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

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Researchers at Georgia Tech Research Institute (GTRI) have been investigating the use of plant-based volatile organic compounds (VOCs) for monitoring the status of peanut plants focusing on *Aspergillus* and aflatoxin detection. VOCs are a class of light-weight chemicals that plants continually release from all parts. The types and amounts of VOCs released change depending on the conditions/stress that the plant is currently under. Over the past several years, the team at GTRI has focused on the use of VOCs for monitoring aflatoxin in both plants and kernels pre and post-harvest. The main goal of this project is the detection, identification and quantification of VOCs unique to *Aspergillus* presence and aflatoxin development. To help accomplish this goal, two separate studies were performed over the past year.

The first study involved a large-scale greenhouse-based trial of peanut plant VOC capture and identification which was conducted June-October 2023. In this experiment, 120 plants of two genotypes, UF150303 (drought tolerant cultivar) and 10x10-3-3-1-1 (drought susceptible cultivar) were allowed to grow in a greenhouse on the University of Florida's campus. At 100 days after planting, plants were inoculated with an *Aspergillus* inoculum. Plant-based VOCs were collected from the plants at time of inoculation with *Aspergillus*, and at three additional times prior to harvest. Twisters® (a bar magnet, encased in glass, surrounded with an adsorbent material) were used to collect VOCs. The Twister® was placed directly on the peanut plant leaf, held in place with a small magnet, and isolated using a plastic lined aluminum bag for one hour. The Twisters® with captured VOCs were then shipped to GTRI for data analysis using gas chromatography–mass spectrometery (GC/MS). Analysis of the GC/MS data was performed using Partial Least Square Linear Discriminant Analysis (PLS-DA) and Random Forest Analysis. Figure 1 shows the results of the PLS-DA 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 Random Forest analysis of 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 genotype. It was found that across the three test days, cross-validation score ranged between 0.9-0.97. These results again indicate that VOCs alone can be used to distinguish between genotypes.

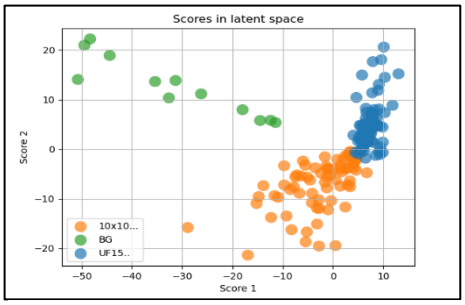

Figure 1. PLS-DA classification based on genotype

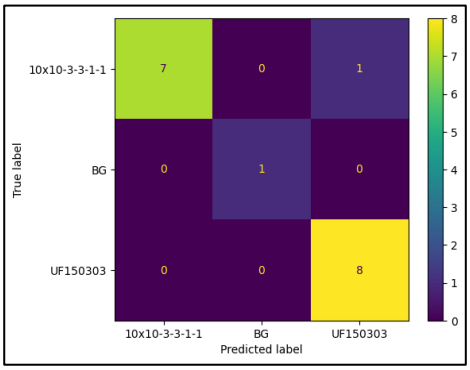

Figure 2. Random Forest classification based on genotype. Test dataset only.

The second study involved the collection of VOCs from inoculated peanut kernels. The samples were inoculated with one of three treatments: water (control), *Aspergillus* (AF13), or Aflaguard (biocontrol agent) on three different genotypes of peanuts: Florida-07, Georganic, and C76-16. Once inoculated, kernels were allowed to incubate for four days before VOCs were collected. VOCs from eight replicates of each genotype per treatment were collected using Twisters®. Kernels were placed in well plates above which, one Twister® was suspended using a magnet. Twisters® containing the collected VOCs were then analyzed using GC/MS. Analysis of the GC/MS data was performed using PLS-DA. Figure 3 shows the results of the PLS-DA classification based on treatment type. Similar to the Random Forest analysis, ten percent of the data was retained and used as the test/verification data set while the model was trained on the remaining 90% of data. Verification was performed for classification based on treatment which scored 0.83. These results again indicate that VOCs alone can be used to distinguish between varying treatments.

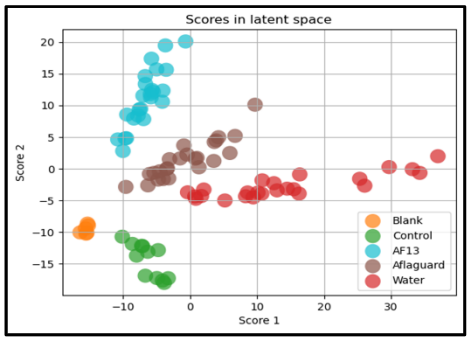

Figure 3. PLS-DA classification based on treatment

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.

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