REPELLENT ACTIVITY OF DEET, ICARIDIN, PERMETHRIN, LEMON EUCALYPTUS OIL (*CORYMBIA CITRIODORA*), AND TEA TREE OIL (*MELALEUCA ALTERNIFOLIA*) AGAINST *ORNITHODOROS TURICATA* NYMPHS

by

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DEDICATION

To my mother, Shirin Remzi, for always standing beside me and lending me her bootstraps.

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LIST OF ABBREVIATIONS

Abbreviation	Description
ASFV	African swine fever virus
DEET	N,N-diethyl-meta-toluamide
PCGS	Purgatory Creek Green Space
TBD	Tick-borne disease
TBRF	Tick-born relapsing fever

ABSTRACT

Tick-borne diseases are among the fastest-growing infectious diseases in correlation with increased interactions with humans, wildlife, and livestock. Although tick-borne diseases have wide-ranging importance for public health and food production, most of the research efforts have been on Ixodid ticks, whereas Argasid ticks have received much less attention. Therefore, there is little knowledge about the efficacy of common deterrents that would prevent bites from these species. Three commercial deterrents (DEET, Icaridin, and Permethrin) and two essential oils (tea tree and lemon eucalyptus) were evaluated for repellency against nymphs of *Ornithodoros turicata,* an Argasid tick that is a vector for Tick-Borne Relapsing Fever, and a putative vector of African Swine Fever. Repellency was assessed using a petri-dish bioassay on nymphal ticks collected from caves in the Purgatory Green Space of San Marcos, Texas. Lemon Eucalyptus Oil and Tea Tree Oil performed well, being able to deter 90% of ticks at 20% concentration. DEET effectively repelled 50% of ticks consistently, even at low concentrations. Icaridin only repelled 50% of ticks at the highest concentration. Permethrin showed very low repellency at all concentrations.

I. INTRODUCTION

Tick-borne diseases (TBD) are among the fastest-growing diseases in correlation with increased interactions with humans, wildlife, and livestock (Cançado et al. 2013). For example, Lyme disease is now the most commonly reported arthropod-borne illness in the USA and Europe (Steere et al. 2004). Although Lyme disease has received much attention in the media within the United States, ticks in this country are known to be vectors for multiple other pathogens that cause diseases in humans like anaplasmosis, babesiosis, ehrlichiosis, Rocky Mountain Spotted Fever and tularemia (Perez de Leon et al. 2014). Moreover, ticks can also transmit pathogens to livestock in cases such as cattle fever (bovine babesiosis), which has the potential for significant economic impacts in this industry (Pérez de Leon et al. 2012). Moreover, although TBD have a wide-ranging importance for public health and food production most of the research efforts have been on ticks from the family Ixodidae (hard ticks), whereas pathogens transmitted by ticks from the family Argasidae (soft ticks) have received much less attention (Lopez et al. 2016).

***Ornithodoros turicata* as a Disease Vector**

Tick-borne relapsing fever (TBRF) is a disease vectored by the soft tick *Ornithodoros spp.,* caused by a *Borellia* spirochete, most commonly *Borellia hermsii* and *Borrelia turicata* (Dworkin et al. 2008). *Borellia* is a gram-negative bacterium measuring about 0.2 to 0.5 microns in width and about 5 to 20 microns in length. After an incubation period of1-2 days, the pathogen causes recurring febrile episodes lasting about three days with a range of non-specific signs and symptoms, followed by a seven-day afebrile period (Cutler 2010). Each three-day episode ends in a set of symptoms, generally referred to as a “crisis”. During this time, patients will experience extremely high fevers up to 41.5ºC, delirium, and tachycardia. Without treatment, it is common for these episodes to reoccur several times (CDC 2015).

Borrelia hermsii and *Borellia turicata* are closely related to *Borellia burgdorferi*, the causative agent of Lyme disease. Because of their close relation, diagnostic testing for Lyme has been shown to test false positive when *B. hermsii* or *B. turicata* are present (Schwan et al. 1996, Cutler et al. 1997). This diagnostic issue, combined with having very general, non-specific symptoms, likely leads to widespread misdiagnosis, which complicates ascertaining the overall prevalence of the pathogen in humans (Wilder et al. 2015).

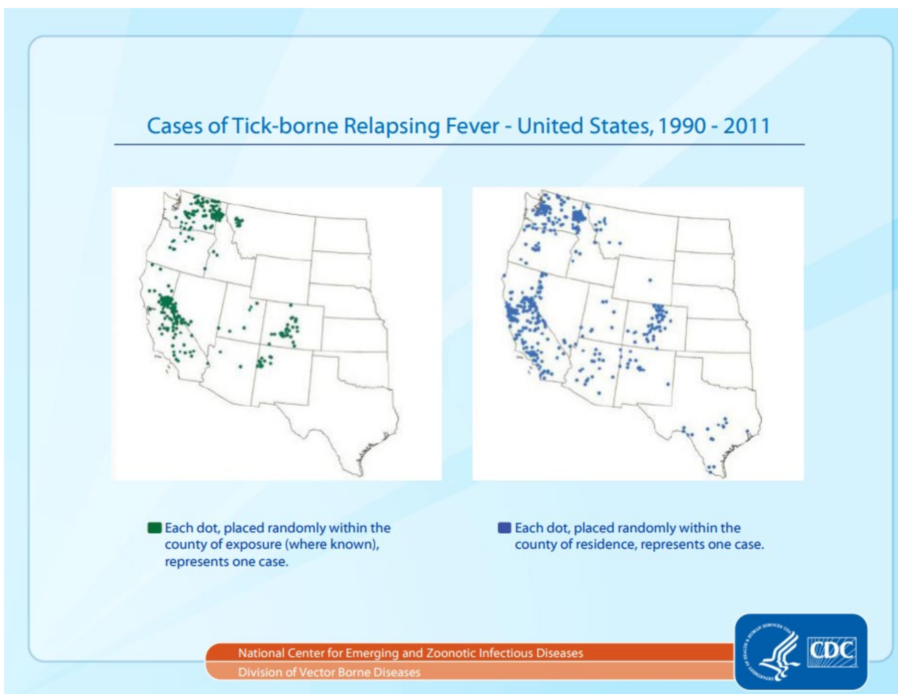

Figure 1. Distribution of TBRF in the United States between 1990 and 2011. 1990 is shown on the left, and 2011 is on the right (CDC 2015).

TBRF is considered relatively uncommon and generally associated with rodent infestations, with 483 cases reported in the U.S. between 1990 and 2011 (Figure 1).

However, it has been hypothesized that it is a largely underreported and under-identified disease in the United States (Dworkin et al. 2008, Cutler 2010, Cutler et al. 2010). Recently, there has been an outbreak of cases in the Austin, Texas metropolitan area, suggesting that the risk of infection may be higher than currently indicated (Bissett et al. 2018). The fact that these infections were contracted within a city park suggests that the at-risk demographics may be different than previously assumed.

In addition to transmitting the pathogen that causes TBRF, *Ornithodoros turicata* is a putative vector for the African Swine Fever Virus, an emergent infectious disease of pigs with up to 100% mortality rate (Higgs 2018). While this pathogen has yet to arrive in the United States, large outbreaks have been reported across Asia and Eastern Europe in recent years (Cwynar et al. 2019, Dixon et al. 2019). These outbreaks were centered mainly in China, the largest importer of goods that could potentially carry the pathogen. Understanding the ecology of competent vectors for such a deadly pathogen could lead to the prevention of large-scale economic losses to the livestock industry if an outbreak does occur in the United States (Hess et al. 1987, Brown and Bevins 2018, Higgs 2018, Golnar et al. 2019, Wormington et al. 2019).

Natural History of *Ornithodoros turicata*

Ornithodoros is a genus of soft ticks belonging to Family Argasidae, containing about 37 species. They usually live within rodent burrows, caves, dens, and cliffsides, where they feed briefly and intermittently on their hosts (Figure 2). As such, contact with humans is generally limited to instances of rodent infestation, entering caves for work or recreation, or when in contact with the few tick species that feed on livestock (Donaldson et al. 2016). *Ornithodoros spp.* do not display traditional questing behavior of Ixodes ticks; rather, they stay inside the burrows and dens of their host to feed rapidly and periodically (Teel 2016). They use their Haller's organs to detect heat, CO_2, and movement that are associated with host-seek within the nests. Three species of *Ornithodoros* have been identified in the United States: *O. turicata*, *O. dugesi*, and *O. coriaceus*. Among these species, feeding is rapid, usually lasting less than 30 minutes. The bite is generally painless, and as such, human hosts are often unaware of the exposure until the onset of disease symptoms (Boyle et al. 2014, Zheng et al. 2015). These hardy ticks have very long lifespans, often up to 20 years, and can survive prolonged periods of starvation (Beck et al. 1986). Because soft ticks are relatively understudied species, there is little knowledge about the effectiveness of even common repellents that would prevent bites from this species (Lane and Anderson 1984, Mehr et al. 1986).

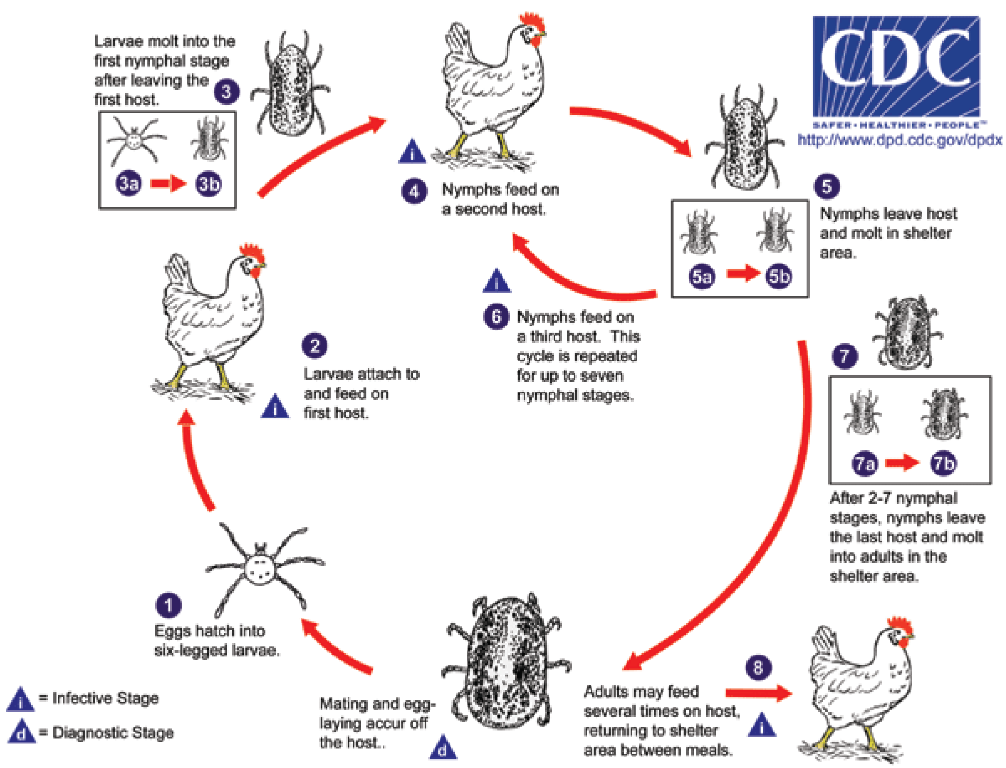

Figure 2. Life Cycle of *Ornithodoros spp.* (CDC 2017)

Repellents

DEET (N,N-diethyl-meta-toluamide) is now the most common active ingredient used in insect repellants. Although chemical resistance to DEET has become more common in several groups of arthropods, it is effective against a large variety of insects and arachnids. There are some concerns about the safety of DEET, as sensitivities have been recorded, and it is toxic to some aquatic organisms (Briassoulis et al. 2001, Mischke et al. 2015). DEET is generally not considered as effective at repelling ticks as pyrethroid repellents. DEET may dissolve some watch crystals, plastics, rayon, spandex, other synthetic fabrics, and affect painted or varnished surfaces (NPIC 2009a). Despite this, DEET remains the top standard to which all other repellents are compared (Kröber et al. 2013, Diaz 2016, Meng et al. 2016). Icaridin (also known as Picaridin, KBR 3023, or Hydroxyethyl isobutyl piperidine carboxylate) is a newer repellent derived from black pepper that is as effective as DEET against a similarly wide range of insects and arachnids. Icaridin is very popular among those who have experienced sensitivity to DEET. It is less toxic than DEET and will not melt plastic, making Icaridin popular among sport fishers (NPIC 2009b). Permethrin ((3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate) is a synthetic pyrethroid and neurotoxin that has been repeatedly shown to be a repellent and acaricide of hard tick species. It is used as a long-term pre-treatment for clothing that is often recommended for all species of ticks. Permethrin is widely used in agriculture and has been repeatedly shown to be a powerful acaricide and tick repellent (Schreck et al. 1980, Lane and Anderson 1984, Evans et al. 1990, Miller et al. 2011, Cisak et al. 2012, Vaughn et al. 2014, Diaz 2016). However, Permethrin is highly toxic to fish and other aquatic animals, bees and other important insects, and mildly toxic to birds (NPIC 2009c).

Plants and arthropods have a long-shared history of coevolution, and many plants harbor natural deterrents (Prajapati et al. 2005, Rehman et al. 2014, Rosado-Aguilar et al. 2017). Many different plant-derived essential oils have been explored as alternative repellants, including anise, oregano, thyme, peppermint, clove, vetiver, sandalwood, juniper, ginger, rosemary, cinnamon, celery, lavender, and others (Isman 2000, Papachristos and Stamopoulos 2001, Prajapati et al. 2005, Nerio et al. 2014). These studies have resulted in limited success, depending on the individual compound and the targeted arthropod. Many studies have been done specifically on hard ticks in Family Ixodidae, with some success (Iori et al. 2005, Meng et al. 2016, Tabari et al. 2017, Benelli and Pavela 2018). Tea tree oil is an essential oil derived from the *Melaleuca alternifolia* plant. It is an acaricide when applied directly and left to sit for a half-hour to an hour. Lemon eucalyptus oil is derived from the *Corymbia citriodora* plant. It is the only essential oil to be currently recommended by the CDC to prevent mosquito and other flying insect bites. A proprietary repellent blend can be purchased at every major retailer right alongside DEET.

***In vitro* Repellency Bioassays**

In the earliest stages of screening repellents for a species without any data, it is useful to know whether a chemical displays any repellency at all. This helps to determine what substances can be explored further, since there is a large time and financial cost to *in vivo* bioassays that involve host cues (Dautel 2004). There is a myriad of *in vitro* bioassay techniques that do not use host cues, but for these initial sorts of tests, it is best to select a bioassay that is simple, quick, and cost effective (Adenubi et al. 2018, Benelli and Pavela 2018). This narrows the selection down to two main bioassay systems: a petri dish bioassay, or a climbing bioassay (Dautel 2004, Sonenshine and Roe 2013). There are benefits and limitations to both tests, but it ultimately must be decided by the target species being tested.

A petri dish bioassay is the simplest test of repellency and is extremely fast and cheap to perform. Ticks are placed in a walking arena with treated and untreated zones, and repellency is determined by the proportion and degree that ticks are found on the untreated portions of the arena (Dremova and Smirnova 1970, Sonenshine and Roe 2013). The limitations of this bioassay are that due to lack of host cues, it is difficult to filter out weak repellents, as even a weak repellent might seem to work if the concentration is high enough (Dautel 2004, Adenubi et al. 2018). Ticks may also move in a random direction, and this would be difficult to determine, resulting in a level of uncertainty about the exact repellency of the chemicals. Lastly, the petri dish test space can quickly become saturated with repellent vapors (Adenubi et al. 2018). Nevertheless, petri dish bioassays remain a quick, cost-effective, commonly used test to achieve a basic understanding of what repellents may offer protection against a specific species (Bissinger, Apperson, et al. 2009, Bissinger, Zhu, et al. 2009, Mkolo et al. 2011, Ferreira et al. 2017).

The climbing bioassay is a test system that seeks to control for the host seeking (questing) behavior of ticks. When seeking out hosts, Ixodid ticks will climb a blade of grass and sit at the top with the first pair of legs extended (Sonenshine and Roe 2013). The *in vitro* climbing bioassay takes advantage of this behavior, where the ticks climbing up the assay eventually come to rest at the top and display the questing pose (Dautel

2004). A repellent is then placed on the test surface to prevent the ticks from climbing, and repellency is deduced by a reduced number of ticks reaching the top of the assay (Meng et al. 2016). Unfortunately, this test system suffers from similar limitations to the petri dish bioassay such as an inability to filter out weak repellents due to no host cues (Adenubi et al. 2018). Random behavior is reduced to some degree in this bioassay. However, this bioassay relies on the questing behavior of Ixodid ticks, and is therefore unsuitable for Argasid ticks, which do not seek out hosts but rather inhabit the burrows of their hosts. Due to this consideration, a petri dish bioassay was determined to be a superior method for *Ornithodoros turicata* nymphs, who would normally be crawling across burrow or cave floors towards their hosts (El-Ziady 1958, Sonenshine and Roe 2013).

Research Objectives

This present project seeks to explore the repellent activity of three conventional repellents (DEET, Icaridin, and Permethrin) and two essential oils (tea tree oil and lemon eucalyptus oil) against nymphs of *Ornithodoros turicata*. There is no current understanding of which chemicals would offer protection from these ticks, and therefore a simple petri dish bioassay is appropriate to gain a basic understanding of which repellents may be further explored for these taxa.

II. MATERIALS AND METHODS

Ticks

Ornithodoros turicata soft tick nymphs were collected from a cave located the Purgatory Creek Green Space (PCGS) from San Marcos in Hays County, which is in Central Texas. Initially, adult specimens were collected from PCGS and Live Oak Cave in Travis County, Texas. Ultimately it was decided that there was not a sufficient number of adults collected to conduct tests, and thus repellency bioassays were restricted to the more abundant nymphal stages. Initially, ticks were collected using CO_2 traps made up of a dish dug into soft soil areas of the daylight areas of the cave. Inside the dish, a piece of white flannel was placed to aid visibility, and then a Styrofoam cup was set in the center, and a block of roughly 0.5kg of dry ice was set on top of the cup. Various CO_2 traps were tested, including the standard vented Styrofoam box (Gray 1985, Caiado et al. 1990). However, they were found to be less effective as having the dry ice fully open to allow maximum dispersal. Traps were placed inside the cave for one hour and checked afterward, with all ticks collected from inside the tray and in the nearby soil.

Trapping from the fall of 2018 to the fall of 2019 was unfruitful due to the fluctuation of soft tick populations at the collection sites. Less than 100 ticks were obtained during this time, all of the adults. In February and March of 2020, a population explosion at the cave in PCGS occurred, and large numbers of nymphal ticks were present at this cave entrance and as far out as 3m. As such, most of the nymphs collected for the study were collected with an entomological aspirator straight from the ground or from the surface of the personal protective equipment being used (Figure 3).

Collected unfed ticks were maintained prior to testing in plastic vials at 20-22 ºC and 85% relative humidity (Schuhardt 1940, Gray 1985, Caiado et al. 1990). Ticks used for the repellency bioassay ranged in size and likely represented all seven nymphal instars. Following testing in the repellency bioassays, all ticks were killed by dropping them in vials with 95% ethanol that were then stored in cryopreservation.

Figure 3. The majority of nymphal ticks were collected by aspiration from the PPE worn during the population explosion at the PCGS in March of 2020.

Repellents

Two essential oils included in this study were obtained from commercial sources. Tea tree oil (*Melaleuca alternifolia)* was obtained from Garden of Life (Palm Beach Gardens, FL, USA). *Melaleuca alternifolia* was sourced from South Africa and extracted from the leaf of the plant. Lemon Eucalyptus oil (*Corymbia citriodora*) was obtained from Aura Cacia (Norway, IA, USA). *Corymbia citriodora* was sourced from Brazil and extracted from the leaf of the plant. Technical DEET (97% active ingredient) and solid technical permethrin (>90%) were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA). A commercial proprietary blend of 20% Icaridin was obtained from Sawyer (Safety Harbor, FL, USA).

Essential oils, DEET, and Icaridin were serial diluted in ethanol to generate the following percentual test concentrations: 10.0, 5.0, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, and 0.039 (Meng et al. 2016). A volume of 3mL of test solution was applied by pipette to half of a 24cm Whatman No. 4 filter paper (226.19 cm^2) for a complete coverage, resulting in surface concentrations of 1.326, 0.663, 0.332, 0.166, 0.083, 0.041, 0.021, 0.010, 0.005, 0.003 mg/cm^2, respectively (Meng et al. 2016). Due to the fact that it is known to be active at much lower concentrations, technical permethrin was dissolved and serially diluted in ethanol to the following percentual test concentrations: 1.0, 0.5, and 0.25, resulting in surface concentrations of 0.133, 0.067, and 0.033 mg/cm^2, respectively (Schreck et al. 1980, Lane and Anderson 1984, Mehr et al. 1986, Lane 1989, Evans et al. 1990, Miller et al. 2011, Vaughn et al. 2014).

Repellency Bioassay

Ticks were tested using a modified petri dish bioassay (Dautel 2004). The testing surface consisted of a 24 cm filter paper circle delineated in half with a pencil. Half of the filter paper was treated with the chemical being tested by pipetting. The other was treated with a negative ethanol carrier control, and both allowed to dry for 15-20 minutes. The filter paper was then placed on a white tray to aid visibility, the indented edge of which was moated with water to prevent tick nymphs from escaping (Figure 4). Two researchers sat facing the repellent side of the paper so that if any encouragement were provided by the CO_2 or heath signals from the researchers, it would require movement across the surface treated with repellent.

Figure 4. Bioassay setup showing marked filter paper on a white tray to aid visibility. The edge of the tray was moated with water to prevent tick escape.

Ten nymphal ticks were released from the rim of a storage vial in the center of the filter paper dividing the treated and untreated zones. Their subsequent locations were recorded every five minutes for a total of thirty minutes. Any ticks who escaped the paper were removed to prevent them from returning to the filter paper. For the remainder of the trial, their location was recorded as their escape point. Each concentration was tested three separate times. Distance repellency was recorded as the distance from the zero lines in cm, with positive values assigned to those specimens found on the control half of the filter paper and negative values assigned to those on the test substance. Additionally, percentage repellency was calculated according to the formula: Percentage repellency = [number of ticks on the control side of the paper/ number of ticks on the test] X 100 (Mkolo et al. 2011). For example, if seven of the ten ticks were found on the control side of the filter paper after five minutes, then repellency would be calculated at 70%.

Data Analysis

The concentration-response data from the two-choice bioassay were analyzed using RStudio v3.5.1. First, data were separated categorically into repelled and not repelled based on which side of the filter paper they were found on at five minutes. Percentage repellency was calculated for each concentration of each chemical and used for the graphical display of results. A two factor ANOVA was done on the distance repellency continuous data (distance traveled by nymph ticks from zero lines) for DEET, Icaridin, Permethrin, tea tree oil, and lemon eucalyptus oil to determine if there were significant differences between dosages, between repellents, and if there were statistically significant interactions between these factors. A Tukey's HSD posthoc analysis was done to determine differences among repellents. Concentration data for Permethrin against distance repellency was analyzed separately using a single-factor ANOVA test since the concentrations differed from the other repellents, and only three concentrations were tested.

III. RESULTS

Most ticks moved very quickly after being placed on the filter paper and escaped from the edge. As such, only 17% of the data points changed values after the five-minute measurement. Therefore, it was determined that time was not a significant factor to be analyzed. All data analysis was done on the data collected at five minutes. Although it is likely that if there was some movement after that time, it did not necessarily represent repellency in a meaningful way. Percentage repellency for each substance was plotted on a graph to visualize the efficacy of the dosages (Figure 5). Among all chemicals tested, the tea tree oil and lemon eucalyptus oil displayed the best repellency (90%) at the greatest concentration (1.326 mg/cm^2). Tea tree oil began exhibiting around 50% repellency at about 0.166 mg/cm^2, while lemon eucalyptus oil was 50% repellent at a lower concentration (0.083 mg/cm^2). DEET performed surprisingly poorly and with an erratic response. Only 80% repellency was reached with two of the greatest strengths (0.663 mg/cm^2 and 0.332 mg/cm^2), although not with the highest concentration tested. Even more, at an intermediate concentration (0.083 mg/cm^2), the response was weaker than the repellency at the lesser concentrations. Icaridin also performed poorly, with only the highest concentration (1.326 mg/cm^2 and 0.663 mg/cm^2) being able to repel 50% of ticks, the maximum amount reached for this chemical. Permethrin also scored low, just reaching 50% repellency. Also, the response was erratic with the intermediate concentration (0.067 mg/cm^2) having a weak (20%) response whereas the lowest (0.033 mg/cm^2) and highest (0.133 mg/cm^2) concentrations reaching 50 and 40% repellency, respectively.

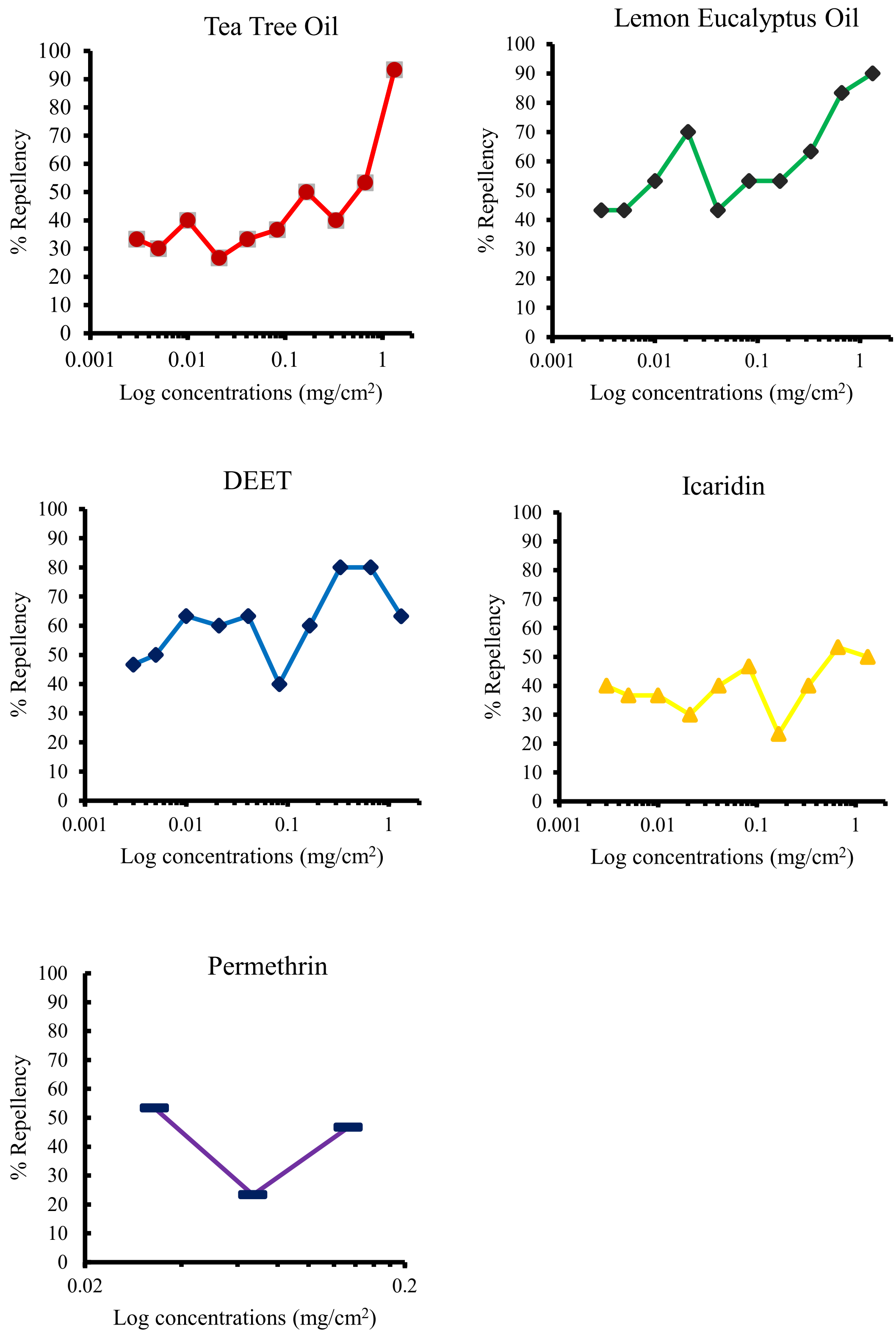

Figure 5. Response of percentage repellency against concentration for the three commercial repellents and two essential oils.

Data followed a normal distribution and was heteroscedastic. Statistical analysis of all repellents, except for Permethrin, which was analyzed separately, revealed significant differences between concentrations and repellents (Table 1). No statistical interaction between the concentrations and repellents was found (P = 0.58). Twenty percent of the variance was due to the repellent, indicating that the type of chemical used as a repellent was the most statistically significant facto

Table 1. Two factor ANOVA analysis of distance repellency comparing DEET, Icaridin, Tea tree oil, and Lemon eucalyptus oil across concentration, repellent, and determining if a significant interaction is present between concentration and type of chemical being used.

Source of Variation	S.S.	df	M.S.	F	P-value	F crit
Concentration	202.391	9	22.487	3.720	**0.001**	1.999
Repellent	209.923	3	69.974	11.576	**2.221E-06**	2.719
Interaction	150.457	27	5.572	0.922	0.581	1.626
Within	483.586	80	6.045			
Total	1046.357	119				

The ” Tukey’s HSD post hoc analysis revealed significant differences in three pairwise comparisons of repellent chemicals: between lemon eucalyptus and Icaridin (P=2.2002E-06), between lemon eucalyptus and tea tree (P=0.0116), and between DEET and Icaridin (P=0.0004) with the rest of the pairwise comparisons being non-significant (Figure 6).

The single-factor ANOVA analysis of Permethrin response data showed no statistical differences between concentrations (P=0.18) for a distance repellency range that went from about 20-50% (Table 2).

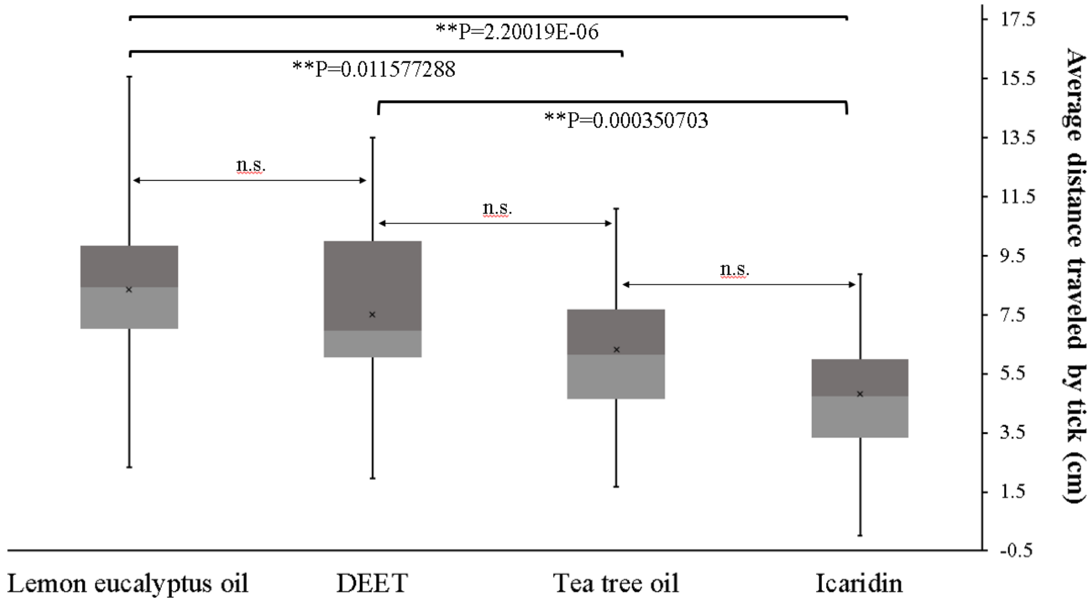

Figure 6. Boxplots of distance repellency response data for each chemical showing where the significant differences exist. n.s. = not significant (all p-values from posthoc Tukey test). Boxplots generated from the mean measurements in cm of tick movement during the bioassay testing.

Table 2. Single-factor ANOVA analysis comparing the variation in the trial data and among three different concentrations of Permethrin.

Source of Variation	*S.S.*	*df*	*M.S.*	*F*	*P-value*	*F crit*
Between Groups	27.504	2	13.752	3.298	0.175	9.552
Within Groups	12.509	3	4.170			
Total	40.0133	5				

IV. DISCUSSION

The current study sought to evaluate the relative effectiveness of DEET, Icaridin, Permethrin, Lemon Eucalyptus oil, and Tea Tree oil against *O. turicata* ticks using a two-choice bioassay between repellent-treated and untreated filter paper surfaces (Dautel 2004, Bissinger, Apperson, et al. 2009, Mkolo et al. 2011, Meng et al. 2016, Adenubi et al. 2018). To our knowledge, the tested repellents have not been evaluated for their efficacy against *O. turicata*, a primary vector of TBRF, and a putative vector of ASFV (Boyle et al. 2014, Wormington et al. 2019). As ethanol is a solvent of many commercial repellent formulations, it was used as a control in repellency bioassays for the current and previous published studies (Bissinger, Apperson, et al. 2009, Meng et al. 2016). Obtained results evinced that both concentration and the type of chemical affect the efficacy (i.e., repellency), with no statistical interaction between these factors detected, indicating that focus needs only be placed on the main effects.

Tea tree oil and lemon eucalyptus oil are effective repellents at concentrations of 1.326 mg/cm^2 (20% repellency) and up. Tea tree oil has been shown to have acaricidal properties against Ixodes ticks; there are no known studies of the effectiveness against the *Ornithodoros* genus (Iori et al. 2005, Carson et al. 2006). A previous study done with a 3% solution of tea tree oil against the Ixodid tick, *Rhipicephalus microplus,* showed that said concentration was ineffective (reported no repellency whatsoever) against *R. microplus* (Pazinato et al. 2014). In comparison to this past research, the present study revealed that a 2.5% tea tree oil only effectively repelled about 50% of *O. turicata* nymphs. In sharp contrast, the results for lemon eucalyptus oil were consistent with the literature, which has shown it to be effective against a variety of Ixodid tick species at equivalent concentrations (Gardulf et al. 2004, Jaenson et al. 2006, Bissinger, Zhu, et al. 2009).

Results for DEET were highly variable. While there was an overall upward trend as concentration increased, but at 0.083 mg/cm^2 and the highest concentration (1.326 mg/cm^2), there was decreased repellency. Two of the most concentrated test solutions (0.663 mg/cm^2 and 0.332 mg/cm^2) repelled 80% of the ticks, making DEET the most efficacious repellent at these concentrations. Overall, the response of *O. turicata* to DEET was unforeseen based on the literature, where it is documented to be extremely effective at minimal dosages for other tick species (Kröber et al. 2013, Meng et al. 2016, Ferreira et al. 2017, Benelli and Pavela 2018). However, a few studies indicate that this could be highly dependent on the species tested (Solberg et al. 1995, Semmler et al. 2011).

Icaridin was not as effective as the other test substances, although the highest tested strength was an undiluted commercial proprietary blend that is intended to spray on to skin and clothing in its existing condition. Icaridin's repellency success against Ixodid ticks has produced inconsistent responses that depend on the proprietary formulation of the repellent; researchers have found Icaridin to be more effective than DEET in some trials, and less effective in others (Semmler et al. 2011, Abdel-Ghaffar et al. 2015, Büchel et al. 2015). Disparity across Icaradin efficacy results within the literature suggests the need for future study. An investigation and comparison of various major proprietary blends of Icaridin from different manufacturers would provide the opportunity to better understand any potential inequality of effectiveness across brands. This could reveal which formulations would provide the most successful protection against *O. turicata.*

Post hoc analysis showed significant differences between tea tree and lemon eucalyptus oil, with lemon eucalyptus oil performing better out of the two. Significant differences were also detected between lemon eucalyptus and Icaridin, with lemon eucalyptus again performing better of the two. Lastly, significant differences were found between Icaridin and DEET, with DEET being the superior repellent. Finally, DEET and lemon eucalyptus were equally efficacious.

Tested concentrations of Permethrin proved to be mostly ineffective; more concentrations would need to be tested for more definitive results. These results were highly unexpected since Permethrin is recognized as an extremely useful acaricide and tick repellent even at minimal concentrations (Schreck et al. 1980, Lane and Anderson 1984, Mehr et al. 1986, Lane 1989, Bissinger, Zhu, et al. 2009, Miller et al. 2011, Cisak et al. 2012, Diaz 2016). It should be noted that the current study initially sought to test 10 concentrations of Permethrin. Yet, it quickly became apparent that there was likely an error in the dosage formulation from solid Permethrin we tested. This is further bolstered by the fact that no statistical differences were found in tick response to the three concentrations. The ticks had no strong aversion to the solution, even at a dosage twice as potent as is generally sold for the treatment of clothing and shoes. This further indicates that there was an error in the formulation of the test concentrations Re-experimentation for Permethrin was not pursued due to the limited supply of solid Permethrin and dwindling quantity of accessible ticks for testing. It was ultimately determined that the data being collected was not valuable, resulting in only three concentration conditions tested instead of then intended ten.

Besides the main effects, several factors might have potentially contributed to introducing noise in the dataset; while these were likely minor, it is relevant to discuss them for future tests. First, there are limitations within the chosen bioassay that are well documented (Dautel 2004, Adenubi et al. 2018). Generally, in a petri dish bioassay, the repellent vapors can quickly saturate the arena, making it difficult to know how much repellent the ticks are experiencing. This was avoided in this study by removing the petri dish, using a large test surface (24cm diameter circle) that was placed directly onto a larger tray to allow vapors to disperse rather than saturating the arena. However, the largest disadvantage of such tests remains: the researcher does not know how much of the movement of the ticks in the arena is due to randomness (Dautel 2004, Sonenshine and Roe 2013, Adenubi et al. 2018). Previous studies of *Ornithodoros turicata* indicate that a wide variety of factors can influence tick behavior (El-Ziady 1958). Of these factors, ones that were not controlled during the bioassay tests were light and humidity. Soft ticks are generally active at night, and bioassay testing was done indoors during the day with the lights on (Sonenshine and Roe 2013). It was anecdotally noted that on several particularly rainy days of testing when relative humidity was likely higher even indoors, ticks appeared to be more active than on drier testing days. Many observations were made of sluggish ticks that would curl in their legs and not move at all unless aggressively disturbed. This extreme immobility could often seem as if the ticks were dead. Upon transporting them into ethanol, it would be discovered that the animal was, in fact, alive. These responses have been documented in a previous behavioral study on a closely related species (El-Ziady 1958). The error related to this phenomenon could be that ticks which were not repelled and did not choose to move at all, could have been mistaken as deceased and replaced with a visibly mobile tick, who would then be more likely to move about and produce a measurable reaction. However, the extent of this behavior in the results of the study are likely minimal as this only occurred a handful of times (< 20) out of the 1,500+ ticks tested.

Additionally, tests were done in the absence of any hosts other than the two researchers conducting the studies sitting nearby, and as such, additional research would need to be conducted to determine what effects host-cues might have on repellency. Field study bioassays could provide useful data about the efficacy of the repellents in a more natural setting to better understand the level of protection offered by the test solutions (Evans et al. 1990, Gardulf et al. 2004, Jaenson et al. 2006, Miller et al. 2011, Kröber et al. 2013, Benelli and Pavela 2018). Likewise, a study designed to highlight efficacy over time to determine how long each repellent is effective would be highly useful for public health recommendations (Dautel 2004, Nerio et al. 2010).

Based on the current study, there are both commercial and essential oil options for protecting against *O. turicata* bites. For best results, DEET or lemon eucalyptus oil of at least 20% is recommended for use by individuals exposed to soft tick habitat to ensure protection from tick bites. Those who are camping, staying in places with rodent infestations, or spending time in caves for work or recreation should take care to use one of these repellents to prevent being inoculated with the TBRF causative agent (Whitney et al. 2007, Dworkin et al. 2008, Igreja 2011, CDC 2015, Donaldson et al. 2016, Lopez et al. 2016). In addition, the recent outbreak of cases in a public park in Austin, Texas, highlights the importance of using some form of repellent any time that an extended period will be spent outdoors in areas with high probabilities of *O. turicata* occurrence

(Bissett et al. 2018). Based on this study's data, Icaridin may not be recommended for effective prevention against *Ornithodoros turicata* nymphs. The results of this study highlight the importance of testing repellents against a wide variety of species to obtain a clearer understanding of the efficacy of individual compounds overall. A broad-spectrum protectant is objectively a better choice when spending time in habitats that pose a risk from a multitude of species, so adding new knowledge to the understanding of the range of protection that a repellent provides is an insightful contribution of the present study.

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