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Coupling a branch enclosure with differential mobility spectrometry to isolate and measure plant volatiles in contained greenhouse settings

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ABSTRACT

Volatile organic compounds (VOCs) are off-gassed from all living organisms and represent end products of metabolic pathways within the system. In agricultural systems, these VOCs can provide important information on plant health and can ordinarily be measured non-invasively without harvesting tissue from the plants. Previously we reported a portable gas chromatography/differential mobility spectrometry (GC/DMS) system that could distinguish VOC profiles of pathogen-infected citrus from healthy trees before visual symptoms of disease were present. These measurements were taken directly from canopies in the field, but the sampling and analysis protocol did not readily transfer to a controlled greenhouse study where the ambient background air was saturated with volatiles contained in the facility. In this study, we describe for the first time a branch enclosure uniquely coupled with GC/DMS to isolate and measure plant volatiles. To test our system, we sought to replicate our field experiment within a contained greenhouse and distinguish the VOC profiles of healthy versus citrus infected with *Candidatus* Liberibacter asiaticus. We indeed confirm the ability to track infection-related trace biogenic VOCs using our sampling system and method and we now show this difference in Lisbon lemons (*Citrus×limon* L. Burm. f.), a varietal not previously reported. Furthermore, the system differentiates the volatile profiles of Lisbon lemons from Washington navels [*Citrus sinensis* (L.) Osbeck] and also from Tango mandarins (*Citrus reticulata* Blanco). Based on this evidence, we believe this enclosure-GC/DMS system is adaptable to other volatile-based investigations of plant diseases in greenhouses or other contained settings, and this system may be helpful for basic science research studies of infection mechanisms.

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1. Introduction

Plants can experience multiple physiological and biochemical responses when exposed to pathogens or injury [1]. For instance, citrus trees infected with *Candidatus* Liberibacter asiaticus (CLas) bacteria exhibit altered gene expression [2,3] and the host response to infection appears to modify certain metabolic signatures of the trees [4,5]. It is no surprise then that an affliction-initiated cascade of biological change can alter the end products of certain metabolic pathways, such as the volatile organic compounds (VOCs) emitted by the trees. All living organisms naturally produce VOCs, and these chemicals have been shown to closely associate with plant health [6,7]. We recently demonstrated the ability to differentiate VOC profiles of healthy citrus from those of CLas-infected citrus in field Hamlin sweet orange (*Citrus sinensis* L. Osbeck) and have reported putative VOCs accounting for these differences [8]. Using in situ VOC sampling and detection, our diagnostic capability preceded conventional diagnostic methods. Our gas chromatography/mass spectrometry (GC/MS) and gas chromatography/differential mobility spectrometry (GC/DMS) methods even preceded visual symptom recognition. Diagnosis using GC/MS allows for the shipping of citrus VOCs trapped onto polydimethylsiloxane sorbents (such as SPME fibers or Twisters®) from field trees to a laboratory for analysis, but GC/DMS allows for a direct field-deployable, real-time diagnostic method for CLas [8].

Abbreviations: CLas, *Candidatus* Liberibacter asiaticus; CV, compensation voltage; DMS, differential mobility spectrometry; HLB, Huanglongbing; PCR, polymerase chain reaction; PCA, principal components analysis; VOCs, volatile organic compounds

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The result of *C*Las infection is Huanglongbing (HLB), currently the world's most destructive citrus disease. In the United States, *C*Las either infects trees via the Asian citrus psyllid (ACP, *Diaphorina citri*) vector or grafting with infected plant material [9]. Afflicted trees produce asymmetric, bitter fruit that tend not to color properly and are unmarketable and of very limited value to growers [9]. In Florida alone, HLB is attributed with the loss of over 60,000 jobs and decreased industry output of nearly eight billion dollars [10]. At present, the only accepted methods for *C*Las detection are polymerase chain reaction (PCR) assays that detect *C*Las DNA. These assays require sufficient bacterial titers in plant tissue, and this is somewhat problematic as the bacteria are not evenly distributed within a tree [11]. The time between initial infection and symptom development (latent period) varies from 6 months to as long as 5 years. In many instances PCR results in false negatives [11]. Rapid early detection methods, such as field-deployable GC/DMS systems, are apposite for combating HLB.

DMS, also known as High Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS), allows for gas-phase ion separation and detection of trace chemicals of interest [12,13]. The DMS sensor is a small and portable technology due to its low energy consumption and ability to operate under normal atmospheric pressure and temperature. A brief description of the technology is as follows: two parallel plates surround a channel. Analytes are introduced on one end of the channel via a carrier gas after being ionized by any common ionization source and flow toward a detector on the opposite end. A voltage is applied to the plates as an asymmetric square wave form, also called dispersion voltage. Different chemicals have unique ion mobilities based on mass [14] and ion structure [15], and in the case of DMS, the ion mobilities also depend on the high electric field [16]. As the analytes traverse the channel, the dispersion voltage allows certain ions to hit the detector while other ions collide with the channel's walls and go undetected [15]. When a mixture of ions flow through the channel, an appropriate small DC voltage, or compensation voltage (CV), is applied in addition to the dispersion voltage to allow certain ion species to reach the detector. A visual representation of this processed is shown (Fig. 1) [17]. In short, DMS is an ion filter that, when coupled with gas chromatography (GC), yields orthogonal separation and detection [18].

GC/DMS is advantageous for research required by law to occur in biocontainment facilities, such as the study of the emerging *C*Las infection in California. While plant pathogens and their vectors may be grown inside contained greenhouses, government permits may be required to remove plant tissue for outside analysis. Often it is too dangerous to remove any type of material for fear of accidental outbreaks. Even laboratory tools are to be quarantined for months before they can be transported out of these facilities. In consequence, it is sometimes too inconvenient or too expensive to install analytical tools, such as traditional GC/MS systems, in these facilities. However, GC/DMS has many advantages that make it ideal for these environments. The devices are small and easily transported; they operate under normal atmospheric conditions and temperatures, even without the need for supplemental gases; they can be used in in situ investigations that avoid having to remove plant material from quarantine facilities or elaborate sample preparations; they allow for diagnostic developments that may be adaptable to field studies for real-time disease analysis; GC/DMS has been in increasing demand for biological applications [13].

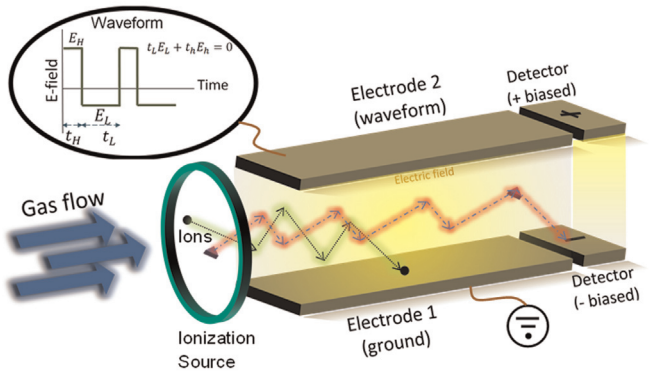

Fig. 1. A planar view of the DMS technology. Ions are forced between two charged plates by a carrier gas toward a detector on the opposite end. An oscillating electric field is applied perpendicular to the ions' trajectory, allowing certain analytes, depending on their ion mobility, to reach the detector. A compensation voltage (CV) may be applied in addition to the dispersion voltage to alter which ion species reach the detector. Reprinted with permission from Creative Commons Attribution [17].

Our previous GC/DMS work exclusively sampled citrus trees in environments with relatively low abundances of ambient background VOCs, such as citrus groves. Thus, by simply placing the GC/DMS sampling inlet into the tree canopy we were able to capture and analyze citrus VOCs [8]. But contained greenhouses, especially Biosafety level 3 facilities, often use recirculated air, allowing high background VOC concentrations to accumulate from the myriad of plants within. Work in a contained greenhouse required us to employ branch enclosure systems to isolate a particular plant's volatiles from the high and inconsistent background noise level. Often these enclosures incorporate adsorbent cartridges to trap plant volatiles and are then sent to outside laboratories for analysis. However, it is not always possible to ship material, including adsorbent cartridges, out of containment facilities. Branch enclosures have been reported using field-portable GC/MS units [19,20], which may be easily installed in such greenhouses, but DMS allows for detection of VOCs not resolved by GC/MS, such as the separation of certain isomers [21]. Given the importance of isomers in biological signal transduction pathways, resolving these metabolite compounds is frequently important. For the first time, we report an alternative branch enclosure system uniquely coupled with GC/DMS to allow for plant VOC measurements in contained spaces. While branch enclosure systems are well known in plant volatile investigations, they have not yet been combined with GC/DMS. We confirm our novel system's ability to measure plant volatiles by comparing volatile profiles of healthy citrus and trees infected with *C*Las; we also examine the volatile profiles of three citrus varieties. We then examine the effect of humidity in our enclosure-GC/DMS system. Our methods confirm that an enclosure-GC/DMS system can be used in contained settings to investigate volatile-based diagnostic methods of plant diseases.

2. Material and methods

2.1. Plant materials

Citrus trees were maintained in a greenhouse at the Contained Research Facility at the University of California, Davis (Davis, CA). The greenhouse was held at a constant 27 °C and the plants received supplemental lighting (high pressure sodium lights) from 07:00 to 23:00. Varietals included Washington navel oranges [*Citrus sinensis* (L.) Osbeck], Tango mandarins (*Citrus reticulata* Blanco) and Lisbon lemons (*Citrus* × *limon* L. Burm. f.) all grafted onto Carrizo rootstock [*Citrus sinensis* × *Poncirus trifoliata* (L.) Raf.]. Plants were inoculated with *C*Las by grafting PCR-confirmed material onto the scion 15 months prior to this study ("*C*Las-infected"). Control plants were grafted with healthy plant material ("controls") at the same time as *C*Las inoculation. At the time of this study, the *C*Las-infected lemons and navels demonstrated visual symptoms of HLB. Infected

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