Volatile Characterization of American Foulbrood Disease Using Comprehensive Two-Dimensional Gas Chromatography

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Background

American foulbrood (AFB) is a deadly disease spread by *Paenibacillus larvae* bacterial spores, targeting the brood stage of *Apis mellifera* honey bees. Broods perish in a matter of days after exposure to the spores, forming infected colloidal scales in the hive that adult bees cannot remove.[1,2] The spores are extremely persistent and resistant to typical treatment methods, which standardized the immediate euthanasia of bees and destruction of the hive and associated equipment by burning.[3]

In an effort to expedite identification of the spores, scent-detection dogs have been trained for use in apiaries. Furthermore, gamma-irradiation has been tested as a proposed treatment method as it would offer a non-destructive alternative to sterilization.

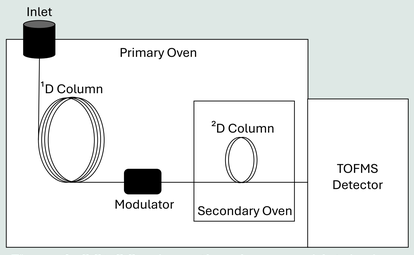

Figure 1. GC×GC schematic using a quad-jet dual-stage cryogenic modulator and TOFMS detector.

The goals of this study were to use comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS) to characterize the volatiles associated with AFB disease for understanding scent detection and to measure the effectiveness of gamma-irradiation in sterilizing these volatiles.

Results and Discussion

Each of the four main sample types are represented in the chromatograms below (Figure 2), with the tentative identification of the most abundant peak labeled. The healthy hive material showed the lowest overall intensity of volatiles, while the gamma-irradiated sample showed the greatest overall intensity.

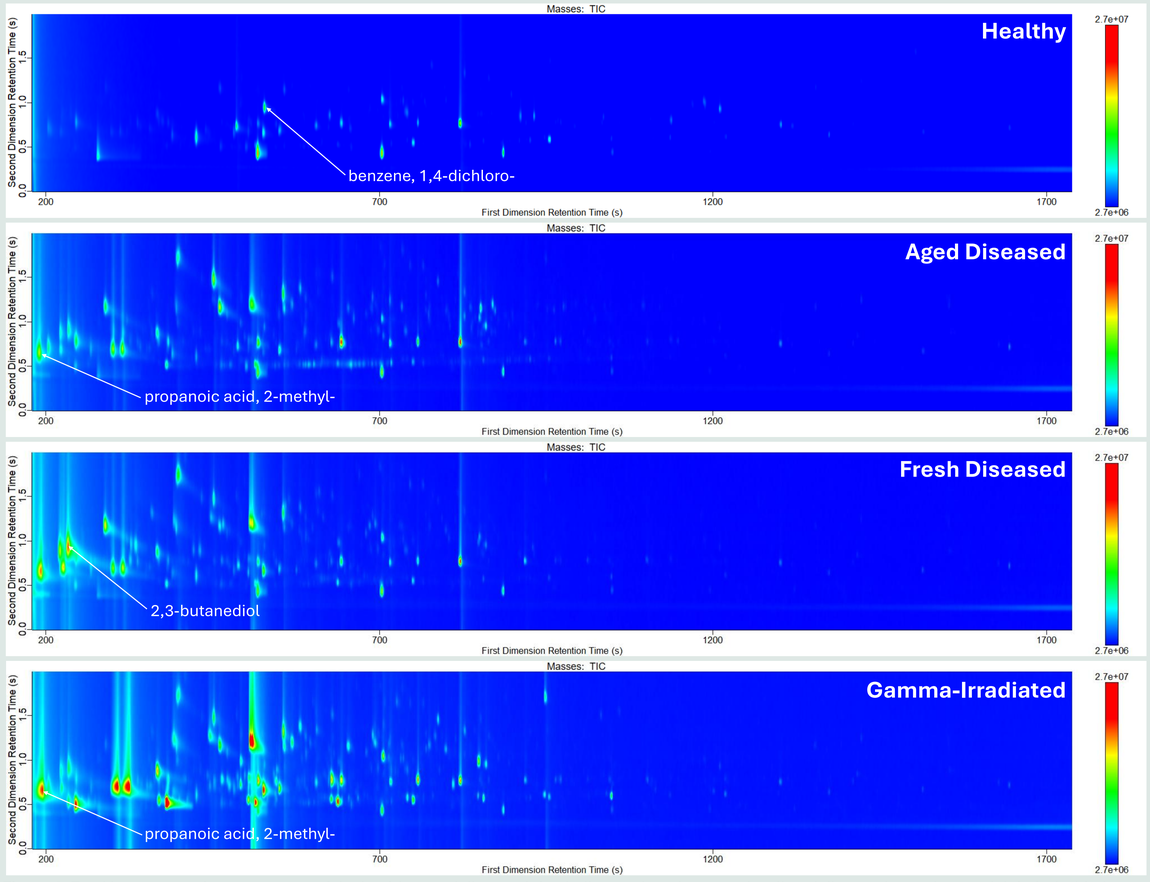

Figure 2. Total ion current chromatograms of the healthy, aged diseased, fresh diseased, and gamma-irradiated diseased samples, each labeled with the most abundant peak present.

Methods

Samples included blanks, material from healthy comb, aged unsterilized diseased scales, fresh unsterilized diseased scales, and diseased scales sterilized with the proposed treatment method of gamma-irradiation. These samples were stored in headspace vials (Figure 3) in the freezer when not in use. No sample preparation was required before injection with solid-phase microextraction using the LPAL-3 (CTC Analytics).

Figure 3. *A. mellifera* samples from left to right: healthy material, aged AFB scales, fresh AFB scales, gamma-irradiated AFB scales.

Analysis was performed using a Pegasus BT4D (LECO Corporation) according to the parameters described below in Table 1. ChromaTOF software (LECO Corporation) was used to analyze the chromatographic data. Preliminary identifications were assigned to peaks using a minimum signal to noise of 200 and a minimum stick count of 3.

Table 1. GC×GC-TOFMS parameters used for analysis.

GC×GC Parameters	
Inlet	Split flow of 62.5 mL/min at 250 °C
Columns	^{1}D: HP-5Q column (29.3 m × 250 µm ID × 0.25 µm d_f) ^{2}D: DB-17MS column (0.975 m × 250 µm ID × 0.25 µm d_f)
Modulator	Quad-Jet Dual-Stage Cryogenic
Modulation Period	3.0 s (0.60 s hot pulse, 0.40 s cold pulse)
Oven Temperature Program	40 °C (hold 3 min) to 240 °C at 8 °C/min (hold 1 min)
Secondary Oven Offset	+5 °C
Modulator Offset	+15 °C
Carrier Gas	Hydrogen at 1.25 mL/min
TOFMS Parameters	
Ion Source Temperature	250 °C
Mass Range	29 to 500 amu
Acquisition Rate	200 spectra/s

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Conclusions

GC×GC-TOFMS can be used to distinguish the volatile profiles of *A. mellifera* samples at different stages of infection with AFB disease.

Future Directions for Research:

- Discrimination of volatiles specific to AFB from non-specific volatiles
- Characterization of AFB-specific volatiles for scent-detection purposes
- Measurement of gamma-irradiation sterilization success in removing AFB volatiles

References

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