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## SCIENTISTS USE BEES AND WASPS TO SNIFF OUT THE ILLCIT AND THE DANGEROUS

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A honeybee receives a fragrant reminder of its target scent each morning and responds by sticking out its proboscis. Leroy N. Sanchez, Los Alamos National Lab

It's the ultimate way to pull off a sting: Teach a group of ordinary honeybees to ignore flowers and, instead, focus on vapors from explosives used in bombs. Then send the bees off in teams to sniff out terrorists. Or track the molecular trail of illicit drugs, or even point police to a rotting corpse.

In recent years, researchers have shown that with just a few minutes of training, undercover bees can detect the smell of TNT, methamphetamine or almost any other scent just as the bees would respond to pollen. Wasps' sniffing abilities may also be put to use finding bodies in search-and-rescue missions or helping farmers track infestations that, unchecked, could lead to crop failure or foodborne illness.

Based on these findings, scientists have begun devising an array of chemical detection devices that exploit the insects' powerful sense for scents. At Los Alamos National Laboratory in New Mexico, researchers are putting bees to work in a portable system that, like a trained police dog, could sniff out drugs and bombs at airports, border crossings, military installations and schools. A similar device, called the Wasp Hound, is under development at the University of Georgia. And a British company is working on insect-based detection systems to find explosives in luggage or minefields.

"The general premise is, if it smells, we believe we can train our bees to detect it," says chemist Robert Wingo of Los Alamos' Stealthy Insect Sensor Project.

Wingo says insects are not only cheaper to keep and quicker to train than dogs, but also can pick up scents that canines can't detect. In some cases, the bees perform better than instruments used in the lab.

While these insect abilities have long attracted interest from military and security personnel on the lookout for highly sensitive and portable devices, the concept has been slow to gain favor in the scientific community. Even though insect-based devices have performed well in laboratory settings and controlled field studies, some scientists question whether these devices can be used as reliably as other sensors.

Glen Rains of the University of Georgia's Tifton campus, who co-leads the Wasp Hound project, outlined these concerns in the June TRENDS IN BIOTECHNOLOGY. "Not only is there a laugh factor in working with insects, but biological systems are sometimes known to be unreliable," he says. "Even search dogs—which are considered the gold standard in the industry—can get tired, bored or cold."

Still, no one denies that insects have a phenomenal sense of smell. Their antennae are covered with thousands of microscopic sensors, allowing them to pick up the faintest odors. Bees, wasps and even moths can learn and remember a wide range of target odors, making them ideal for use in chemical detection systems.

Now several laboratories are stepping up efforts to test insect devices in real-world conditions. Scientists say the studies will provide empirical evidence needed to make the devices more widely accepted as biological sensors. If all goes well, commercial insect sniffing devices may become available within a year.

### **Buzz bombs and stinger missiles**

Military uses for honeybees and other insects date back to ancient times. The Romans used catapults with beehives as projectiles, to unleash the fury of angry bees on the enemy. During World War I, beehives were rigged to topple with trip wires to thwart an approaching enemy.

More recent studies on honeybees and other foraging insects show these small, winged creatures possess other traits that can sting enemy agents. For example, despite their tiny brains, honeybees are quite intelligent and can be easily trained using classical conditioning techniques. Just like Pavlov's dogs, which learned to associate a ringing bell with dinner, bees and wasps can be trained to associate a smell—vapors, say, of a liquid explosive or decaying corpse—with a sugary treat.

In the early 1990s, the U.S. military began studying ways to use free-flying bees to help search for hidden explosives. The idea was to train the bees to prefer the scent of a particular explosive, and then set them free in the hope that they would hover over any nearby threats.

"The problem was, it's hard to track a bee whizzing by at 15 miles per hour," Wingo says. Never mind tracking a large group. "Technologically, it's an extraordinary challenge. Number two: How do you prove the associative conditioning?"

Through Pavlovian conditioning, wasps can learn to associate odors with food. These wasps display food-searching behavior when presented with the odor alone. Rains et al.

Enter Tim Haarmann, an entomologist working at Los Alamos at the time. With Wingo and microbiologist Kirsten Taylor-McCabe, also of Los Alamos, Haarmann found a more practical way to harness honeybees' scent-abilities by restraining and individually training bees in a box. The team then combined insect behavior and

technology to track the bees' responses. The result: a portable device with a team of five bees as its sensor.

The bee bomb detector is about half the size of a shoe box and weighs roughly two kilograms (four pounds). From the outside, it looks like a plain box with a few air holes. Inside, lined up in a row and strapped into strawlike tubes, bees are exposed to puffs of air as a video camera monitors their reactions. The camera is tied to pattern-recognition software that signals when a bee responds to a scent.

### **A, Bee, C's of detection training**

Though bees can't bark when they encounter a target scent, they do have a way of communicating that the camera can catch. It turns out that a hungry bee will stick out its tongue in anticipation of a meal. Bees will also stick out their tongues when a drop of sugar water is touched to their antennae. Pair the sugar drop with the scent of TNT or C-4 plastic explosives half a dozen times, and the bees will extend their tongues at a whiff of the explosives alone. This response, called the proboscis extension reflex, can assess the bees' reaction to a particular scent.

"A honeybee will not stick out its tongue for any other reason than to eat. So we can train bees to associate food with a particular scent," Taylor-McCabe says. "It's an unambiguous signal that the honeybee gives us to indicate yes or no."

Wingo and Taylor-McCabe are using this approach to train forager bees to detect a wide range of compounds, including methamphetamine and cocaine. The honeybees can even detect triacetone triperoxide, or TATP, an explosive that canines often have trouble detecting. TATP was the detonator carried by the "shoe bomber" in his attempt to destroy a commercial aircraft with plastic explosives in his shoe.

Bees can be trained to detect multiple scents and taught to pick up a single scent from a bouquet. The bees can also pick up the scant molecular trail of vapors too faint to be detected by lab instruments. In trials at Los Alamos, the bee detectives performed better at detecting minute traces of explosives than ion mobility spectrometers, which are used to swipe luggage and clothing in airports.

"We haven't quantified exactly how low their threshold is, but the bees are able to detect the explosives at concentrations below that stated of the detection instruments in our lab, and that's generally in the low parts per trillion," Wingo says.

A British firm, Inscintinel Ltd., is developing a bee-based detection device that relies on a team of 36 bees. Mathilde Briens, research and development manager, says the company is investigating ways to pack twice that many bees in a single unit, allowing them to screen up to a dozen chemicals at once.

"It's mainly an engineering issue," she says. "We need to make sure all the bees are exposed to the scent and find ways to manage all the bees so we know when they are responding."

### **Dances with wasps**

While wasps don't stick out their tongues in response to a scent, they do communicate with each other—through dance. The University of Georgia's Rains and his collaborators have developed a small, portable odor detector that relies on the body movements of tiny black wasps called *MICROPLITIS CROCEIPES* to sense odorants. Also known as parasitic wasps, these social bugs use their keen sense of smell to seek out meals and find a host in which to implant their offspring. If their efforts prove successful, they signal the news to peers through a series of carefully choreographed movements—with different dances to signal food versus host.

Rains' group is exploiting the wide range of such movements to build biological sensors with wasps capable of detecting more than one odor. For example, a wasp trained to associate a specific odor with a food reward will press its antennae down onto the source of an odor. If scientists present a different target odor while the wasp is stinging its host, the wasp will display coiling behavior, rearing up on its hind legs and bending its antennae the next time it encounters that scent.

Similar to the bee-based detector, the Wasp Hound houses a team of trained wasps in a handheld, ventilated cartridge. At one end of the cartridge, a small fan draws outside air through a hole. If the wasps don't recognize an incoming odor, they continue flying about. If they do recognize the scent, they cluster around the opening, where a miniature video camera records their movements and sends images to a laptop for analysis.

In an early field trial designed to compare the detection limits of the Wasp Hound to an "electronic nose," the insect detector proved to be 74 times more sensitive to fungi than the mechanical device, and 94 times more sensitive to plant odors. That study appeared in *TRANSACTIONS OF THE ASAE* in 2004.

Rains and his collaborators are now working to make the device even more sensitive. Don Kulasiri of Lincoln University in Christchurch, New Zealand, is developing mathematical models that will enable him and his peers to better understand and interpret insect responses.

By analyzing the wasps' responses to chemical stimuli at different concentrations and tracking any resulting changes in their behavior, the scientists aim to develop a device that not only detects a specified chemical but also can accurately measure its concentration.

"What that holds for us is potentially developing a device that's not just yes-or-no but is concentration-specific to some level," says Rains, who along with his colleagues, reviews the efforts in the August *ENTOMOLOGIA EXPERIMENTALIS ET APPLICATA*.

Ideally, the scientists say, the device could be carried into farm fields and grain stores to check for contaminants and disease. The group is now using a prototype Wasp Hound to detect aflatoxin, a toxin produced by a fungus that grows on peanuts, corn and other plants. Trials suggest the device may provide a better way of detecting the toxin before crops enter the food supply, Rains says.

“Current detection methods rely on just a subsample of a large quantity of material, so there’s a possibility of missing it when it’s there,” he says. The group is also investigating ways to detect infestations of E. COLI, salmonella and other food contaminants. The Wasp Hound may also be used for security and forensics, and has the potential to detect volatile compounds in human breath associated with diseases such as cancer and tuberculosis, Rains says.

### **Giving it the sniff test**

Though insect sensing systems have performed well in laboratory settings and controlled field studies, the devices have yet to prove themselves to be reliable in real-world applications.

This summer, scientists began putting the devices to the test. In a field trial in July, the Wasp Hound went nose-to-nose with a team of five nationally certified human rescue dogs to detect soil contaminated with the scent of human remains. The results will be presented in February at a meeting of the American Academy of Forensic Sciences.

This fall, the bee bomb-detection device is being used in a blind test by a small-town police force to sniff out explosives during a training exercise, and will be done in concert with a canine team.

Jeffery Tomberlin, an entomologist at Texas A&M University in College Station, is working with Rains to carry out the field trials with rescue dogs. He says using insects as biological detectors offers several advantages over dogs. For example, insects can be used to detect chemicals in situations such as arson, where toxic fumes may pose a danger to the dog or its handler. Living-insect sensors can also be put in the hands of someone who is not trained to work with dogs.

“Another advantage is that you don’t have to worry about the wasp trying to provide a response simply because it wants to please its owner,” he says. Still, not all scientists are convinced that the insect sniffing devices will fly. University of Hawaii biologist M.E. “Jeff” Bitterman, a pioneer in the field of honeybee learning, says insects such as bees are constantly picking up on new chemical cues in the environment. Even when trained to only one scent, the bees will generalize and begin to respond to other similar scents, he says.

“In order to get a bee to respond only to the odor you’re interested in, you have to do what’s called differential reinforcement, which means you present some other odor without sucrose until the animal responds differentially, and that may take several trials,” Bitterman says.

Moving to the real world presents other obstacles, Bitterman notes. "It is one thing to assert that a forager from an established hive can detect explosives in a dish under standard field conditions but quite another to decide how to use that ability in screening the contents of a shipping container at a pier or on a highway."

Still, scientists working to build the insect devices say these obstacles can be overcome. Wingo and his group reinforce their bees' learning every day with a "breakfast boost," providing the scent of interest with the bee's morning meal.

Despite the repeated training sessions, and the occasional sting, Taylor-McCabe says the effort is worth it.

"Bees are wonderful insects for detection devices," she says. "They give us an unambiguous answer, and they work until the minute they die."