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THE EFFECTS OF NATURAL AND SYNTHETIC ATTRACTANTS AND REPELLENTS ON *IXODES PERSULCATUS*

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ABSTRACT: Tick-borne diseases have posed a serious threat to human health and life in recent decades as the number of cases of vector-borne diseases is rising steadily. Taiga ticks (*Ixodes persulcatus*) are among the most hazardous species of the Ixodidae family; they have spread from Siberia to China, Japan, Scandinavia and Poland. While repellents constitute a conventional line of defense against arthropod assaults, attractants are also employed in arthropod traps and may serve as the foundation for future protective technologies. The purpose of our study was to determine whether synthetic and natural substances of different classes have repellent or attractant effects on the taiga ticks. The substances included: DEET, IR3535, icaridin (KBR 3023), 2-undecanone, nootkatone, squalene, methyl salicylate, benzaldehyde and guanine. Choice trials (treated vs. untreated textile materials) were conducted across a broad concentration range. The results have shown that the effective dose of IR3535 was 66.0 g/m^2, while DEET and icaridin repelled taiga ticks at 17.3 g/m^2. The most effective repellent for *I. persulcatus* was nootkatone, at the concentration of 1.0 g/m^2. Undecanone's repellent ability was weaker compared to nootkatone, with an effective concentration of 2.0 g/m^2. None of the chemicals under study exhibited a 100% attractant effect. Nevertheless, benzaldehyde and guanine exhibited the highest levels of attractant activity.

KEY WORDS: taiga ticks, attractants, repellents, nootkatone, 2-undecanone, squalene, benzaldehyde, guanine, DEET

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INTRODUCTION

According to the World Health Organization (2020), vector-borne diseases account for more than 17% of all infectious diseases and cause over 700,000 deaths annually. Ticks, as vectors, can spread parasites (Luu *et al.* 2020), bacteria (Shpynov 2012), or viruses (Jääskeläinen *et al.* 2016), which can cause diseases as diverse as Crimean-Congo hemorrhagic fever, Lyme disease, relapsing fever (borreliosis) (Dworkin *et al.* 2008), rickettsial diseases (e.g., spotted fever and Q fever) (Mum *et al.* 2008), tick-borne encephalitis (Czarnowska *et al.* 2024) and tularaemia (Zellner and Huntley 2019). The spread of taiga ticks, or *Ixodes persulcatus*, has been documented throughout northeastern Europe, as well as some regions of Russia, China, Korea, Japan and other countries (Shah *et al.* 2023). The habitat of taiga ticks is still growing; studies (Wang *et al.* 2023) indicate that it may spread to the Scandinavian countries, the Balkans and Alaska, as well as to some provinces of Canada.

An *I. persulcatus* bite would greatly increase the risk of transmitting a pathogen to the human body. In Mongolia, screening studies have revealed a tick-borne encephalitis virus (TBEV) infection in 1.7% of ticks, *Borrelia burgdorferi*—49.4%, *B. miyamotoi*—4.9%, Anaplasma—13.6% and Ehrlichia—16.2%. In addition, ticks were infected with other pathogens in 58.8–70.2% of cases (Lagunova *et al.* 2022). Long-term studies (Bugmyrin *et al.* 2022) conducted in Karelia since 2007 show that the average proportions of *I. persulcatus* ticks infected with TBEV and *B. burgdorferi* are 4.4 and 23.4%, respectively. The same studies have shown that for *I. ricinus*, these rates are 1.1 and 11.9%, respectively.

To prevent tick-borne diseases, personal protection is the most effective strategy. There are two ways to achieve such protection: using textiles treated with pyrethroids that have acaricidal properties (Zverev *et al.* 2023); or applying repellents on skin and clothing (Eisen 2022; Schwartz *et al.* 2022). Additionally, baits containing systemic poisons can be used to control tick populations. A substance that has an attractive effect is used in such products (Poché *et al.* 2020).

Traps with attractants can serve as a useful tool in the timely detection of ticks, as they can additionally help track tick population numbers and identify which diseases they carry. Most attractant research is carried out on Ixodidae, which have high populations in North America and Europe, including *I. scapularis* and *I. ricinus* (Lupi *et al.* 2013). However, attractants and repellents of taiga ticks have not been the subject of any systematic investigation.

Our paper describes the investigation of the attractant properties of squalene, methyl salicylate, benzaldehyde and guanine, as well as the repellency properties of DEET, IR3535, KBR 3023, 2-undecanone and nootkatone (Fig. 1) on *I. persulcatus*.

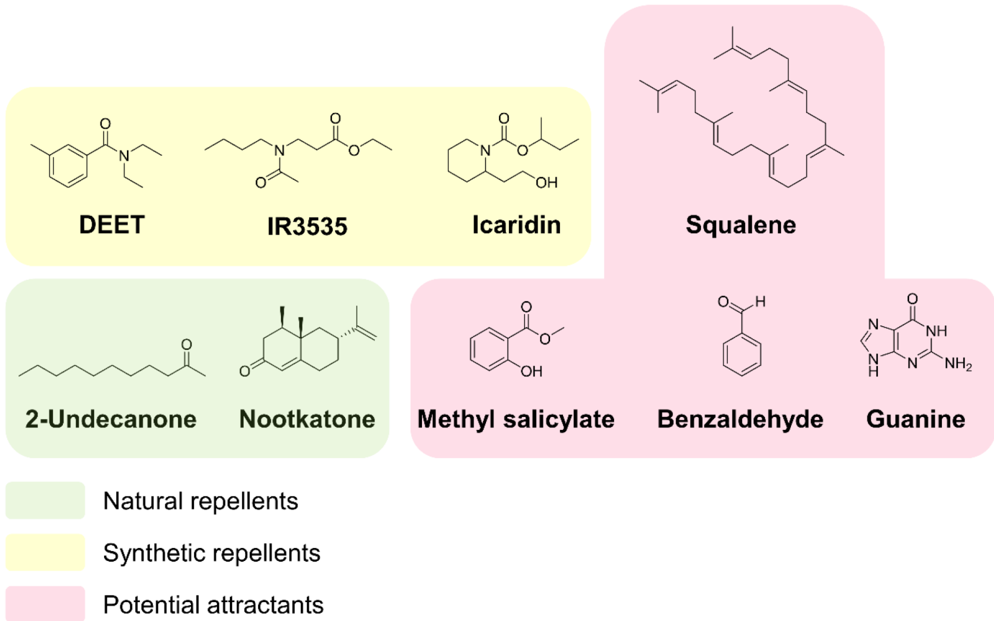

Fig. 1. Attractants and repellents used in this study.

MATERIALS AND METHODS

Chemicals

DEET, benzaldehyde, methyl salicylate, squalene and 2-undecanone were purchased from Merck KGaA (Darmstadt, Germany). IR3535 and icaridin (KBR 3023) were purchased from Upeco Ltd. (Moscow, Russia). Nootkatone was purchased from the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All other reagents were of analytical grade or higher and were used without further purification.

Preparation of textile samples

Samples of white calico (Shuiskie Sitsy Ltd., Shuya, Russia) in the shape of a half of a 90-mm-diameter Petri dish were used in the tests. To obtain the desired substance concentration, the fabric was kept in an alcoholic solution of the substance until complete saturation. Preliminarily, under laboratory conditions, it was determined that for solutions of all substances in different concentrations, the fabric should be soaked for 5 mins. The concentrations of substances on the fabric C (g/m^2) were calculated using the formula

$$C = \frac{m_s \cdot X}{100\% \cdot S},$$

where m_s is the mass (in g) of an alcoholic solution of the substance (the maximum absorbency of alcohol solutions containing different concentrations of test substances was previously determined under laboratory conditions); *X*—substance content in the solution (w/w, %); *S*—fabric surface area, m^2.

After soaking in the solution for three hours, the fabric samples were dried for 3 hours at 22–25 °C. The above time interval was chosen based on the preliminary studies that have shown that the samples of all substances reach a constant mass during this period. The fabrics with the applied substances were then used on the day of the impregnation.

The following textile samples were prepared: 0.17, 1.73, 17.3, 66.0 and 110 g/m^2 for DEET, IR3535 and icaridin; 0.5, 0.7, 1.0 and 2.0 g/m^2 for 2-undecanone; 0.1, 0.3, 0.5, 0.7 and 1.0 g/m^2 for nootkatone; 0.314, 31.4, 314, 31,447 and 314,465 mcg/m^2 for squalene; 0.314, 3.14, 314 and 15,702 mg/m^2 for guanine; 3.145, 31.447, 314.465 and 628.930 mg/m^2 for benzaldehyde and methyl salicylate. The standard deviation of the mass gain after impregnation was <5% (n=10).

The study area and the collection of ticks

Taiga ticks were collected along forest roads and along forest edges near the Taltsa River, Irkutskaya Oblast, Russia (52.013653, 104.673896), in May–June 2023, during warm and dry weather (air temperatures of 20–25 °C and relative humidity of 50–70%), under mixed coniferous-leaved forest canopy. Ticks were collected on white cotton cloth (flags) with no chemicals applied and transported to the laboratory in damp gauze tape the same day they were collected. Female ticks were identified by the size of their body and by red alloscuta on their backs, while males were identified by the size of their capitulum (Estrada-Peña *et al.* 2017). The microscopic identification of microscopically-aspirated individuals was carried out after the experiment in laboratory conditions. The ticks were used on the day of the collection or the next day. Wet gauze was used to store them at 4–8 °C. One to two hours prior to testing, ticks were placed in room temperature.

Choice trials

Behavioral bioassays were conducted at 23–25 °C and 50–60% relative humidity (RH) (Fig. 2).

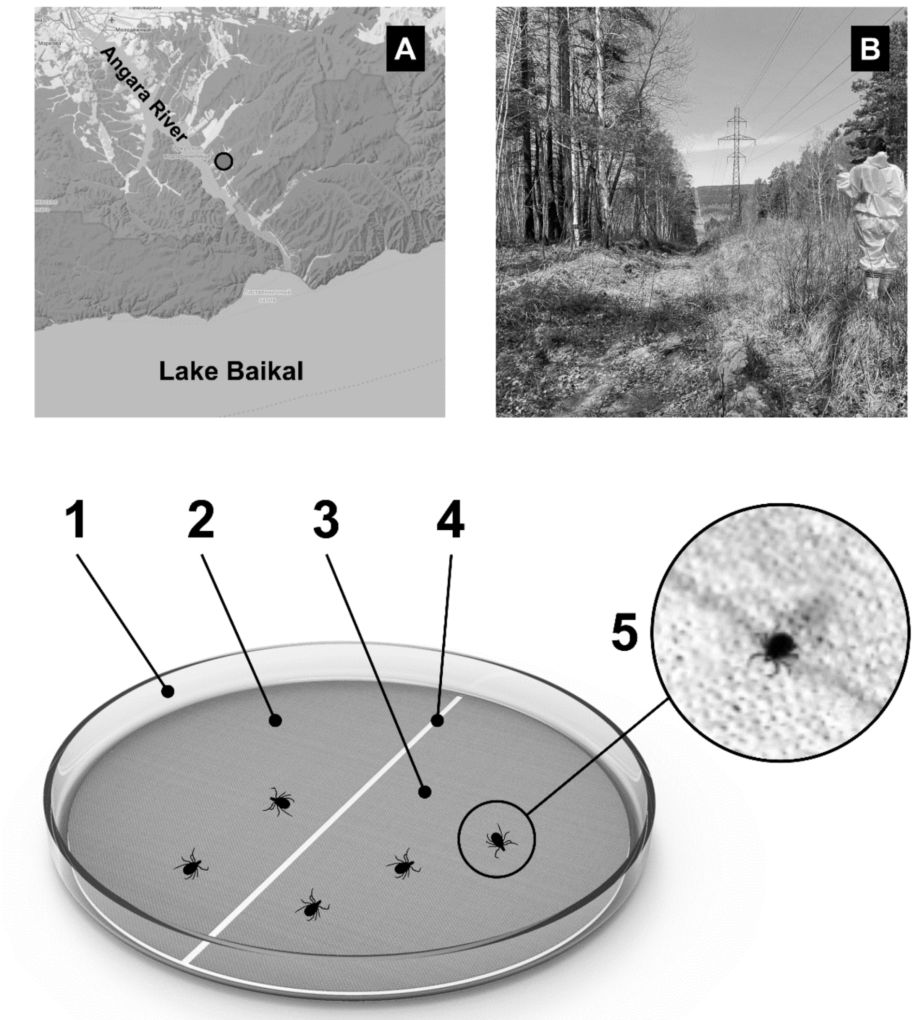

Fig. 2. Behavioral bioassays setup. 1—Petri dish, 2—blank fabric piece, 3—impregnated fabric piece, 4—start line, 5—*Ixodes persulcatus* ticks; A—study area, B—collection site.

There was a choice between untreated and treated calico fabrics for ticks. In this experiment, two 31.8 cm^2 semi-circular pieces of calico fabric were layered into a 63.6 cm^2 petri dish lid. One fabric piece was soaked in a solution that contained the active ingredient diluted in ethanol, while the other section (control) was soaked in pure ethanol. They were allowed to dry for 3 h at room temperature. Five ticks were then placed along the line formed by the intersection of the treated and untreated fabrics. After ticks were introduced into the test area, their distribution with respect to the line was recorded every 5 mins, up to 30 mins total. The experiment was repeated three times (N=15).

Data analysis

Mean repellency percentages (R, %) for bioassay samples were calculated separately for each substance as follows:

$$R = -200 \cdot \frac{N_{tr}}{N_{tr}+N_{un}} + 100 \ ,$$

where N_{tr} is the number of ticks on the treated fabric, and N_{un} is the number of ticks on the untreated fabric.

Pairwise mean comparisons were performed to identify statistically significant differences in the mean repellency percentages of distinct substances across all time points and at each individual time point. Tukey's adjustment was used to assure an experiment-wise significance level of P=0.05 for all comparisons (Agbangba *et al.* 2024). Null hypothesis was that the probability of tick location on one half of the Petri dish is 0.5 at any given time (H_o: Repellency=0). The 95% confidence intervals were estimated by the method of Robertson and Preisler (Robertson *et al.* 2007). Calculations were made in Excel 2019 (Microsoft Corporation) and OriginPro 2021 (Origin Corp.).

RESULTS AND DISCUSSION

Numerous substances have been described as repellents against ixodid ticks. They include traditional repellents like DEET or IR3535, as well as naturally occurring substances like nootkatone and 2-undecanone. In this study, we compared the effects of different substances on taiga ticks under identical conditions. Additionally, we calculated the minimum concentration of the substance that must be applied to the fabric to achieve repellency. Several possible attractants were also examined.

The compounds were divided into repellents and attractants, based on which features became more noticeable at higher concentrations under identical experiment conditions. Since the substances showed mixed results during the experiments, we decided to present the results on a single "Repellency (R, %)" scale, where positive values correspond to the percentage of repellency, and negative values—to the percentage of attractancy.

The study of repellency

There are three main synthetic repellents that are active against arthropods: DEET, IR3535 and icaridin (Nguyen *et al.* 2023). Even though these substances have been known for quite a while, only DEET and icaridin were found to be effective against taiga ticks (Abdel-Ghaffar *et al.* 2015; Ogawa *et al.* 2016). We compared the repellent effects of all three substances. Fig. 3 shows how the repellent effect varies with time for various concentrations.

All substances were studied at concentrations ranging from 0.17 to 110 g/m^2. We may classify these compounds' repelling impact as "moderate" based on these data. Only DEET reached 100% repellency at the concentration of 17.3 g/m^2 after 15 mins. Icaridin exhibited maximum repellent activity at the concentration of 17.3 g/m^2, resulting in a maximum effectiveness of 87%. The efficiency of IR3535 was even lower: it remained at around 73% at the concentration of 17.3 g/m^2. A noticeable decrease in repellency was observed for DEET, icaridin and IR3535 at the concentration of 110 g/m^2. Ticks became less mobile, essentially remaining in one place at such concentrations. A noteworthy fact is that when ticks were placed on the experimenter's hand after the experiment, their mobility was restored.

A correlation between the repellent effect and the concentrations of DEET, icaridin and IR3535 is shown in Fig. 3. We plotted the graph based on the R value at the end of the experiment, which lasted 30 mins in total. Assuming 80% as the conditional threshold for effective action, the necessary DEET and icaridin concentrations are 63 and 15 g/m^2, respectively. IR3535 did not achieve 80% repellency, as previously mentioned.

The statistical analysis (Table 1) shows that DEET at concentrations below 110 g/m^2 had a repellent effect. At the same time, IR3535 and icaridin at the concentrations of 0.17 g/m^2 had no repellent effect (P>0.05). Weaker repellent properties of icaridin are confirmed by the fact that no sig-

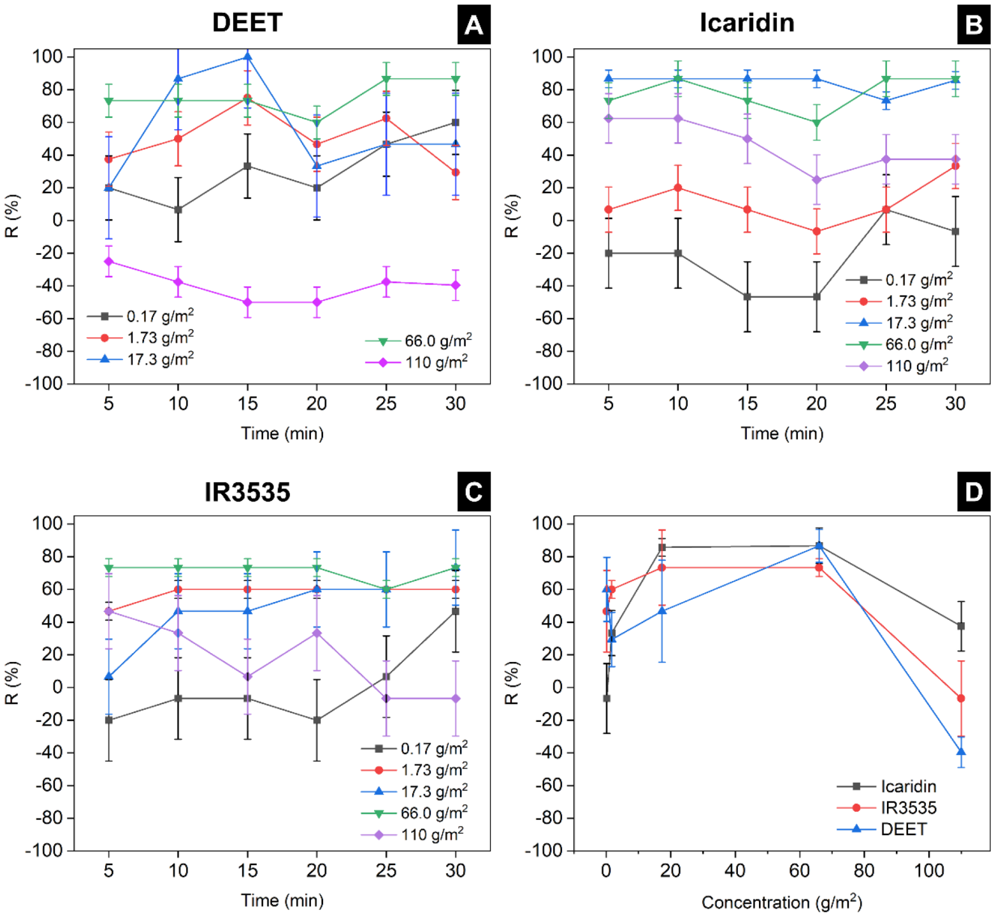

Fig. 3. Mean percentage repellency of DEET (A), Icaridin (B), IR3535 (C); D—their dose-response curves after 30 min of exposure.

Table 1

Statistical analysis of DEET, icaridin and IR3535 repellency data (n=6, α=0.05)

Substance	Concentration, g/m²	|t| value	P	Mean Repellency (R, %)	95% LCL	95% UCL
Control	No substance	1.11	0.465	5.56	−12.5	23.6
DEET	0.17	5.49	0.0116	31.1	10.5	51.7
	1.73	10.5	7.00E-4	50.2	32.8	67.6
	17.3	6.17	0.00725	55.6	22.8	88.3
	66.0	26.1	8.74E-6	75.6	65.0	86.1
	110	14.8	1.39E-4	−39.9	−49.7	−30.1
IR3535	0.17	4.03E-16	1.00	0.00	−26.2	26.2
	1.73	36.8	1.69E-6	57.8	52.1	63.5
	17.3	7.37	0.00343	48.9	24.8	73.0
	66.0	45.3	6.97E-7	71.1	65.4	76.8
	110	2.68	0.116	17.8	−6.32	41.9
Icaridin	0.17	3.60	0.0514	−22.2	−44.6	0.195
	1.73	2.80	0.105	11.1	−3.34	25.6
	17.3	54.3	3.71E-7	84.3	78.6	89.9
	66.0	24.7	1.13E-5	77.8	66.4	89.2
	110	10.5	7.02E-4	45.8	29.9	61.7

nificant distribution was found in the presence of ticks on one or the other half of the Petri dish for the sample with the concentration of 1.73 g/m^2. At the maximum concentration of 110 g/m^2, all three substances affected the mobility of the ticks.

Humans are not the only ones who protect themselves from harmful arthropods. Certain species of plants produce repellents against many species of pests and parasites. More than 80 natural substances have been described as tick repellents (Benelli *et al.* 2016). We chose to test 2-undecanone in this study, since it exhibits comparable repellent efficacy to DEET against *Amblyomma americanum*, *Dermacentor variabilis* and *I. scapularis* (Bissinger *et al.* 2009). Nootkatone, which has been shown to be effective against *A. americanum* and *I. scapularis* (Jordan *et al.* 2012), was also tested. The repellency values of these substances at different concentrations are compared in Fig. 4.

The statistical treatment of the results of the evaluation of the repellency of 2-undecanone and nootkatone is summarized in Table 2.

2-undecanone is moderately repellent, but at lower concentrations than typical repellents such as DEET, IR3535 and Icaridin. At the concentrations up to 2 g/m^2, its repellency is around 50%. For nootkatone in the concentration range of 0.1 to 1 g/m^2, all states from inactive dose to active dose were passed. 2-undecanone and nootkatone are both more effective repellents than synthetic DEET, icaridin and IR3535. Both substances had a repellent effect of 100%; 2-undecanone was required to be applied at the concentration of 2 g/m^2, while nootkatone needed to be applied at the concentration of 1 g/m^2, which is an order of magnitude less than the required DEET concentration. Using dose-response curves for nootkatone and 2-undecanone, we can conclude that for 80% repel-

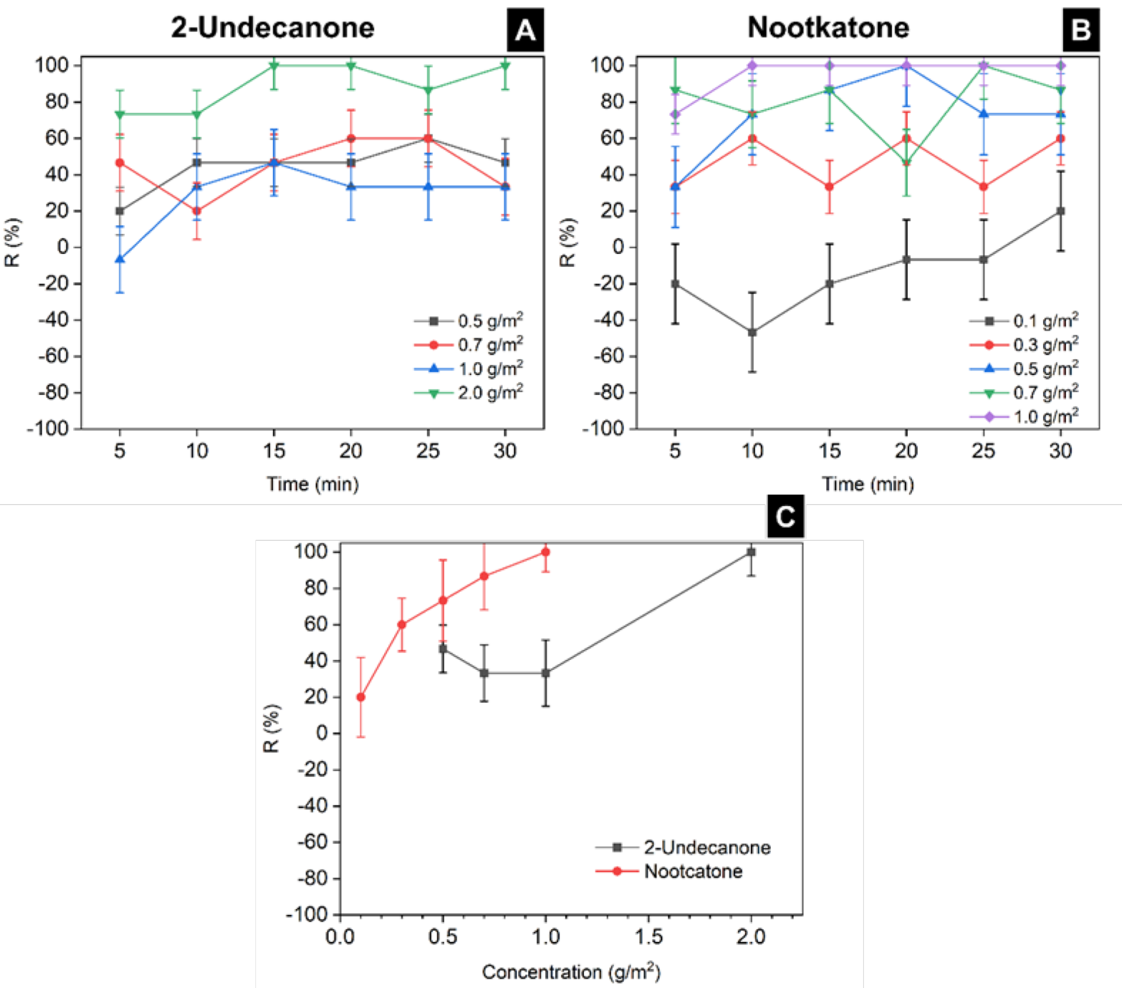

Fig. 4. Mean percentage repellency of 2-undecanone (A), Nootkatone (B); C—their dose-response curves after 30 min of exposure.

Table 2
Statistical analysis of 2-undecanone and nootkatone repellency data (n=6, α=0.05)

Substance	Concentration, g/m²	|t| value	P	Mean Repellency (R, %)	95% LCL	95% UCL
Control	No substance	1.11	0.465	5.56	−12.5	23.6
2-undecanone	0.5	11.7	4.14E-4	44.4	30.7	58.2
	0.7	9.88	9.26E-4	44.4	28.1	60.8
	1.0	5.49	0.0116	28.9	9.77	48.0
	2.0	23.4	1.45E-5	88.9	75.1	102
Nootkatone	0.1	2.11	0.196	−13.3	−36.3	9.66
	0.3	11.1	5.46E-4	46.7	31.3	62.0
	0.5	11.4	4.78E-4	73.3	49.9	96.7
	0.7	15.1	1.26E-4	80.0	60.7	99.3
	1.0	30.4	4.16E-4	95.6	84.1	107

lency, 0.7 g/m^2 of nootkatone and 1,8 g/m^2 of 2-undecanone are required, which is 90 and 38 times less compared to DEET, respectively. In terms of repellents against taiga ticks, nootkatone and 2-undecanone both have an exceptional potential.

The study of attractant properties

The effects of tick attractants are studied much less frequently than the effects of repellents. Additionally, the attractants have not yet found industrial applications (Carr and Roe 2016). Squalene, methyl salicylate, benzaldehyde and guanine were chosen as potential ixodid tick attractants (Yoder *et al.* 1998; Carr and Roe 2016). The concentrations of attractants were selected based on literature review.

Squalene and methyl salicylate did not exhibit repellent or attractant effects (Fig. 5). For methyl salicylate, the R value was in the range of −4,44 to 42,2%, almost identical to the values obtained in our control experiment, but there was a significant scatter for squalene. Meanwhile, the median R value of squalene was about 50% as concentration increased, indicating a repellent effect.

The attractant effect of benzaldehyde and guanine was more prominent than that of squalene and methyl salicylate. The obtained findings are independent of concentration; nevertheless, based on Fig. 5, it may be inferred that the R coefficient is about −60% at the concentration of 628,930 mcg/m^2.

For attractants, the picture altered over time, but the repellent effect showed a rapid onset after about 10 mins of viewing. Our analysis of guanine at the concentration of 3.14 mg/m^2, for example, showed that when ticks remained on the treated piece of fabric for 15 mins, their distribution nearly returned to 50:50 after 15 mins. Lower drug concentrations in the test might be the cause of the observed effect. Despite our attempts to significantly increase the concentration of potential attractants, they began to act more like repellents. As a result, the attractant effect of any drug is limited to a certain range of concentrations.

CONCLUSION

In this study, different substances were tested for their repellent and attractant properties on the taiga tick, *I. persulcatus*. We employed a two-choice experiment comparing textiles that had been treated and those that had not. The proposed approach enables the testing of many compounds at various concentrations in a comparatively short time.

The results have confirmed previous studies, which indicated that DEET and icaridin repel taiga ticks. This may indicate that the proposed method for studying repellent activity is agreeing with previously-published methods. The repellent effect of IR3535, nootkatone and 2-undecanone, as well as the attractive effect of benzaldehyde and guanine, on taiga ticks were demonstrated for the first time.

The best repellent properties were demonstrated by the substances of natural origin: nootkatone and 2-undecanone. For their potential, these substances may be considered as active ingredients in commercial repellent formulations. Among the traditional synthetic repellents, icaridin demonstrated greater effectiveness than DEET and IR3535.

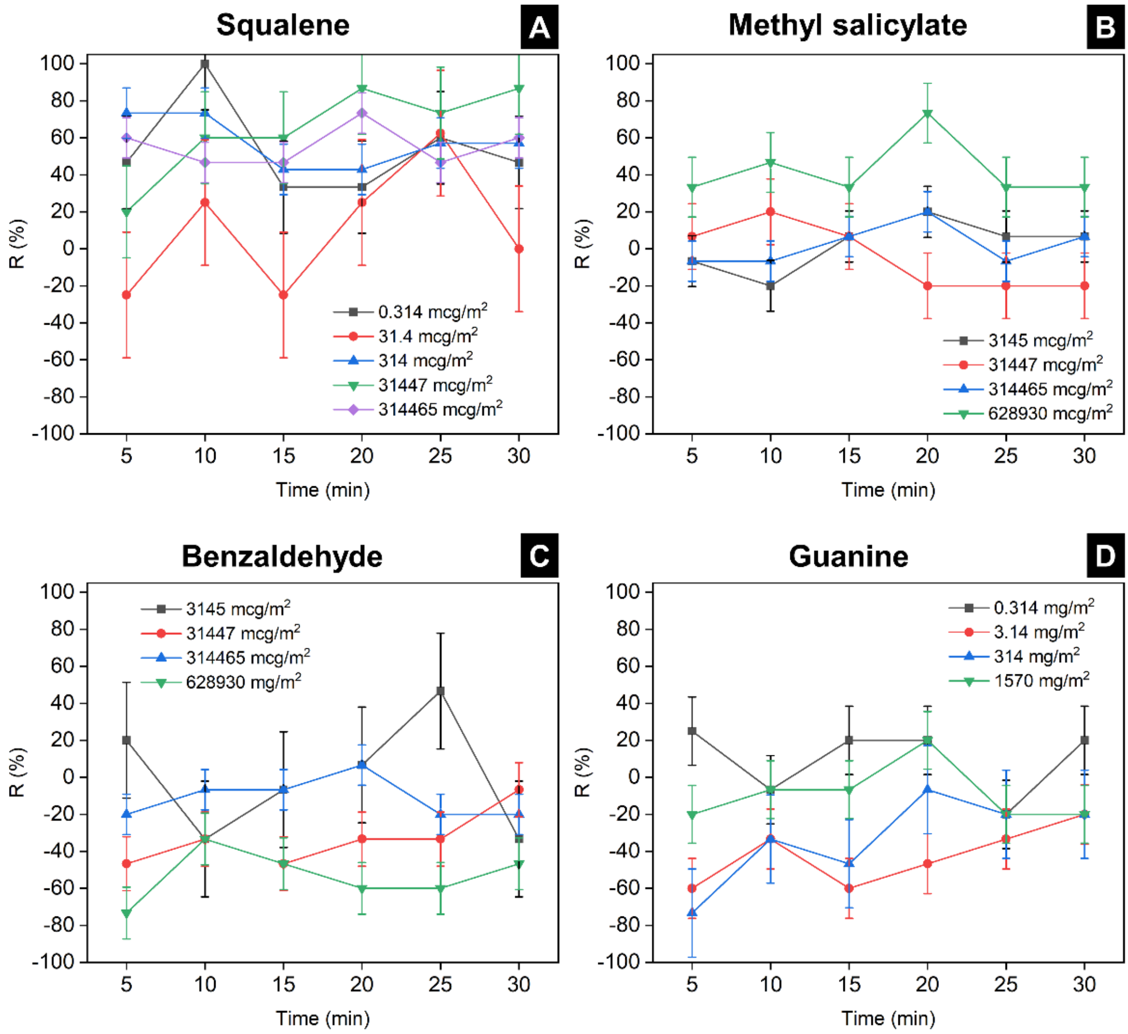

Fig. 5. Mean percentage repellency of squalene (A), methyl salicylate (B), benzaldehyde (C) and guanine (D).

Among the chemicals under investigation, we have discovered that only benzaldehyde and, to a lesser extent, guanine can be used as possible attractants. Further research should be conducted to identify more effective attractants.

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Credit authorship contribution statement

S.V. Andreev: formal analysis, writing the original draft, conceptualization, conceivement of the presented idea, project administration. **K.A. Sakharov**: conceptualization, reviewing and editing the manuscript; **S.A. Zverev**: investigation; **E.A. Lapina**: investigation; **D.V. Savraeva**: investigation; **M.B. Akhmetshina**: data curation; **E.V. Ushakova**: conceptualization; **A.S. Kuzovlev**: conceptualization, formal analysis, conceivement of the presented idea, reviewing and editing the manuscript.

Declaration of competing interest

The authors declare that there is no conflict of interest in this study.

Data availability

The data used in this study is confidential.

REFERENCES

Abdel-Ghaffar, F., Al-Quraishy, S. and Mehlhorn, H. 2015. Length of tick repellency depends on formulation of the repellent compound (icaridin=Saltidin®): tests on *Ixodes persulcatus* and *Ixodes ricinus* placed on hands and clothes. *Parasitology Research,* 114: 3041–3045. https://doi.org/10.1007/s00436-015-4506-z

Agbangba, C., Sacla Aide, E., Honfo, H. and Glèlè Kakai, R. 2024. On the use of post-hoc tests in environmental and biological sciences: a critical review. *Heliyon,* 10: e25131. https://doi.org/10.1016/j.heliyon.2024.e25131

Benelli, G., Pavela, R., Canale, A. and Mehlhorn, H. 2016. Tick repellents and acaricides of botanical origin: a green roadmap to control tick-borne diseases? *Parasitology Research,* 115: 2545–2560. https://doi.org/10.1007/s00436-016-5095-1

Bissinger, B., Apperson, C., Sonenshine, D., Watson, D.W. and Roe, R.M. 2009. Efficacy of the new repellent BioUD® against three species of ixodid ticks. *Experimental and Applied Acarology,* 48: 239–250. https://doi.org/10.1007/s10493-008-9235-x

Bugmyrin, S.V., Romanova, L.Yu., Belova, O.A., Kholodilov, I.S., Bespyatova, L.A., Chernokhaeva, L.L., Gmyl, L.V., Klimentov, A.S., Ivannikova, A.Y., Polienko, A.E., Yakovlev, A.S., Ieshko, E.P., Gmyl, A.P. and Karganova, G.G. 2022. Pathogens in *Ixodes persulcatus* and *Ixodes ricinus* ticks (Acari, Ixodidae) in Karelia (Russia). *Ticks and Tick-borne Diseases*, 13(6), 102045. https://doi.org/10.1016/j.ttbdis.2022.102045

Carr, A. and Roe, M. 2016. Acarine attractants: chemoreception, bioassay, chemistry and control. *Pesticide Biochemistry and Physiology,* 131: 60–79. https://doi.org/10.1016/j.pestbp.2015.12.009

Czarnowska A., Groth, M., Okrzeja, J., Garkowski, A., Kristoferitsch, W., Kułakowska, A. and Zajkowska, A. 2024. A fatal case of tick-borne encephalitis in an immunocompromised patient: case report from Northeastern Poland and review of literature. *Ticks and Tick-borne Diseases,* 15: 102273. https://doi.org/10.1016/j.ttbdis.2023.102273

Dworkin, M., Schwan, T., Anderson, D. and Borchardt, S. 2008. Tick-borne relapsing fever. *Infectious Disease Clinics of North America,* 22: 449–468. https://doi.org/10.1016/j.idc.2008.03.006

Eisen, L. 2022. Personal protection measures to prevent tick bites in the United States: knowledge gaps, challenges and opportunities. *Ticks and Tick-borne Diseases,* 13: 101944. https://doi.org/10.1016/j.ttbdis.2022.101944

Estrada-Peña, A., Mihalca, A. and Petney, T. 2017. *Ticks of Europe and North Africa*. Springer International Publishing, Cham.

Jääskeläinen, A., Tonteri, E., Pieninkeroinen, I., Sironen, T., Voutilainen, T., Kuusi, M., Vaheri, A. and Vapalahti, O. 2016. Siberian subtype tick-borne encephalitis virus in *Ixodes ricinus* in a newly emerged focus, Finland. *Ticks and Tick-borne Diseases,* 7: 216–223. https://doi.org/10.1016/j.ttbdis.2015.10.013

Jordan, R., Schulze, T. and Dolan, M. 2012. Efficacy of plant-derived and synthetic compounds on clothing as repellents against *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae). *Journal of Medical Entomology,* 49: 101–106. https://doi.org/10.1603/ME10241

Lagunova, E., Liapunova, N., Tuul, D., Otgonsuren, G., Nomin, D., Erdenebat, N., Abmed, D., Danchinova, G.A., Sato, K., Kawabata, H. and Khasnatinov, M.A. 2022. Co-infections with multiple pathogens in natural populations of *Ixodes persulcatus* ticks in Mongolia. *Parasites and Vectors*, 15: 236. https://doi.org/10.1186/s13071-022-05356-x

Lupi, E., Hatz, C. and Schlagenhauf, P. 2013. The efficacy of repellents against *Aedes, Anopheles, Culex* and *Ixodes* spp.—a literature review. *Travel Medicine and Infectious Disease*, 11: 374–411. https://doi.org/10.1016/j.tmaid.2013.10.005

Luu, L., Bown, K., Palomar, A., Kazimírová, M. and Bell-Sakyi, L. 2020. Isolation and partial characterisation of a novel *Trypanosoma* from the tick *Ixodes ricinus*. *Ticks and Tick-borne Diseases,* 11: 101501. https://doi.org/10.1016/j.ttbdis.2020.101501

Mum, A., Masala, G., Tola, S., Satta, G., Fois, F., Pirns, P., Rolain, J.-M., Raoult, D. and Parola, P. 2008. First direct detection of rickettsial pathogens and a new rickettsia, "*Candidatus* Rickettsia barbariae", in ticks from Sardinia, Italy. *Clinical Microbiology and Infection,* 14: 1028–1033. https://doi.org/10.1111/j.1469-0691.2008.02082.x

Nguyen, Q., Vu, M. and Hebert, A. 2023. Insect repellents: an updated review for the clinician. *Journal of the American Academy of Dermatology,* 88: 123–130. https://doi.org/10.1016/j.jaad.2018.10.053

Ogawa, K., Komagata, O., Hayashi, T., Itokawa, K., Morikawa, S., Sawabe, K. and Tomita, T. 2016. Field and laboratory evaluations of the efficacy of deet repellent against *Ixodes* ticks. *Japanese Journal of Infectious Diseases*, 69: 131–134. https://doi.org/10.7883/yoken.JJID.2015.038

Poché, D., Franckowiak, G., Clarke, T., Tseveenjav, B., Polyakovam L. and Poché, R.M. 2020. Efficacy of a low dose fipronil bait against blacklegged tick (*Ixodes scapularis*) larvae feeding on white-footed mice (*Peromyscus leucopus*) under laboratory conditions. *Parasites and Vectors,* 13: 391. https://doi.org/10.1186/s13071-020-04258-0

Robertson, J., Savin, N. and Preisler, H. 2007. *Bioassays with Arthropods*. CRC Press, Boca Raton. 224 pp.

Schwartz, A., Mackeprang, J., Mead, P. and Hinckley, A. 2022. Effectiveness of personal protection measures against Lyme disease: a review of epidemiologic studies from the United States. *Zoonoses and Public Health,* 69: 777–791. https://doi.org/10.1111/zph.12984

Shah, T., Li Q., Wang, B., Baloch, Z., Xia, X. and Xia, X. 2023. Geographical distribution and pathogenesis of ticks and tick-borne viral diseases. *Frontiers in Microbiology,* 14: 1185829. https://doi.org/10.3389/fmicb.2023.1185829

Shpynov, S. 2012. *Ixodes persulcatus*, a major vector of Alphaproteobacteria in Russia. *Ticks and Tick-borne Diseases,* 3: 305–307. https://doi.org/10.1016/j.ttbdis.2012.10.029

Wang, S.-S., Liu, J.-Y., Wang, B.-Y., Wang, W.-J., Cui, X.-M., Jiang, J.-F., Sun, Y., Guo, W.-B., Pan, Y.-S., Zhou, Y.-H., Lin, Z.-T., Jiang, B.-G., Zhao, L. and Cao, W.-C. 2023. Geographical distribution of *Ixodes persulcatus* and associated pathogens: analysis of integrated data from a China field survey and global published data. *One Health,* 16: 100508. https://doi.org/10.1016/j.onehlt.2023.100508

Yoder, J., Atwood, A. and Stevens, B. 1998. Attraction to squalene by ticks (Acari: Ixodidae): first demonstration of a host-derived attractant. *International Journal of Acarology,* 24: 143–147. https://doi.org/10.1080/01647959808684143

Zellner, B. and Huntley, J. 2019. Ticks and tularemia: do we know what we don't know? *Frontiers in Cellular and Infection Microbiology,* 9: 00146. https://doi.org/10.3389/fcimb.2019.00146

Zverev, S.A., Andreev, S.V., Sakharov, K.A., Akhmetshina, M.B., Istomina, L.I., Verzhutskaya, Y.A. and Shashina, N.I. 2023. Evaluation of the efficacy of permethrin- and cypermethrin-based textile against taiga tick, *Ixodes persulcatus*. *Experimental and Applied Acarology,* 89: 275–286. https://doi.org/10.1007/s10493-023-00785-4

WHO. 2020. *Vector-borne diseases*. Retrieved Aug. 21, 2023, from https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases