P1.2 HONEYBEES SCAVENGE AIRBORNE BACTERIA FROM THE ATMOSPHERE

Bruce Lighthart[1,2]*, Kevin Prier[1,2], Gerald Loper[3], Jerry Bromenshenk[2]
[1]Microbial Aerosol Research Laboratory LLC, 10975 Doll Rd., Monmouth, OR 97361
[2]Division of Biological Sciences, University of Montana, Missoula, Montana 59812
[3]USDA, Agriculture Research Service, Carl Hayden Bee Center, Tucson, AZ 85719

1. INTRODUCTION

Honeybees (***Apis mellifera***) are electrostatically charged during flight (Erikson, 1975; Yes'kov & Sapozhnikov, 1976) This charge is useful for the bee in collecting pollen (Corbet ***et al.***, 1982), as the charge on the bee attracts particles of opposite charge. It is our hypothesis that a flying bee will tend to collect any oppositely charged particulate material that they fly through, including aerosolized bacterial spores.

We have therefore been studying whether honeybees can be used to collect measurable quantities of bacterial spores from the air through which they fly. The purpose of this research is to investigate the possibility of using honeybees to detect the presence of biowarfare/bioterrorism agents or to detect the presence of plant pathogens before the appearance of disease symptoms.

2. METHODS

We have designed a wind tunnel system in which we can tether a single bee and measure its electrostatic charge as it flies. In addition to the charge on the bee, we measure the temperature, wind speed, relative humidity, and concentration of airborne particles in the system. Controllable parameters in the wind tunnel are wind speed, temperature, and the timing and duration of exposure to a continuously generated bacterial spore aerosol (***Bacillus subtilus*** var. ***niger*** (BG)). A typical experiment is at a temperature of 22ºC, a wind speed of 9.5–10km/hr, and 3 pulses of aerosol of 45 seconds each. After exposure to the aerosol, the bee is removed from the wind tunnel and sonicated in two rinses of 7ml sterile deionized water. The rinse water is heated to 70ºC for 10 minutes to kill any vegetative bacteria and then filtered. The filter is placed spore side up on a nutrient agar plate and incubated at 30ºC overnight. The number of colonies on the filter is then counted and compared to the particle count from the wind tunnel to determine what percentage of the bacterial spore exposure adhered to the bee. All wind tunnel measurements and controls are controlled through a computer using LabVIEW.

* ***Corresponding author address***: Bruce Lighthart, Microbial Aerosol Research Laboratory LLC, 10975 Doll Rd., Monmouth, OR 97361.
e-mail: lighthab@open.org

3. RESULTS

3.1 *Bees adsorb bacterial spores in proportion to their static charge*

We have discovered that aerosolized bacterial spores are adsorbed onto flying bees and that the percentage of the spore exposure dose adsorbed is proportional to the electrostatic charge on the bee. We hypothesize that the static charge produces an attractant field around the bee and that the greater the static charge, the larger the attractant sphere around the bee, and therefore the bee has a larger effective scavenging volume as it flies (Lighthart ***et al.***, 2000).

We have also discovered an as yet unidentified seasonal effect on the bees' ability to adsorb bacterial spores from the air, independent of the charge on the bee. "Winter" bees were taken from inside the hive, whereas "Summer" bees were caught as they left the hive. One contributing factor may be the age of the bees, as wintering bees tended to be older than summer foraging bees and had fewer hairs for spores to get "caught" in.

3.2 *A model for adsorption of aerosolized bacterial spores in the field*

The foregoing discoveries, along with the need to predict the amount a bee will adsorb in field tests, led us to develop a model to predict the amount of spores that can be collected from bees that have flown through a continuously generated plume of aerosolized bacterial spores originating from a point source. In field tests, the bees will be trained to fly across the aerosol plume to a feeder target and back to the hive. The model uses a normal distribution model (Pasquill) for the cloud particulate distribution, including a settling factor for the particles and adjusting for the bees flying perpendicular to the wind (thus increasing their flight path length). An additional factor calculates how many of the particles in the plume will adhere to the bee during each meter of flight, based on laboratory observations. The formula is integrated over the flight path, crossing the plume twice (once on the way to the target and once on the way back). It is further assumed that a certain percentage of the particles adsorbed onto the bee will be desorbed during each meter of flight, and this is included in the calculation.

3.3 *Collection of spores from bees returning to the hive*

The difficulty in assaying bacteria in the field is the recovery of spores from the bees as they return to the hive. The most efficient method would be to capture all returning bees and rinse them as a batch and then culture the rinse water as in the laboratory experiments. The obvious disadvantage of this approach is the death of all the returning bees. This may be acceptable in an emergency scenario where maximum recovery/sensitivity is necessary, but it would be impractical for hives used as long term monitoring stations.

We have designed a non-invasive method of collecting spores from returning bees by modifying a standard under-hive pollen trap. Returning bees must work through two mesh screens to enter the hive. The screens knock off pollen balls and other particles as the bees go through. A cyclone sampler is hooked up to the back of the hive and air is being continuously pulled through the bottom of the hive. The small spore particles will be caught in the air flow and pulled into the sampler while the heavier pollen balls will fall to the bottom of the pollen trap.

3.4 *System disadvantages*

The main problem in the system is contamination: once a positive result is obtained, it is impossible to tell if future positives are from the environment or from residual contamination from the hive. In most cases, though, this should not be a problem. In an acute scenario, for example looking for anthrax, the hive would be destroyed after a positive result. In domestic use in monitoring for crop pathogens the field would be treated after a positive result and by the time the treatment regimen was finished the hive should have been cleaned out by the bees and any remaining bacteria immobilized in wax or propolis.

For military applications, a styrofoam nucleus hive could be filled with five frames from a clean stock hive and then transported to the test site. After a period of flight, the entire nucleus hive could be sacrificed to test for the suspected pathogen without sacrificing the queen from the stock hive, which would then be able to recover the lost workers.

4. CONCLUSIONS

Honey bees may make a effective tool for the detection of harmful bacterial spores in the atmosphere. Because of their long flight range (up to 3km), and large numbers, one hive can cover a significant area. In addition to military use in detecting the presence of biowarfare agents, the honey bee system shows good potential for domestic applications such as monitoring crops for plant pathogens. Especially during pollination periods, the bees would have a close association with the crop plants, and would tend to pick up particulates, including pathogens, from the plants themselves as well as the air around them, making them an even more effective collection and detection tool.

5. REFERENCES

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