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# Linking Animal Behavior to Useful Natural Repellents

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STEPHEN AUSMUS (D2783-8)

In a lab test, this lone star tick nymph *(Amblyomma americanum)* was exposed to a citrus rind chemical, placed upside down inside an untreated filter paper cylinder, and observed to see whether it could right itself.

**A little monkey business is revealing a few clues** about natural remedies that animals use to protect themselves against biting insects and arthropods.

Certain species of animals, such as monkeys and birds, anoint themselves with citrus, other plants, and creatures like millipedes. To find out more about this behavior and to determine if any chemicals in the anointing substances effectively deter ticks and mosquitoes, scientists are examining responses to natural compounds.

Scientists at the Agricultural Research Service Henry A. Wallace Beltsville [Maryland] Agricultural Research Center (BARC) and the Smithsonian Conservation Biology Institute (SCBI) at the National Zoological Park in Front Royal, Virginia, compared the effects of citrus compounds on lone star ticks and yellowfever mosquitoes. They also investigated compounds found in millipedes.

### Citing Citrus Effects

"We tested a number of components known to be abundant in all citrus extracts, not just lemons, limes, and oranges, but all the fruits that are used in anointing—including citrus leaves," says SCBI researcher Paul Weldon.

Of the more than 20 citrus compounds they evaluated, the scientists found that 10 deterred ticks and/or mosquitoes, and 9 impaired basic tick behavior.

Weldon used a feeding membrane module that he developed to test citrus compounds against mosquitoes. Some compounds were very effective. But the same compounds were not effective at all when mosquitoes were exposed to them in a wind tunnel module by chemist Ulrich "Uli" Bernier, in the Mosquito and Fly Unit at the ARS Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida.

"We viewed the results of the wind tunnel as being more authentic," Weldon says. "The compounds didn't affect mosquitoes that much, but mainly affected ticks. It was a step forward in pinpointing what we believe is the reason that animals anoint themselves with citrus substances."

### Ticking Off Ticks

Mosquitoes, ticks, and other blood-feeding arthropods are attracted to certain chemicals, such as carbon dioxide in an animal's breath. One behavior of host-seeking ticks when a host draws near is to climb up a plant to reach the passing host and then find an attachment site on the host's body.

Entomologist John Carroll, of BARC's Invasive Insect Biocontrol and Behavior Laboratory, and colleagues conducted several experiments to evaluate citrus compound repellency against ticks. One test involved putting lemon rind exudates and various citrus chemicals on paper strips. When they sensed a host cue, the ticks

ARS entomologist John Carroll conducts tests to show the effects a 1-hour exposure to citrus rind chemicals has on ticks.

STEPHEN AUSMUS (D2782-9)

GREG ALLEN (D2799-1)

In Gainesville, Florida, ARS chemist Uli Bernier uses an olfactometer to evaluate the efficacy of a chemical to inhibit mosquitoes from being attracted to humans.

started climbing the paper. Information was recorded on whether a tick crossed a line into chemically treated zones, continued crawling, turned around, crawled back down, or fell off the paper. On average, 9 out of 10 ticks rushed to the top of the paper strip treated with acetone—the control. Stopping, retreating, and falling off the paper indicated repellency, Carroll says.

"In another experiment, we put ticks inside filter-paper packets treated with citrus chemicals," he says. "After an hour, ticks were removed from the packets, placed on their backs, and timed to see whether they could turn themselves right side up, walk, and climb out of a low enclosure."

Some of the chemicals that had repellency also had a big effect on tick behavior, but so did some of the nonrepellent ones, Carroll says. Some ticks did not crawl out and appeared uncoordinated. Of 24 ticks exposed to 1 chemical, only 1 tick righted itself. Of more than 20 chemicals tested, only 1 killed ticks exposed to it for an hour. Several other chemicals appeared potentially useful in deterring tick attachment.

### Milling Millipedes

While some animals use citrus to ward off parasites, others roll on or rub themselves with crushed millipedes.

"Certain millipedes discharge chemicals to protect themselves," Carroll says. "If you pick up some species of millipedes, you'll notice the characteristic smell of cyanide."

Carroll and his colleagues tested the responses of lone star ticks to three benzoquinone chemicals found in millipedes and to permethrin, a commercial insecticide and repellent. Ticks were confined in filter-paper packets treated with each chemical for 1 hour.

Only one of the benzoquinone chemicals killed ticks, but it was not as toxic as permethrin, Carroll says. In the behavioral tests, all three benzoquinones inhibited righting and climbing. At higher concentrations, they impaired tick climbing for months.

"Some of the experimental methods that we used are kind of simple, but they can provide a lot of information," Carroll says. "In fact, one of the things that came out of the citrus chemical study was a much-needed method for statistically analyzing repeated behavior."

### Measuring Multiple Behaviors

Although scientists had compiled data on many different host-seeking behaviors, they needed a simple method to determine how to assess repellency.

Based on data collected on the effects of five chemicals on lone star ticks, BARC statistician Matt Kramer devised a method to collapse several tick behaviors into one score when tested with different chemicals.

"The idea is to use the behavioral differences observed as ticks are tested on different compounds to find optimal weightings of these behaviors," Kramer says. "The sum of these weighted behaviors produces a single score for each tick." These scores are the best single numbers that could be used for discriminating among the compounds, he adds.

"We knew different compounds should produce different behaviors," Kramer says. "We just didn't know which behaviors were the most important to use in the score and how much weight each should get before summing them."

A technique called "canonical discriminate analysis" tells how much to weight each measure or behavior to best separate known groups—animals tested on different compounds, Kramer says. With some minor changes, this technique was used to create the composite scores.

The new method allows scientists to determine which chemicals are most effective in tests, greatly reduces the complexity of the analysis, and provides a valuable tool for measuring animal behaviors.

"It can be applied not only to other animals, but also to plants and in many situations where you have multiple measurements or dependent variables for a single individual," Kramer says.

Behavioral studies are valuable in understanding organisms that affect the health of animals, humans, and plants. With the new scoring system, scientists can obtain more accurate data that will benefit the producers of repellents and, ultimately, the people who use them.—By **Sandra Avant,** ARS.

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