Large Peaks Of *Aspergillus Flavus* Propagules Observed At Cotton Fields During Corn Harvest

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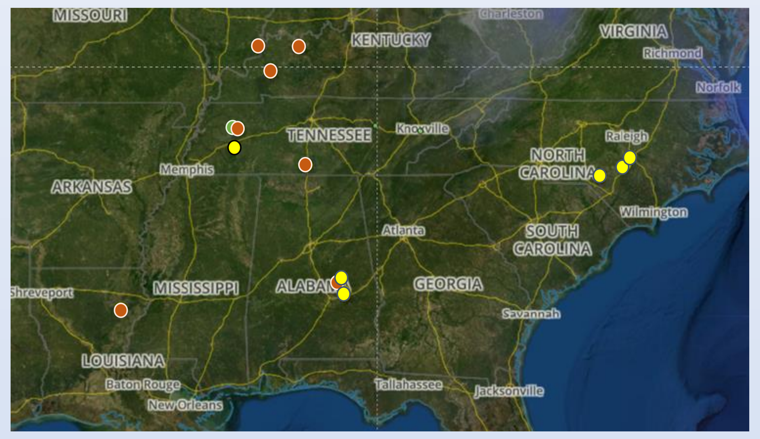
Introduction

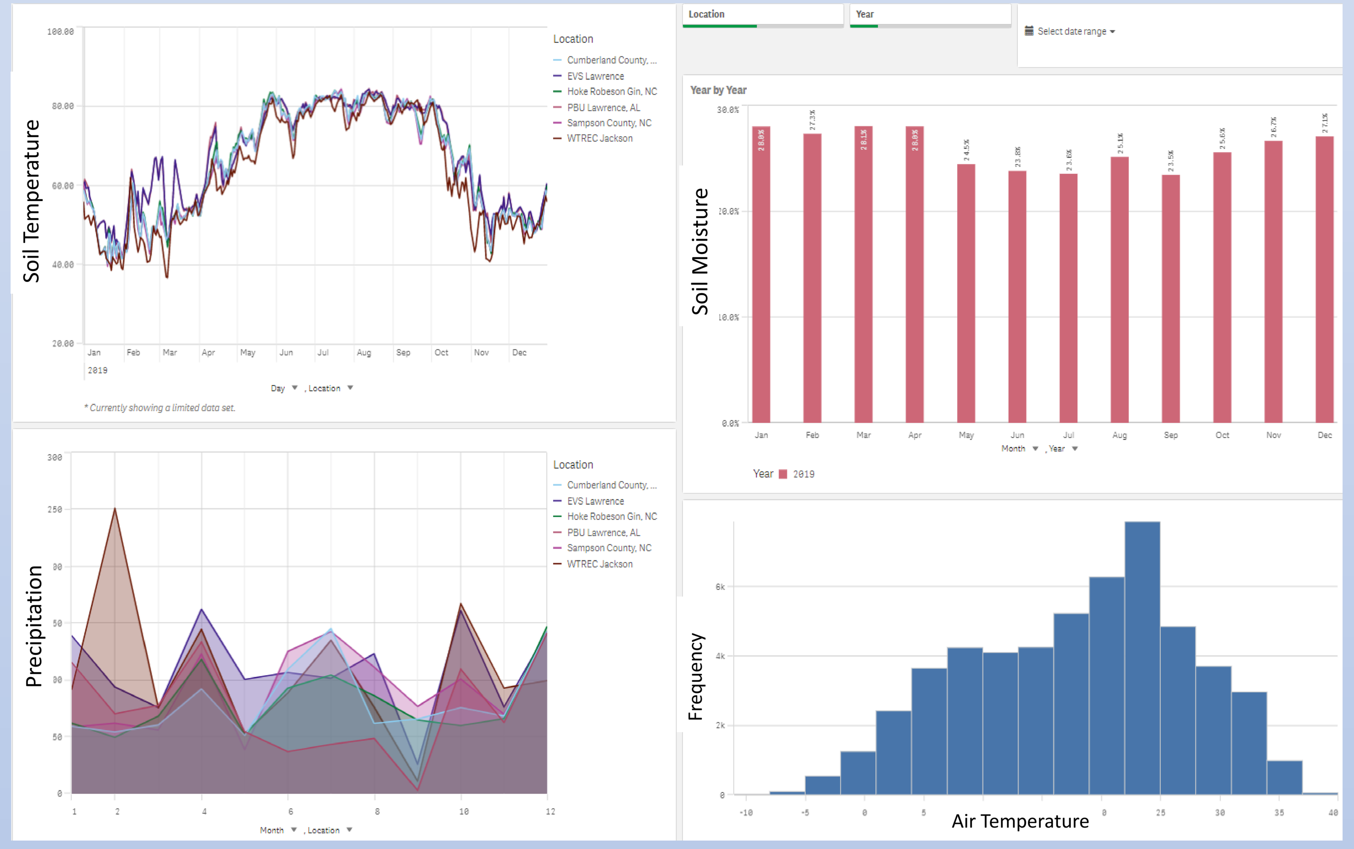
Fourteen row crop sites were monitored biweekly across the southeastern United States for five air-borne pathogens, *Alternaria alternata*, *Corynespora cassiicola*, *Cercospora sojina*, *Cercospora zeae-maydis* and *Aspergillus flavus*. The sites included seven soybean sites, one corn site, and six cotton sites. All but three sites were University research sites. The remaining three sites were commercial cotton fields.



Spore Monitoring Locations

Molecular methods were used to identify captured propagules. Probe and primer sets were evaluated from the published literature in order to also quantify spore counts. Tissue samples were submitted from our university collaborators to isolate and propagate spores for use in quantification calibration. In the end, we were able to both identify all five pathogens to the species level, and to quantify two pathogens *Alternaria alternata*, and *Aspergillus flavus*.

The Earthstream data analytic service (Mesur.io) was used to integrate nineteen local climatic measurements with spore counts from each site (an example subset, soil temperature, soil moisture, precipitation and temperature histogram is shown below for our six cotton sites.



Various Representations Of Local Climatic Data From The Earthstream System

The environmental data were used to develop a predictive sporulation model. The output of the sporulation model was compared with measured spore counts as a means of data validation. One inconsistency was the increase in spore counts of *Aspergillus flavus* in an Alabama cotton site at a time when the environmental conditions did not favor sporulation.

Aspergillus flavus is an allergen, a human pathogen, and is also pathogenic to important crops in the US including peanut, cotton, and corn.

Aspergillus flavus contamination on peanut (left), Cotton (middle) and corn (right)

Aspergillus flavus is particularly troublesome as it produces a plethora of dangerous mycotoxins which can infect food and feed.

Investigating the source of this unexpected increase in Aspergillus flavus spore counts at the Alabama site is the focus of this poster.

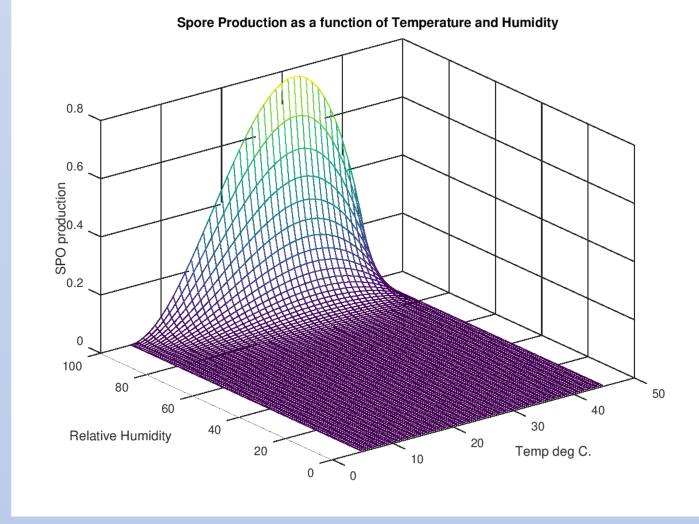
Methods

Low cost, windsock-based spore samplers were deployed at each site. Within the throat of each windsock is a removable, high-efficiency, spore capture cassette. The cassette's filter material is coated with a patented bioprotein to disrupt normal air flow and maximize spore capture efficiency. New cassettes are installed periodically (typically weekly) and the removed cassette is sent to our partner Assured Bio Labs for molecular analysis. All organic and inorganic material is extracted from the filter material, DNA was isolated and then processed using qPCR methods to identify and/or quantify specific target organisms.

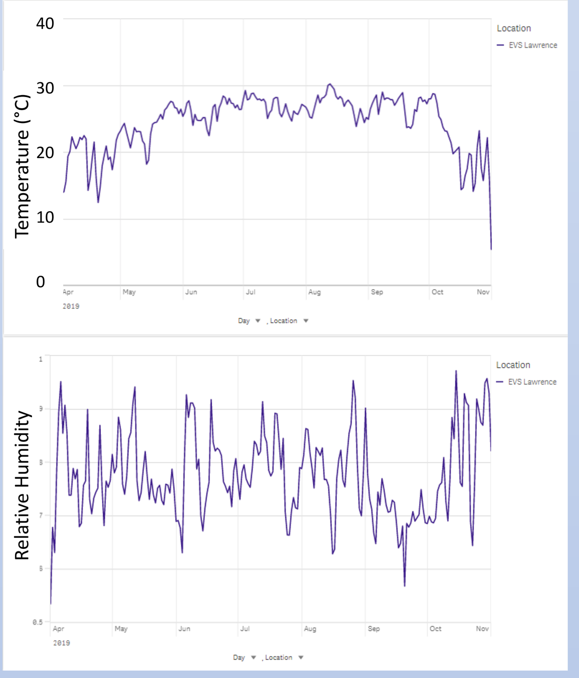
Tall and Short Passive Windsock Spore Samplers

Spore Capture Cassette with Coated Filter Material

A fundamental premise in pathology is that pathogen's presence alone is insufficient to manifest disease. The pathogen must infect a susceptible host and be in an environment that supports it growth. Many mathematical models have been developed to predict various aspects of pathogen development. We used a simple model (Battilani 2013) to estimate *Aspergillus flavus* sporulation rates based upon two environmental variables, air temperature and air humidity.



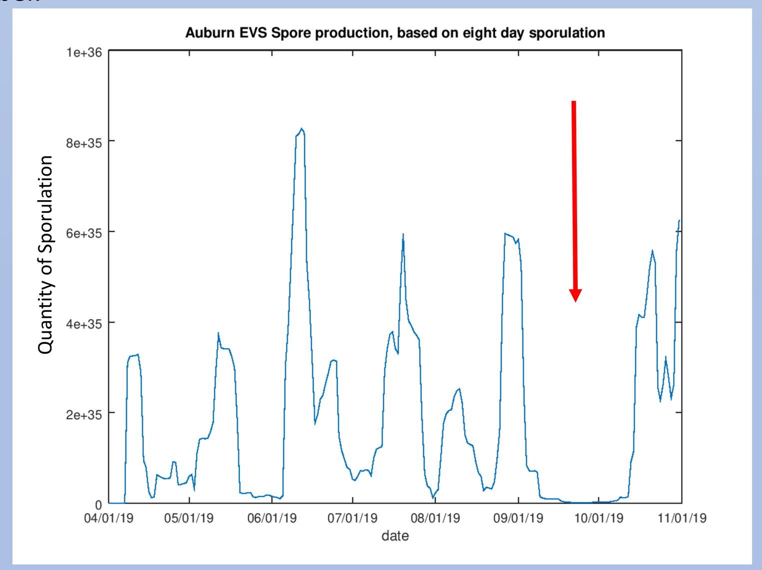
Mathematical Representation of Battilani Model of Spore Production as a function of temperature and humidity.



Local Temperature and Humidity Measurements

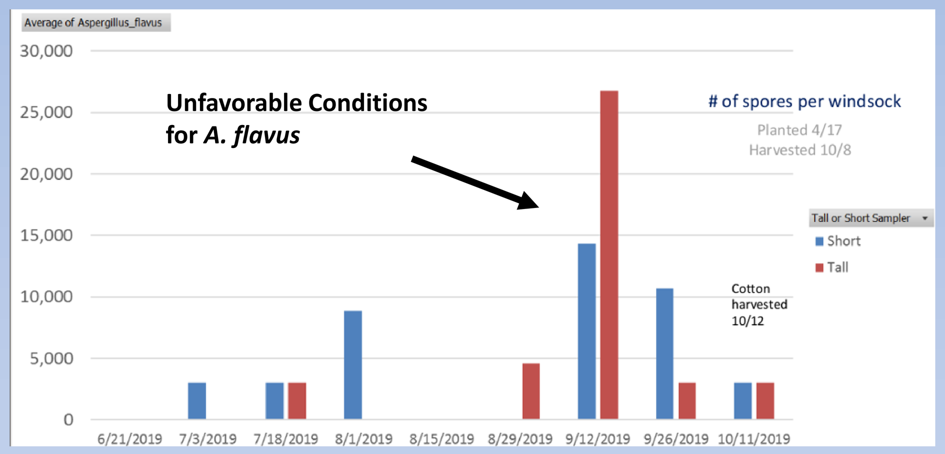
Results

Applying the model to the weather data for each site predicts local fluctuating environmental suitability for *A. flavus* sporulation. The EVS site in Alabama was predicted to have near zero spore production during the month of September.



Predicted Spore Production From Battilani Model.

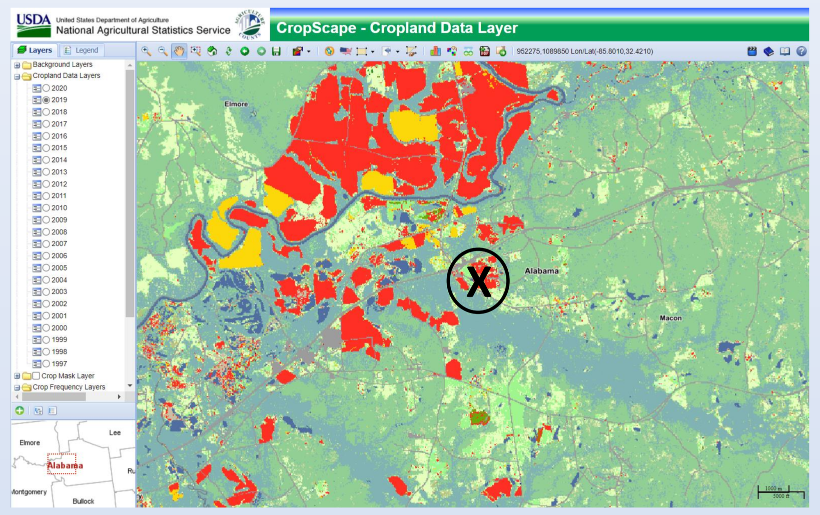
Note while the model predicts near zero environmental suitability at the EVS site for the month of September, the spore trap data (below) was indicating a spike in *A. flavus* spore counts.



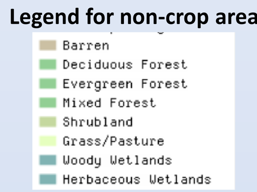
Measured Spore Counts

Results (Continued)

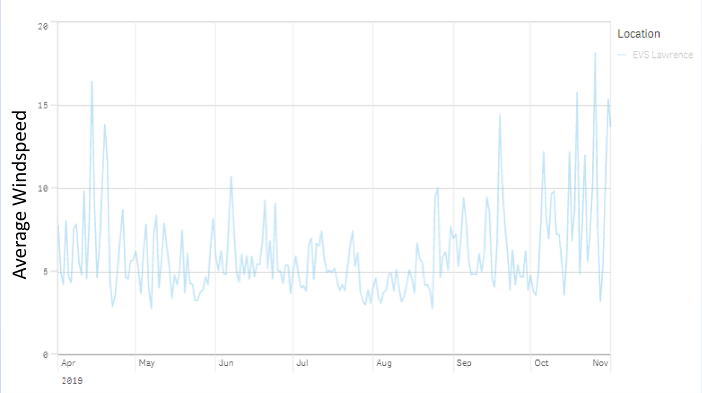
The Earthstream system is integrated with georeferenced data bases. We used the USDA CropScape database to look at other crops planted near our EVS cotton site in 2019.



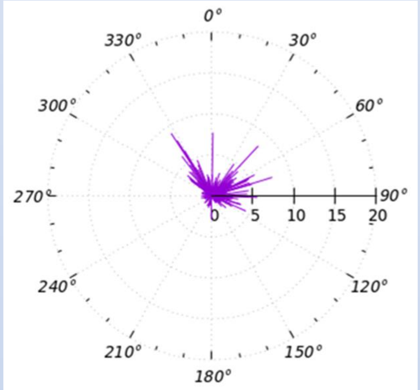
USDA Crop Scape Representation Of Various Crops In 2019. Red area represents cotton fields and gold represents corn fields. X represents location of EVS site.



The prevailing winds for this area is out of the south-southeast. As can be seen from the image above the area to the south-southeast of our location is devoid of large crop acreage. The Earthstream wind speed and wind bearing data indicated the wind direction and intensity during the September 2019 sampling timeframe was frequently out of the north.

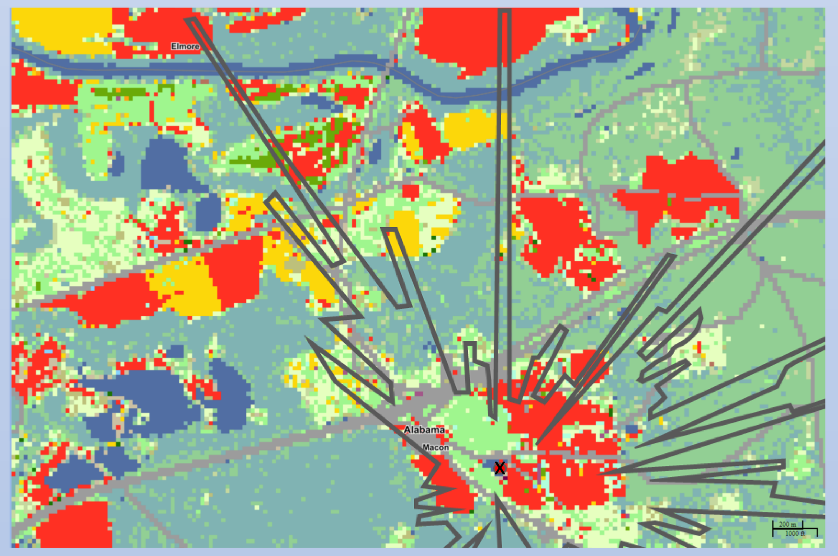


Wind Speed at EVS site during 2019 growing season.



Custom Wind-Speed Rose Histogram Which Sums Average Hourly Wind Speed In Each Compass Direction for the Two Weeks Period of High Counts.

Superimposing the custom wind-speed histogram rose over the crop scape image outlines a shape of the wind history during the sampling period.



Custom Wind-Speed Histogram Rose Superimposed Over EVS Sampler Location. Custom wind speed histogram rose gives a visualization of wind history over sampling period. Sampler can capture propagules from the directions indicated by the outer rose pedals. The longer the distance from the sampler the lower the probability of capture. The top to bottom distance of this graphic is around 3 Km. Average wind speed was 8km per hour. The typical corn harvest time in this area of Alabama is mid-August to mid-September.

Conclusions

Simple spore propagation models were used to validate data in a large, regional, crop-pathogen monitoring experiment. A data anomaly was discovered at one of our sites. Combining spore count measurements, wind, humidity and temperature data with the USDA Crop Scape data base suggests that the apparent data anomaly could be a valid data point. It is possible that suspended particles (and propagules) generated by corn harvesting could have been carried by the wind to our cotton site.

References

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[11] Jeffrey W. Cary, Matthew K. Gilbert, Matthew D. Lebar, Rajtilak Majumdar, Ana M. Calvo. Aspergillus flavus Secondary Metabolites: More than Just Aflatoxins. Food Safety 2018; Vol. 6, No. 1, 7–32.

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