DETECTION OF HONEY BEE DISEASE THROUGH MONITORING SHIFTS IN VOLATILE PROFILES

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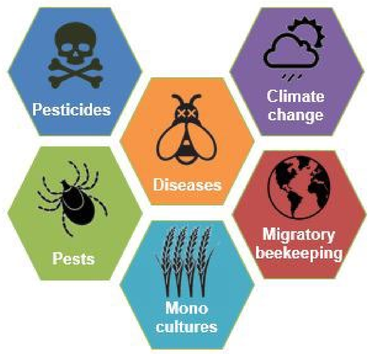
Introduction

Significant declines in honeybee numbers have been reported globally, which cost the economy an estimated $15 billion a year. Honeybees face many disease and pest pressures, and early detection and treatment significantly improves honeybee colony survival. The analysis of volatile organic compounds is used for the early detection of human diseases. The aim of this research is to identify VOCs associated with honeybee diseases and pests using head space analysis and GC×GC-TOF MS to develop sensors for earlier disease detection which could facilitate more efficient treatment and control methods.



Honey bee diseases and pests

The term colony collapse disorder (CCD) was coined in the United States to describe unexplained large-scale ongoing losses of honey bee colonies. The declining trend in honey bee numbers and CCD have yet to be attributed to any one cause, but multiple factors such as pesticide use, agricultural and bee keeping practices and climate change have been shown to contribute. Honey bees face numerous disease and pest pressures, and the early detection and treatment of diseases and pests have been shown to significantly improve honey bee colony survival. Details of some of the more significant diseases and pests are outlined below.

American foulbrood (AFB) – Paenibacillus larvae

A rod-shaped spore forming gram positive bacteria. Larvae become infected when fed brood food containing spores. The infection kills the larvae at the pro-pupal stage of development and symptoms include wax capping's which are greasy, sunken and dark with perforations, and the formation of a mucus rope when the cell contents is agitated with a small stick and withdrawn. Destruction of infected colonies is currently the only effective method of eradication, and due to this P. larvae is a notifiable disease in most countries worldwide.

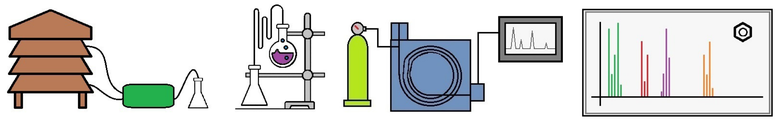
European foulbrood (EFB) – Melissococcus plutonius

A gram-negative lance shaped bacteria. Larvae become infected when fed brood food containing the bacteria, where the bacteria multiply in the larval gut and competes with the larvae for food. The infection does not always kill the infected larvae if there is sufficient food to feed both, and symptoms include melted looking discoloured larvae which lie in the cells in unnatural positions. EFB infections are problematic to resolve as antibiotic treatments contaminate hive products and biotechnological methods are time consuming and complicated to complete.

Varroa – Varroa destructor

A parasitic mite of honey bees which reproduce in comb cells and feed on the honey bee pupae, weakening the pupae and the colony as a whole. Mites feeding on pupae also increase transmission of viruses such as deformed wing virus (DWV). Treatment of infested colonies is problematic as mites can develop resistance to some chemical treatments, which can also contaminate hive products. Other methods of control are either costly, time consuming or complex making varroa a significant threat to honey bee populations.

There are also numerous minor diseases and pests which affect colonies of honey bees, and whilst singularly they may not be detrimental to the colony, in combination with other diseases, pests and environmental pressures they contribute significantly to honey bee declines.



Volatile collection and analysis

A variety of sampling techniques, such as head space analysis and dynamic flow systems using both MonoTrap™ and Pye entrainment with tenax tubes will be used in laboratory conditions to collect VOCs from isolated samples of diseases, pests, bees, equipment and hive products. The same equipment and techniques will be used to collect VOCs from healthy and infected honey bee colonies in the field.

Volatile profiles of all samples will be obtained on a PEGASUS® BT GC-TOFMS. Splitless injection is typically used in honey bee VOC research as the proportion of analytes of interest in the sample in generally small (<0.01%), and splitless injection maximises the amount of analytes that will reach the column. To help prevent the thermal degradation of unstable compounds from the extremely high temperatures used in the injector ports, cool-on-column injector ports can be used. A DB-35 column is suitable for the analysis of honey bee VOC which tend to be comprised of polar compounds.

TOFMS has a very high mass resolving power which allows for a retrospective look at potential small molecule analytes that may be present in samples and is useful when screening for unknown analyte which is useful for honey bee VOC research.

Multivariant statistical analysis of peak / ion pairs will be used to identify volatile profile which will allow for the identification of disease and pest biomarkers.

Sampling undertaken over multiple seasons to monitor shifts in the natural variations of the volatile profiles of healthy and diseased colonies which will enable the development of field based diagnostic tools for early detection of honey bee diseases and pests.

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