A., Goundar, P., Leighton, P., Lee, M.K., Morshed, M., Ripoché, M., Savage, J., Smadi, H., Smolarchuk, C., Thivierge, K., Koffi, J., 2023. Surveillance for *Ixodes scapularis* and *Ixodes pacificus* ticks and their associated pathogens in Canada, 2020. *CCDR/CANADA* 49, 288.
- Weeks, J.A., Guiney, P.D., Nikiforov, A.I., 2012. Assessment of the environmental fate and ecotoxicity of *N,N*-diethyl-*m*-toluamide (diethyltoluamide). *IEAM* 8, 120–134. <https://doi.org/10.1002/ieam.1246>.
- Xing, H., Hu, Y., Yang, L., Lin, J., Bai, H., Li, Y., Tanvir, R., Li, L., Bai, M., Zhang, Z., Xu, H., Wu, H., 2023. Fumigation activity of essential oils of *Cinnamomum loureirii* toward red imported fire ant workers. *J. Pest Sci.* 96, 647–662. <https://doi.org/10.1007/s10340-022-01540-1>.
- Xu, W., Cornel, A.J., Leal, W.S., 2010. Odorant-binding proteins of the malaria mosquito *Anopheles funestus* sensu stricto. *PLoS One* 5. <https://doi.org/10.1371/journal.pone.0015403>.
- Xu, P., Zeng, F., Bedoukian, R.H., Leal, W.S., 2019. DEET and other repellents are inhibitors of mosquito odorant receptors for oviposition attractants. *Insect Biochem. Mol. Biol.* 113, 103224. <https://doi.org/10.1016/j.ibmb.2019.103224>.
- Zhu, T., Deng, J., Xu, M., Cai, A., Ye, C., Li, J., Li, X., Li, Q., 2020. DEET degradation in UV/monochloramine process: kinetics, degradation pathway, toxicity and energy consumption analysis. *Chemosphere* 255, 126962. <https://doi.org/10.1016/j.chemosphere.2020.126962>.