

Sandia National Labs Microneedle and microGC Tech

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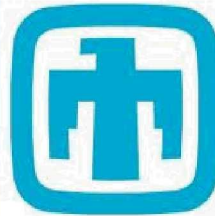
Outline

- Sandia Background
 - Biology departments
 - Technology development
- ROOTS Program (DOE/APRAe)
 - Microneedle
 - Background
 - Plant phenotyping with impedance spectroscopy and metabolite sensors
 - MicroGC platform
 - Background
 - Plant/soil phenotyping
- Disease Detection
 - Literature on VOC detection
 - Literature on microneedle based methods

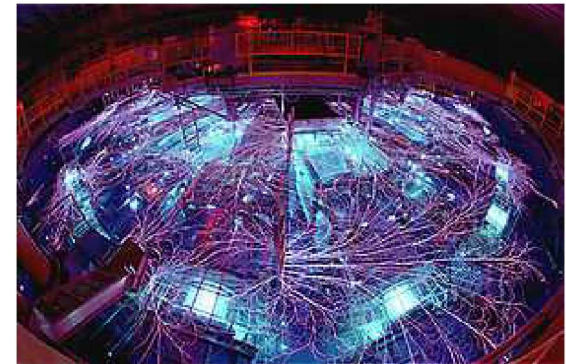


Sandia National Laboratories

- At Sandia, national security is our business. We apply advanced science and engineering to help our nation and allies detect, repel, defeat, or mitigate national security threats. Our national security mission has grown from responding to the threat of the Cold War to countering a range of threats - some nuclear, others involving chemical and biological weapons of mass destruction, and other acts of terrorism.



**Sandia
National
Laboratories**

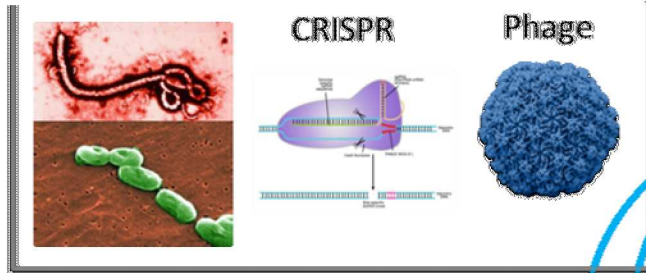


- Largest national lab
- ~10,000 employees at 2 main sites (Albuquerque, NM and Livermore, CA)
- FFRDC (federally funded research and development center), \$3.1 billion in FY16
- Research areas include NW, energy, cybersecurity, national security, and chem/bio detection

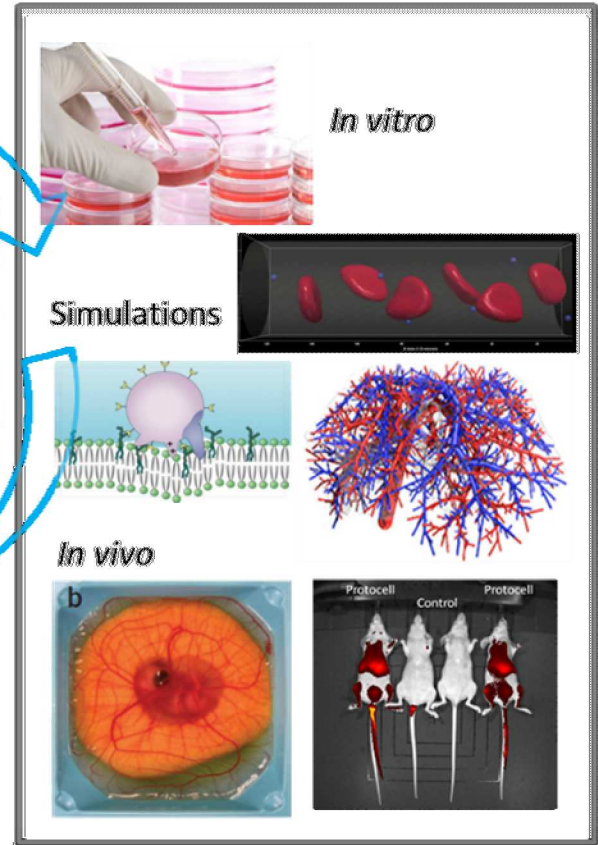


Biology Work at Sandia: NanoCRISPR Grand Challenge

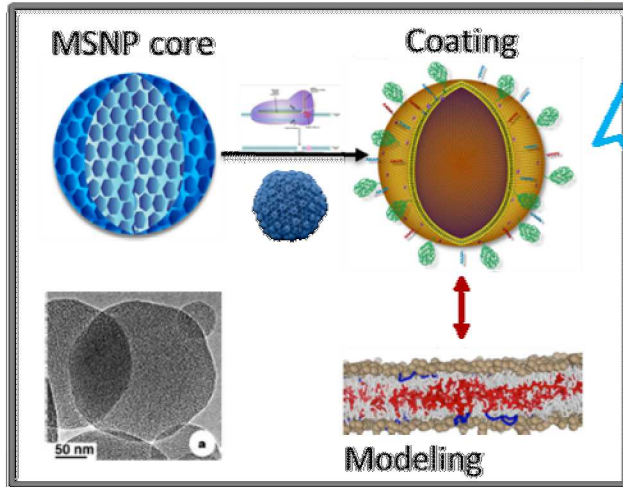
Targeting pathogens with CRISPR



Biodistribution and targeting

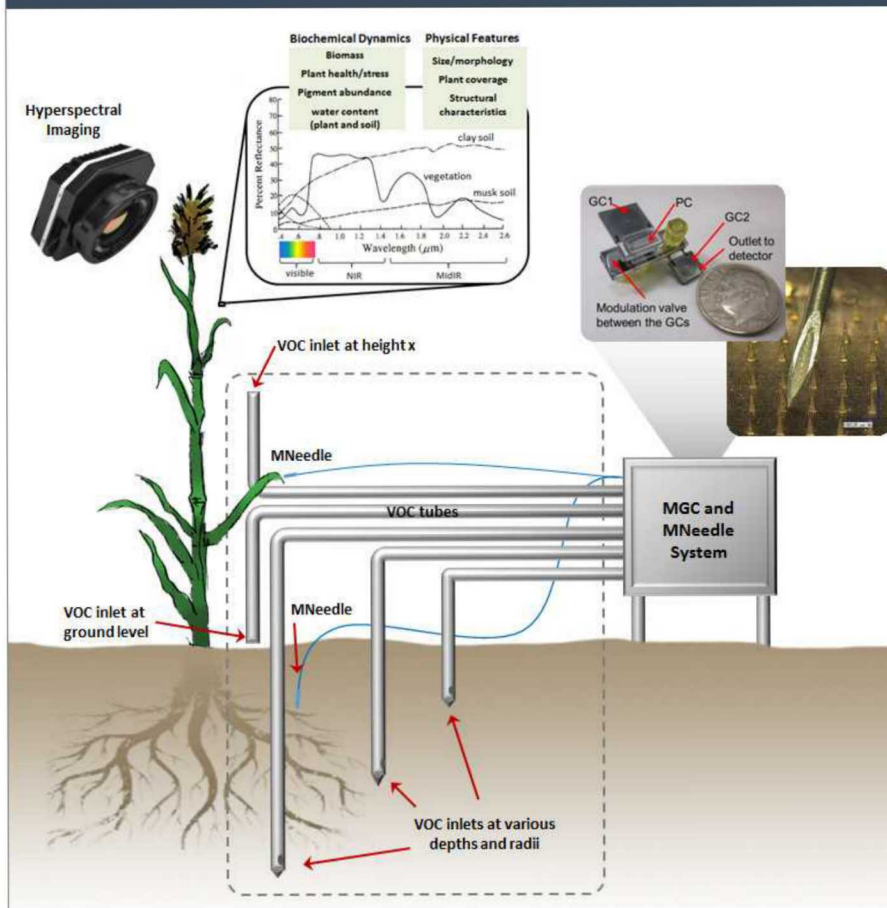


NanoCRISPR assembly



Vision of ROOTS Program

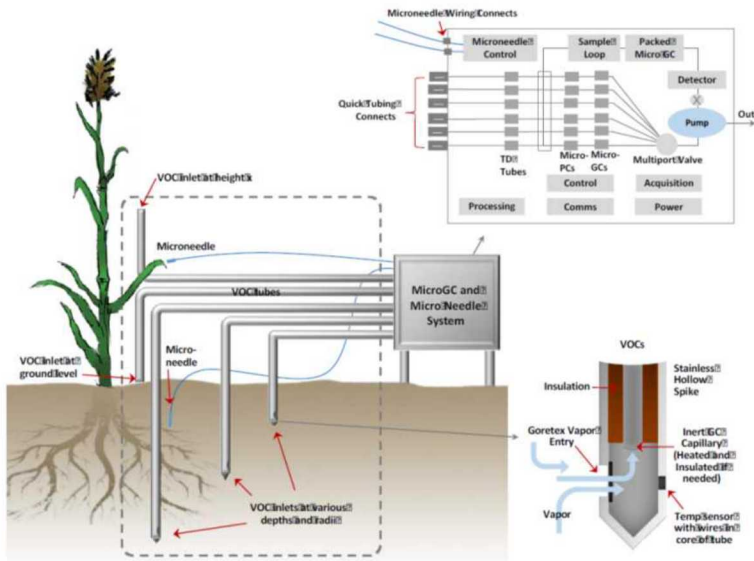
Data, Computation, Modeling, Toolkits



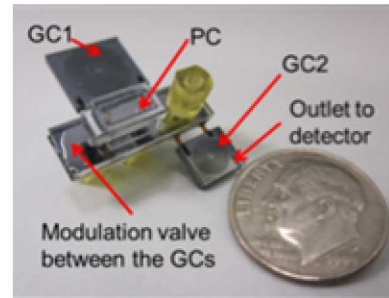
- ROOTS program funded via DOE/ARPAe
- ROOTS: rhizosphere observations optimizing terrestrial sequestration
- Goal is to increase both CO₂ uptake in soils and agricultural productivity

Current ROOTS Project Overview

Proposed Sensor Suite

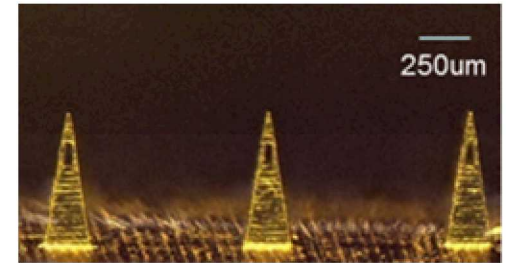


Portable Chemical Sensing



Smallest wearable GC sensor system

Wearable Bio/Chemical Sensing



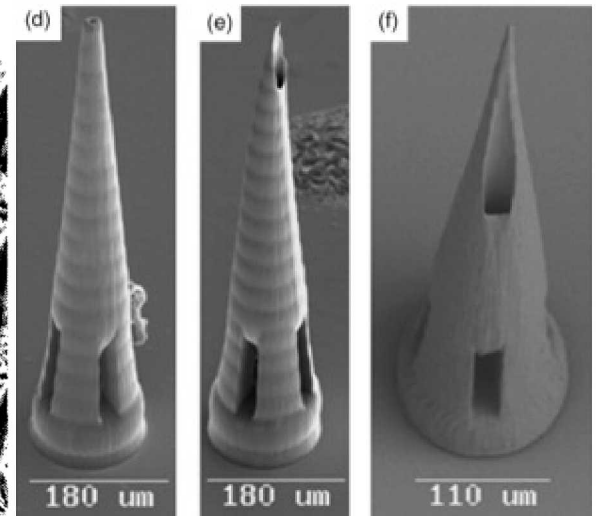
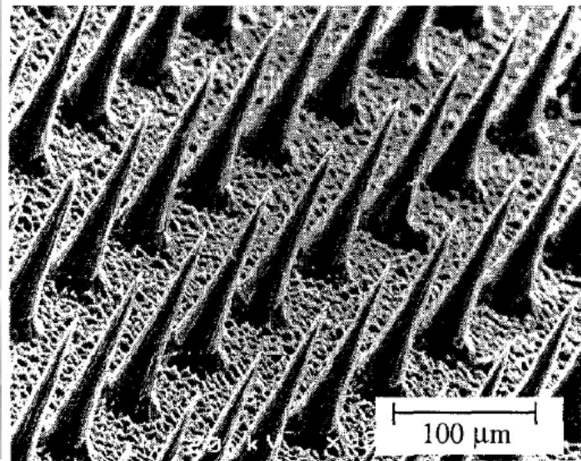
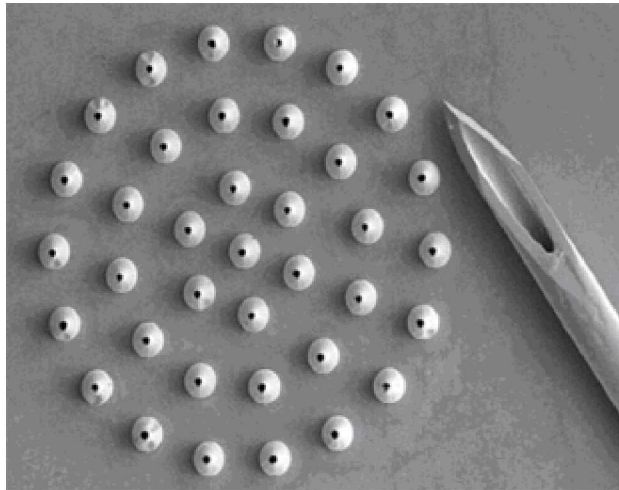
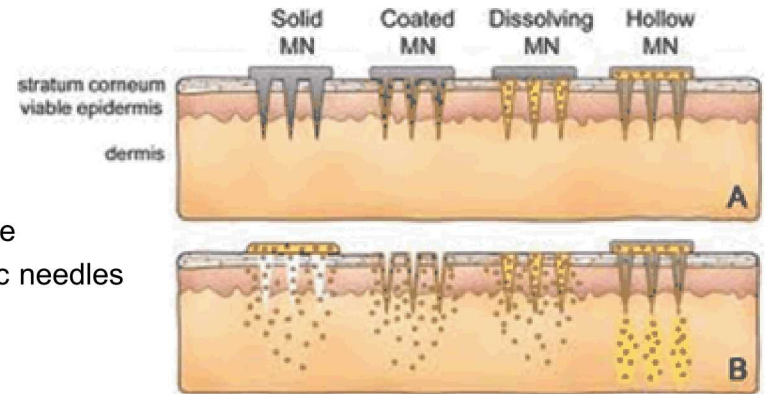
In-situ detection and fluid extraction systems

- Funded by ARPA, ROOTS program
- Micro-needle sensors, gas phase VOC detection, and hyperspectral imaging
- Monitoring sorghum plant health and root system in drought conditions
- Micro-needle goal: conductivity sensor, photosynthesis monitoring, and turgor pressure



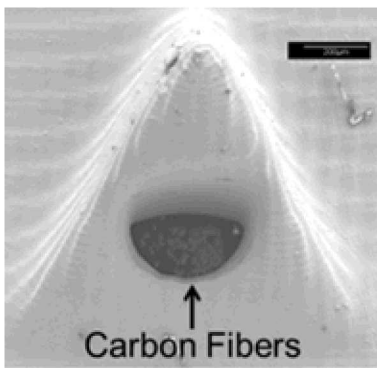
Microneedles

- Initially developed for drug delivery to overcome stratum corneum barrier
- General requirements: height <1000um and aspect ratio > 3:1 height: base
- MN's are associated with significantly less pain than 26 gauge hypodermic needles
- Can be self-administered

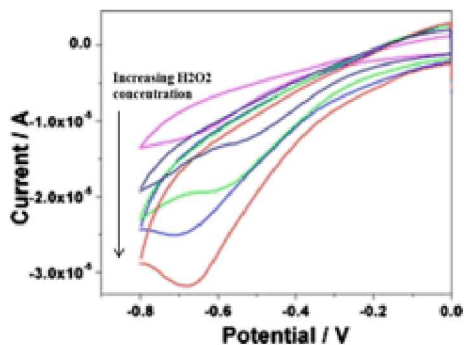


Our Previous Microneedle Work

Small Molecule Detection

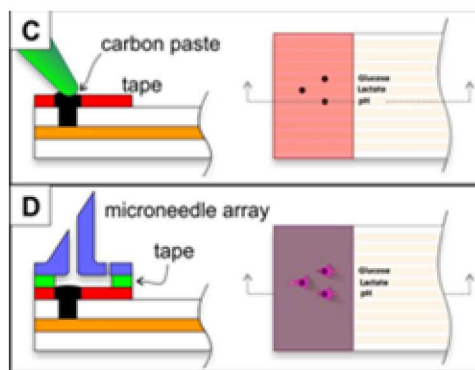


Fabricated with Microstereolithography

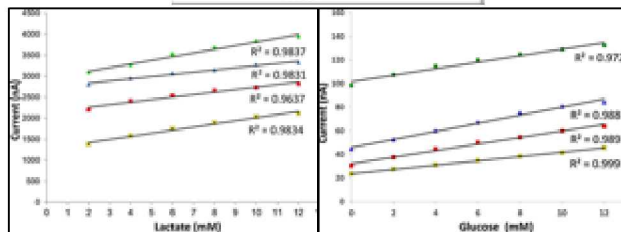
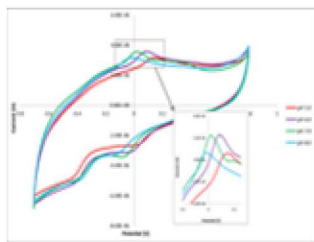


Detecting reactive oxygen species (H₂O₂)

Enzymatic Sensors

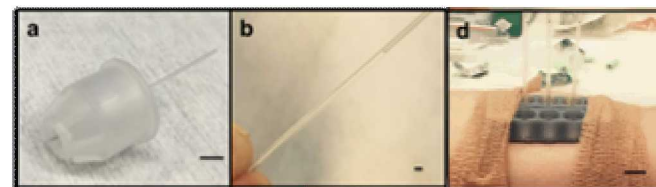


Schematic of device

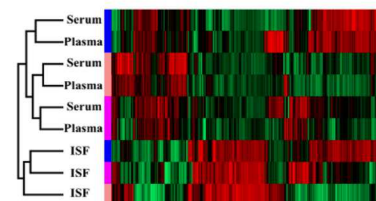


Multiplexed detection of lactate, pH, and glucose

Fluid Extraction and Analysis



Extraction of interstitial fluid (ISF) from humans



B Proteomic Analysis of ISF

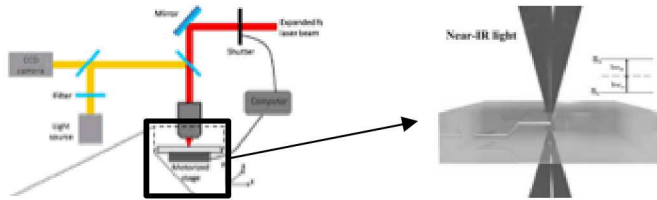


RNA Analysis of ISF



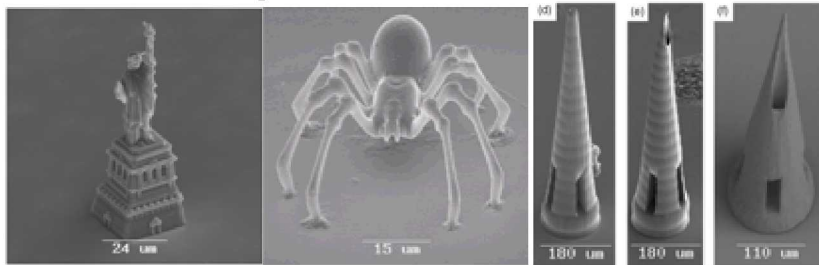
Microneedle Fabrication

Additive Manufacturing Method: Two Photon Polymerization (2PP)



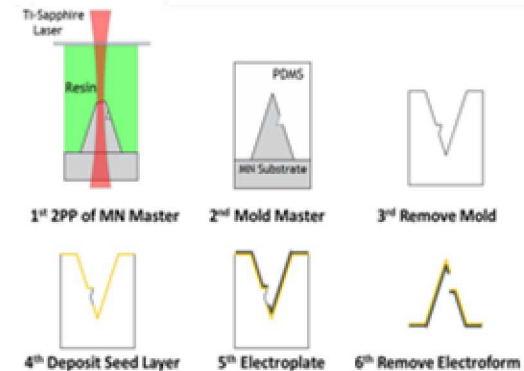
-Two photon polymerization with (2PP) with laser direct write makes the microneedle master structures

Examples of 2PP Resolution



- Hollow microneedle fabrication of metal needles is very challenging
- Most material used for hollow microneedle (polymers, SiO₂) cannot withstand repeated insertion in plants.
- Fabrication process combines two photon polymerization, molding, and electroplating for creation of user defined metal microneedles
- Larger scalability, one master can make dozens of molds

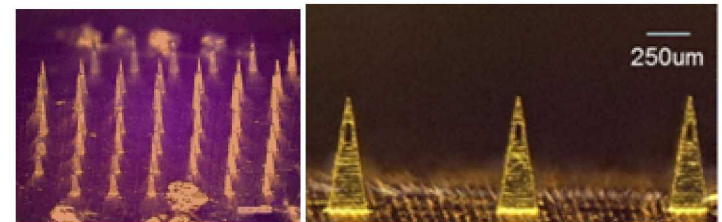
Hollow Metal Microneedle Fabrication Process



Flexibility of Metal Microneedle Array Substrate



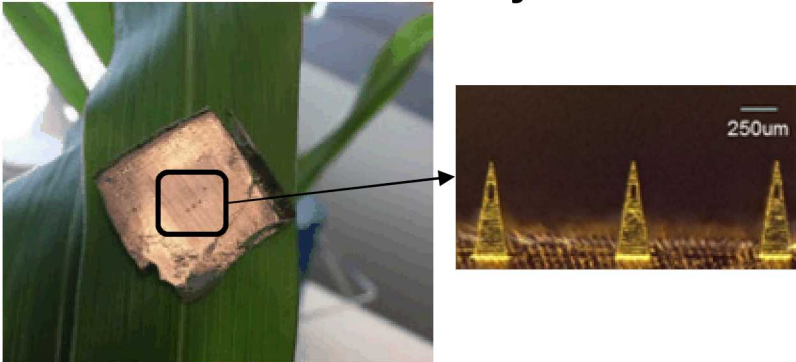
Hollow Metal Microneedle Array



Microneedle Insertion Kinetics and Adhesion to Sorghum Leaves

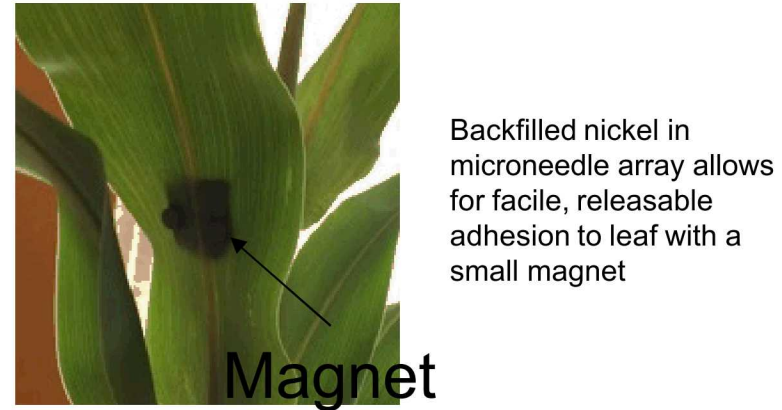
In collaboration with Dave Hanson's group (UNM)

Microneedle Array Size



Array facing up on sorghum leaf to show scale

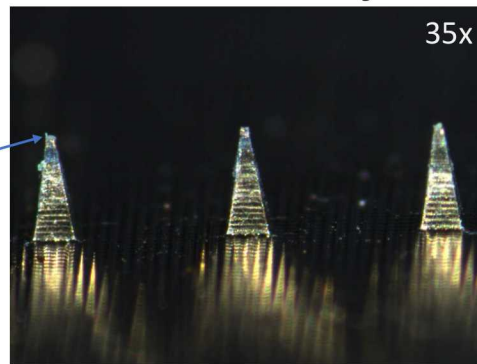
Magnetic Adhesion to Leaf



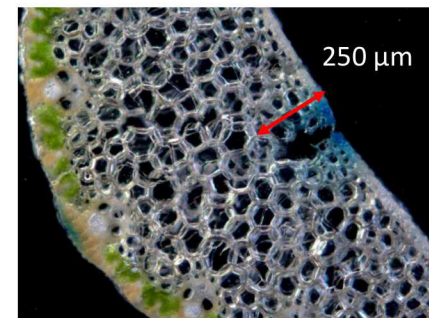
Insertion in Leaf Mid-Rib



Microneedles Left in Leaf for 10 days



Cross-section of Leaf Post Microneedle Insertion

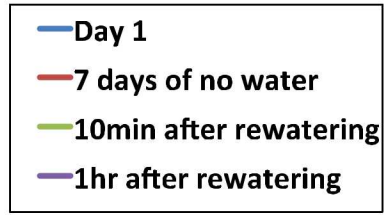


*Needles coated with blue dye to help visualize Insertion site during sectioning

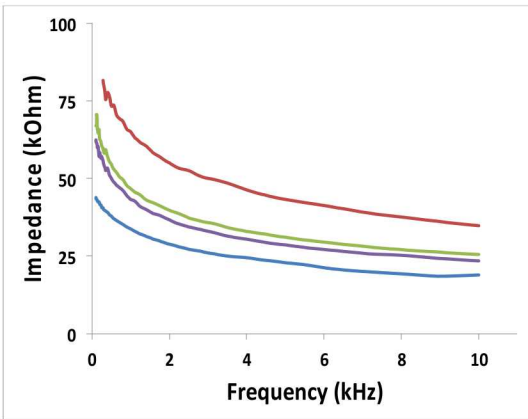
Microneedles were capable of repeated insertion into sorghum tissue without deformation and the microneedle array shown above has been used >30 times without significant damage.



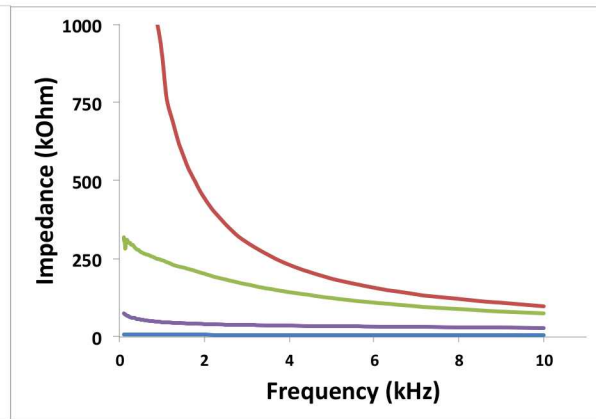
Microneedle Impedance Sensor: Plant experiencing drought and rehydration



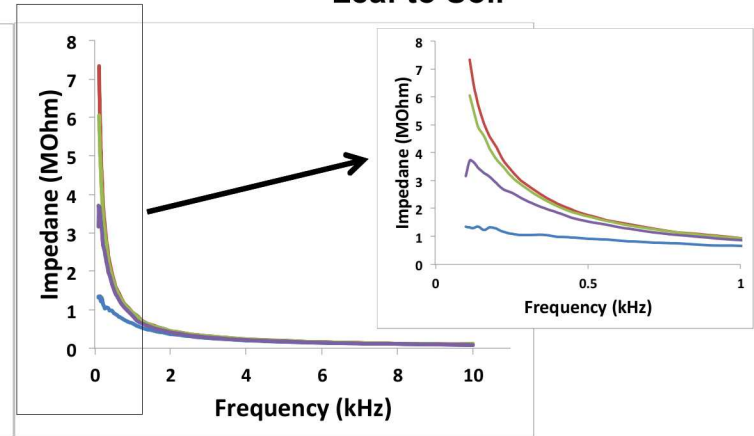
Stalk to Soil



**Microneedle:
Root crown to Soil**



**Microneedle:
Leaf to Soil**



-A typical impedance scan took 2min and since the magnetic holder held the microneedles in position we put the sorghum plant through a week long drought in the greenhouse, with the probes in their original positions. The impedance scans were retested at each location and then the plant was watered. Impedance scans were measured again at two time points following watering.

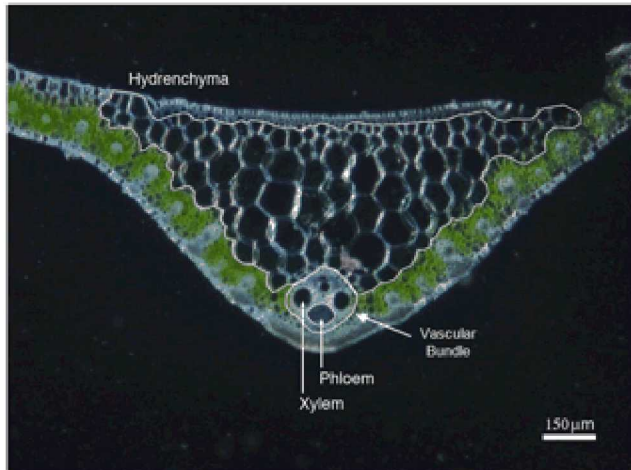
-Compared to the standard method each microneedle probe and location was capable of the drought conditions and the rehydrating process. Leaf measurements showed a large range of impedances at low frequency, possibly due to the slow rehydrating process through cell walls, which may be useful for assessing plant health during stress.



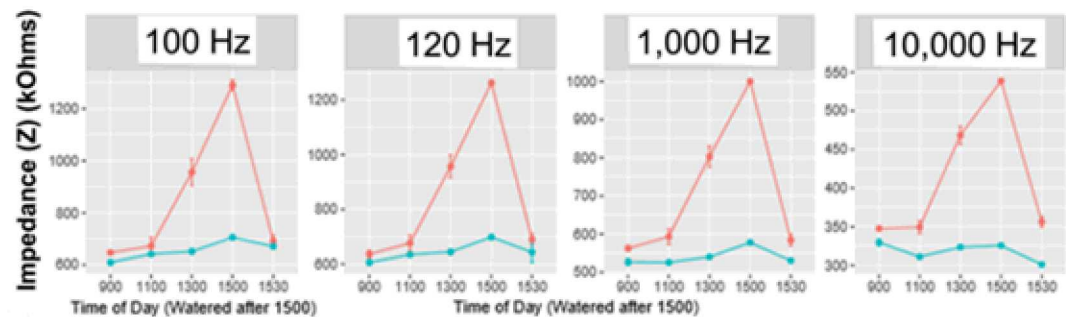
Targeted Tissue Impedance Measurements

- Electromolded microneedles were used to probe the adaxial (AD) and abaxial (AB) surfaces of sorghum leaves during a short-duration drought experiment. AD-to-AD and AB-to-AB impedance measurements were made to measure the vascular bundles and the hydrenchama and preliminary results indicate an impedance increase in the vascular bundles while the hydrenchama remained relatively stable. Future experiments will extend the duration of the measurement and attempt to correlate the impedance values with water potential.

Cross-section of Leaf Stem



Frequencies Tested

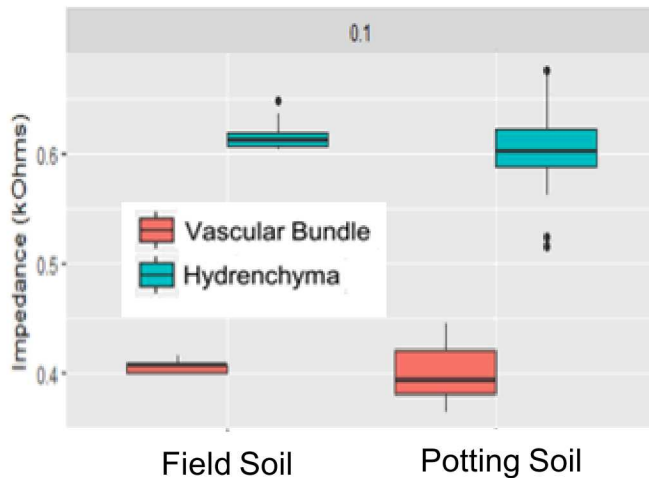


Red: Vascular Bundles
Blue: Hydrenchama

Targeted Impedance Methodology

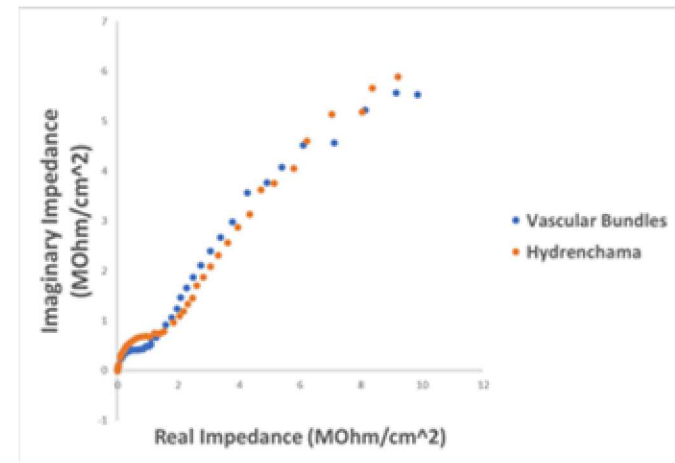
- Our current microneedle impedance experiments are focusing on increasing the sample size of the drought experiments, testing the effect of different soils on measurements, working on the equivalent model circuit, and building a portable microimpedance analyzer to begin taking data in the field and at a larger scale.

Soil Influence on Impedance



-Initial results indicate the targeted impedance method can eliminate the influence of soil type, which is a challenge for some existing plant impedance methods

Experimental Data for Model Circuit



-Initial full frequency scans have been taken on each tissue type and indicate the equivalent circuits are similar
-Fitting the data to a model will help determine what plant physiology or environmental is driving the impedance change (ie capacitive shift > cell membrane degradation) which is vital for field tests

Coaxial Microneedle Sensor Design and Fabrication

Schematic of Coaxial Microneedle Top-Down View

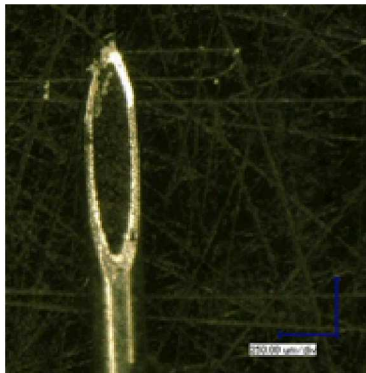


- Biosensor
- Dielectric
- PEN Needle
- Reference Electrode

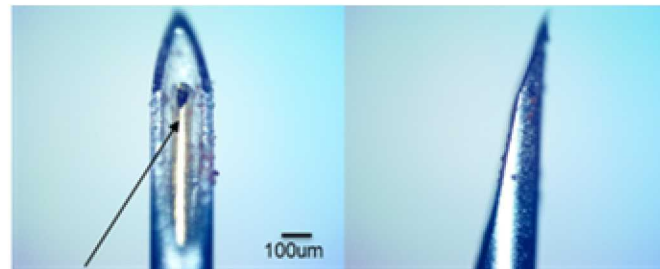
- Coaxial microneedle sensors are designed to place the sensor as close to the analyte source as possible while using the needle to protect the sensor
- Interior biosensor can be either a wire or paste/ink
- Reference and counter electrode made with same fabrication methodology
- Puncture tests in sorghum will determine optimal electrode material

Two Coaxial Sensor Configurations Tested

Carbon Ink/Paste Packed Needle



Microneedles w/Au Microwire



Electrically insulated
60um Au wire

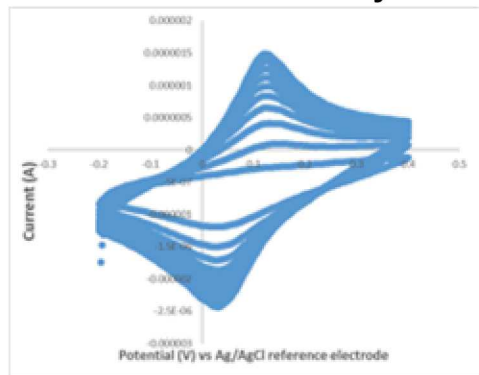
Front view

Profile view

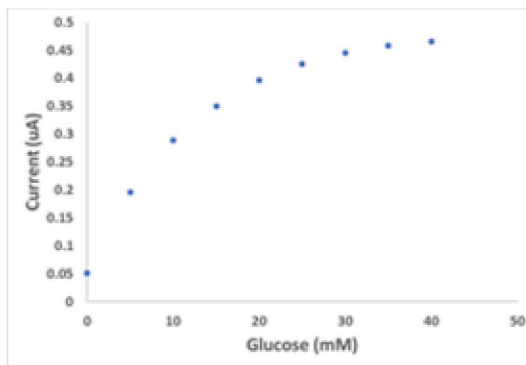
Metabolite Microneedle Sensor

- The carbon ink coaxial microneedle glucose sensor was tested in solution across a concentration range based on the literature cited values of glucose in sorghum leaves (1-40mM). We suspect hole punch measurements average the glucose concentration across the leaf and that values in the phloem will be much larger and future experiments will test a serial dilution across a wider range of concentrations.
- Detection of glucose was performed by a multi layer enzymatic biosensor. In the first layer, a mediator (prussian blue (PB)) was electrochemically deposited (left plot showing layer growth) on the carbon ink working electrode that acts as a catalyst for hydrogen peroxide reduction, which is a by-product of the glucose oxidase (enzyme) glucose interaction. The outer layer is an electrochemically polymerized polymer (polyphenylenediamine) to entrap glucose oxidase.

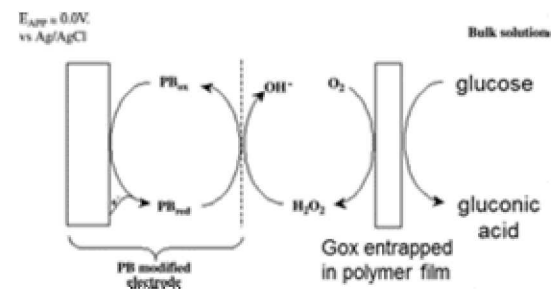
Electrochemical Deposition Of Prussian Blue Layer



Glucose Detection Across Relevant Sorghum Range



Electrochemical Detection Cascade



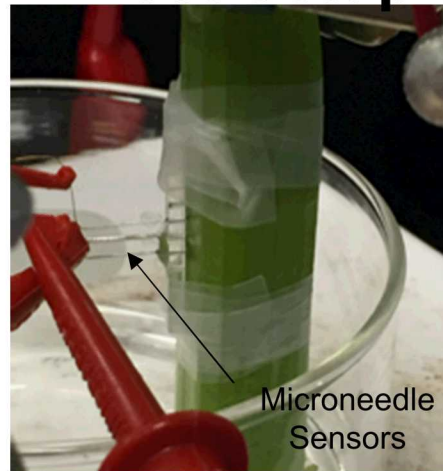
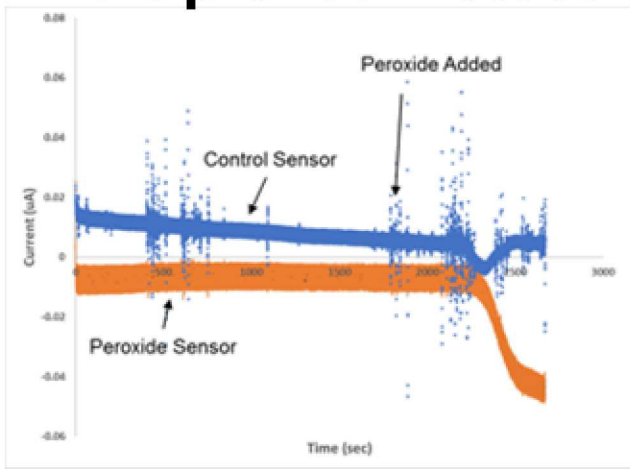
Modified from: [//doi.org/10.1016/j.bios.2004.12.001](https://doi.org/10.1016/j.bios.2004.12.001)

Plant Testing

-Initial in-situ detection experiments of the coaxial needle peroxide sensor layer were performed in celery due to its availability and ability to wick solution through its veins such that the target compound could be passed through microneedle sensors that were either functionalized to detect peroxide or to act as a control. Sensors were allowed to baseline prior to addition of peroxide to solution and delayed response on the functionalized sensor was seen in 7min. Typically response times of the sensor in solution are <20sec.

-Puncture test of glucose microneedle biosensor were performed in sorghum leaves with in solution tests following insertion to determine viability of enzymatic film

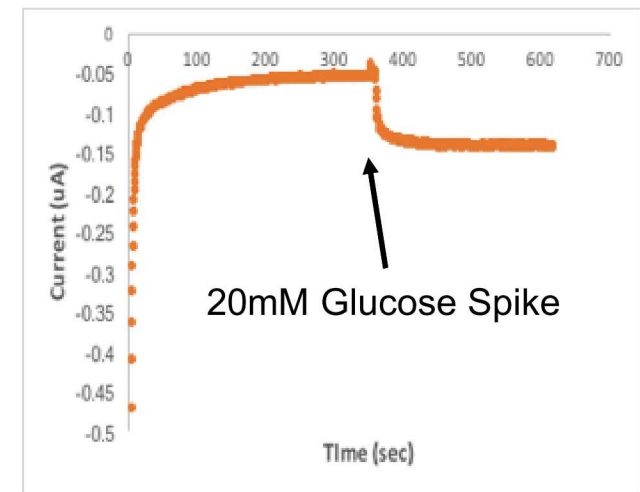
Multiplexed Detection Test Setup



- In plant measurement of peroxide at a microneedle sensors with and without surface functionalization

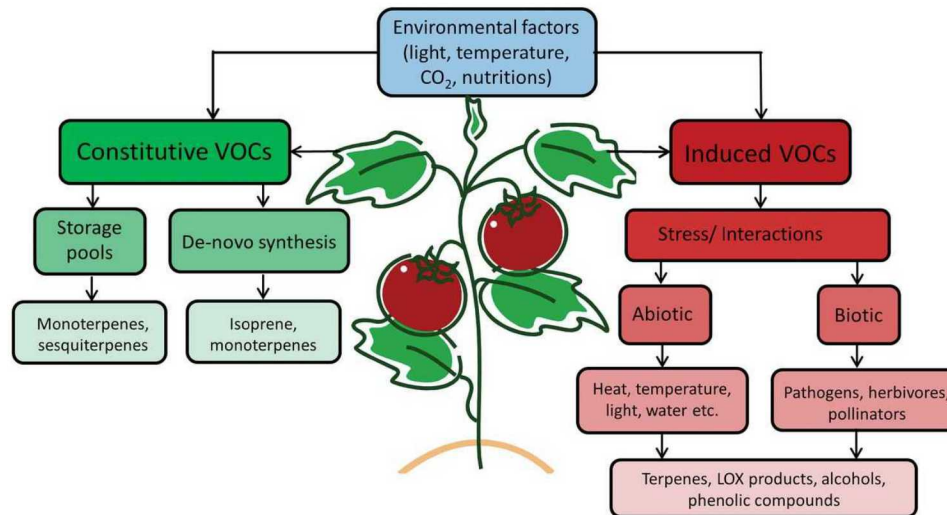
- Using naturally capillary effect in celery vein to transport known compound to sensors

Puncture Test Stability of Biosensor



- Glucose microneedle sensor tested in solution post insertion into sorghum leaf

Biogenic Volatile Organic Compounds



- VOCs emitted from all plant material (fruit roots, leaves, etc.)
- Biotic and abiotic stresses induce different VOC profiles

Micro Gas Analyzer Development

Commercial GCxGC-MS



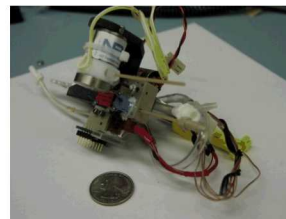
$V \sim 2 \text{ m}^3$
 $t \sim 10^3 \text{ sec}$
 $P \sim 2 \text{ kW}, E \sim 7 \text{ MJ}$
GCxGC
Peak capacity $\sim 10^3$
Peaks/sec ~ 1

Sandia Handheld GC-SAW



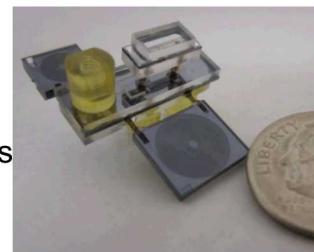
$V \sim 1000 \text{ cm}^3$
 $t \sim 10^2 \text{ sec}$
 $P \sim 2 \text{ W}, E \sim 100 \text{ J}$
Single GC
Peak capacity ~ 10
Peaks/sec ~ 0.1
(low FAR by limiting target chemistry)

Micro Gas Analyzer



Phase II
Single GC
 $V < 20 \text{ cm}^3$

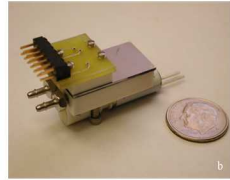
Phase IV
 $V < 4 \text{ cm}^3$
(Analytical components only)



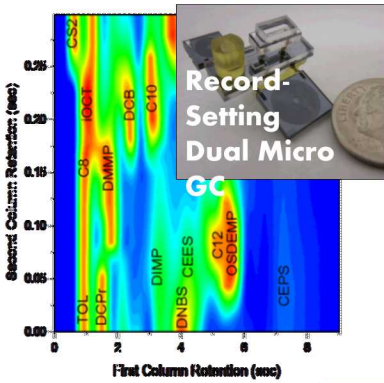
$t \sim 4 \text{ sec}$
 $E \text{ goal} < 1 \text{ J}, \text{ actual } 10\text{-}100 \text{ J}$
GCxGC
Peak capacity > 300
Peaks/sec ~ 50

Nano and Micro Sensors Department, 08634

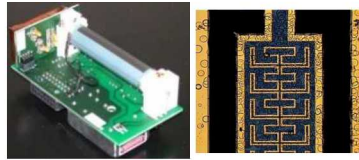
Vapor Detection Thrust



- **Purpose:**
- **Extraordinary sensors & systems addressing:**
 - **Homeland, national/global security & defense**
 - **U.S. commercial competitiveness**
- **Science, engineering, systems & micro/nano expertise utilized to create superior solutions**



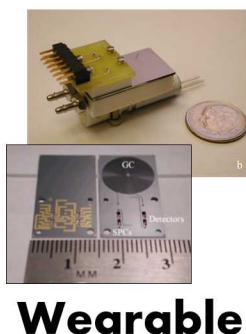
Adapting our latest to new platforms



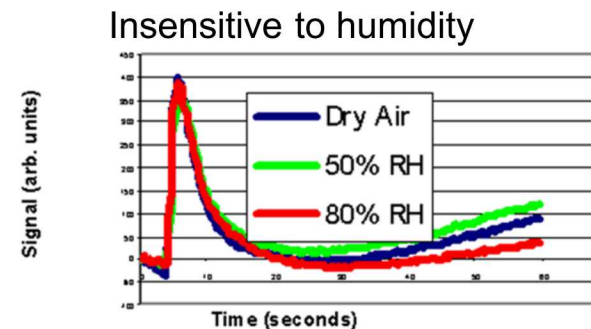
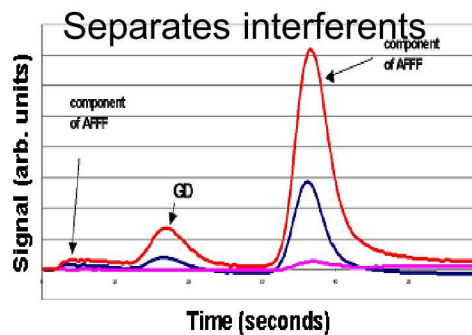
- **Advanced Capabilities:**
- **Mini CBRNE & biogenic volatile compound sensing**
- **Lower false alarm rates (FAR), power & size**
- **Handheld, wearable & mini UAV systems**
- **Affordable, adaptable, high performance**
- **Test and evaluation**
- **Vision:**
- **Work seamlessly with customers to agilely adapt our latest technology to advanced:**
 - **UAVs**
 - **Next-gen wearable detection**
 - **Facility protection**
 - **Medical Dx and human performance**
- **Integration of sensors & modeling for optimization and to create enhanced response architectures**



Original MicroChemLab TRL8

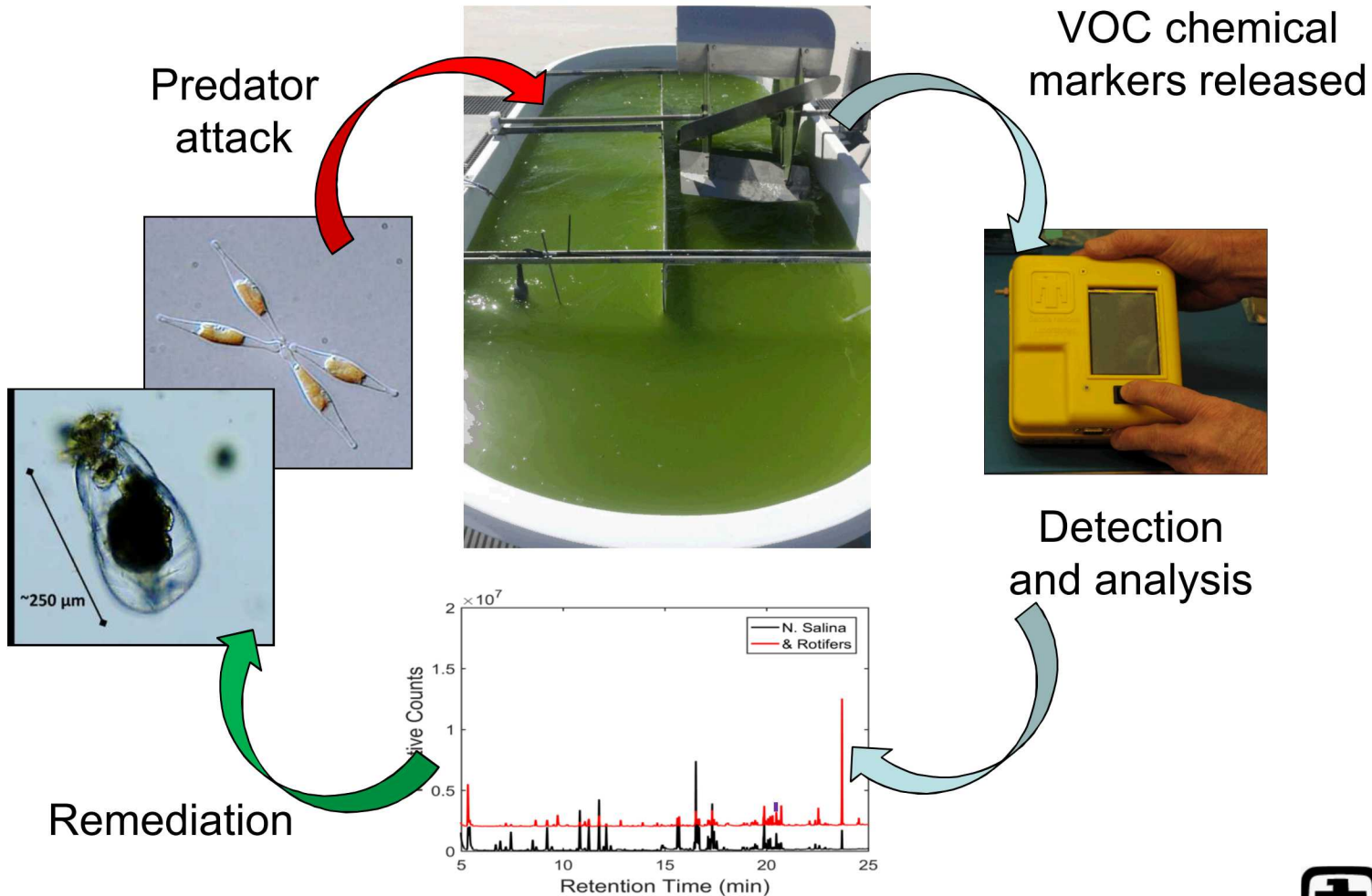


- 22 months in Boston South Metro air-handling system
- 450,000 analyses, **no false alarms**
- 17,000 *remote* tests
- Repeatable response below IDLH
- G, V and blister agents in air 5 sec to 90 seconds
- Insensitive to humidity, diesel, JP8
- Non-expert use, reusable, non destructive
- Also tested at ECBC, Dugway, NTS, SFO...



R&D
100
54 Years of Invention

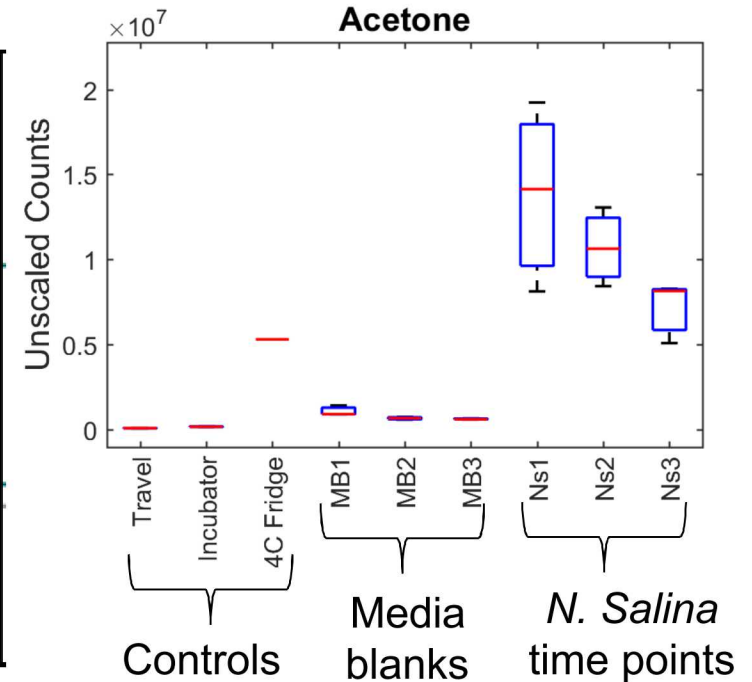
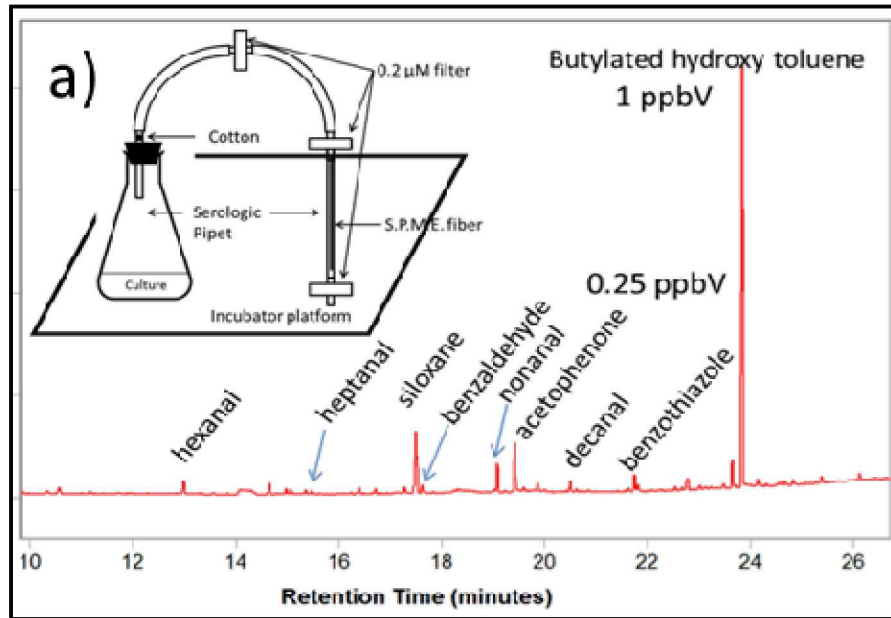
Algal Pond Monitoring in the Field Using VOCs



LDRD funded, Matthew Moorman (PI)



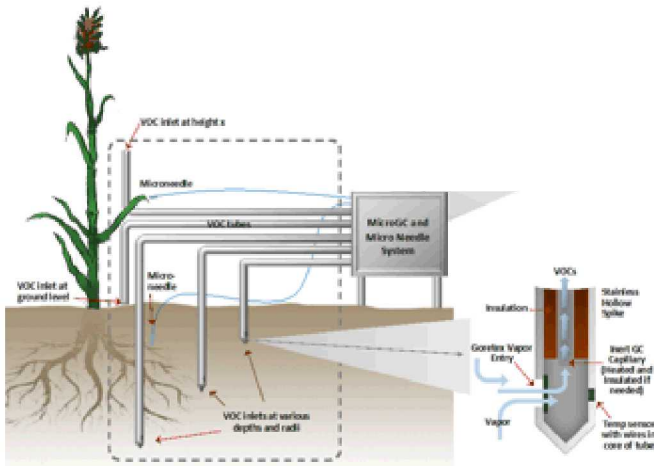
Early Monitoring Results



- Several VOCs have been determined to be produced by the growth of *Nannochloropsis salina*, a common algal biofuel species.
- Compounds produced by predation of *N. salina* by *Brachionus plicatilis* (rotifer) being identified as well.
- Potentially allow VOC-based state-of-health monitoring of biofuel ponds.

ROOTs VOC Outline

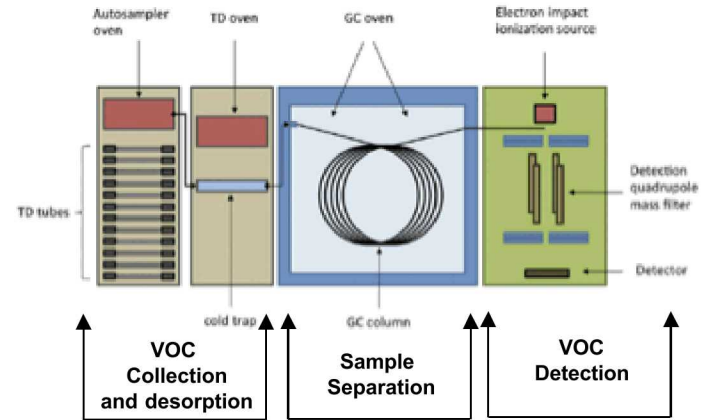
VOC Collection at Plant/Soil



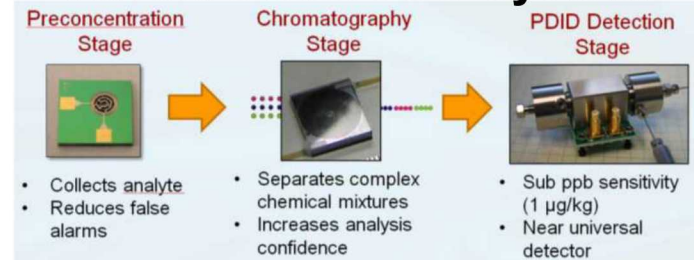
Year 1 VOC Analysis Method

Year 2 VOC Analysis Method

Benchtop System



Sandia's MicroGC System

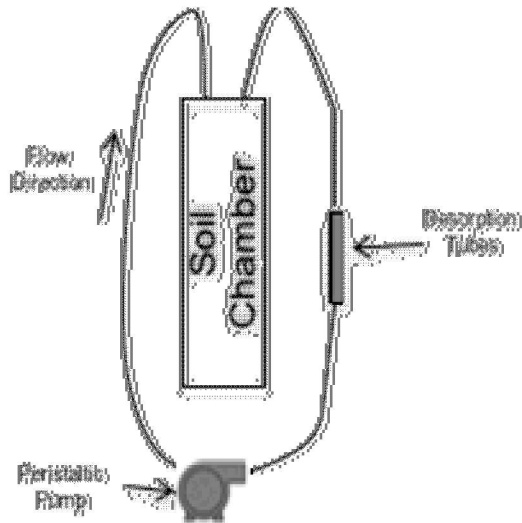


- Schematic of VOC analysis process

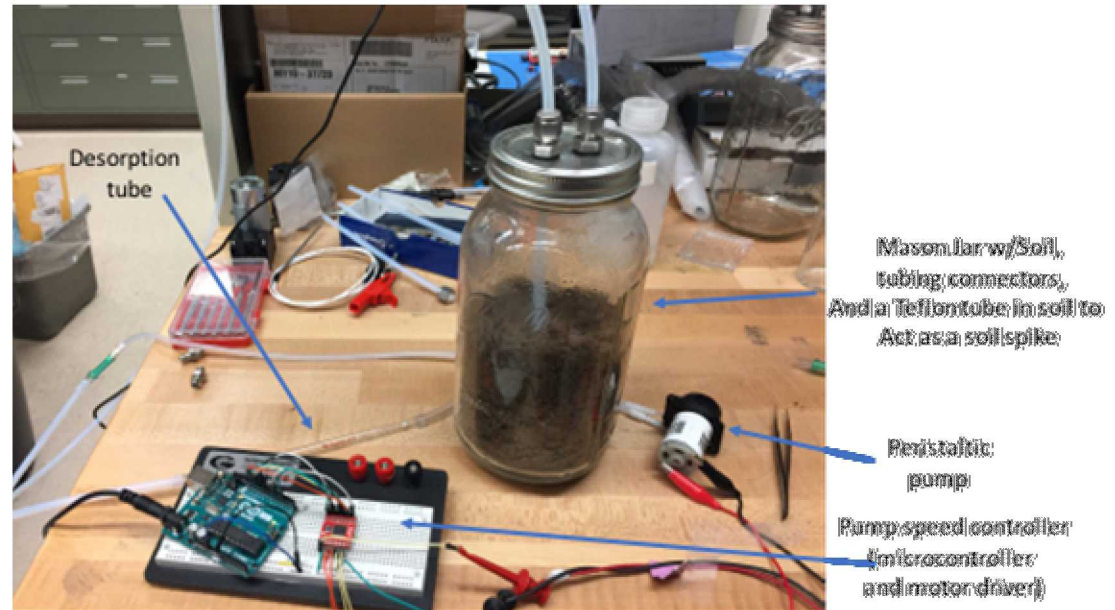
Recirculating VOC collection setup

- Based on a series of initial experiments and results from another internal VOC project, a recirculating collection system was created to collect VOCs on desorption tubes using a peristaltic pump. Our initial results showed little signs of a VOC profile and sampling for longer intervals (24hrs vs 1hr) with recirculation improved the collection method. The collection system could be run in either headspace or below ground sampling configurations to mimic our soil spike prototypes.

Recirculating System Schematic



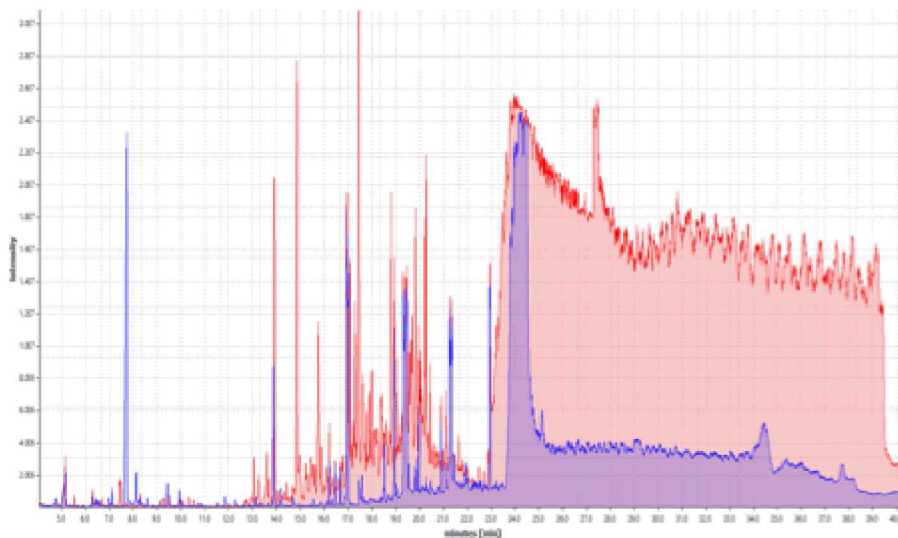
Recirculating System Setup



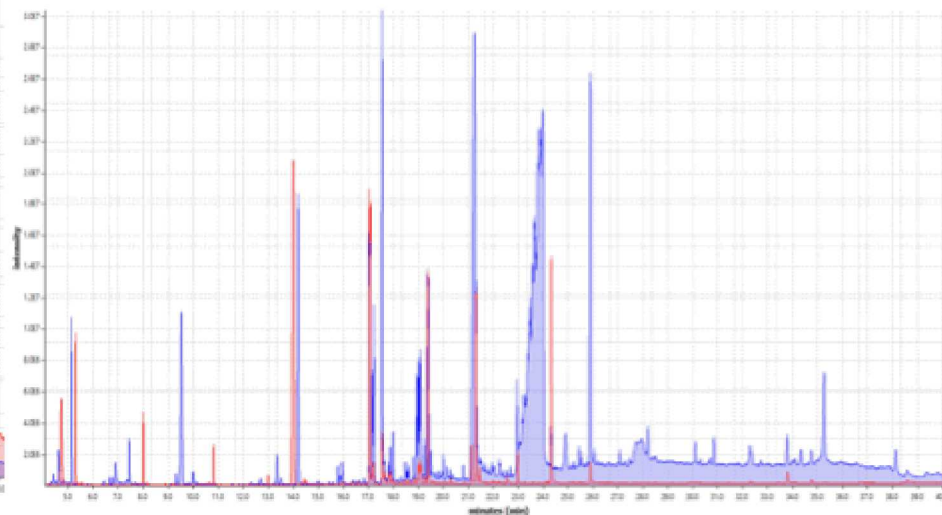
Soil VOC Characterization

- Soil collected from the Los Lunas field site was added to the recirculating system and sampled both in its dry state and during rehydration with sterilized tap water. 48 hours post hydration VOC profiles became apparent and mainly consisted of hydrocarbons, some of which have been referenced in the literature as emitting from some soil types. Initial results run at the same conditions indicate that more VOCs were collected through the soil spike than in the headspace of the jars. 65 compounds were identified in the soil sample collection vs 31 VOCs in the headspace. Miracle grow samples were also tested as a positive control.

Los Lunas Field Soil



Miracle Grow Control



Blue: in-soil collection
Red: headspace collection



Data Processing and Common Compounds

- Analysis of the chromatographs is done by using ANDIS (automated mass spectral deconvolution and identification system, developed at NIST) to identify peaks through a NIST library and then our expert filter that data set down to compounds we suspect are real based on mass spectra mass and reverse match scores. For instance, the Agilent GC/MS software found 2200 compounds in the chromatograph for the watered Los Lunas field soil, ADMIS found high confidence matches for 66, and our team believes that 31 compounds are real based on mass spectra match and reverse match scores. An example list is plotted below (headspace collection) with retention times and NIST match and reverse match scores.

Common Los Lunas VOCs

Decane
Toluene
Heptane
Limonene

Common Miracle Grow VOCs

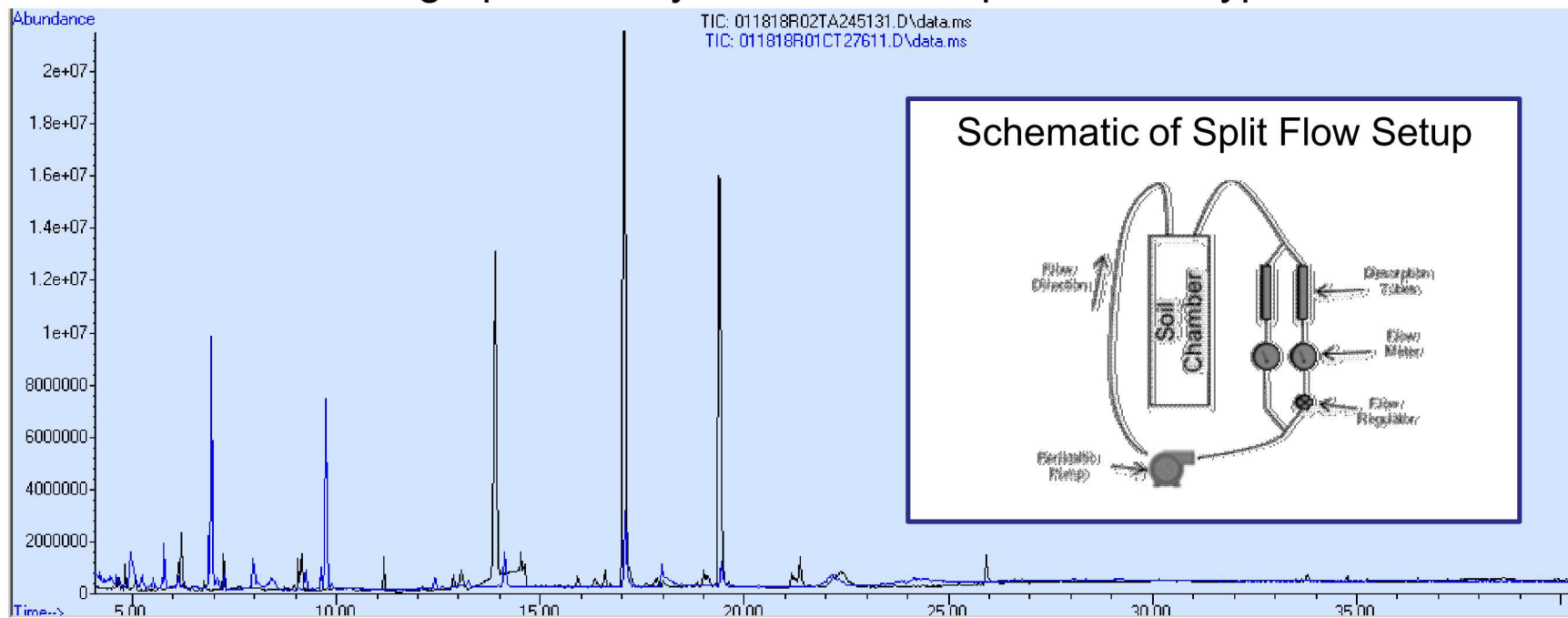
Hexanal
Benzaldehyde
Acetophenone
Benzoic Acid



Desorption Tube Comparison

- The recirculating VOC collection system is being outfitted with the hardware necessary to split the stream of incoming VOCs and evenly disperse them across two different desorption tube types. Initial results showed that the two tube types collected different compounds so the system is being modified in order to capture as many of the compounds as possible. Data set is currently being processed.

Chromatograph Overlay of Each Desorption Tube Type

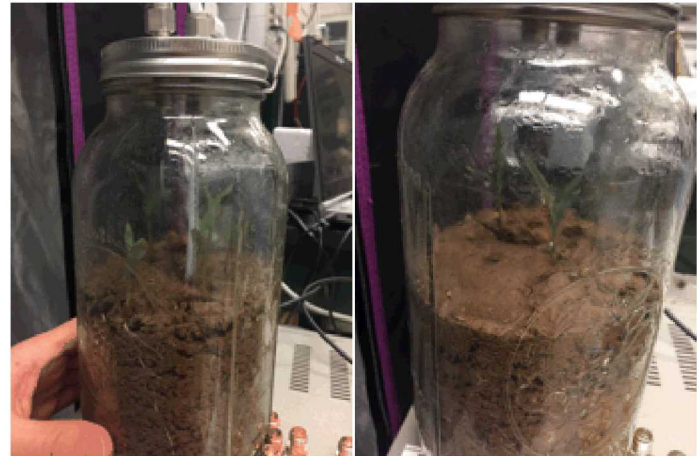
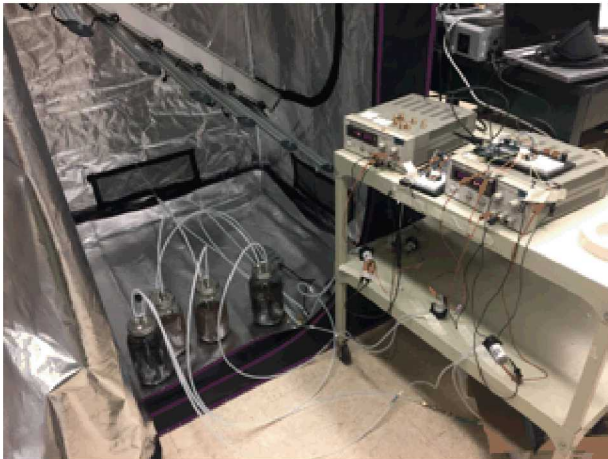


Tenax Tube – Miracle Grow Watered 3 Days Prior to Sampling, Headspace
CarboTrap Tube – Miracle Grow Watered 3 Days Prior to Sampling, Headspace



Sorghum Seedling Grow and VOC Collection

- Sorghum (dwarf variety) are currently growing in Los Lunas soil with above and below ground collection in the recirculating system. Seeds sprouted within 48 hours and growth chamber has been programmed to run for 16 hours of continuous light exposure with a controlled temperature.
- A small sample size of plants was started to understand the effect of relative humidity on VOC collection in the desorption tubes, which is caused problems in the past.





Contributions and Funding

- **Sandia**

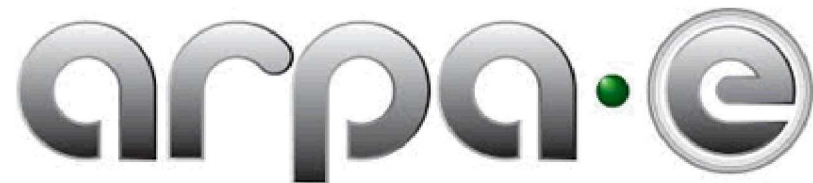
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