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Detection of Methyl Salicylate Transported by Honeybees (Apis mellifera) Using Solid Phase Microextraction (SPME) Fibers

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Detection of Methyl Salicylate Transported by Honeybees (Apis mellifera) Using Solid Phase Microextraction (SPME) Fibers

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Abstract

The ultimate goal of many environmental measurements is to determine the risk posed to humans or ecosystems by various contaminants. Conventional environmental monitoring typically requires extensive sampling grids covering several media including air, water, soil and vegetation. A far more efficient, innovative and inexpensive tactic has been found using honeybees as sampling mechanisms. Members from a single bee colony forage over large areas ($\approx 2 \times 10^6$ m^2), making tens of thousands of trips per day, and return to a fixed location where sampling can be conveniently conducted. The bees are in direct contact with the air, water, soil and vegetation where they encounter and collect any contaminants that are present in gaseous, liquid and particulate form. The monitoring of honeybees when they return to the hive provides a rapid method to assess chemical distributions and impacts (1).

The primary goal of this technology is to evaluate the efficiency of the transport mechanism (honeybees) to the hive using preconcentrators to collect samples. Once the extent and nature of the contaminant exposure has been characterized, resources can be distributed and environmental monitoring designs efficiently directed to the most appropriate locations. Methyl salicylate, a chemical agent surrogate was used as the target compound in this study.

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Introduction

The Defense Advanced Research Projects Agency (DARPA) has recognized that biological or chemical toxins are a real and growing threat to troops, civilians and the ecosystem. Sandia's Explosive Component Facility (ECF) personnel have been involved in numerous projects aimed at detecting land mines, unexploded ordnance and chemical warfare agents by characterizing and modeling their degradation in the environment. Recently the ECF has been supporting the Controlled Biological and Biomimetric Systems (CBBS) program of DARPA, Defense Sciences Office, by evaluating the feasibility of using honeybees from environmental sampling of biological and chemical "agents of harm".

Long-term sampling and subsequent analysis demonstrated that using honeybees as sample collectors to transport contaminants to the hive for detection is a viable field analytical methodology, especially in areas where physical access is limited (2). This report describes the possibility of using honeybees as near real-time collectors for immediate analysis. A single colony of honeybees forage over large areas ($\approx 2 \times 10^6$ m^2), making tens of thousands of foraging trips per day, returning to a fixed location where sampling can be conveniently conducted. They are in direct contact with most environmental media (air, water, soil and vegetation) and encounter contaminants in gaseous, liquid and particulate form. Multiple hives can be used to map the distribution and concentration of the target compounds. Research to develop novel methods of measuring these threats is aimed at early warning, monitoring and remediation. Methyl salicylate is a standard reference used as a surrogate for chemical warfare (CW) agents. This paper describes results that show the success of using of solid phase microextraction (SPME) fibers in preconcentrating a CW simulant collected by bees and transported back to the hive. SPME fibers used for this study are commercially available and are designed to capture chemical vapors at a remote or field site. For this study, the fiber is transported to a laboratory for analysis, although fieldable sensors can be used.

Experimental Details

A sugar-water feeder doped with methyl salicylate, also known as oil of wintergreen (WG), was placed on a platform 20 feet from the entrance of the hive. The concentrations of WG used were 100, 50, 25, 12.5, 6, 3 and 1.5 parts per million (ppm). Background samples were collected from inside and outside the hive each day before experimentation began. Control samples of ambient air were taken during experimentation to ensure that sampling equipment was not contaminated (3). Samples were collected by placing the SPME fibers in a groove along the doorway of the hive. The SPME fibers were protected from breakage by mounting them in a protective sampling cover. (Figure 1) The bees were forced to walk across the sample holder as they entered the hive (Figure 2). A pumping station was used to draw air (1.3 liters per minute) across the SPME fiber as the bees crossed the sampler (Figure 3).

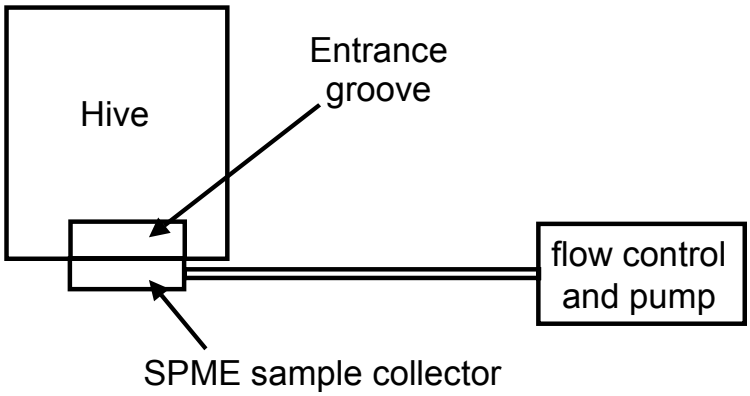

Figure 1: Schematic of sampling arrangement.

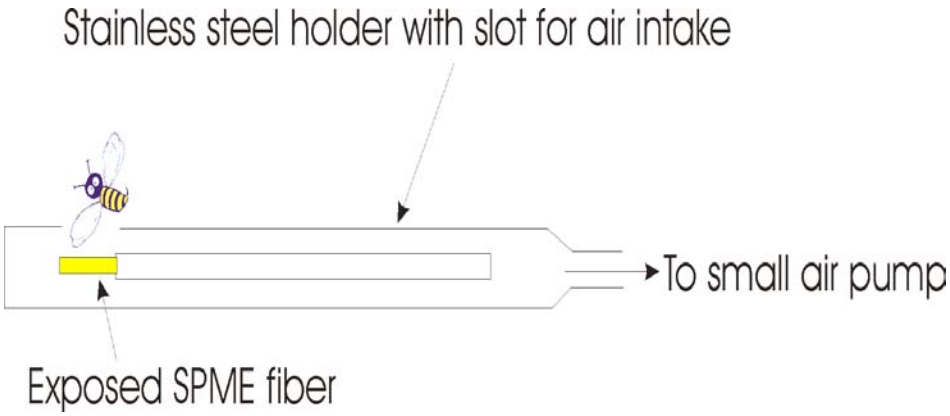

Figure 2: Sample holder used for SPME sampling in entrance of hive.

"Dart" containing SPME fiber

Dart installed on hive

Figure 3: Photos of test configuration.

The SPME fiber was then transported to the laboratory where a chemical analysis was performed using a gas chromatograph with a mass spectrometer detector. Each concentration of WG solution was sampled on different days, and several of the concentrations were sampled repeatedly to provide a measure of reproducibility.

The samples were analyzed using a ThermoQuest GCQ gas chromatograph / mass spectrometer (GC/MS) equipped with a 25 meter DB-35 column. GC/MS parameters for SPME analysis were as follows:

injector temperature 225°C;
split/splitless mode (split vent off initially);
split rate 80 cc/minute;
open split vent at 0.75 minutes;
data were acquired using a full scan mode to allow target verification of the compound identity.

Calibration of methyl salicylate was performed by making 1μL injections of methyl salicylate solutions into a split/splitless inlet of a GC. The MS is subsequently eluted from the column and detected using an ion trap mass spectrometer detector. These concentrations of methyl salicylate from the 1μL injections translate directly to nanograms of MS arriving at the detector. The mass reaching the detector is directly proportional to the output voltage of the electron multiplier. The area and/or height of this signal was plotted against the actual concentration of the methyl salicylate to establish a calibration curve (Figure 4).

Results and Discussion

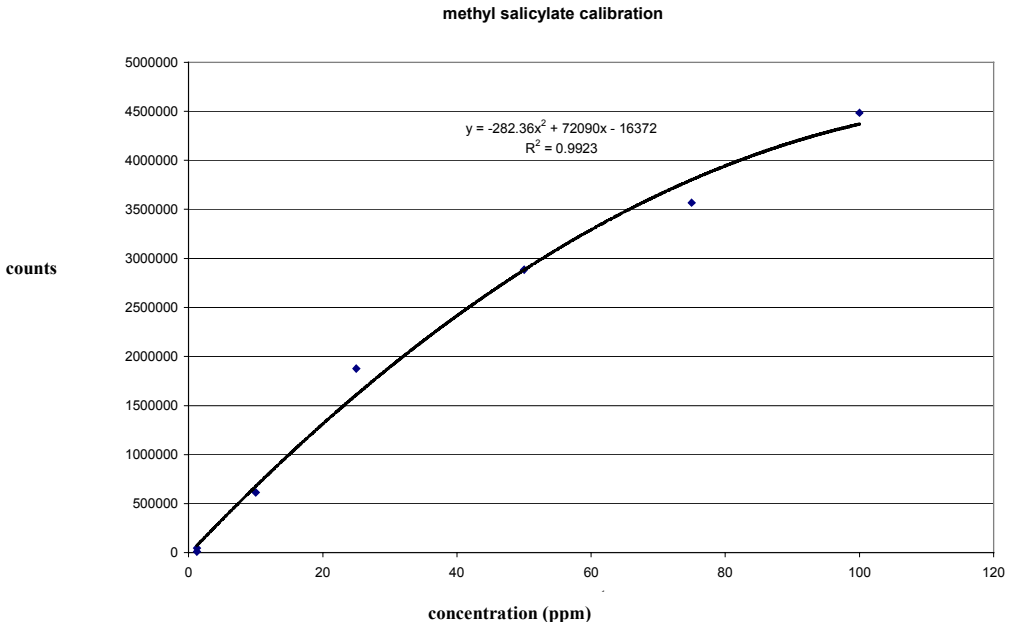

Figure 4: Calibration Curve Methylsalicylate.

A representative chromatogram obtained from a SPME sampling event is shown in Figure 5. In this case, a feeder was contaminated with 1.57 parts per million methyl salicylate and 1.5 parts per million ethyl salicylate. The sample collection time was one hour. The reconstructed ion chromatogram m/z 120 shows three distinct peaks: the methyl salicylate and ethyl salicylate peaks, and a naphthalene derivative. This naphthalene compound was present in all hives sampled, including control hives, and appears to be a naturally occurring product. Its concentration is proportional to the sampling time and flow rates used.

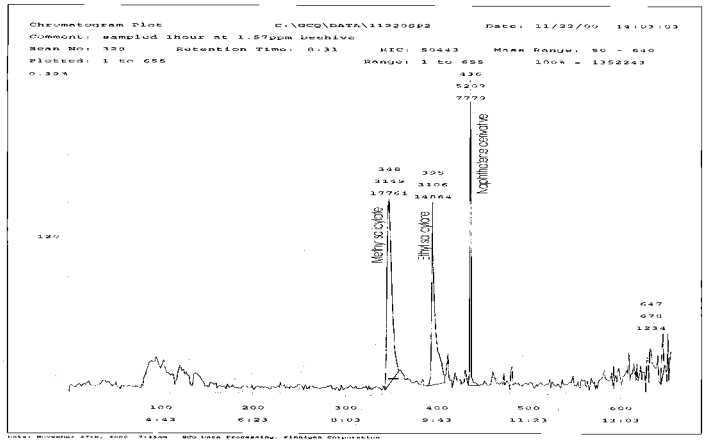

Figure 5: Chromatogram from SPME sampling of hive.

In this experiment, it should be noted that much of the methyl salicylate solution brought back to the hive had been consumed by the bees and was therefore carried internally. This material was not available for collection or analysis. Only trace amounts residing on the bee's external body surfaces were available for sampling. This will tend to bias the collection efficiency towards lower values. However, based on the total amount of methyl salicylate consumed by the insects compared to the amount collected on the SPME fiber, the sampling efficiency of this system is approximately 0.07%. The bulk of the target compound that was consumed will be carried into the hive and regurgitated. Sampling of the hive interior will likely provide a higher sampling efficiency. An improved collector for the hive entrance has been designed to increase collection efficiency, and will be tested upon resumption of insect flight activity.

Conclusions

It has been demonstrated that SPME fibers can be used to collect environmental contaminants as they are brought back to a beehive by foragers. The calculated efficiency of the existing system is approximately 0.07%. Based on a signal to noise ratio of 10:1, it is estimated that contaminants present at the 2 ppm level could be detected within 6 minutes. By attaching a simple autosampler to the SPME system and utilizing a low-cost fieldable sensor, a system capable of rapid, on-site monitoring could be developed.

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