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.

KEYWORDS

volatile organic compounds (VOCs); differential mobility spectrometry (DMS); field asymmetric ion mobility spectrometry (FAIMS); Huanglongbing (HLB); *Candidatus Liberibacter asiaticus* (CLas)

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

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).

The result of CLas infection is Huanglongbing (HLB), currently the world's most destructive citrus disease. In the United States, CLas 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 CLas detection are polymerase chain reaction (PCR) assays that detect CLas 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 process is shown (**Figure 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 CLas 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 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).

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 CLas; 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.

MATERIAL AND METHODS

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 CLas by grafting PCR-confirmed material onto the scion 15 months prior to this study ("CLas-infected"). Control plants were grafted with healthy plant material ("controls") at the same time as CLas inoculation. At the time of this study, the CLas-infected lemons and navels demonstrated visual symptoms of HLB. Infected mandarins did not show visible symptoms of HLB. All plants were screened and confirmed negative for other possibly confounding citrus diseases, including citrus stubborn and citrus tristeza, which have been reported to also alter the volatile profile of citrus trees (22).

Branch Enclosures

We designed a branch enclosure as a variation of enclosures that are common in atmospheric biogenic organic compound emissions studies (19) (23). All tubing in the system was composed of ¼ in. (6.35 mm) outer diameter PTFE. Connections were made with Swagelok® stainless steel fittings. The air flow was generated by an oil-less air compressor (Model LAM-200, Mido USA Inc., Roselle, IL). To control the temperature and level of humidity inside the enclosures, the air passed through 3 m of coiled copper tubing resting in an ice bath, then passed through a moisture separator (McMaster-Carr, Chicago, IL). The flow was passed through a hydrocarbon trap (Part 22013, Restek Corp., Bellefonte, PA) to scrub the air of background greenhouse VOCs. Brass needle valves controlled the flow to four individual purge lines, and the lines were monitored and re-calibrated as necessary to each maintain a flow of 1 L/min.

Roughly a 30 cm length of a single citrus tree branch was placed inside a previously conditioned PTFE bag with dimensions 0.0025 in. × 24 in. × 12 in. (0.0635 mm × 610 mm × 305 mm) (Welch Fluorocarbon Inc., Dover, NH). Although translucent, the bags allowed photosynthetically active radiation to pass through, encouraging normal plant photosynthesis (23). Bags only contained one tree branch before being reconditioned in a vacuum oven at 160 °C for ~12 h overnight. The purge line was placed along the branch and the bag was closed around the branch using a zip tie. A 1/8 in. (3.175 mm) PTFE tube connected the bag to the GC/DMS sampling inlet (**Figure 2**). Care was taken to avoid damaging branches or leaves during set up. Branches were chosen to have approximately the same number of leaves of the

same age to avoid experimental biases in this methods optimization study (24) and were marked with flagging tape to allow resampling the same branch over long duration experiments. We also were careful to ensure there was enough leaf mass placed into the enclosures to generate adequate VOC signals (25). All plants were allowed to equilibrate in their enclosure overnight before sampling to remove any installation artifacts (26).

To monitor the environment inside the bags, custom temperature and humidity dataloggers were built. Digital temperature and humidity sensor probes (Part AM2315, Aosong Electronics Co, Ltd., Guangzhou, China) were attached to microcontrollers and recorded data to micro SD cards. Measurements were taken inside the enclosure continuously every 1 min. Collecting temperature and humidity data, as reported in other branch enclosure publications (19) (23), helped ensure the branch enclosures experienced similar temperature and humidity levels as those in the surrounding greenhouse.

Volatile Sampling and Analysis

We used a custom built GC/DMS analysis system with a Carbopack X/Carbopack B sorbent trap preconcentrator connected to a DB-XLB 10 m $\times$ 0.25 mm $\times$ 1.0 μ m GC column interfaced to a commercial DMS sensor (Sionex Corporation). A detailed description of the DMS technology is given elsewhere (8). The custom GC/DMS applied to citrus VOC detection has also been described previously (8).

The detailed GC/DMS chemical separation and detection method used was as follows. The initial step was the cleaning of the system by setting the sorbent trap set to 330 °C and the GC column to 200 °C for 200 s. Afterwards, the column was cooled to 30 °C and the trap to

80 °C. The sample was then collected for 15 min via a sampling pump. Once the sampling step was finished, the trap heated to 330 °C while the column held at 30 °C for 30 s. The column then took 130 s to ramp to 200 °C, holding at this temperature for 80 s, followed by a cool down to 75 °C. The DMS sensor had a 500 µm gap size and had the following settings applied: temperature 55 °C; carrier gas flow 300 mL/min; asymmetric waveform amplitude 1300 V; RF frequency 1.2 MHz with the asymmetric waveform 34% high field and 66% low field; CV scan range from -37 to +15 V with a step of 0.52 V; 101 steps across the CV scan with 0.505 ms per complete CV scan. Including the initial system cleaning, the 15 min sampling sorption and finally the desorption and subsequent GC/DMS measurement, one complete sample collection lasted 1450 s.

GC/DMS Data Analysis

A total of 81 individual VOC recordings (samples) were collected for 10 plants over 12 days. Prior to any analysis, a visual inspection of the spectra was performed and 8 spectra were removed due to user error or instrument malfunction. Analysis was only applied to the non-system purge, non-sample sorption section of the method (namely, between 1250 and 1380 s). DMS spectra contain a reactant ion peak (RIP), which can fluctuate independent of the sample introduced (27) and was not included in the analysis, therefore only the CV range from -20 to 15 V was analyzed. The chemometric analysis was performed using MATLAB (The Mathworks Inc., Natick, MA, 2011). In order to reduce background noise and device inconsistencies within the sample spectra, the baseline of each sample set was analyzed and removed using asymmetric least squares with a smoothing regularization parameter of 10^2 and an asymmetry regularization

parameter of 10^{-2} (28). The processed data set was then standardized using weighted normalization before finally being centered to the mean and reduced to one standard deviation across samples. Following this preprocessing, principal components analysis (PCA) was performed to visualize similar and dissimilar VOC profiles (29).

RESULTS AND DISCUSSION

Overcoming Greenhouse VOC Measurement Constraints

Previously we reported a GC/DMS method for VOC analysis under field conditions from citrus tree canopies (8). We anticipated using this same experimental procedure for VOC studies in greenhouse conditions; however, this attempt yielded spectra that showed little difference between background air samples and the VOCs from the plant of interest. The signal from the greenhouse background air was taken in a corner of the greenhouse as far from our measurement trees as possible (**Figure 3A**) in an attempt to measure the volatile background. We then compared this air blank to a sample taken by placing the GC/DMS inlet in the tree canopy, per our previously reported method (8) (**Figure 3B**). The lack of visual discrimination between the background and tree VOC profiles was apparent, and higher order statistical analysis of the data failed to separate these two data types (not shown). This was due to the high abundance levels of background odors that are frequently encountered in containment greenhouse facilities, especially given the recirculating air handling systems. The experimental greenhouse housed nearly 200 citrus trees of 4 varieties of citrus. As the building recirculates air to control the greenhouse temperature, it was not surprising that the ambient greenhouse air was saturated with volatiles off-gassed by the plants within (perceivable to the human nose). Therefore, it is

consistent that the VOC profiles of the greenhouse background citrus should overlap with the individual plant volatiles of interest. A few volatiles were unique to greenhouse background spectra and may be attributed to other research tools (e.g. fertilizer or soil) found within the facility.

To overcome this limitation, we sought to isolate plant volatiles from the ambient air. By constructing the enclosure system described in the **Branch Enclosures** section, we were able to isolate volatiles off-gassed by a specific plant at a specific time point. We first examined the VOC signatures of enclosures that did not contain plants (“enclosure blanks”, **Figure 3C**). Enclosure blank spectra were found to be mostly featureless when compared to greenhouse background spectra (**Figure 3A**), suggesting our enclosure effectively scrubbed the purge air of the greenhouse background odors. We next compared enclosure blanks to enclosed healthy Lisbon lemons (“control Lisbon”, **Figure 3D**) and found a clear visual difference between blanks versus samples. Volatiles from Lisbon lemons have been previously measured (30) and so we anticipated a unique lemon VOC profile when compared to enclosure blanks. We then employed PCA to visualize variance between spectra from empty enclosures (n=6) and spectra from 4 individual healthy control lemon trees (4 control Lisbons, n=15 total) (**Figure 4**). The PCA scores plot of enclosed tree samples shows separation along the first principal component (PC1). Thus, we confirmed that the enclosure system was efficient in generating clean purge air for our plant volatile study. Volatiles trapped and analyzed by our enclosure-GC/DMS system almost exclusively originated from the plants.

CLas-Infected Lisbon Lemon Study

Our previous field study demonstrated the ability to use VOC profiles of citrus to identify trees infected with CLAs (8). We anticipated our enclosure system would reproduce such results and investigated CLAs-infected and control lemons. Using our enclosure setup, we then collected GC/DMS volatile profiles of both healthy and CLAs-graft inoculated Lisbon lemon trees (2 controls, n=16 samples; 2 CLAs-infected n=19 samples). CLAs-infected trees were grafted with PCR-confirmed plant material and exhibited symptoms of citrus greening, including malformed leaves, vein corking and asymmetrical chlorosis (9). PCA was applied to explore the possible differences of these spectra, and the scores plot of these samples shows separation along the first principal component PC1 (**Figure 5**). There was a small tendency for the CLAs-infected lemons to cluster away from control lemons along the first principal component (PC1), confirming the difference in VOC profiles related to the health status of the trees. The distinct VOC signatures were directly related to the citrus host response to CLAs infection and did not appear to result from any confounding health factors. All trees were managed identically under controlled greenhouse conditions and underwent multiplex testing for possibly confounding citrus diseases, such as the *Spiroplasma citri* bacterium, the causal agent of citrus stubborn disease that also inhabits the phloem. This study corroborated our previously reported VOC profile difference (8) among CLAs-infected and healthy citrus using GC/DMS and was novel to report that this separation was apparent in the Lisbon lemon cultivar. We also witnessed that healthy citrus generate higher abundances of VOCs when compared to CLAs-infected, as seen in the average GC/DMS profiles of healthy and infected trees (**Supplemental Figures S1 and S2**). This suggests the metabolic changes of HLB lead to a cessation of volatile emissions throughout disease progression.

Volatile-Based Separation by Citrus Variety

Another study has reported differences in the volatiles emitted by Meyer lemon (*Citrus × meyeri*) and Clementine mandarins (*Citrus × clementina*) (25) and thereby different VOC profiles for Lisbon lemon and Tango mandarin cultivars were anticipated using our new enclosure system. We collected samples from control Tango mandarins (2 controls; n=11 samples) and control Lisbon lemons (2 controls, n=16 samples). A distinction was observed between control mandarins and control lemons VOC profiles (**Figure 6**). The PCA scores showed a separation by cultivar among the first principal component (PC1). Thus, our branch enclosure-GC/DMS set up was able to distinguish the nuances in volatiles off-gassed by Lisbon lemons and Tango mandarins.

High Humidity Enclosures

We then removed the ice bath from the experimental setup in order to investigate humidity-induced differentiation between control and CLas-grafted trees. Without the ice bath, enclosures rapidly reached 100% relative humidity due to the moisture generated from plant respiration. In high humidity testing, we observed Washington navels (2 controls, n=5 samples) and Lisbon lemon trees (2 controls, n=16 samples). The GC/DMS differentiated navel and lemon VOC profiles. PCA scores show this varietal-based separation along the second principal component (PC2, **Figure 7**). A different study revealed that monoterpenes are among the major VOCs emitted by oranges whereas lemons emit negligible amounts of monoterpenes (25), which may account for the separation of VOC profiles seen here.

Examining the average GC/DMS spectra of lemon trees sampled in humidity-controlled enclosures versus high-humidity enclosures (**Supplemental Figures S2 and S3**, respectively), the trees emitted higher abundances of VOCs when sampled in humidity-controlled environments with a relative humidity between 50-70%. Abiotic factors are known to attribute to plant volatile emissions, and these factors have varied effects on the different chemical classes of volatiles. Monoterpenes, for instance, are stored in secretory structures inside the leaf. Higher kinetic energies allow these reservoirs to naturally evaporate and thereby emission rates of certain monoterpenes are temperature dependent. Sesquiterpenes are suspected to behave similarly, showing strong correlations to temperature. Other terpenoids derive from *de novo* synthesis pathways related to the amount of light available to the leaf (25). Meanwhile a study on corn plants in similar branch enclosures and a field study of apple trees reveal that high humidity environments decrease the overall amount of volatiles off-gassed by these plants (31) (32). Thus, our high-humidity enclosures are a combination of two conflicting environment types: (i) increased temperature, which increases certain emissions, and (ii) increased humidity, which decreases certain emissions. According to visual inspection of the average GC/DMS spectra (**Supplemental Figure S3**), volatile measurements were decreased in our high-humidity enclosures. Measurements were significantly reduced, as PCA scores of high-humidity samples and humidity-controlled samples showed separation along the first principal component (**Supplemental Figure S4**). We attempted to distinguish between healthy and CLas-infected lemons in high-humidity enclosures, but signals were suppressed to such an extent that it inhibited our ability to detect CLas.

Both the sorbent traps and the DMS unit may be affected by humidity, but we did not see evidence that high humidity affects CLas detection in our field work. High humidity levels may

saturate the sorbent traps with water molecules, and less VOCs are collected. Additionally, DMS is known to be affected because of unfavorable changes to the reactant ion peak in high humidity environments (15). However, in our field work in Florida the relative humidity would reach 100% throughout the day. That particular GC/DMS model was most successful during the summer, high-humidity months compared to other seasons. We suspect this is largely attributed to the size of the orchard trees and thus the quantity of volatiles emitted. Field trees in Florida were > 2 m high with areas of dense foliage; the GC/DMS sampling inlet was placed directly up to the canopy. Greenhouse trees used in this study were pruned to be no taller than 1 m, and only a relatively small portion of a single branch was enclosed. Even if volatile emissions in citrus are decreased in high humidity environments, field trees may be relatively large enough that this does not affect our CLas detection in orchards. Overall, humidity-controlled enclosures seem appropriate for a volatile-based diagnostic method for CLas using our enclosure-GC/DMS method; otherwise, humidity does not seem to have the same effect in enclosure-less field studies.

CONCLUSION

In our study we conducted *in situ* VOC profiling of Washington navel oranges, Lisbon lemons and Tango mandarins using branch enclosures that were paired with a GC/DMS system for the first time. We confirmed a difference in the VOC profiles of healthy and CLas-infected citrus trees and for the first time noted this distinction in Lisbon lemons. We also noted VOC profile differences among Lisbon lemons and Washington navel oranges, as well as between Lisbon lemons and Tango mandarins. This set up will allow for further controlled investigations

on the specific time course of HLB development and its effect on citrus volatile emissions while working in a controlled greenhouse. Furthermore we will continue to examine the potential of GC/DMS to help growers detect HLB. Future studies aim to monitor tree decline over the course of CLas infection against conventional diagnostic methods under controlled greenhouse conditions. We anticipate results from this enclosure-GC/DMS system inside a contained facility may provide insight for even earlier detection of CLas using our field-deployable, enclosure-less GC/DMS method (8) in citrus orchards.

By no means is this enclosure-GC/DMS system limited to the CLas pathogen nor to citrus trees. Our setup allows for easy “fingerprinting” of volatile biological markers of possibly any type of plant and could serve to differentiate healthy from afflicted plants in a myriad of biotic and abiotic studies. Most importantly, employing GC/DMS instead of GC/MS distinguishes our enclosure system and alleviates certain restrictions when measuring plant volatiles in quarantined laboratories. We foresee similar enclosure-GC/DMS setups helping advance volatile-based diagnostic methods of other plant pathogens.

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B&W/COLOR INDICATIONS:

For online versions, we request all Figures and Supplementary Material be in color.

For print, we request all Figures and Supplementary Material be in black and white.

CAPTIONS:

Figure 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).

Figure 2: A schematic of the enclosure-GC/DMS system. Purge air is generated by an oil-less air compressor. To ensure enclosures do not exceed the normal temperature and relative humidity levels of the greenhouse, the purge air passes through 3 m of copper tubing resting in an ice bath and then a water trap, which cools the air and removes excess moisture. The purge air then filters through a hydrocarbon trap to reduce the concentration of background volatiles. Enclosures are made of PTFE bags and allow photosynthetically active radiation to pass through for normal plant respiration. Temperature and humidity probes monitor the environment inside the enclosures. Gas samples from the enclosures pass to the GC/DMS system for chemical separation, detection, and analysis.

Figure 3: Examples of raw GC/DMS spectra of A) the ambient greenhouse background, B) a Lisbon lemon sample without an enclosure, C) an empty enclosure and D) a control Lisbon lemon sample from the enclosure system. Greenhouse background and non-enclosed trees showed no visual differences (A and B), whereas trees inside enclosures were easily distinguishable from the enclosure blanks (C and D).

Figure 4: PCA showed separation along the first principal component (PC1) of enclosure blanks and enclosures containing control Lisbon lemon branches, demonstrating that any possible artifacts of the enclosures did not interfere with plant volatile collection.

Figure 5: Plant samples of control and CLas-infected Lisbon lemons showed separation along the first principal component (PC1), confirming previous results (8) that our GC/DMS system can differentiate the volatile profiles of healthy and citrus infected with *Candidatus Liberibacter asiaticus* bacteria.

Figure 6: PCA of VOC profiles of Lisbon lemons and Tango mandarins showed separation along the first principal component (PC1), indicating our enclosure-GC/DMS system can differentiate these cultivars.

Figure 7: PCA of Lisbon lemon and Washington navel orange VOC profiles differentiate along the second principal component (PC2), showing our enclosure-GC/DMS's ability to distinguish these varieties.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Graphical Abstract