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**BIOLOGICAL SYSTEMS (PAPER II)**

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**OVERVIEW**

Honeybees offer the potential of using free-flying organisms to search wide areas for the presence of explosives, unexploded ordnance (UXO), and landmines. The use of bees is analogous to dogs for mine clearance, except that a colony of tens of thousands of bees can be trained in about one hour to fly over and search a field for explosives, does not require a leash, and will not set off any mines. Like dogs, bees can be trained to search for either the odors of individual explosives or suites of these chemicals.

Initial tests indicate that bees are capable of detecting these odors at concentrations below those detectable by most instruments. Bees approach, if not match, the odor sensitivity of dogs, i.e., low ppt to ppq, and possibly lower. Field trials with honeybees at ppb and ppt vapor concentrations of 2,4-DNT (a residue in military-grade TNT) showed a detection probability of 97–99 percent. We calculated a 1.0–2.5 percent probability of false positive, and less than 1 percent probability of false negative, based on three different statistical sampling strategies [1,2]. Calculated receiver operating characteristic (ROC) curves for 10 ppb through 0.001 ppb indicated that for doses higher than 0.01 ppb (10 ppt), the bee system behaves like a very fine-tuned, nearly ideal, detector. In addition, bees have mop-like, electrostatically charged hairs that enable them to bring back to their colonies samples of explosive chemicals as well as biological agents and other harmful materials [3,4,5].

## **PHYSICAL PRINCIPLES AND MINE FEATURES EXPLOITED FOR AREA REDUCTION**

Like dogs, the use of bees exploits the ability of an organism to detect landmine chemical signatures at very low concentrations. Unlike most instruments, bees and dogs can be trained to search for either specific chemicals or suites of chemicals. However, dogs are usually kept on a leash, and most chemical-sensing instruments have to be carried across minefields. Because free-flying bees search outward from their hives and then return to their hives, fairly large areas can be searched and samples recovered without risk to humans. In addition, bees continually cross and recross forage areas, ranging as far as 1–2 km. Consequently, bees are likely to encounter explosive vapor plumes multiple times, amplifying low concentrations. In other words, the bees autonomously and intensively search each region.

## **STATE OF DEVELOPMENT OF BEE LANDMINE FINDER**

Under the leadership of the University of Montana (UM), a consortium of Defense Advanced Research Projects Agency (DARPA)–sponsored investigators has been exploring ways to leverage the natural foraging behavior of honeybees for military applications, including detection and localization of explosive materials and landmines.

Over the past three decades, demonstrations by UM at U.S. Environmental Protection Agency Superfund sites, Department of Energy reservations, and Department of Defense installations have shown that honeybees effectively gather chemical information over ranges extending several kilometers. Honeybees have been used to monitor trace elements and heavy metals, volatile and semivolatile organic chemicals, radioactive materials, and (more recently) explosives, in both the United States and Europe. As honeybees collect water and forage for nectar and pollen, the electrostatic charge on their body attracts dust, pollen, soil, and other particles, including chemicals leaking from explosive devices into soil and water. These materials are then returned to the hive. These capabilities are summarized in a newly released book entitled *Honey Bees: Estimating the Environmental Impact of Chemicals* [6].

The U.S. Army Center for Environmental Health Research contracted with UM to complete a seven-year series of applications of honey-

bees for surveying and monitoring industrial and militarily unique chemicals at Aberdeen Proving Ground (APG), Md. These investigations pioneered examination of volatile and semivolatile chemicals (including explosives) in the air inside beehives and developed electronic hives capable of continuously monitoring colony dynamics and of controlling chemical sampling equipment associated with the hives. The objective was to trigger a sampling event when critical bee performance traits displayed a significant change, such as altered forager flights, reduced numbers of bees returning to the hive, or a breakdown of the complex cooperative behaviors, such as maintaining constant temperature in the brood nest. Also, all this information was ported from remote field sites via communication links to a central facility in Montana.

A DARPA program that began in 1999 brought together a team of scientists from UM, Sandia National Laboratories (SNL), Oak Ridge National Laboratories (ORNL), and Southwest Research Institute (SwRI) to focus on developing bees for use in detecting chemical and biological warfare agents as well as landmines. Partners at the Air Force Research Laboratory (AFRL) and the Air Force Force Protection Laboratory joined the research team in 2000.

As a result of work by UM, SNL, and ORNL, accompanied by field trials at APG, honeybees, pollen, wax, honey, and the air inside the hive can now be analyzed for explosives. Analytical technologies including Solid Phase Microextraction fibers (SNL), and a new generation of sorbent sol-gels (ORNL), combined with gas chromatography and mass spectrometry have been field validated for detection of explosives. Tested compounds include nitroglycerine (NG); diethyleneglycoldinitrate (DEGN); 2,6-DNT; 2,4-DNT; TNT; RDX; and pentaerynitoltetranitrate (PETN) in bees and their hives [7,8].

### **BEES TRAINED TO FIND TRACE LEVELS OF EXPLOSIVES**

In addition to this passive collection of materials by untrained bees, more recent field trials have shown that honeybees can also be trained to actively search for and detect explosive materials. The training technologies were developed by UM. During field trials at SwRI in 2001, sand-explosive mixtures on the ground provided point-source targets and generated plume vapor concentrations of 2,4-DNT in the 0.7–13.0 ppb range. Honeybee flights over blanks,

sand controls, and the targets were continuously monitored with video cameras and analyzed off line. Using detection algorithms designed for radar applications (AFRL), honeybees were shown to locate the DNT within minutes of when the colony began to forage. The false positive rate was 2.5 percent, while the false negative rate was less than 1 percent. Concurrent statistical data analysis of cumulative counts of bees over blanks, controls, and targets by UM and SwRI yielded a detection probability of 98.7 percent, with less than 1 percent false positive and 1 percent false negative results. Continuing 2002 field trials have pushed thresholds of detection an order of magnitude lower, with bees reliably detecting targets in the low ppt range and well within the range of measured vapor concentrations above active landmine fields. *An informal comparison at SwRI of the Nomadics Fido sensor, under identical field conditions showed equal performance by Fido and by bees in detecting 2,4-DNT vapor plumes—with the threshold of detection virtually the same for the instrument and for the bees. Bees correctly located and identified DNT targets in an area 200 m across in less than one hour with no need for a person to enter the test area. For Fido, the operator had to be led to the target so that it could be “sniffed.” Searching the 200-m-diameter test area for a small DNT target would have required hours or days.*

This initial bee project, which began as an unproven concept, has in three years developed into a system for which preliminary performance capabilities and operating characteristics can be quantified. Overall, the costs of the combined bee testing have averaged about \$1 million per year. This includes laboratory assessment of odor detection, greenhouse flight experiments, development of training protocols for bees at a one-acre tent and under open field conditions at SwRI, and improved analytical methods and devices for measuring explosive residues and vapors in bee systems.

Because of the encouraging results from recent field trials, UM proposed additional technology demonstrations to DARPA for spring 2002. These included detection of UXO at weapon ranges, tracking of honeybees with Lidar or digital imagery, and detection of buried explosives and actual landmines at landmine test beds.

A primary goal is to determine minimum detection limits for honeybees, comparing those to minimum detection levels exhibited by trained dog teams. If successful, honeybees would provide an eco-

nomical and effective method for wide area monitoring and localization of UXO and landmines. *As mentioned, preliminary results from the new series of audited field trials at SwRI have shown that bees can easily locate, in less than an hour, a 2,4-DNT target placed approximately 100 m from the hive. The targets emitted trace vapor plumes measured in the low ppt range.*

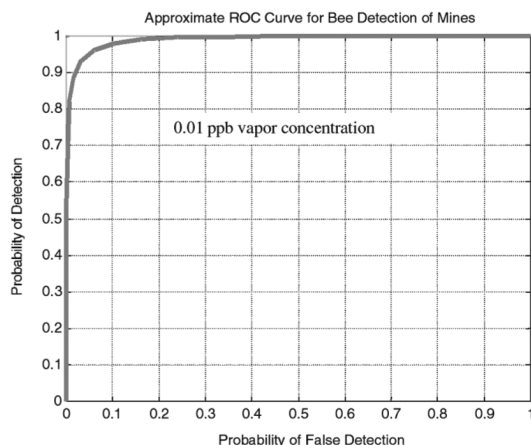
### **CURRENT CAPABILITIES AND OPERATING CHARACTERISTICS**

At UM, using a benchtop bioassay called the Proboscis Extension Response System, individual bees were trained to discriminate between a small puff of air containing no scent and a puff containing an explosive's odor. The bee proved to be able to reliably detect (p less than 0.001) the presence of 2,4-DNT at 20 ppb. Experiments conducted by ORNL revealed that surface chemistry interactions in glass tubes of the dose-delivery system—not the bees' sensitivity to odor—limited the level to which the bees could respond. As such, the minimum detection limits of bees could not be established using this system. Follow-up studies with free-flying bees in a greenhouse again showed that bees could detect a 10-percent mixture of DNT in soil and would search for and hover over DNT-spiked targets. The bees' ability to discriminate between blanks and DNT was almost perfect (p less than 0.001).

In summer 2002, UM researchers supported by SwRI scientists completed a series of double-blind, fully audited trials. UM trained the bees and SNL explosives experts worked with SwRI chemists to estimate and then measure the dose delivered at each target. UM and SwRI statisticians analyzed the cumulative count data, while AFRL digitized and subsampled the video data and then calculated the probability of detection (PD) and approximate ROC.

The ROC curve shown in Figure S.1 is the relationship between the PD by the receiver when the target or agent is present and the probability that the device or receiver will register the target or agent present, when it is in fact absent [9]. In biological terms, the ROC curve relates the probability of a true positive to the probability of a false positive. The ROC curve presented is for honeybees trained to

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**Figure S.1—ROC Curve for Honeybees Trained to Associate the Smell of DNT with Food**

associate the smell of DNT (the most common signature of explosives in landmines) with food. The diagram plots 0.01 ppb lower level in the vapor plume above DNT placed under dry sand in petri dishes. The ROC curves for these concentrations are very near 1.0 and 0, suggesting that at these vapor concentrations, honeybees perform as virtually an ideal detector.

Bees had little difficulty detecting 0.7–13.0 ppb DNT vapor in the controlled experiment at SwRI in 2001. The 2001 levels of DNT are likely to be higher than those found above most buried landmines, but the experiment was designed to characterize the potential use of bees for landmine or explosive detection and not to simulate use of honeybees in mine removal operations. Subsequent trials in Montana reduced the amount of DNT to 0.5 g of explosive in 300 g of sand, with an estimated vapor concentration from 50–70 ppt. There was little change in the bees' ability to detect the target out to 100 m from the hive (DNT detection,  $p$  less than 0.0001 at an observed power of 1). The curve in the ROC diagram above is calculated from these Montana trials. Although these results represent two-hour trial

periods, data subsampling showed high statistical significance from as little as four minutes of data. The audited trials of low DNT concentrations recently concluded at SwRI confirm our findings in Montana. Targets containing 0.1, 0.5, and 1.0 g of DNT were evaluated, and plume concentrations from 20 to 300 ppt were measured. Bees consistently detected targets generating 50–80 ppt vapor and under moist conditions detected plumes down to 30 ppt.

The experimental conditions for the SwRI trials were optimized for good bee performance. Ground cover was limited and wind conditions were mild. Terrain was flat, and there were few or no flowering plants to divert the attention of the bees from the target. Operating temperature was high, thereby increasing vapor pressure above the target. However, the sand and DNT were dry—conditions that tend to decrease probability of detection by dogs and many instruments. In Montana, the temperatures were cooler, and the vapor pressure was much lower. Based on actual plume measurements combined with models of the effects of wind, DNT concentrations were estimated by Sandia explosives experts to be in the low ppt, and possibly ppq, depending on how far from the target the bees were detecting the leading edge of the vapor plume.

The ROC calculations should not be interpreted to mean that honeybees are perfect detectors of landmines in real-world conditions. However, these structured and audited field trials demonstrated significant detection capability.

## LIMITATIONS

Bee detection trials using real landmines have yet to be performed. Bees do not fly at night, in heavy rain, or in cold weather (below 40°F). The effects of such environmental conditions as dry versus wet soil, open areas versus dense forests, different climatic conditions, and interference or complicating factors (e.g., multiple targets, mine fragments, exploded ordinance, chemical smokes) have not been examined. Bees perform well in open fields under hot and dry conditions. Whether they would perform as well in dense forests is unknown. The principal factors limiting performance are lack of knowledge about the search patterns of bees in different settings and the inability to track bee movements.

### **POTENTIAL FOR IMPROVEMENT (TWO TO SEVEN YEARS)**

Many of the aforementioned complicating factors can be tested and evaluated within two years. Practical experience and testing at real minefields is essential. Conditioning protocols need to be fine tuned to accomplish signal amplification (i.e., more bees over a target—estimated one-year effort). However, for use in demining applications, a tracking system that can locate and follow bees, either by their own chemical signatures, explosives adsorbed onto the bees, or some form of an inexpensive marker, such as a reflective powder, is needed. Systems to be tested range from high-resolution cameras that could be flown over the site or to those used to look down from satellites to Lidar, which could discriminate bees by chemical signatures 1–2 km away from their hive. The tracking problem should be solvable in two to three years, maybe five at the most. Assuming that bees can detect real landmines without a serious loss in the PD and in the ROCs, it is conceivable that the bee system might provide a near ideal detector. Combined with an imaging or tracking system capable of discriminating bees over targets (e.g., landmines) and the software to automate the process, a Lidar or camera system combined with bees, in the best of conditions, should be able to survey a 1–2 sq km area within a few hours.

There may be utility in also developing a decidedly low-technology counterpart. In theory, it should be feasible to teach local beekeepers to train their own bee colonies to survey fields as part of an area reduction program for the price of some syrup, some training standards, a ladder, and a pair of binoculars. Even this crude setup could track and count bees at distances equal to or farther than the leash currently used on minefield dogs.

### **SENSIBLE RESEARCH AND DEVELOPMENT PROGRAM LEADING TO A FIELD-USABLE DEMINING SYSTEM WITHIN 24 MONTHS**

Surveying areas by examining chemical residues brought back to the hive by bees is based on 30 years of proven technology. In less than four years, bees have been shown to be trainable and to detect explosives vapors at very low concentrations with a very high probability of detection and a very low probability of false negatives. Developing



this system into a usable tool for landmine detection, especially area reduction, should be possible within two to five years at a cost of \$1 million per year, based on previous research.

Critical research needs include

- Examining the effects of environmental factors, multiple targets, and interferences on odor discrimination and PD with respect to wide area search. Anecdotal evidence from the 2002 SwRI trials indicate that simply wetting the soil may dramatically improve bee detection of minute amounts of explosives. Also, bees can be kept continually conditioned and able to find trace amounts of explosives for more than 36 days, despite changing climatic conditions ranging from a drought to a 500-year flood.
- Determining the limits of detection (Are they really as good as the dog, or are they just better than most instruments?):
  - Test under controlled conditions (e.g., dry soil versus wet soil).
  - Test at real minefields in different parts of the world.
  - Compare competing technologies, such as Nomadics' Fido (informal trials, 2002 demonstrated similar detection thresholds for Fido and bees).
- Defining how bees search and their movement patterns in different habitats, settings, and climates:
  - Use Lidar, radar, video imaging, or taggants and pigments to establish foraging patterns and bee densities.
  - Assess residue/breakdown chemistry of explosives and chemical agents for other promising training odors.
  - Optimize/enhance at-the-hive analytical protocols for quick identification of agents in the area to be searched.
- Developing improved methods for tracking/spotting of bees as they search and when they are over targets (e.g., landmines), including
  - Technology improvements for the military to provide order of magnitude improvements in the speed at which antipersonnel mines can be cleared (e.g., Lidar or high-resolution cameras mounted on aircraft or satellites).

—Low-cost approach enabling local beekeepers to help effect area reduction (e.g., bees, binoculars, ladders). On average, there is a colony of bees per every 0.5 km around the world.

Finally, the potential use of bees for humanitarian demining has begun to result in inquiries from demining centers and companies situated around the world. To date, no other system offers the potential of standoff detection and surveying of areas ranging from 100 m to 1 km or more across. All lines of evidence indicate that bees should be able to find real landmines (or at least, those that leak trace amounts of explosives). The missing aspects are experience on real minefields, so that the system can be optimized and thoroughly calibrated, and a tracking system, such as a portable Lidar, that would provide standoff tracking of bees so the minefields could be mapped.

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