Annual update / Summary

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NATIONAL PEANUT BOARD/SOUTHEAST PEANUT RESEARCH INITIATIVE
~~QUARTERLY PRO~~GRESS REPORT FOR WORK DONE UNDER RESEARCH AGREEMENT

Quarter ending 9/30/17

INSTITUTION: Georgia Tech

PROJECT TITLE: USING PEANUT VOLATILE ORG

RES. AGR. NO.: D7984 PROJECT LEADER: Dr. Doug Britton
GACCP Budget No.: N/A

EXPIRATION DATE: December 31, 2017 NPB CONTACT: Bob Parker/Maria Mehok
NPB Budget No.: PID 462

REPORT OF PROGRESS:

General Activities

- Measured and started analysis on the first set of VOC samples, 30DAP.
- Investigated the use of Twister adsorbent stir bars.
- Developed extraction and GC-MS recipe for VOCs adsorbed onto Twister collection material.
- SPME results from test peanut plant.

VOC analysis of 30- DAP:

VOCs were collected from peanut plants under three types of conditions: Control, Moderately Stressed and Highly Stressed. Fully irrigated plants form a Control group while plants that undergone two levels of drought treatment form Moderately Stressed and Highly Stressed groups. The VOC data for the field's background was also collected to make sure that there is no interference with the data collected from the plants.

Figure 1 represents the preliminary analysis of VOC levels related to emission of Cis-Hexen-1-ol – a chemical associated with water stress in peanut plants. Variable Collection Date denotes if data was collected before (1) or after (2) the treatment was applied. As one can tell, while VOC's levels for all three types of plants are not very different among themselves they are definitely higher than ones produced by the background. Low levels of VOC's emitted by the plants can be explained by plants' immaturity at this stage of the experiment.

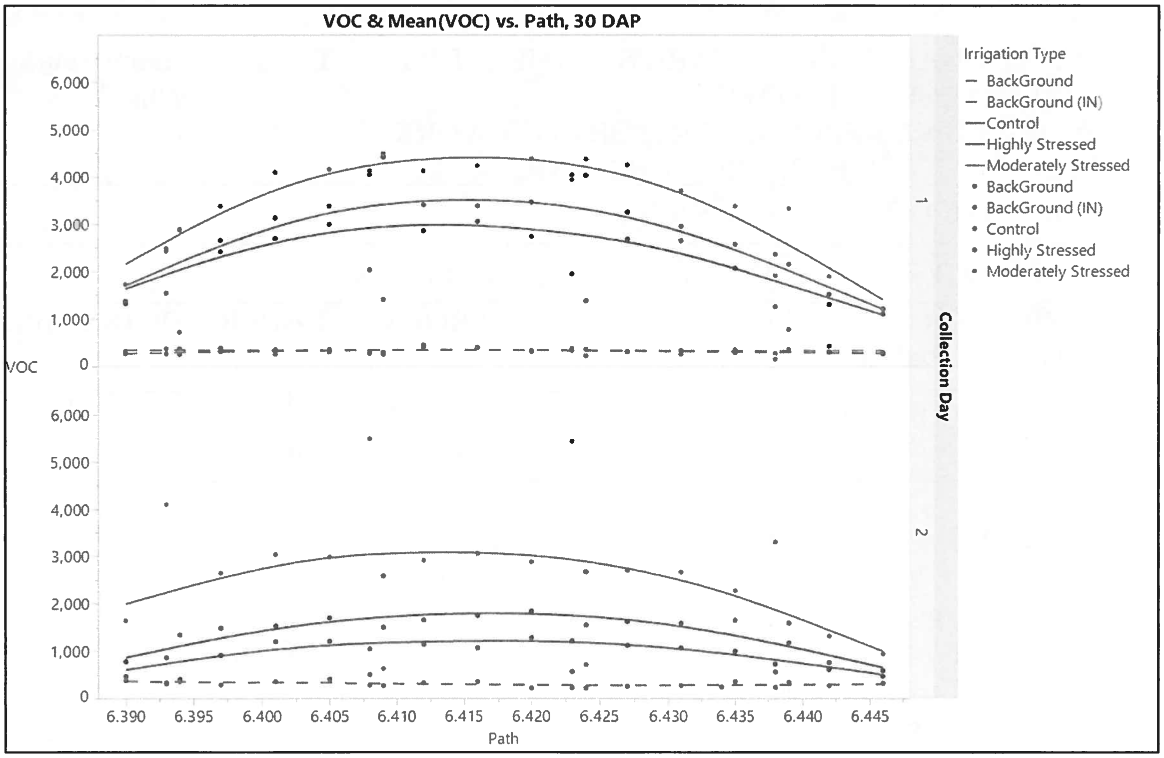

Figure 1. Comparison of VOC's levels produced by peanut plants and background

Initial correlation to moisture readings were also performed, however, at this stage of experiment no immediate conclusions can be made due to the immaturity of the plants. It should also be noted that for the 30 DAP moisture readings, the probe depths were measured at 8 and 16cm. The probe depths were changed to 4 and 8 cm for the 50, 70, and 90 DAP readings.

<u>Use of Twister (absorbent material) stir bars:</u>
It was suggested to us by Dr. John Dimandja at the FDA that we use a special type of stir bars coated with absorbent material termed Twisters for the VOC collection and analysis for several reasons. One of which is that the Twisters are stable and can be reused >50 times. In order to be able to use them in the field we first had to develop a collection method and desorption method for analysis using a GC-MS. The collection method is quite simple in that a Twister is exposed to the VOC producing leaves for one hour in the same sample bag used previously. The sample bag is then sealed and shipped back to GTRI for measurement on the GC-MS.

<u>Development of extraction and GC-MS recipe for Twister collection method:</u>
In order to use the Twisters we had to also develop an extraction of the VOCs from the twisters. Several standard extraction techniques were tested. They included hexane extraction, isopropanol extraction, and thermal desorption. In the hexane extraction, the absorbent material on the stir bar became swollen with hexane, resulting in damage to the Twister. This would prevent the reuse, as well as the collected sample was quite dirty. In the isopropanol extraction, the absorbent material did not swell, however when injected into the GC-MS, the signal was quite low, due to a dilution effect from the excess

isopropanol required. The last method that was tested was a thermal desorption of the VOCs from the Twister using a specialized piece of equipment that applied heat to the Twister, resulting in the release of VOCs. This last method works quite well for the Twister samples and provides good signal response from the VOCs, Figure 1. Twister samples for 50, 70, and 90 DAP samples are planned to be collected and measured using the GC-MS.

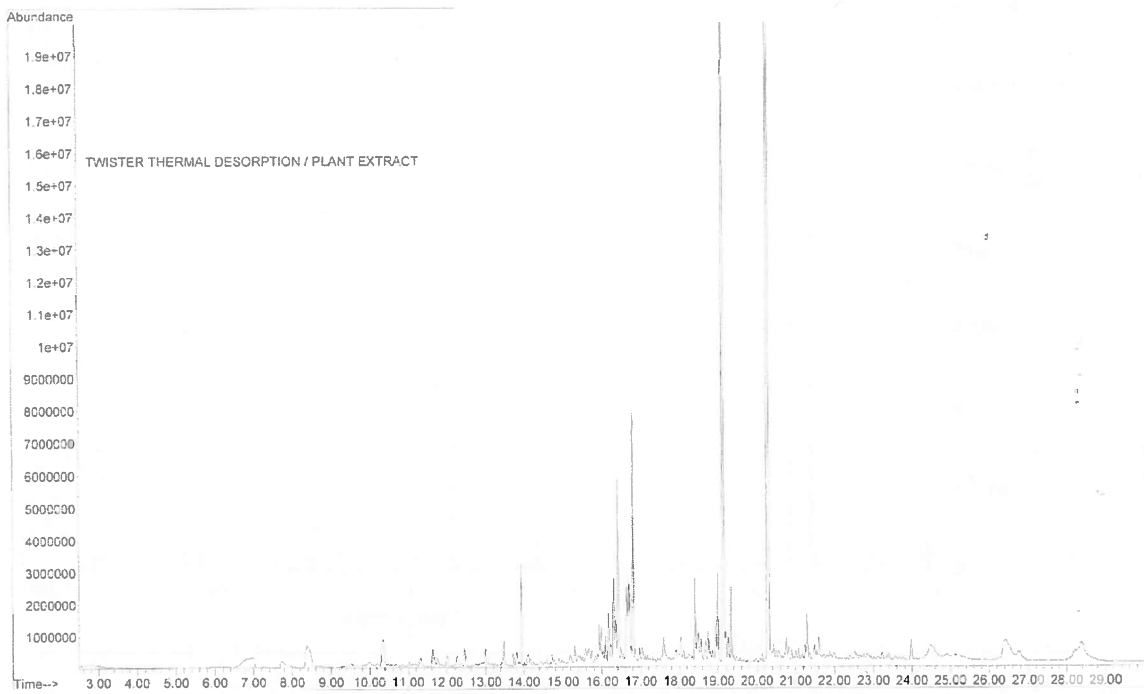

Figure 2. Thermal desorption of Twister with VOCs from test peanut plant.

<u>SPME Results of test peanut plant:</u>

Another sampling tool under investigation is Solid Phase-Microextraction (SPME). After collecting the headspace VOC samples using the collection bag in the field, the SPME were placed inside the collection bag for 10 minutes to absorb VOCs, then it was analyzed by Leco Pegasus 4D-LN2 GC-MS-TOF time of flight gas chromatography mass spectrometer. This sophisticated and fully automated GC/MS system is dedicated to the analysis of Volatile Organic Compounds (VOC). The following graphs are one of the VOC results of a 30DAP peanut plant in the field.

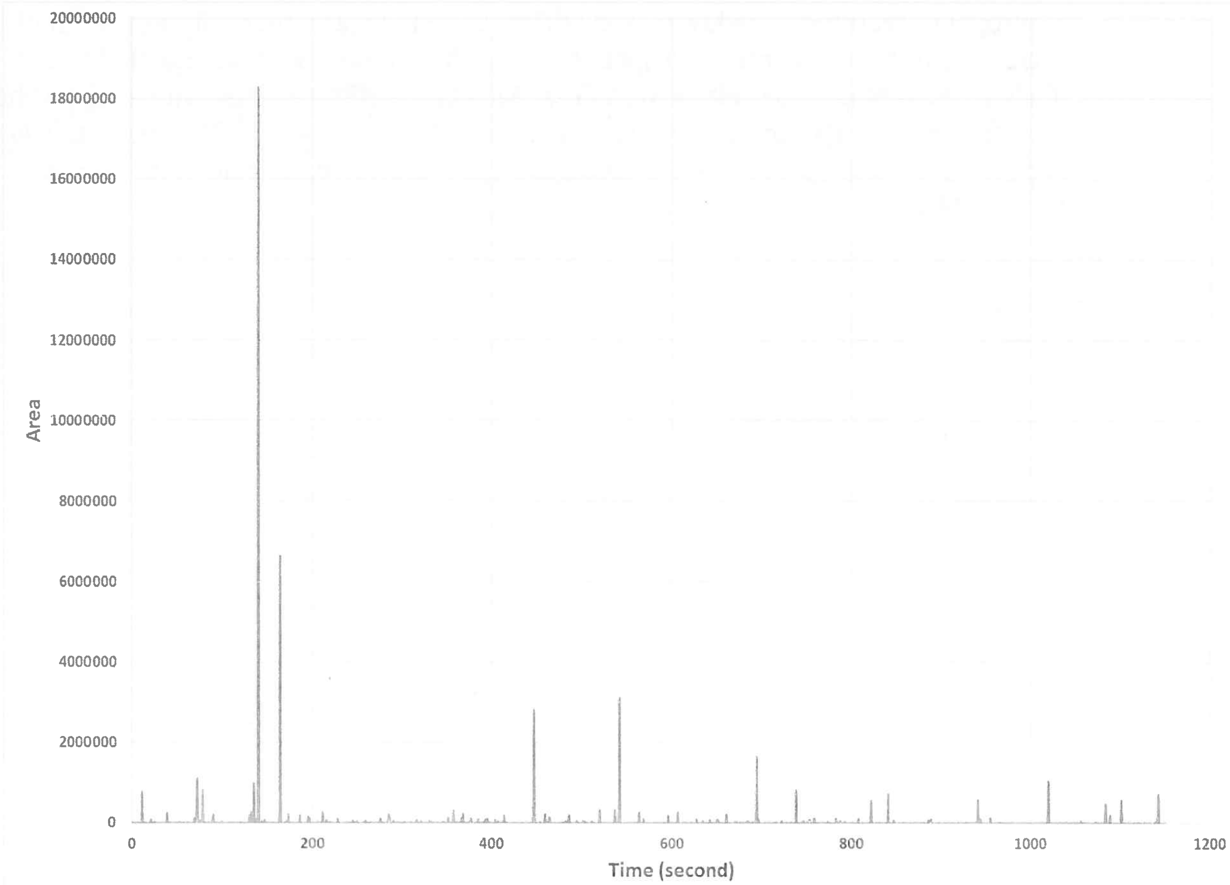

Figure 3. Gas Chromatogram of 30DAP peanut plant VOC

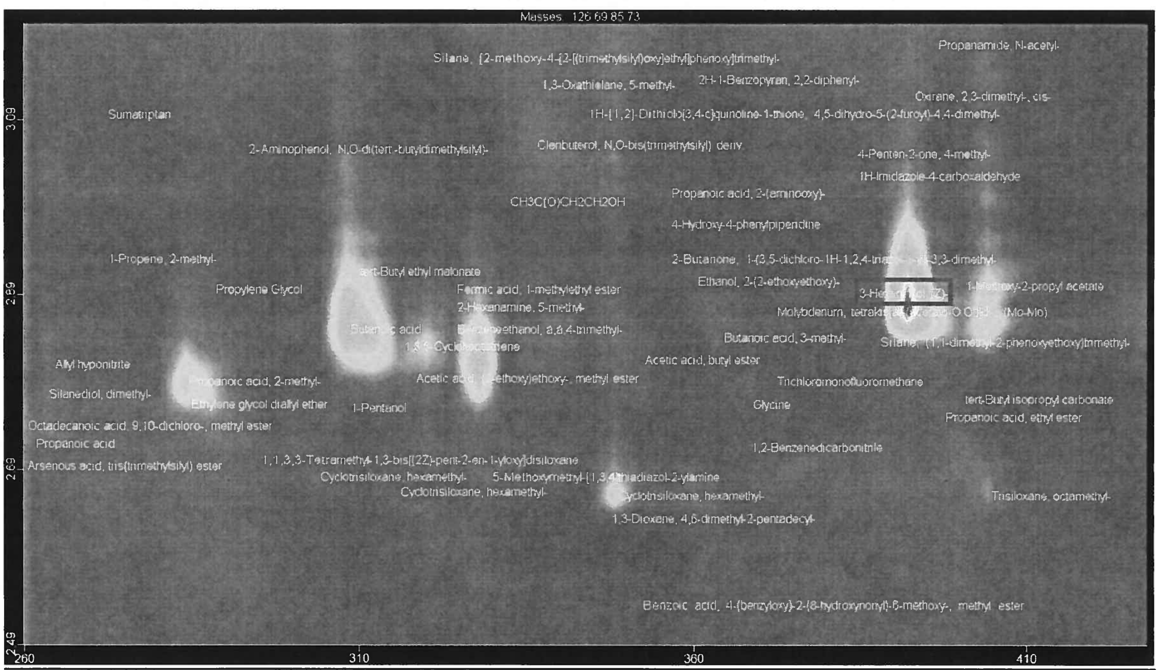

Figure 4. Contour plots of 30DAP peanut plant VOC (part 1)

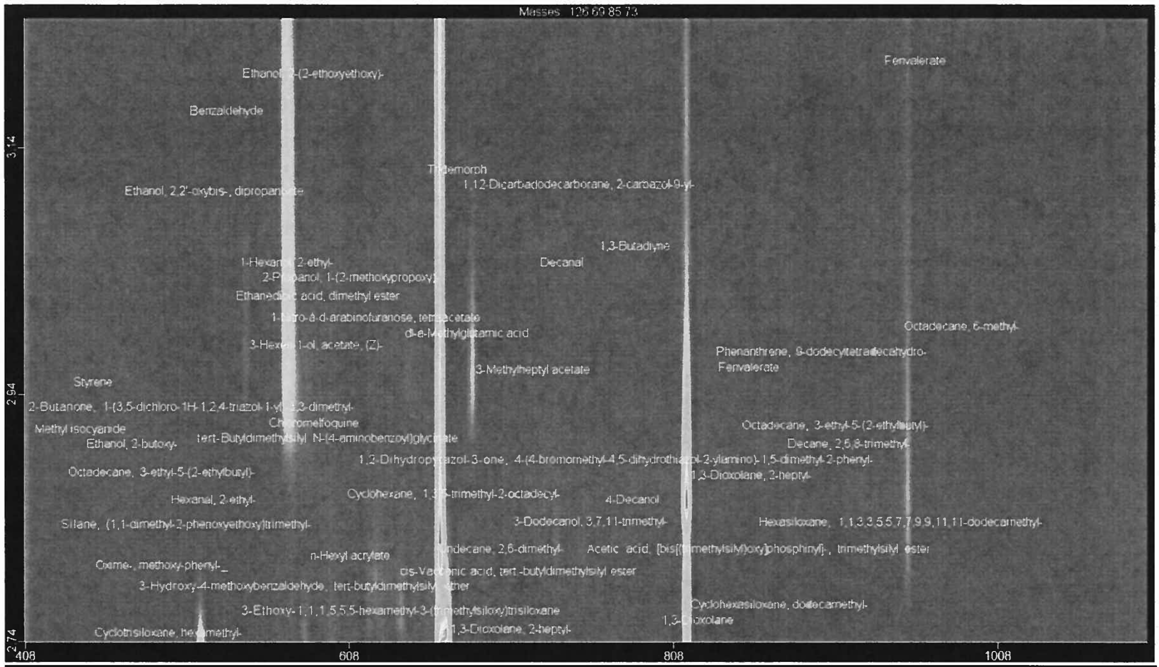

Figure 5. Contour plots of 30DAP peanut plant VOC (part 2)

Figure 3 is a one-dimensional gas-chromatogram of a 30DAP peanut plant VOC analysis. It separates various (>1000) VOCs based on column retention time. Figure 4 and Figure 5 are the comprehensive analysis of the two-dimensional gas chromatogram coupled with Time-of-Flight mass spectrometry. This data resulted in unequivocal compound identifications through spectral similarity comparisons to large, well-established databases and formula determinations for high-resolution accurate mass fragment and molecular ions. The distinct advantage of GCxGC–high-resolution TOF-MS for complex material analysis is a direct result of its ability to reduce coelutions through superior chromatographic separation and minimizes mass spectral interferences with higher mass analyzer resolving power. Including the stress marker (z)-3-Hexen-1-ol (red box in Figure 4) that has been identified, this analysis could provide us an even more detailed VOCs list that relates to peanut plant water stress we may have overlooked using the traditional GC/MS analysis. Further data processing and interpretation is under investigation.

<u>Summary & Next Steps</u>

This quarter we have collected and analyzed samples collected at 30 DAP using vial collection technique developed during the previous growing season. Initial correlation to moisture readings were performed. It should be noted that for the 30 DAP moisture readings, the probe depths were measured at 8 and 16cm. The probe depths were changed to 4 and 8 cm for the 50, 70, and 90 DAP readings. During this time we have also explored the use of specialized absorbent material that coats a magnetic stir bar, Twisters. This exploration includes the absorption of VOCs onto the bar and developing special GC/MS recipes for sample injection with the help with Dr. Dimandja at the FDA. In the next quarter the team will take the following steps:

- Analyze 50 and 70 DAP data collected with vials

- Process and start analysis of samples collected 50 and 70 DAP collected using Twisters
- Develop collection and analysis plan for aflatoxin samples in field and at collection points