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# SHORT COMMUNICATION

## Avoidance of rosemary oil by scorpions

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**Abstract.** Scorpion stings are prevalent and sometimes deadly. Rosemary oil deters phytophagous insects, so we investigated whether scorpions, which prey on insects, might also be deterred. We tested the repellant properties of rosemary oil on adult *Centruroides vittatus* Say, 1821 scorpions in an arena lined with sand infused with rosemary oil on one side and mineral oil on the other. A middle, neutral zone consisting of dry sand ensured independence between the two sections. Scorpions spent significantly more time on the mineral oil sand ($P = 0.0031$). Next, we tested 1,8-cineole, the primary component of rosemary oil, against mineral oil and found no preference ($P = 0.789$). These findings suggest that scorpions may be repelled holistically by rosemary oil, rather than the tested primary component. Future studies could test other components of rosemary oil in varying combinations to determine the critical ingredients responsible for the avoidance behavior induced by the complete mixture.

**Keywords:** Repellent, behavior, Buthidae, 1,8-cineole.

Worldwide, scorpions are estimated to deliver more than 1.2 million stings a year, leading to over 3000 deaths annually (Chippaux & Goyffon 2008). The situation is particularly acute in areas where deadly species coincide with increasing urbanization (Reckziegel & Pinto 2014; De Araújo et al. 2017). Our goal was to find a way to reduce the frequency of scorpion stings by identifying a substance that scorpions avoid. This chemical could then be used to create a household-safe scorpion repellent.

Rosemary oil has been shown to repel plant-eating insects such as onion thrips, armyworms, and cabbage loopers (Koschier & Sedy 2003; Isman et al. 2007). Since scorpions prey on insects, we speculated that natural selection may have favored scorpions that avoided the low density of insects around rosemary plants. The objective of this study was to test the repellency of the rosemary oil on scorpions rather than the effect of the plant as a whole.

We tested eight adult male and eight adult female striped bark scorpions (*Centruroides vittatus* Say, 1821) that we collected from Lake Thunderbird State Park in Norman, Oklahoma on September 20, 2017. We kept the animals in sixteen separate Mason jars lined with a layer of Eco Earth coconut fiber and exposed them to a 15:9 hour light:dark cycle, mimicking the natural environment. Temperature and humidity were held constant (25°C and RH 55–60%). We gave each scorpion approximately 10 mL of water twice a week and one live cricket (from Rainbow Mealworms Inc. Compton, CA) every two weeks.

We conducted the trials in a rectangular Plexiglas® box measuring 22.5 cm by 9.0 cm by 6.0 cm (Fig. 1A). A pair of 1 cm tall dividers created three sections for holding the treatment, neutral, and control substrates. We placed the arena on a foam platform (Stalwart) to reduce vibrations and placed a rectangular piece of Plexiglas® on top as a lid to reduce air currents. In our first set of experiments, we used 100% pure *Rosmarinus officinalis* oil (NOW Essential Oils) as our treatment. The treatment oil consisted of 0.8 mL of rosemary oil mixed with 0.2 mL of unscented mineral oil (ScholAR Chemistry). We added this mixture to 100 mL of sand (from Monahans, TX) in a $13.0 \times 8.0 \times 7.5$ cm plastic container (Ziploc) and stirred it with a plastic ruler. Similarly, the control sand substrate consisted of 1.0 mL of mineral oil mixed with another 100 mL of sand using a separate ruler and plastic container. The neutral substrate was plain, dry, untreated sand; it created independence between the treatment and the control substrates.

We recorded each trial with an infrared camera (Nest cam indoor NC1104US), which captured an overhead view of the arena. We ran the trials after sunset in the first hour of the dark cycle (around 2000 h), when scorpions are most active. Between trials, we rotated the arenas 180° to minimize effects of light, temperature, magnetic fields, or other unforeseen variables.

To prepare for each trial, we placed a scorpion beneath an inverted film canister in the middle of the neutral zone of the arena, leaving the scorpion for 30 s to acclimate. We initiated the trial by lifting the canister to release the scorpion and covered the arena with the Plexiglas lid. The trials ran for 2 min each, and the infrared camera recorded the scorpion's movements for later analysis. Between trials, we leveled the sand in all three parts of the arena to disperse any chemicals left behind by the previous scorpion. We repeated this process for each individual tested; the 16 scorpions were tested from November 12$^{th}$ to November 15$^{th}$.

We measured time spent on each substrate and ran a two-tailed paired t-test to analyze scorpion behaviors. Our null hypothesis was that there would be no difference between the amount of time the scorpions spend on mineral oil substrate and rosemary oil substrate. We set a statistical significance level of $P<0.05$. Our data suggest that scorpions are repelled by rosemary oil. Only two of the sixteen scorpions spent more time on the rosemary than on the treatment oil substrate. Scorpions spent significantly more time on the mineral oil substrate than on the rosemary oil substrate ($P = 0.0031$).

Rosemary oil's primary component is 1,8-cineole (Hori & Komatsu 1997). If scorpions are repelled by rosemary oil, it seems reasonable that a majority of the avoidance behavior would be caused by 1,8-cineole. In a second set of trials, we therefore used 0.8 ml of 1,8-cineole mixed with 0.2 ml of mineral oil as the treatment substrate. Dry sand and mineral oil treated sand constituted the neutral zone and control substrate, respectively. All procedures were followed as per the rosemary oil trials, and the same animals (except for one female that died between experiments) were used in these trials. The 1,8-cineole trials were conducted during the evenings from November 16$^{th}$ to November 18$^{th}$.

We observed no difference between the 1,8-cineole and mineral oil sand ($P = 0.789$), and we failed to reject the null hypothesis. In these trials, two of the animals did not move from the neutral zone. Of the remaining 13, five spent more time on the 1,8-cineole than on the mineral oil substrate.

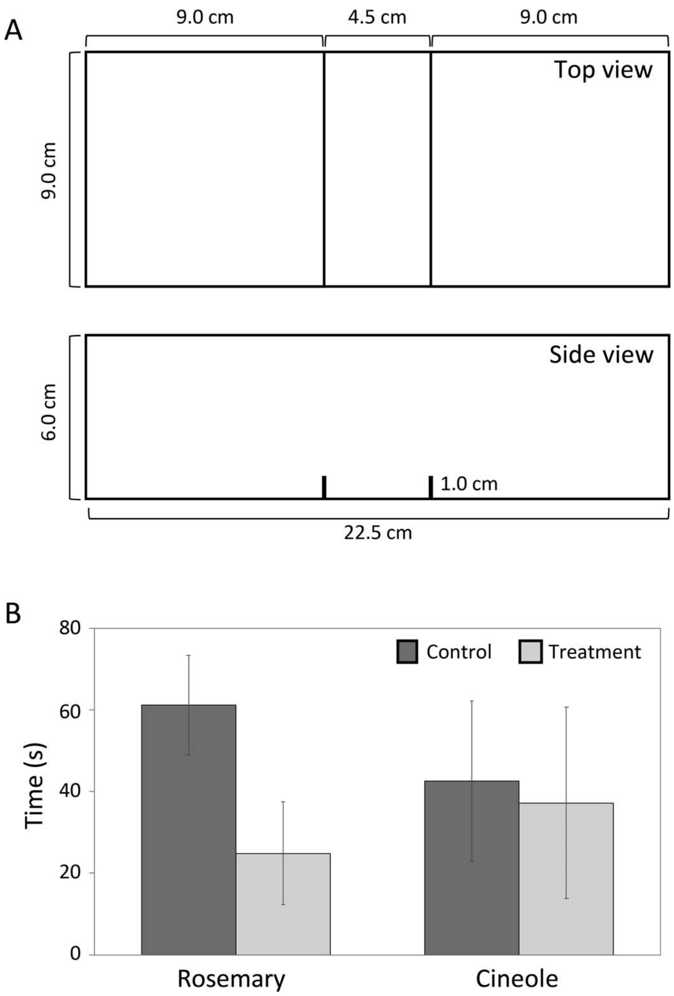


Figure 1.—**A** Diagram of top and side view of arena. The 1 cm ridges served as dividers to ensure the same amount of sand filled the chamber's compartments for each trial. The central neutral zone was filled with dry sand. **B** Average time spent on each substrate (treatment or control) for rosemary oil and 1,8-cineole experiments; error bars are 1.96*SE.

The means (± 1.96 SE) of both the rosemary oil and 1,8-cineole trials are summarized in Figure 1B. We also conducted a post-hoc analysis of animal behavior in both experiments based on sex. Overall, there was no apparent difference between the average time spent on the treatment substrates by males and females. An additional post-hoc review of a subset of videos in both experiments revealed no difference in average velocity of scorpions in the neutral zone vs. the mineral oil zone (data not shown), suggesting that the mineral oil did not induce lethargy that might confound the results.

Our behavioral evidence suggests that scorpions avoid rosemary oil as a mixture, but are not deterred by its prime component, 1,8-cineole. This finding is different from behavioral studies of aphids, which were strongly repelled by both rosemary oil and 1,8-cineole (Hori & Komatsu 1997). In addition, an enhanced insecticidal effect was observed when 1,8-cineole and camphor (another main rosemary oil component) were applied together topically to cabbage loopers (Tak & Isman 2015). A synergistic combination of chemicals may be required to elicit the scorpion sensory response to rosemary oil.

We did not test the mechanisms by which scorpions detect rosemary oil. The primary chemosensory organs of scorpions are the ventromedial pectines, which morphologically (Foelix & Muëller-Vorholt 1983) and physiologically (Gaffin & Brownell 1997; Gaffin & Walvoord 2004; Knowlton & Gaffin 2011) appear to be contact chemoreceptors. Other candidate receptors include tarsal sensilla, such as the tarsal organs and taste hairs (Foelix & Schabronath 1983; Gaffin et al. 1992), and constellation array sensilla on the pedipalps (Fet et al. 2006). Future research could use sensory ablation to isolate the chemoreceptors responsible for the avoidance behavior, coupled with electrophysiology to assay the kinetics and threshold sensitivity of putative chemosensory organs to rosemary oil.

These results could lead to an inexpensive, effective, and marketable scorpion repellent that prevents stings and therefore deaths. More research should be done to determine whether rosemary oil could work as a scorpion repellent over a large area. Additional experiments should test whether scorpions adapt to rosemary oil and how long a repellant would be effective. Furthermore, future experiments could also test combinations of different components of rosemary oil, or even the rosemary plant itself, to explore which combination is the most effective repellent.

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