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Analysis of Early Host Responses for Asymptomatic Disease Detection and Management of Specialty Crops

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ABSTRACT: The rapid and unabated spread of vector-borne diseases within US specialty crops threatens our agriculture, our economy, and the livelihood of growers and farm workers. Early detection of vector-borne pathogens is an essential step for the accurate surveillance and management of vector-borne diseases of specialty crops. Currently, we lack the tools that would detect the infectious agent at early (primary) stages of infection with a high degree of sensitivity and specificity. In this paper, we outline a strategy for developing an integrated suite of platform technologies to enable rapid, early disease detection and diagnosis of huanglongbing (HLB), the most destructive citrus disease. The research has two anticipated outcomes: i) identification of very early, disease-specific biomarkers using a knowledge base of translational genomic information on host and pathogen responses associated with early (asymptomatic) disease development; and ii) development and deployment of novel sensors that capture these and other related biomarkers and aid in presymptomatic disease detection. By combining these two distinct approaches, it should be possible to identify and defend the crop by interdicting pathogen spread prior to the rapid expansion phase of the disease. We believe that similar strategies can also be developed for the surveillance and management of diseases affecting other economically important specialty crops.

KEY WORDS: biological regulatory network, differential mobility spectrometry, gas chromatography-coupled time-of-flight mass spectrometry, huanglongbing, induced volatile organic compound, lateral flow microarray, microbe-associated molecular patteru, specialty crops, Twister

I. INTRODUCTION

Rapid, early, and accurate diagnosis in the orchard is essential to counter threats from deadly pathogens. Currently, diseases such as citrus huanglongbing (HLB) are diagnosed by scout teams looking for disease-specific visual symptoms that often appear too late to prevent spread to surrounding trees. To be successful, early diagnosis of diseases such as HLB must recognize complex

ABBREVIATIONS

BRN, biological regulatory network; **DMS**, differential mobility spectrometry; **GC-TOF-MS**, gas-chromatography time-of-flight mass spectrometry; **HLB**, huanglongbing; **IVOC**, inducible volatile organic compound; **LFM**, lateral flow microarray; **MAMP**, microbe-associated molecular pattern; **PCR**, polymerase chain reaction; **PDMS**, polydimethylsiloxane; **PPI**, protein-protein interaction; **SPME**, solid-phase microextraction; **VOC**, volatile organic compound

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interactions among the infectious pathogen, the insect that vectors the pathogen, and the innate host plant response at an asymptomatic stage.[1] Once HLB bacteria have systemically infected a specific tree, it is too late to prevent additional spread of the disease in adjacent acreage by its insect vector, the Asian citrus psyllid *Diaphorina citri*.[2,3] In the case of HLB, it has been shown that high incidence of the pathogen, *Candidatus liberibacter asiaticus*, in *D. citri* can be found in an area well before the onset of symptoms in citrus plants are found and secondary spread has occurred.[4] In parts of Florida where HLB is widespread, growers struggle to afford the cost of regular scout teams, vector control measures, and tree removal. Unfortunately, these practices usually are not sufficient to prevent disease spread, because bacteria can exist in an orchard or vector well before they can be detected by existing polymerase chain reaction (PCR)-based technologies.[4-10] The discovery of presymptomatic biomarkers that track the primary phase of disease infection is essential for detecting primary sources of infection and for controlling secondary infestation with cost-effective and robust surveillance and management of HLB.

Traditional approaches rely on pathogen detection in the field, which is problematic for a disease such as HLB, in which the pathogen may escape detection because: i) the organism is not uniformly disttibuted within the tissues of infected ttees and is therefore easily missed,[11] and ii) long-distance, highly virulent, asymptomatic primary spread of the disease is associated with very low titers undetectable with current real-time PCR technologies.[12] Our strategy focuses on the analysis of host responses that are triggered during infection with specific emphasis on the plant innate immune responses that are induced early during infection and manifest locally at the site of infection and also at a distance.[13] Biomarkers that are associated with such responses are present both locally and at a distance. The innate immune system found in plants, fungi, insects, and primitive multicellular organisms constitutes an evolutionarily conserved defense strategy against diseases and pests.[14]

Recent findings have highlighted remarkable similarities in the innate pathogen defense systems of plants, animals, and insects, components of which are evolutionarily conserved across kingdom borders.[15] Recognition molecules structurally related to microbe-associated molecular pattern (MAMP) receptors discovered in animals are now being discovered in plants, suggesting a common evolutionary origin of pathogen defense systems in higher eukaryotes.[16] In particular, plants have evolved receptors for numerous microbial surface-derived compounds, which induce plant defense responses in both host and non-host plants.[14] MAMPs, the eliciting molecules of microbes, include (glyco)proteins, peptides, carbohydrates, and lipids, all of which can trigger plant defense responses comparable to those observed upon *R*-gene-mediated pathogen recognition in resistant host-plant cultivars.[17] These elicitors bind to recognition receptors that trigger expression of immune response genes and the production of antimicrobial compounds.[18] Intriguingly, many of these elicitors act as general elicitors of defense responses in many plant species.[19] For example, some structural elements of lipopolysaccharides from gram-negative bacteria are potent inducers of plant defense reactions.[20] These findings strongly suggest that plants have acquired and maintained the ability to recognize MAMPs (both lipopolysaccharides and flagellins that decorate gram-negative bacteria).

An example of an early-stage event of the host-pathogen interaction is a "stress condition" similar to the "inflammatory response" that occurs in the host in response to pathogen-associated and -induced virulence factors. These early asymptomatic responses are associated with changes in both host plant and pathogen at the transcriptional (mRNA)[21-23] and the post-transcriptional (proteins, volatile and nonvolatile metabolites) levels.[24-26] They precede the onset of symptoms, when plants activate more self-destructive physiological responses, leading to disease-associated phenotypic changes and metabolic dysfunction due to pathogen virulence factors.[27] The plant response itself leads to negative effects, exaggerating the direct effects of the pathogen or activating mutually destructive responses.

Plants release large quantities of volatile organic compounds (VOCs). In unperturbed leaves, isoprenoids (isoprene and monoterpenes) are the most abundant VOCs.[28] Methanol, acetaldehyde, and C-6 compounds are also emitted in large quantities.[29] However, when plants are subjected to stress, there is a substantial increase in

novel inducible VOCs (IVOCs) that regulate plant responses to their environment, including defense responses to disease, plant-to-plant communication, tritrophic interactions, and ozone quenching.[30,31] In some instances, unique compounds are produced during stress, but more commonly, IVOCs differ from VOCs only in scale and compositional complexity. The above-mentioned classes of VOCs are also generally associated with mechanical wounding or with stress due to insect pests, disease, or abiotic conditions.[32,33]

The analysis of the plant response during this stress condition could provide important clues for: i) designing an early detection at a presymptomatic stage, thus avoiding secondary spread; ii) developing novel therapeutic strategies for rapid recognition and clearance of pathogens before irreversible damage has occurred; and iii) identifying pathways and bioactive compounds that can stimulate repair and restoration of the innate immune response associated with the healthy state. Our sttategy is to identify biomarkers that are derived from either host or pathogen and that are induced at very early stages of infection. We are examining transcripts by expression profiling using a deep sequencing approach with the Genome Analyzer II (Solexa Ltd., now Illumina, San Diego, CA) and the Solid System (Applied Biosystems, Foster City, CA) and volatiles using spectrometry-based systems. At the same time, we are developing in-field detection methods including immunosensory devices to detect infection-associated changes in the profile of both host and pathogen responses using lateral flow microarray (LFM), and for volatiles using Twister sampling with gas-chromatography time-of-flight mass spectrometry (GC-TOF-MS) detection. We are also developing mobile sensors that rely on differential mobility spectrometry (DMS) to detect specific volatile compounds from complex environments such as the tree canopy. These sensors should allow detection at very low levels and at nearly real time. The objective of this article is to present a novel multidisciplinary and integrated approach to developing efficient, cost-effective, and easy-to-use surveillance systems that can identify infected plants by the unique pathophysiology associated with the early, asymptomatic phase of disease.

II. ANALYSIS OF HOST-PATHOGEN RESPONSES

A. Transcriptome and Biological Regulatory Network Analysis

The first step in the plant innate immune response involves the perception of MAMPs or pathogen-associated molecular patterns through pattern recognition receptors at the plant's cell surface.[13,34,35] This system is evolutionarily conserved across kingdom borders.[15-17] In plants, two types of resistance to pathogens can be considered: basal and adaptive. The first, the innate defense, is not pathogen specific and is also sometimes referred to as non-host resistance. Innate defense includes passive defenses such as preformed barrier or toxic compounds synthesized by the plant, as well as defenses based on the recognition of MAMPs. These defenses may include the hypersensitive response and frequently involve basal defensive responses to biotic stress.[17] The second, adaptive resistance, based on the gene-for-gene concept, constitutes the interaction of pathogen-specific resistance (R) plant proteins that interact, directly or indirectly, with avirulence proteins of the pathogen.[34,35]

When plants are subjected to stress, they manifest an "induced stress response" similar to the host "inflammatory response" to pathogen-associated and -induced virulence factors. If the induced stress response is sustained, then injury can occur, leading to irreversible cell and tissue damage. It is critical to identify stresses and act at a presymptomatic stage, because it is often too late to prevent injury and the resulting tissue damage once symptoms appear. During the early stages of this condition, there is activation of certain stress-related genes and pathways commonly associated with biotic stress (host responses to pests and pathogens) and with abiotic stress—environmental extremes such as drought, heat, salt, and wounding.[36-38] These genes are involved in the general early state of stress response.

Other differentially regulated genes at the presymptomatic stage are stress specific and serve as useful biomarkers for the early diagnosis of the plant's health status. These responses include changes in the key metabolic pathways that can be considered important indicators of early stages of infection of known pathogens or abiotic stress.[39,40]

Usually, this stress condition precedes manifestation of disease symptoms. These observations led to the idea that a focus on the analysis of key genes, proteins, metabolites, or pathways could be used to monitor the health status of the plant.[41] These could then serve as biomarkers for both disease detection and for recovery. Specific management strategies employing the spray application of specific bioactive compounds or the expression of specific therapeutics may be employed to successfully restore plants to healthy status and to restore crop productivity and product quality. The identification of key genes, proteins, and metabolites that are early host responses associated with a reversible stress condition using in-field devices is the first step to toward presymptomatic diagnosis. Such identification requires analyzing large data sets obtained through different "omic" studies. Web-based applications are available to query large public databases of information on gene expression, proteins, or metabolites in specific biological contexts. For example, the Genevestigator software package contains new, web-based tools that provide categorized quantitative information about elements (genes or annotations) contained in large microarray databases.[42] The PRIDE database (http://www.ebi.ac.uk/pride/) is a public data repository for proteomics data, and Oliver Fiehn's laboratory maintains a database (http://fiehnlab.ucdavis.edu:8080/m1/) of metabolomic experiments obtained from different species.

A biomarker is formally defined as "a biological characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or responses to a therapeutic intervention."[43] In the context of omics, this definition expands to include a "biomarker profile" as a combination of transcriptomic, proteomic, and/or metabolomic features associated with a specific stress stage or condition of interest. Combined, these features may have biomarker potential and thus assist in diagnosis and therapy.[44] A systems-based approach can define the underlying biological regulatory network (BRN) governing interactions between plants and pathogens. Heterogeneous data sets of transcriptome, proteome, and metabolome data can be integrated to build a BRN that identifies early host and pathogen biomarkers for presymptomatic detection in the field. Protein networks are increasingly

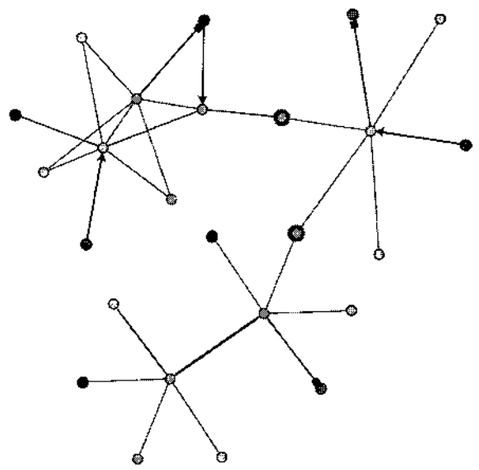

FIGURE 1. A biological network consists of nodes (fixed points) and edges (lines connecting the points). Nodes represent discrete molecular entities: genes, RNA transcripts, proteins including enzymes, and regulatory factors. Edges represent interactions, e.g. activation, inhibition. Hubs are high-degree nodes from which numerous edges radiate. Other important nodes provide crucial links between otherwise disjoint regions of the network, e.g. the two nodes circled in red.

serving as tools to elucidate the molecular basis of identifying disease-related subnetworks.[45,46]

In the complex network made of nodes and edges, nodes are substrates (e.g., genes, proteins, or metabolites) and edges represent interaction types (e.g., expression, inhibition, or catalysis) and have edge weights proportional to the strength or statistical significance of the interactions (Fig. 1). The networks can be visualized using existing software packages (the Bioconductor package of R, Centibin, Graphviz, Pajek, Cytoscape), which also calculate many fine-grained, statistical properties of the networks. Interpreting the significance of statistical features benefits from recent advances in understanding the structure and function of networks, such as the role of feedback during network evolution[47] and the statistical properties of random networks composed of multiple types of nodes and edges (E.A. Leicht and R.M. D'Souza, unpublished data, 2009). Analyzing the properties of constructed BRNs, it is possible to identify "hub" proteins that regulate large regions, feedback loops between pathways, and nodes of

high "between-ness" (information brokers connecting otherwise disjointed regions). A platform-independent software system, "Gaggle,"[48] and a web-based cloud server named "Bioshare" (http://bioshare.genomecenter.ucdavis.edu) integrate various bioinformatics software used to manage and share diverse data sets.

Understanding stress-related BRNs allows the identification of biomarkers that include key proteins, genes, and/or metabolites suitable for early diagnostics and possibly as targets for therapeutic treatments. Comparing healthy and asymptomatic plants will aid in the identification of genes regulated at the early stages of infection. These genes would serve as important biomarkers for early disease detection. Comparing transcriptome profiles from asymptomatic and symptomatic fruits will lead to the identification of genes that report the onset of disease symptoms. After a decade of analysis in model organisms, protein-protein interaction networks have been used to gain insight into the ability of cells to continue their physiological functions under stresses.

We have deduced a protein-protein interaction (PPI) network for citrus based on Arabidopsis.[49] Additionally, we have analyzed differentially regulated genes from diverse microarray data sets in citrus derived from mature leaves affected by HLB disease,[23,50] and peel tissues of fruits affected by "puff," a disorder stimulated by abiotic stress (our unpublished data).

Our analysis focused on the PPI networks involving these differentially regulated genes using Cytoscape software to visualize the network (Fig. 2).[51]

The PPI network was derived from two diverse citrus microarray data sets, one detailing the citrus response to puff disorder and the other a recently published data set of temporal responses to HLB disease in citrus. The HLB data set was obtained by analyzing differentially regulated genes in two different microarray data sets from the analysis of mature leaves affected by HLB disease.[23,50] The puff microarray data set was obtained by analyzing peel tissues of fruits affected by this physiological disorder stimulated by abiotic stress (our unpublished data). We identified differentially regulated genes encoding highly interactive proteins belonging to the two conditions. Interestingly, the most highly interactive protein, HSP 81-1 (heat-shock protein 81-1), expected to be associated with puff disorder, was also differentially regulated in the HLB response (HSP 81-1). Further, two hubs of differentially regulated genes were related to starch and sucrose metabolism, a pathway that is highly associated with HLB disease. These genes encoded a plant glycogen-like starch initiation protein (ATG18660, PPI number = 97) up-regulated in HLB disease and a carbohydrate transmembrane transporter (AT5G26340, PPI number = 71) commonly regulated in HLB and puff stress response. The latter is linked with the up-regulation of the starch pathway observed in early and late response to HLB.

The down-regulation of the copper chaperone for superoxide dismutase 1 observed during the HLB response[50] could be also associated with citrus susceptibility, because this protein was a hub in the PPI network. Genes encoding for hub proteins or for proteins that link hubs are biomarkers directly detectable in the field with LFM. The network analysis provides important information to test for correlations with observed IVOC profiles. We have multiple chemical-sensing methods that together provide us with a large database of observed volatile profiles from distressed plants. We believe that an integrated approach composed of analyzing emitted plant volatiles and BRNs at the transcriptome and metabolome levels may yield important insights for early disease detection before symptoms appear.

B. Field Detection of Host-Pathogen Transcripts Using LFM

The analysis of transcripts in the field has been greatly facilitated by the development of a novel LFM technology that enables rapid, hybridization-based nucleic acid detection using an easily visualized colorimetric signal (Fig. 3).[52] Patterned by a noncontact picoliter deposition system, oligonucleotide microarrays are fabricated on miniaturized lateral flow chromatography nitrocellulose membrane supports. The resulting LFM devices exhibit remarkably rapid (< 2 min) hybridization times and a 250-amol detection limit comparable to microarray detection schemes requiring elaborate laboratory instrumentation.[53,54] The success of efficient presymptomatic diagnosis relies on the ability to obtain the informative biomarkers from

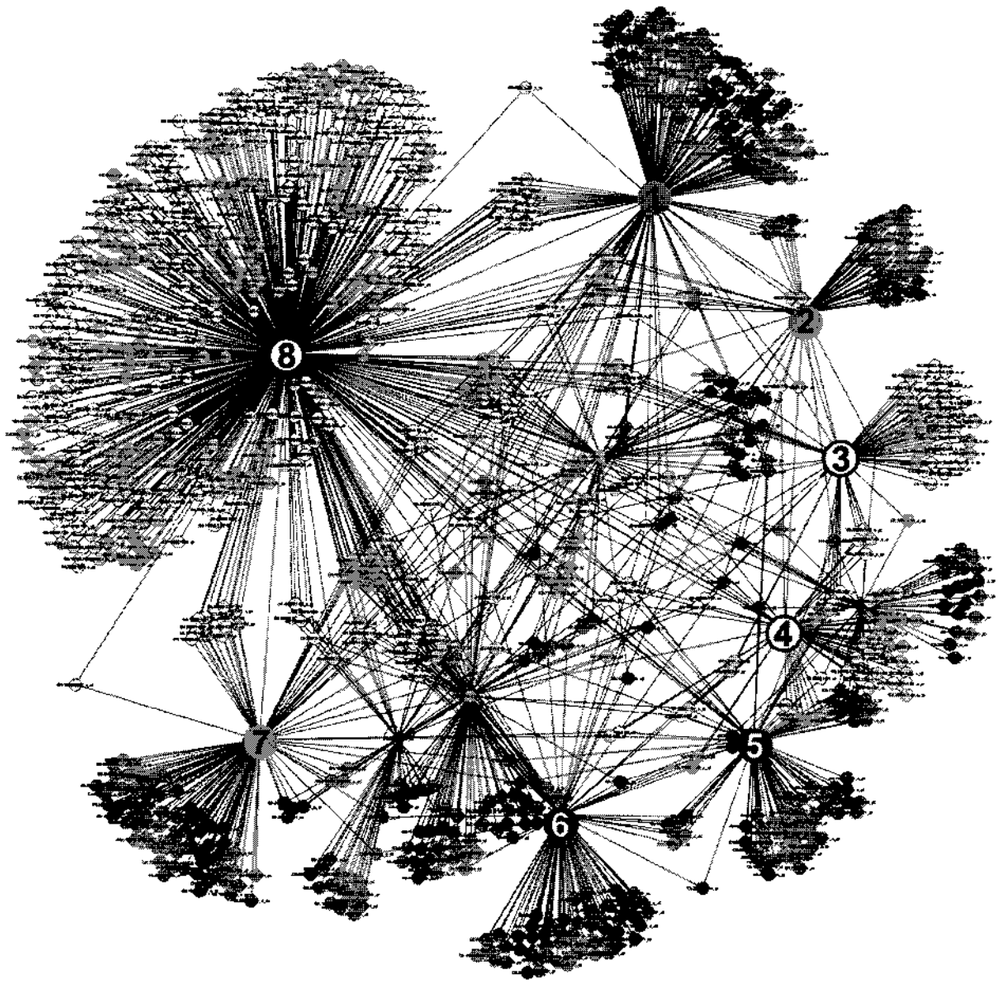

FIGURE 2. PPI network in citrus derived from a knowledge base for Arabidopsis.[49] Microarray analysis related to citrus response to HLB disease[23,50] and to peel tissue of fruits with the puff disorder (unpublished data) were compared and analyzed using Cytoscape software. Highly interacting (hub) proteins were grouped depending on the five groups differentially regulated between puff and HLB disease. Yellow nodes represent "first neighborhood proteins" of commonly regulated genes between HLB disease and puff disorder, and belong to a general stress-related condition.

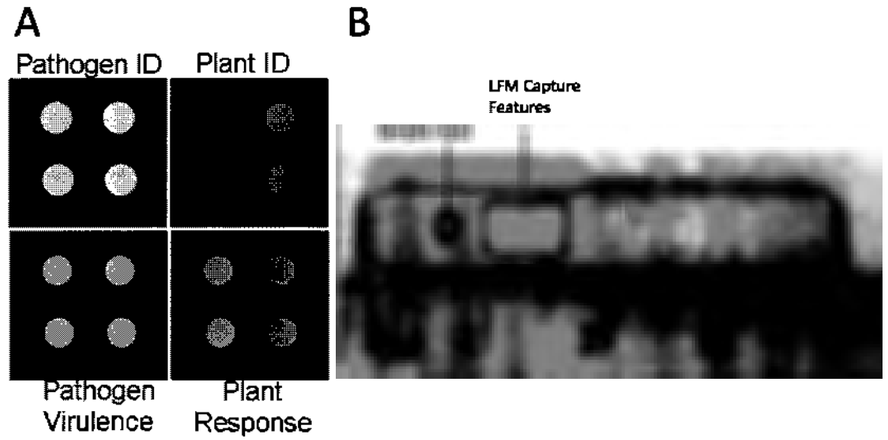

FIGURE 3. A, A simple microarray layout can be employed to simultaneously evaluate a sample for the presence of genetic targets derived from the host as well as from the pathogen. The multiplex capacity of such an approach allows concomitant pathogen identification, virulence determination, and host disease state evaluation through the use of multiple genetic signatures and biomarkers. B, Miniaturized lateral flow devices patterned at microarray density allow sequence-specific, hybridization-based detection of 250 amol of nucleic acid analyte in under 2 min. The speed, multiplex capacity, and low cost of these devices are highly desirable characteristics for a field-deployable yet informative and robust molecular diagnostic test.

plant host and pathogen, and to detect them in a rapid, sensitive, and cost-effective manner.

Coupled with exponential nucleic acid amplification methods employing isothermal reaction schemes, the LFM can be used to eliminate the need for most, if not all, of the laboratory instrumentation traditionally required for molecular diagnostics. Isothermal amplification is accomplished by incubating samples at a constant temperature, allowing complex thermal cycling requirements associated with the polymerase chain reaction to be circumvented. The sensitivity and specificity of this approach allows trace probative nucleic acids to be detected in the presence of a large excess of nonprobative nucleic acid species. Indeed, the LFM employed for the detection of RNA from a bacterial pathogen, when present in a two-million-fold excess of human total RNA, has shown that LFM can rapidly and accurately detect amplicons in complex nucleic acid samples.

The capacity to rapidly accomplish sequence-specific detection of genetic signatures and biomarkers will enable a multifaceted approach to citrus disease detection and identification. By allowing the simultaneous interrogation of host biomarkers for the early assessment of plant health and pathogen genetic markers for identification and virulence evaluation, LFM-based methods will provide robust, early disease management decision support.

C. Field Detection of Plant Volatiles Using Differential Mobility Spectrometry

The DMS is a novel chemical/biological sensor of volatile compounds (Fig. 4).[55-59] This device is quantitative and can detect some chemical and biological materials at parts-per-trillion concentrations, far lower than other ambient detection methods. The DMS is a tunable ion filter that differentiates charged ions due to mobility differences in an electric field. A gas sample is introduced into the spectrometer and ionized using one of several methods. A carrier gas moves the ions through the drift tube portion of the sensor, where the sample is exposed to an asymmetrical, oscillating electric field. A "steering" compensation voltage counterbalances the mobility of the ions so that they can exit the drift tube region, where they move toward a pair of detectors that measure the abundance of positive and negative ion species. The steering or compensation voltage for each ion species yields

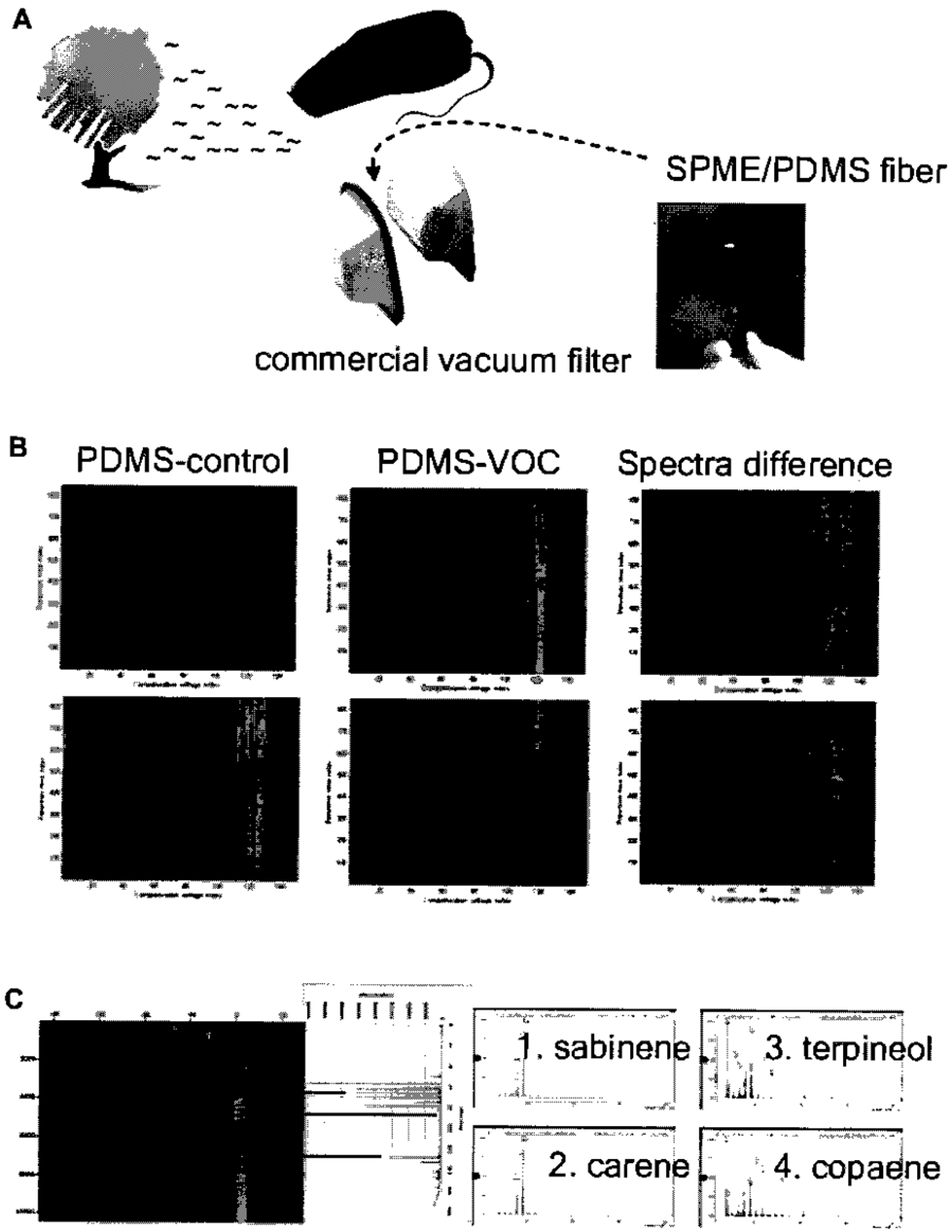

FIGURE 4. A, One potential VOC collection method involves using traditional vacuum sources to pull volatile compounds from tree canopies and using sorbent polymer materials such as PDMS to sequester and concentrate compounds for subsequent analysis. B, PDMS absorption showed that VOCs could be sampled from immature citrus trees after a 10-min sampling regime (n = 3 replicates for each condition). The top (positive ions) and bottom (negative ions) spectral plots were averaged over these replicates, and the control blank background average was subtracted from the VOC sample average (far right). C, GC/DMS spectra can be cross-correlated with traditional GC/MS data to validate compound libraries.

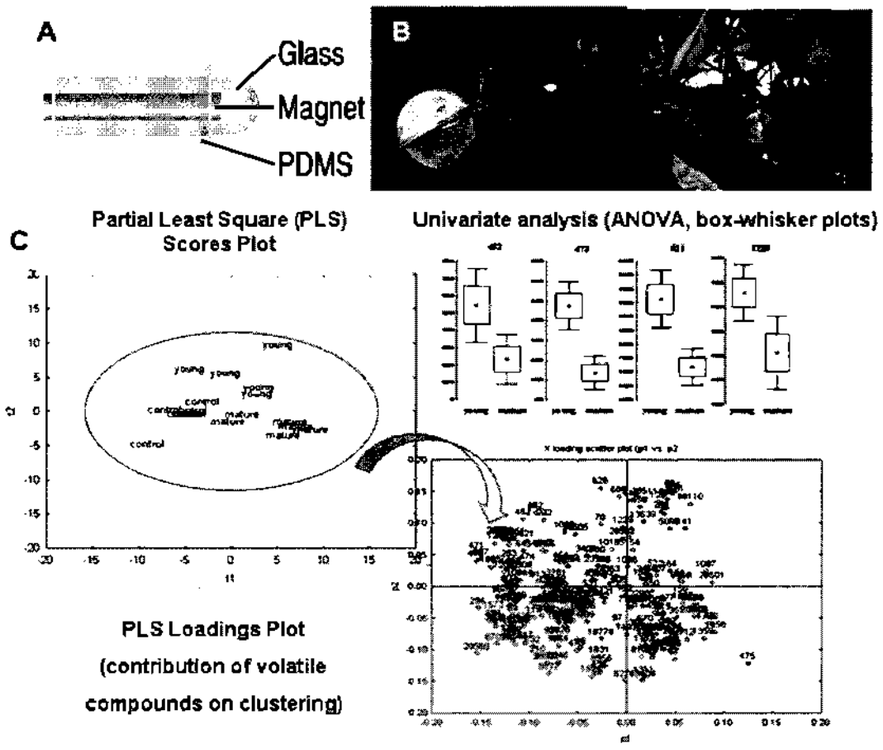

FIGURE 5. A, Representation of the Twister device. B, The instruments used in the field (left panel) and a Twister suspended in a protective mesh holder hung in a tree (right panel). C, Analysis of the data obtained: ANOVA and partial least squares scores and loading plots showed that young and mature leaves were clearly distinguishable by their VOC profiles.

specific information about its chemical identity, allowing compound identification.

In our current sampling method, VOCs are collected by two different sampling regimes from ambient air within the citrus tree canopy. The first method uses commercial vacuum systems to sample large volumes of canopy air, which pass over an absorptive substrate such as polydimethylsiloxane (PDMS) to collect and concentrate volatile compounds (Fig 4A). The second method exposes an absorbent solid-phase microextraction (SPME) polymer fiber to specific citrus leaves within the canopy, absorbing VOCs from a smaller subset of the tree's overall metabolic profile. The PDMS or SPME polymers are analyzed using either GC/DMS or GC/MS instrumentation.

The first method has been implemented in a short pilot trial to test its efficacy. Briefly, we created PDMS thin films that were 5 mm^3 using commercial elastomeric and curing solutions (Sylgard 184, Dow Corning, Midland, MI). To fully prepare the films, the PDMS samplers were heated to 200°C to promote complete polymerization and remove any residual background VOCs absorbed onto the films. The PDMS samplers were then placed into a commercial vacuum system (SPV1800, Black & Decker, Towson, MD) and the vacuum was turned to the highest setting for 5 min to collect VOCs from the ambient canopy of an immature naval citrus tree (n = 3). Each sampler was then immediately placed into a 10-mL borosilicate vial sealed with a Teflon septum and heated to 200°C for subsequent analysis. An 85-μm-thick polyacrylate SPME fiber (Supelco, Bellefonte, PA) was used to sample the headspace above the PDMS for 30 min, and then introduced into a 5 mL/min N_2 carrier gas to be analyzed in the GC/DMS, with a GC-heating protocol that moved

from 30°C to 200°C at a rate of 10°C/min. The DMS compensation voltage was scanned from −43V through +15V every 14 msec. The positive and negative ion spectta were collected for each sample, and the three replicates were signal averaged. Representative spectra traces are shown in Figure 4B.

Figure 4C shows a correlation between the GC/MS spectra and the GC/DMS output for building a biomarker library. Leaves from "Valencia" orange trees were removed and put in two sealed glass vials. Two SPME fibers were used to sample the vial headspace for 1 h to collect the VOCs. After sample collection, simultaneous injections were made on GC/MS and GC/DMS instruments. The GC/MS chromatogram was searched for chemicals using a standard NIST Mass Spectral Database v2.0 library.[69] The individual peaks on the chromatogram were aligned with the output spectra of the DMS. This method allows us to: i) build a library of chemical compounds in the DMS based upon the GC/MS data, and ii) locate biomarkers in the GC/DMS signal space that are not represented in the GC/MS data sets due to sensitivity differences.

GC/DMS data sets can be analyzed a variety of different ways, and we have successfully employed a variety of algorithms and mathematical approaches to interpret our data sets. One method to analyze two-dimensional GC/DMS data is to convert the data into one dimension either based on retention time or compensative voltage.[60] Afterward, various chemometrics and machine-learning methods can be applied to reveal the rules hidden behind a complex biological system. Another method is to apply two-dimensional signal analysis approaches directly to the GC/DMS data. In a recent fruit disorder diagnosis study, we successfully inttoduced two-dimensional wavelet analysis to extract pertinent features of GC/DMS data that accurately diagnosed problems by applying support vector machine to the extracted features.[60] For biomarker detection, we optimize by selecting the most differentiable or representative biomarkers. Genetic algorithm and simulated annealing are two typical approaches for this detection problem.[61] Chemometrics and machine-learning methods, including principal component analysis, principal component regression, partial least-squared regression, neural networks, and a support vector machine, play a key role in this biomarker searching process, evaluating the properties of selected biomarker candidates.[60] Our experimental plan is to examine signal differences in the GC/DMS spectra and confirm those biomarker identities from the corresponding GC/MS signals to build putative biomarker libraries (Fig. 4C). Putative biomarkers will be identified for more extensive field tests. Although DMS testing in the field has not yet been performed, the platform itself can be tuned and redirected for use in different agriculture industries to detect devastating plant diseases.

D. Profiling of Volatiles Using Twister-GC-TOF

Twisters are magnetic stir bars coated with PDMS that can be easily deployed as passive volatile samplers in the orchard (Fig. 5). Twister samplers offer much higher capacity than SPME fibers because of their thicker PDMS coating (25–100 μL PDMS per bar vs. 0.5 μL per fiber), and provide higher sensitivity at shorter sampling times. Another 10-fold increase in sensitivity can be obtained using two-dimensional GC × GC separation in the chemical profiling of emitted plant volatiles for early detection of IVOCs in response to infection by several pathogens. A SetupX/BinBase database for combining study designs with a mass spectral metabolomic database has been developed[64,65] and is currently being expanded to include volatile compounds. Almost 1700 volatile compounds associated with plant defense against pests[24,66,67] have been isolated from more than 90 plant families, and constitute about 1% of plant secondary metabolites, including fatty acid and amino acid derivatives, terpenoids, and phenylpropanoids.[68] We have deployed Twisters to analyze volatile compounds emitted by young and mature leaves in citrus. Partial least squares and analysis of variance showed that young and mature leaves were clearly distinguishable based upon their VOC profile (Fig. 5). Currently, the development of a volatiles database containing host emission data, mass spectra, and other metadata such as biological species and stress to enable correlation of emissions patterns with specific molecules is a priority for the enhancement of the Twister technology.

III. CONCLUDING REMARKS

We have highlighted a translational genomics approach integrating novel rapid detection devices (DMS, Twister-GC-TOF, LFM) to build a comprehensive disease surveillance and management system for high-priority diseases affecting specialty crops. The application of novel detection devices that analyze IVOCs and transcriptomic changes associated with early host responses to pathogen attack can be correlated with the deep transcriptome profiling technologies to create a BRN to interpret and define targets for early detection and management. These integrated methodologies could be part of a real-time platform that can be used to monitor plant health status, determine the presence of a stress state, and verify the effect of crop management actions. Application of this approach is under development for HLB disease, and the expectation is that it will be easily adapted to other high-priority diseases of specialty crops.

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