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Annual report & summary

NATIONAL PEANUT BOARD/SOUTHEAST PEANUT RESEARCH INITIATIVE
QUARTERLY PROGRESS REPORT FOR WORK DONE UNDER RESEARCH AGREEMENT

~~Quarter~~ **ending** 12/31/17

INSTITUTION: Georgia Tech

PROJECT TITLE: USING PEANUT VOLATILE ORG

RES. AGR. NO.: D7984
GACCP Budget No.: N/A

PROJECT LEADER: Dr. Doug Britton

EXPIRATION DATE: June 30, 2018

NPB CONTACT: Bob Parker/Maria Mehok
NPB Budget No.: PID 462

REPORT OF PROGRESS:

General Activities

- Statistical analysis of 50, 70, and 90 DAP data collected with vials
- Preliminary analysis of samples collected 70 and 90 DAP collected using Twisters
- Analyzed data for samples collected over a 24 hour period

Statistical analysis of 30, 50, 70, and 90 DAP data:
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. The data discussed in this section were collected at 30, 50, 70 and 90 DAP and were all collected using vials and a collection bag.

Figure 1 and Figure 2 summarize the mean Cyclohexene and (z)-3-Hexen-1-ol values collected for the study period. Data was collected two times for each DAP: before and after the stress treatment was applied. Results indicate that there is a clear trend and higher amounts of Cyclohexene are emitted as the plant matures while (z)-3-Hexen-1-ol gives the highest signal in the middle of plant's lifecycle. Based on these results we may suggest that both chemicals should be monitored throughout the peanut plant lifecycle. There is no clear evidence that the amount of VOC's emitted by plants change after application of a stress treatment, however, for 50 and 70 DAP one may notice that Cyclohexene gives significantly lower signal during the second collection time.

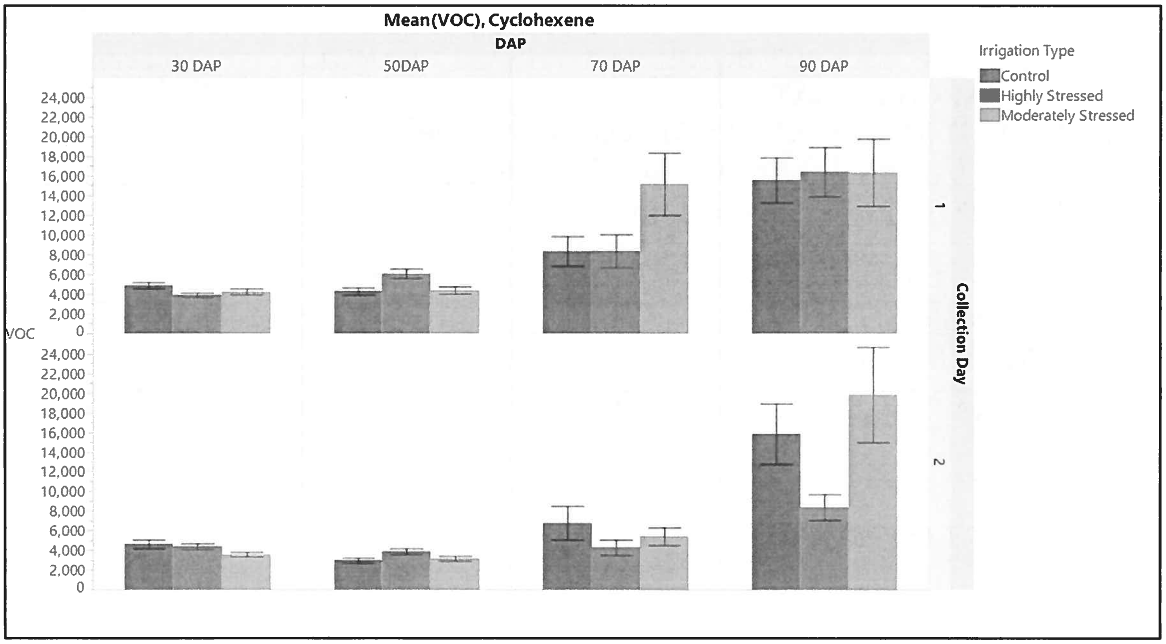

Figure 1. Mean Cyclohexene Levels by Collection Day and Day after Planting (DAP).

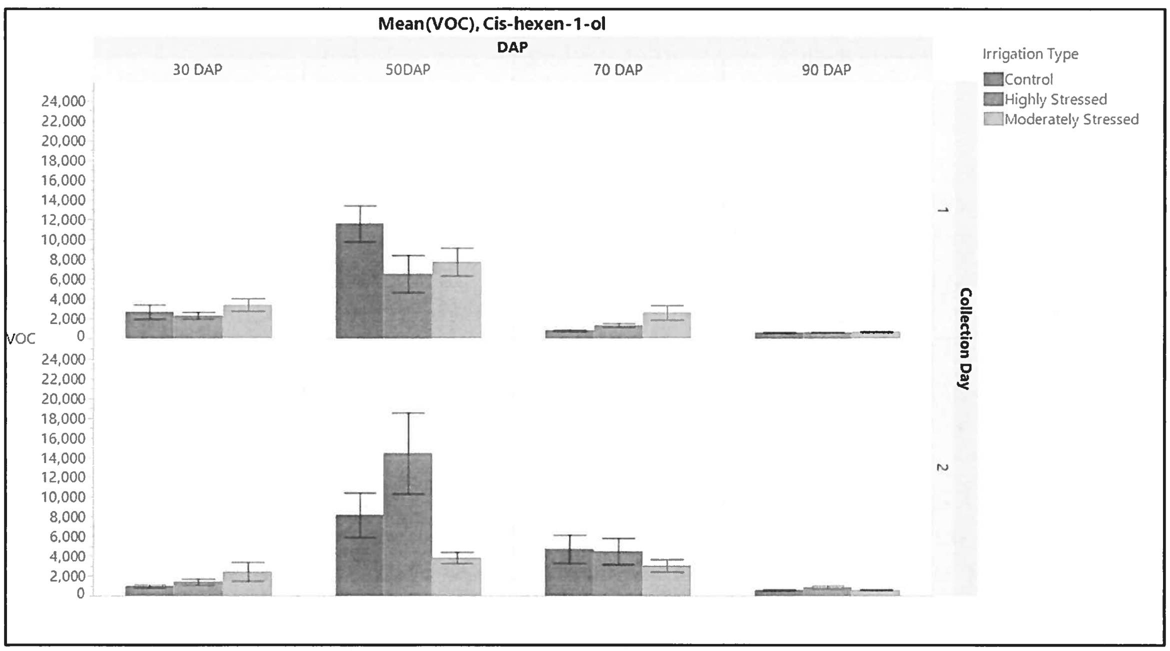

Figure 2. Mean (z)-3-Hexen-1-ol (Cis-hexen-1-ol) Levels by Collection Day and Day after Planting (DAP).

Preliminary Twister data for 70 and 90 DAP:

A Twister is a special type of stir bar coated with an absorbent material used for VOC collection and analysis. The collection method utilizing a Twister is quite simple in that a Twister is exposed to the VOC producing leaves for one hour in a similar sample bag used previously for vial collection. The sample bag is then sealed and shipped back to GTRI for measurement using a GC-MS. A thermal desorption unit designed specifically for the Twisters is used to desorb and introduce the VOC sample to the GC-MS and is a key component to complete the GC-MS VOC analysis. Figure 3 indicates that both Cyclohexane and (z)-3-Hexen-1-ol have been detected from samples collected using the Twister adsorbent material. These two chemical are the ones previously identified as potential VOC stress markers from samples collected using only vials. However, for the samples collected with the Twister, the low peak intensity for cyclohexane compared to (z)-3-Hexen-1-ol reveals that the current Twister adsorbent material may not be the suitable to collection of Cyclohexane. Figure 3 is a GC-MS spectra obtained from the Twister thermal desorption analysis with Cyclohexane and (z)-3-Hexen-1-ol chemicals highlighted.

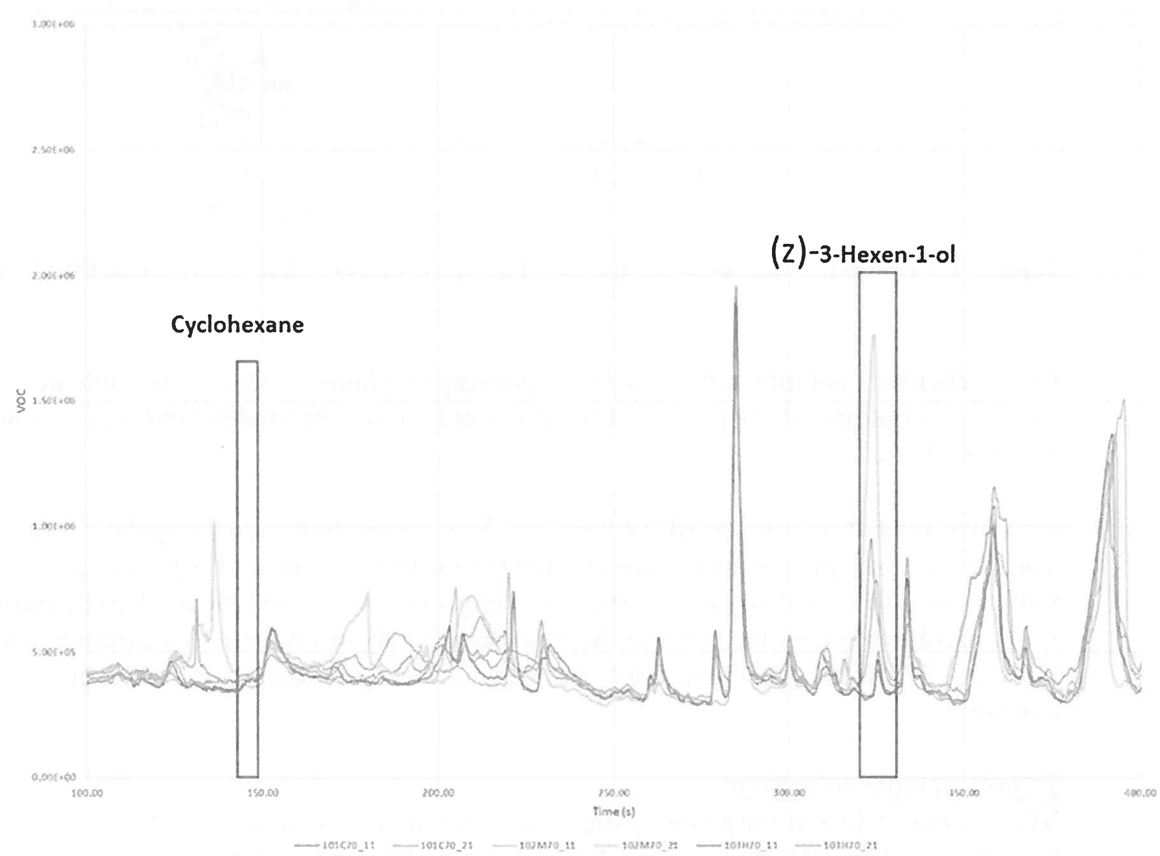

Figure 3. GC-MS spectra for a Twister sample collected from a peanut plant in the field at 70 DAP

Also from Figure 3, it was observed that there are many additional peaks that may be of interest. In order to investigate this, advanced analysis tools are being investigated. One approach is the use of machine learning to pick out trends in the data that may describe the stress level of the plants. The chromatographic data was first aligned and normalized followed by Principal components analysis (PCA) which was used to reduce dimensionally and visualize variance in the data, Figure 4. It can be seen, encircled, the clustering and variance in data for the Twister samples for 70 DAP and 90 DAP. Preliminary analysis indicates that there is a lower variance between the 90 DAP samples compared to 70 DAP.

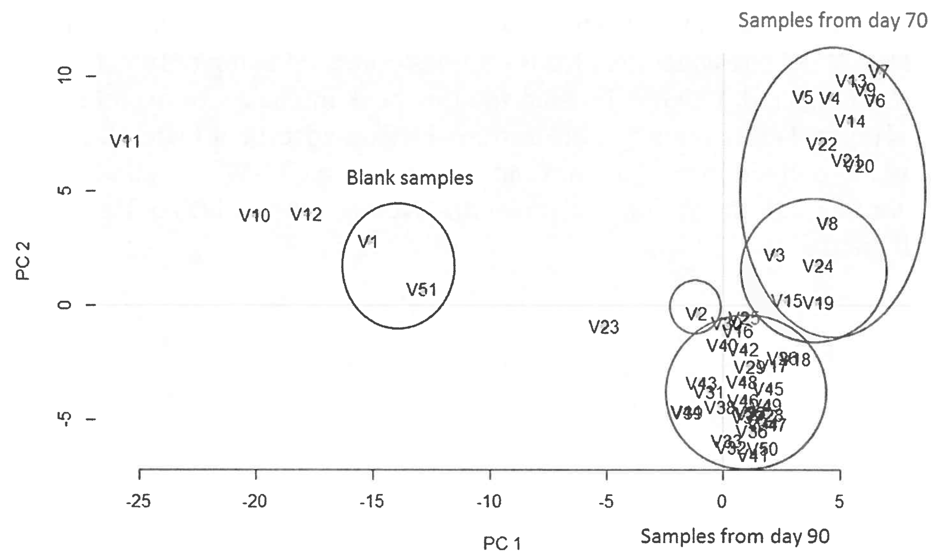

Figure 4. Principal components analysis (PCA) of Twister data from 70 and 90 DAP.

Comprehensive volatile metabolic fingerprinting of plants using advance machine learning techniques show promise for further enhancing our understanding of plant metabolomics.

One final note about the use of Twisters for VOC collections and analysis: While the Twisters do look promising for ease of sample collection and analysis, the group at GTRI will not be able to utilize them as there is a required specialized piece of equipment that we do not have access to. We will therefor perform all subsequent measurements using vials and SPME adsorbent material, until regular access to the equipment is made available.

<u>24 hour sample collection:</u>
VOCs were collected for peanut plants that were under no stress over the course of a 24 hour period. The collection was performed using vials and collection bags, and were collected every 2 hours. 2 samples were collected per plot, with 12 plots in the test field. Background samples were also collected at the same time as plant samples. The results of this collection can be seen below.

Based on observations summarized below researches suggest that the best collection time to gather VOC's associated with targeted chemicals would be between 2pm and 6pm. This is indicated by amount of VOCs released at this time is the highest for both target VOCS. These results can also aid in providing information on the generation and release of VOCs from peanut plants.

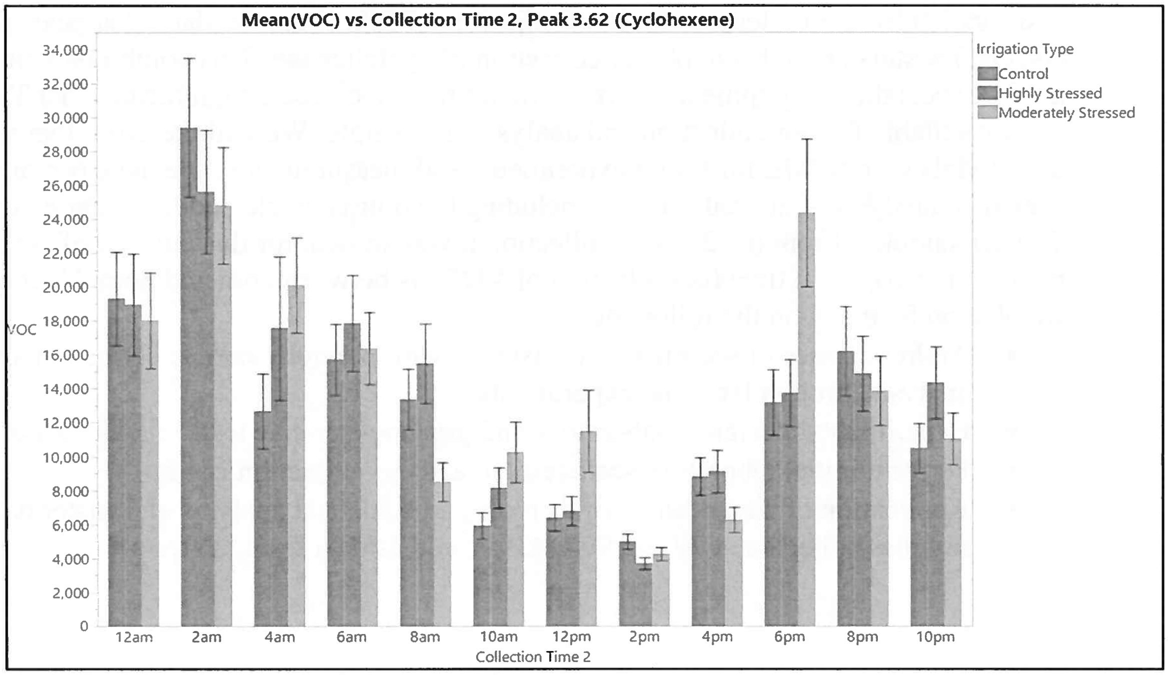

Figure 5. Mean Cyclohexene Levels by Collection Time

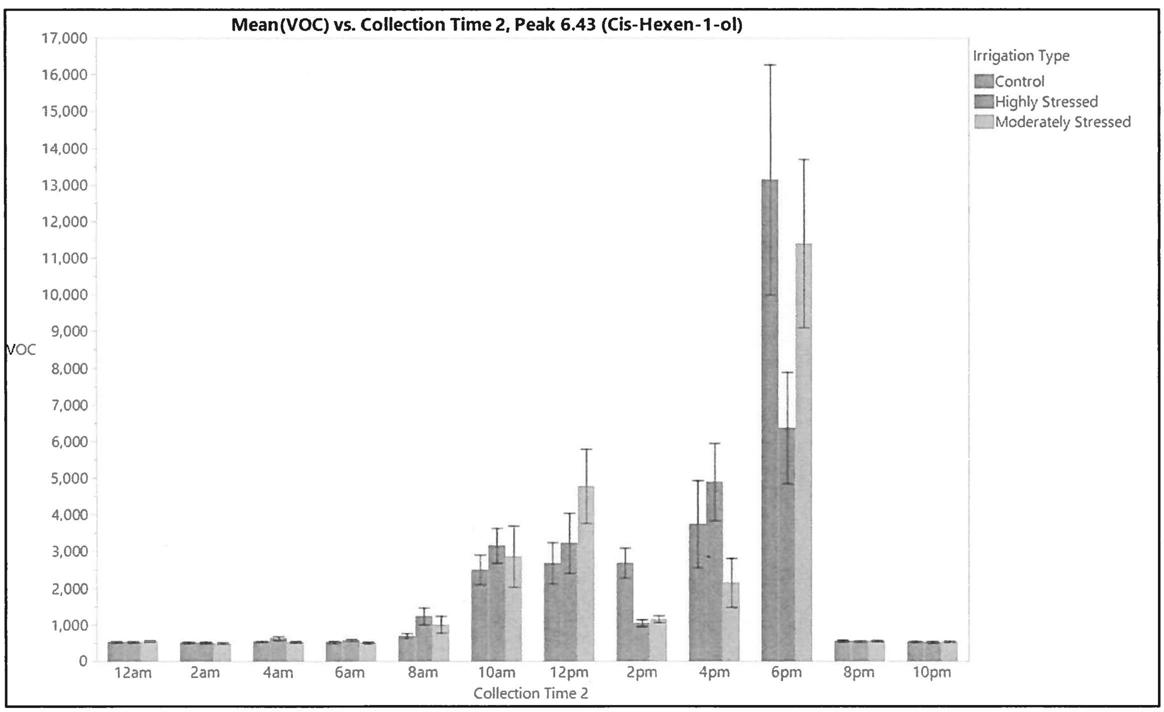

Figure 6. Mean (z)-3-Hexen-1-ol (Cis-hexen-1-ol) Levels by Collection Time.

Summary & Next Steps

This quarter we have finished all sample collections and completed measurement using the GC-MS system for all vials samples. This includes the 24 hour diurnal sample. We also have completed the measurement of the samples collected using Twisters with assistance from our colleague Dr. Dimandja at the FDA. From the data, it appears that the current Twisters are not suitable for collection of cyclohexane. This combined with the need of specialized equipment, of which we do not have access to, indicates that Twisters are not suitable for our collection and analysis at this time. We will therefore focus on the use of vials with SPME for future experiments and measurements. We have performed statistical analysis of all vial samples including the diurnal cycle and have started on the Twister samples. From the 24 hour collection it was shown, for this cultivar of peanut plant that the optimal time for collection of VOCs is between 2pm and 6pm. Next quarter we plan on focusing on the following:

- Address issues of seeming inconsistency with previous sample collection and analysis through lab scale experiments
- Experimental design of laboratory and greenhouse scale tests to address issues
- Perform initial laboratory scale tests to address collection issues
- Application of advanced analysis tools and statistical analysis of Twister data for samples collected at 70 and 90 DAP as well as data from lab tests