E18 Greenhouse Evaluations of Volatile Plant Defense Against an Invasive Agricultural and Environmental Biothreat Agent, *Raffaelea lauricola*, and Possible Implications for Canine Detection

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After attending this presentation, attendees will understand the processes of the biothreat *Raffaelea lauricola* and how avocado trees respond through altering volatile secondary metabolites. Attendees will also understand how research is progressing to characterize these volatiles so that mimic training aids can be created to expand the use of canine detection for this protection of national resources and the economy.

This presentation will impact the forensic science community by presenting a method of strengthening pre-existing canine detection in forensic science and food safety through the chemical analysis of the Volatile Organic Compounds (VOCs) in the headspace of an invasive biothreat agent for the protection of natural resources by characterizing the VOCs of a living target's odor.

Biological threats are increasingly gaining attention because of the hazard they pose to national resources, including agriculture and the environment. The fungus *Raffaelea lauricola* is a biothreat vectored by the invasive beetle *Xyleborus glabratus*, or Redbay Ambrosia Beetle (RAB). RABs are attracted to members of the Lauraceae family, including commercial and private avocado trees as well as six wild species. Avocado trees are of particular interest because they are Florida's biggest tropical fruit crop, comprising $55 million of the state's annual economy. Since the RAB introduction into the United States about a decade ago, the fungus killed more than 12,000 commercial avocado trees and an estimated half million wild trees in the southeastern region of the nation. The introduction of the RAB and similar vectors into the United States can be halted through improved detection techniques using canines handled by the United States Department of Agriculture (USDA), who police agricultural ports of entry. The resulting introduction of the RAB and now-rapid spread of the fungus puts commercial avocado groves at risk in California, Mexico, and Central and South America. The fungus lives in the tree's xylem, or vascular tissue. Once a tree is inoculated, it shuts down the vascular tissue in an attempt to halt the spread of the fungus. Unfortunately, this also stops the spread of water and nutrients vital to the tree's life, resulting in tree death within approximately six weeks in a process referred to as laurel wilt disease (a reference to the disease's symptoms).

Due to the rapid spread of *R. lauricola* and the quick death of trees, early detection is essential. The only current method of pre-symptomatic identification is canine detection. Despite the high risk to food safety associated with biothreats and invasive species, canine detection use has been limited in this field. The lack of widespread application for canines in food safety targets is largely due to the lack of mimic training aids. Without mimic aids for biothreats, live training aids must be used; however, live aids are high risk and often prove difficult to obtain because of rarity, legality of attaining and transporting the species, and method of containment. In the case of *R. lauricola*, containment is difficult because fungal spores are easily spread. In order to create a mimic training aid, the VOCs in the headspace of infected trees must be fully characterized.

The current study evaluated VOCs of inoculated young avocado trees in a greenhouse setting using Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS) in order to follow the progression of infection through VOCs in a controlled environment. Nine compounds were previously detected in greater than

80% of avocado trees, all of which are sesquiterpenes, secondary metabolites produced in indirect plant defense and interactions with fungi. Young trees were inoculated with plugs of *R. lauricola*, while healthy trees were spatially separated from the others. The trees were sampled each day, starting before inoculation and ending with tree death. Inoculated trees change their VOC production as part of their inherent defense system, slowing the production of certain categories of VOCs and producing others not seen in the healthy trees. Notably, these changes are detected before symptoms can be visually observed. The characterization of VOCs in this study will be essential in creating a mimic training aid for avocado trees infected with *R. lauricola*. Use of detector canines can then be expanded to help protect national resources and the economy.

Biothreat, Canine Detection, Food Safety