

# 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.

A small-scale greenhouse-based trial of peanut plant VOC capture and identification was conducted in October-May of 2022/23. In this experiment, 12 plants each of two genotypes, UF150303 (drought tolerant cultivar) and 10x10-3-3-1-1 (drought susceptible cultivar) were allowed to grow in a hoop house on the University of Florida's campus. Ample lighting was applied to offset nonseasonal photoperiod and 14 hours of light a day was applied. Additionally, temperature was maintained at 60F. Due to plants being grown off season resulting in slower development, plants were inoculated mid-January. Plant-based VOCs were collected from the plants at time of inoculation with *Aspergillus*, and at two additional times prior to harvest. Twisters<sup>®</sup> were used to collect VOCs. These Twisters<sup>®</sup> consist of a bar magnet, encased in glass, surrounded with an adsorbent material. The Twister<sup>®</sup> 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<sup>®</sup> with captured VOCs were then shipped to GTRI for signal processing and data analysis. Each Twister<sup>®</sup> was then analyzed via gas chromatography–mass spectrometry (GC/MS). Analysis of the GC/MS data was performed using Linear Discriminant Analysis and Random Forest Analysis. Figure 1 shows the results of the LDA classification based on genotype. As it can be seen, there is clear separation of the two different genotypes of peanut plants as well as background samples. This indicates that VOCs alone can be used to separate plants by genotype. Figure 2 shows the results for LDA classification based on inoculation status: inoculated or non-inoculated. Again, VOCs alone can be used to differentiate inoculation state of the plants before any physical indication can be detected. Random Forest analysis was also applied to the GC/MS data. Ten percent of the data was retained and used as a test/verification data set. The model was trained on the remaining 90% of data. Six-fold verification was performed for classification based on either genotype or inoculation state. For genotype separation, it was found that across the three test days, cross-validation score ranged between 0.9-0.97. For inoculation status prediction, results of the cross-validation score ranged between 0.89-0.93 across the three test days. These results again indicate that VOCs alone can be used to determine inoculation state or distinguish between genotypes. A large-scale greenhouse-based study is currently underway to repeat these results as well as for aflatoxin determination in the plant, pods and kernels.

Overall these results show promise for the use of VOCs for not only the detection of aflatoxin but can be used in other areas of interest such as genotype differentiation.

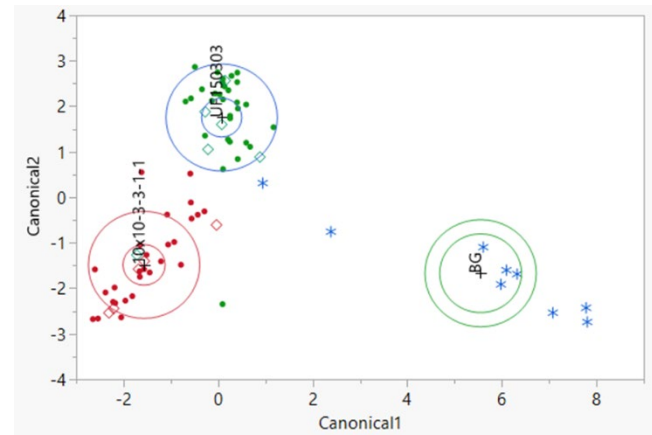


Figure 1. Linear Discriminant Analysis classification based on genotype.

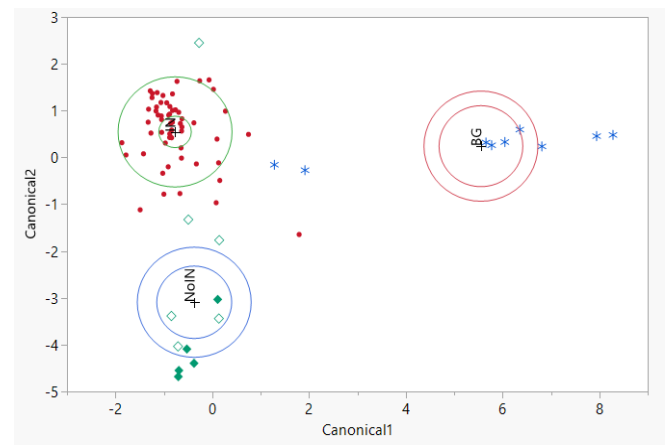


Figure 2. Linear Discriminant Analysis classification based on inoculation status.