

Utilizing Peanut Volatile Organic Compounds (VOCs) to Detect *Aspergillus* in Peanut Plants, Pods, and Kernels

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Over the past few years GTRI has been investigating the use of plant-based volatile organic compounds (VOCs) for monitoring the status of peanut plants. VOCs are a class of light weight chemicals that plants release from all parts on a continual basis. The amount and types of VOCs released vary depending on the conditions that the plant is currently under. Initially, GTRI started utilizing these VOCs to monitor for heat and drought stress in peanut plants. Those studies provided some very promising indications of the ability of VOCs for monitoring the water status of peanut plants. More recently, the team at GTRI has moved to the investigation of using VOCs for monitoring aflatoxin in both plants and kernels pre and post-harvest.

GTRI's first field trial of aflatoxin VOC capture and identification was conducted in August-September of 2019. In this experiment, 48 plots were allowed to grow under normal conditions for 100 days, after which one group of the plants was treated with *aspergillus* fungus; a second group was treated with Afla-Guard (biocontrol agent), and the third group of plants was not treated – acting as a Control group. Plant-based VOCs were collected from the plants before treatment, and once a week for 2 weeks post treatment. Stir Bar Sorptive Extraction (SBSE) devices or Twisters® were used for all VOC collections. These Twisters® consist of a bar magnet, encased in glass, surrounded with an adsorbent material. The Twister® was placed directly on the peanut plant leaf and held in place with a small pill magnet, for 1 hour. The leaf was isolated using a plastic lined aluminum bag. The Twisters® with captured VOCs were then shipped to GTRI for processing and analysis. Each Twister® was then analyzed via gas chromatography–mass spectrometry (GC/MS). Analysis of the data was performed using Linear Discriminant Analysis. See Figure 1 for a sample of the results obtained from this analysis. Separation of all three groups was accomplished using the data obtained from the GC/MS. Once the plants and pods were harvested, a second set of VOCs were collected for the harvested pods. Pods were separated into clean glass jars and sealed with Twisters® magnetically suspended from the cap. VOCs were collected for 24 hours before being analyzed using GC/MS. After initial VOCs were collected, the pods were shelled and the wet kernels collected. VOCs from the wet kernels were collected and analyzed in the same manner as the pods. Next, the kernels were dried out in an oven at 70°C for 72 hours. These dried kernels also had their VOCs collected and analyzed. Linear Discriminant Analysis was utilized for the pod and kernel data collected. Similar separation of the various samples based on treatments was obtained. Overall these results show promise for the use of VOCs for not only the detection of aflatoxin but also differentiation between toxigenic and non-toxigenic aflatoxins.

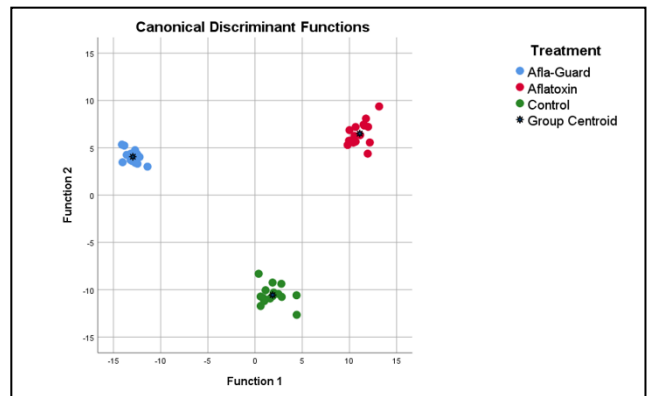


Figure 1. Linear Discriminant Analysis of peanut plants. Data collected on 09/12/2019.

Over the past year GTRI has been focusing on the development of a quantification method utilizing the Twisters®. This would allow for not only for the quantification of the amounts of VOCs released but may eventually allow for the quantification of aflatoxin levels within the plants, pods and kernels at multiple points during the harvest and processing line of peanuts. This quantification method involves the addition of an internal standard to the Twister® in a controlled manner that allows for standardization of data collected from many different GC/MS samples. Using this internal standard, all other chemicals measured can then be quantified and evaluated for their ability to aid in the detection of aflatoxin. To verify this quantification method, GTRI and their partners are in the process of growing two different types of peanut plants (drought resistant and sensitive) in a greenhouse that will be treated with *aspergillus* fungus when the plants reach 75 days after planting. VOCs will be collected with Twisters® (that contain the internal standard), before treatment, before drought is initiated, and before harvest. Pods will be harvested and VOCs will be collected from the wet pods, the wet kernels, and from dried kernels (similar to previous studies). This data will be analyzed using various classification techniques such as Linear Discriminant Analysis, Principal Component Analysis and Random Forest.