Large peaks of *Aspergillus flavus* propagules observed at Cotton fields
Appendix Document

Phenotype Screening Corporation
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Summary

In Phenotype Screening Corporation's (PSC) 2019 airborne pathogen identification and quantification program, sponsored by Cotton Inc., we passively sampled for three Cotton Pathogens, *Alternaria alternata*, *Aspergillus flavus*, and *Corynespora cassiicola* at nine sites. Spore capture data for *A. flavus* were compared to a model of the life cycle of the fungus in order to validate the data. Some capture results appear to be consistent with the model. For most of the sites analyzed, a large peak of spore collection occurred at a time when the model suggested that sporulation and dispersal would not occur. These peaks occur during corn harvest dates for the various sites. A possible mechanism for the aerial dispersal of *A. flavus* spores from corn harvest is presented. Each of the sites is discussed below.

The Project

Project title: "Early Implementation of Passive Microbial Air Sampler and Microbe Identifier for Cotton Field Management Improvement, Phase II"

Funded by Cotton Incorporated: Agreement Number 2019-18-841.

University Collaborators

This project relied upon the deep knowledge and support of our university collaborators. They provided access to their research plots and disease incidence and severity ratings. as well as deploying passive spore collectors at their fields. With their leadership the project was able to monitor corn, soybean and cotton fields over the 2019 growing season for *Alternaria alternata*, *Corynespora cassiicola and Aspergillus flavus*.

Auburn University - Kathy Lawrence, PhD, Edward Sikora, PhD, Marina Nunes Rodon, PhD, Bisho Lawaju

The University of Kentucky - Carl Bradley, PhD

Louisiana State University - Sara Thomas-Sharma, PhD, Nelomie Galagedara,

The University of Tennessee - Heather Kelly, PhD, Rachel Guyer

Background: *Aspergillius flavus* and Aflatoxins

A. flavus is a crop pathogen that causes Aflatoxins in corn, cotton, peanuts, tree nuts, and other crops. Aflatoxins in food and feed products can cause cancer and other potentially fatal health problems.

Relevant Quotes:

“Particularly common clinical syndromes associated with *A. flavus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and inoculation.” [9]

“Fungi are in a constant battle with both predator and competitor organisms that they must be able to coexist, compete and interact with in their particular environmental niche. These niches, be they the human body or an agricultural field are dynamic environments subject to change where fungi must be able to adapt in order to compete for available nutrients allowing them to reproduce and disseminate thus ensuring survival. Fungi have evolved the capability to produce a wide array of SMs [Secondary Metabolites] in response to their environments many of which play important biological roles such as virulence factors, chemical defense agents, insect attractants, protection from abiotic stressors, developmental regulators, and as chemical signals for communication with other organisms.”[10]

“Additionally, little is known about the role of SMs [Secondary Metabolites] on the interaction of *A. flavus* with other microbes that constitute the microbiome of the host plant and surrounding soil environment. These types of studies will provide invaluable insights into the roles of *A. flavus* SMs with respect to fungal virulence, survival, toxigenic potential and modulation of microbial and insect communities, all with the goal of controlling contamination of susceptible crops with *A. flavus* mycotoxins, particularly aflatoxins.”[11]

Spore Collection

Passive spore collectors were used to collect spores. Each orange windsock contains a spore collector cassette at the exit end of the wind sock. The short collector is 3 ft. above surface and the tall collector is 6 ft. above the soil surface.

Tall (6 ft.) and short (3 ft.) Passive Spore collectors

DNA Analysis

DNA analysis work was done by Assured Bio Labs, LLC, an AIHA-LAP, LLC accredited laboratory for testing mold, bacteria, and coliforms using traditional microscopic and DNA-based (MSQPCR) analysis. Assured Bio is located in Oak Ridge, Tennessee. Assured Bio processes and interprets samples from homes, office buildings, schools, hospitals, micro-sensitive manufacturing facilities, and other buildings, as well as agricultural settings.

Weather Data

Mesur.io is a real-time environmental analytics provider. The Earthstream™ platform puts hyper-local data about soil, weather, pests and disease patterns in the hands of growers. Weather data from mesur.io was used to run the sporulation model and to produce the wind roses (shown below).

***Aspergillius flavus* Model**

The Battilani model [1] is a mechanistic model of the life cycle of *Aspergillus flavus*. The entire Battilani model consists of five parts, the sporulation model, the dispersal model, the corn infection model, the aflatoxin growth model, and the prediction of aflatoxin content in corn.

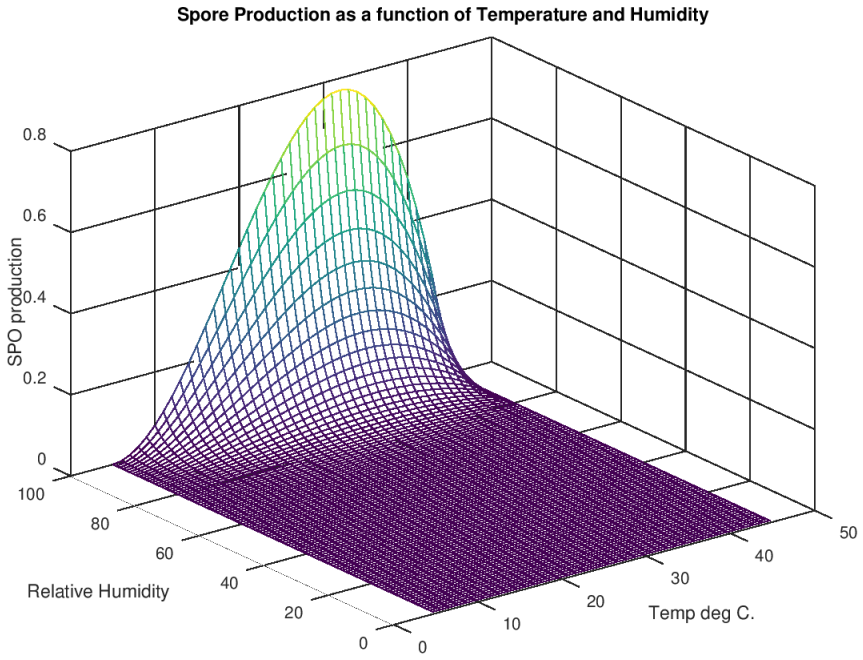
In order to understand and explain the results of detection of *A. flavus* spores, PSC developed a computational model of sporulation based on the Battilani model. This is a mechanistic model in that equations were derived from laboratory data to describe the growth and development of the fungus under a range of steady state conditions. The model was implemented on a piecewise basis, over an

eight day period from the initiation of sporulation to completion of sporulation and availability to disperse. To the best of our knowledge, PSC is the first to implement the sporulation model.

The sporulation model uses the hourly temperature and relative humidity weather data from mesur.io. Under equilibrium conditions, relative humidity and water activity are equal. However, field conditions are dynamic and always changing. The use of hourly piecewise data was used to minimize deviations from equilibrium.

According to Battilani Field Observations, dispersal occurred when spores are available, Relative Humidity was <80 percent and there was no rain [1]. This part of the model was investigated and felt to be insufficiently specific for our purpose. The dispersal model was not used in this study.
Spore dispersal in *A. flavus* is correlated negatively with Relative Humidity and positively with wind speed, but no quantitative data on those relationships were available in the literature. According to Battilani Field Observations, dispersal occurred when Relative Humidity was <80 percent and there was no rain [1].

The Battilani sporulation model is based on temperature and water availability. This model can only predict the environmental suitability for growth of spores based on those variables. The model cannot predict the presence of spores, but only the possibility of spores being present from a local sporulation and dispersal event.



A. flavus spore (SPO) production as a function of relative humidity and temperature

The logic of the model:

Spore dispersal can occur after environmental suitability for sporulation has been high.

It is possible that spores are being dispersed locally and also being brought in from some other location during the same collection period.

If spores are captured during a period of time when sporulation and dispersal are predicted by the model, this suggests that locally produced spores are being collected.

If no spores are collected during a period of time when sporulation and dispersal are predicted by the model, this suggests that no local sclerotia are present to sporulate and disperse, and no spores are brought in from other locations.

If spores are captured during a period of time when sporulation and dispersal are not predicted by the model, this suggests that spores are being dispersed by some other process, either at the site in question or from some other location.

USDA CropScape

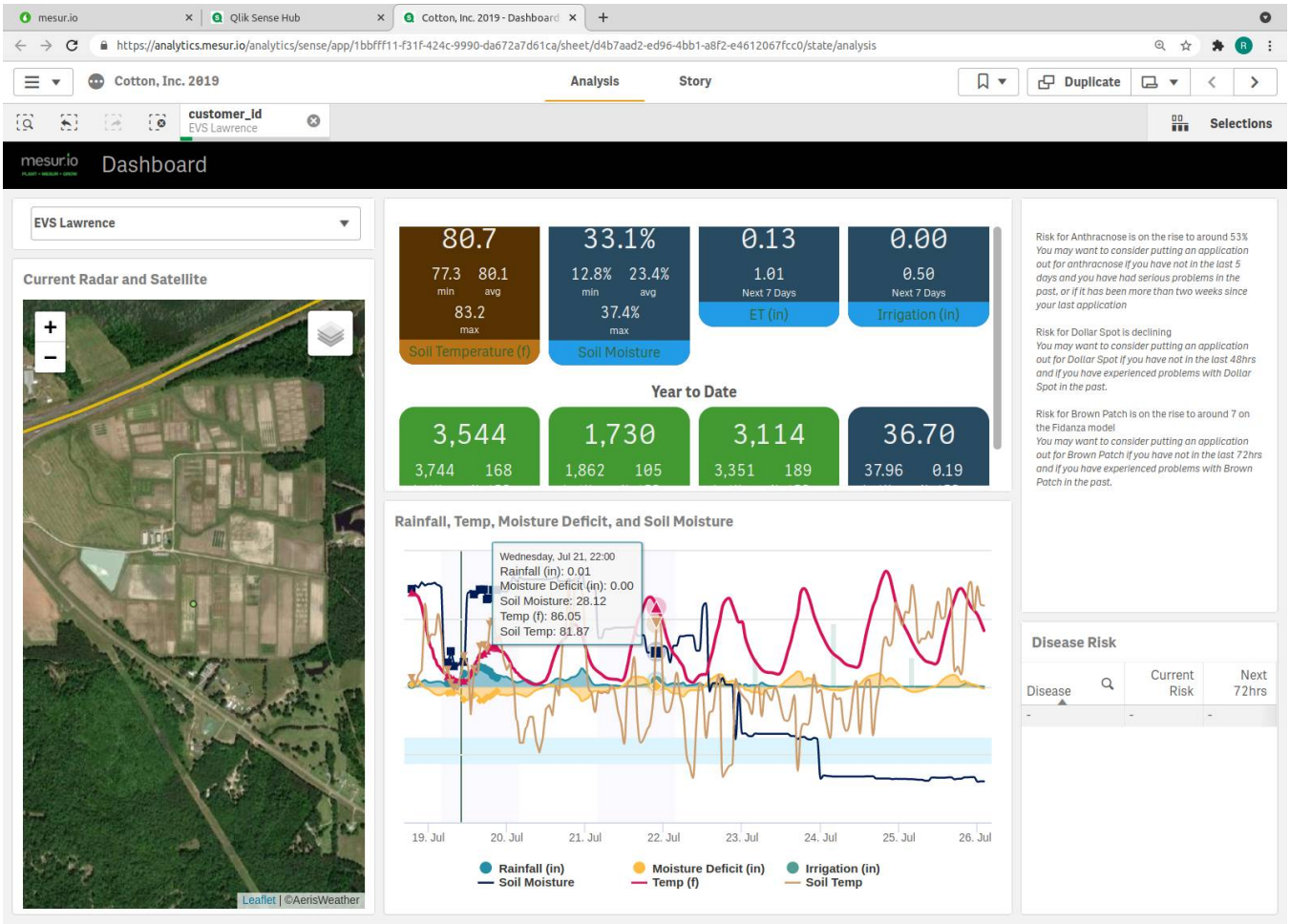
USDA CropScape [4] was used to document the crops in surrounding fields. The wind data from mesur.io was used to make wind roses for those spore collection periods with large spikes of spore count. Corn fields located upwind from the spore collector were then identified at most sites as being potential sources of A. flavus.

Collection Sites and *A. flavus* Detection

A. flavus spore collection results at several sites in the 2019 Cotton Inc. project are discussed below. Dates shown on bar charts are for the last day of a sampling period. Unless noted, The replacement of one collector occurs at the same time the previous collector is removed. For example, in the Auburn EVS [4] bar chart, the bars labeled 9/12/2019 represent spores collected between 8/29/2019 and 9/12/2019.

Analysis of Sites

Figures below show the results of running the Battilani model with mesur.io weather data for each of the nine sites considered. See the Cotton Inc. project report [5] for details of site location. The mesur.io dashboard has site location, soil types, CropScape, as well as weather and other agronomic conditions.

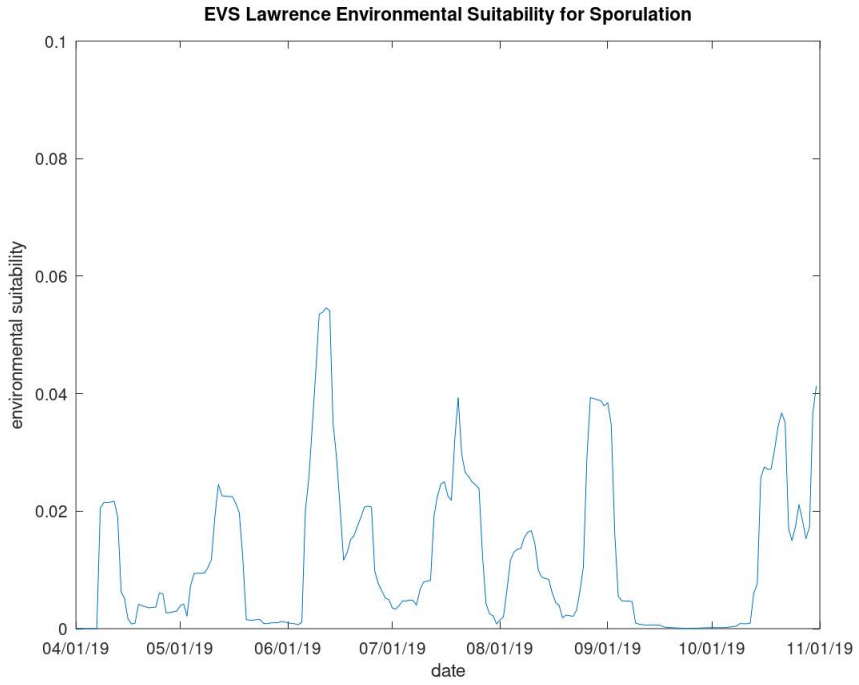


Mesur.io Dashboard, example view

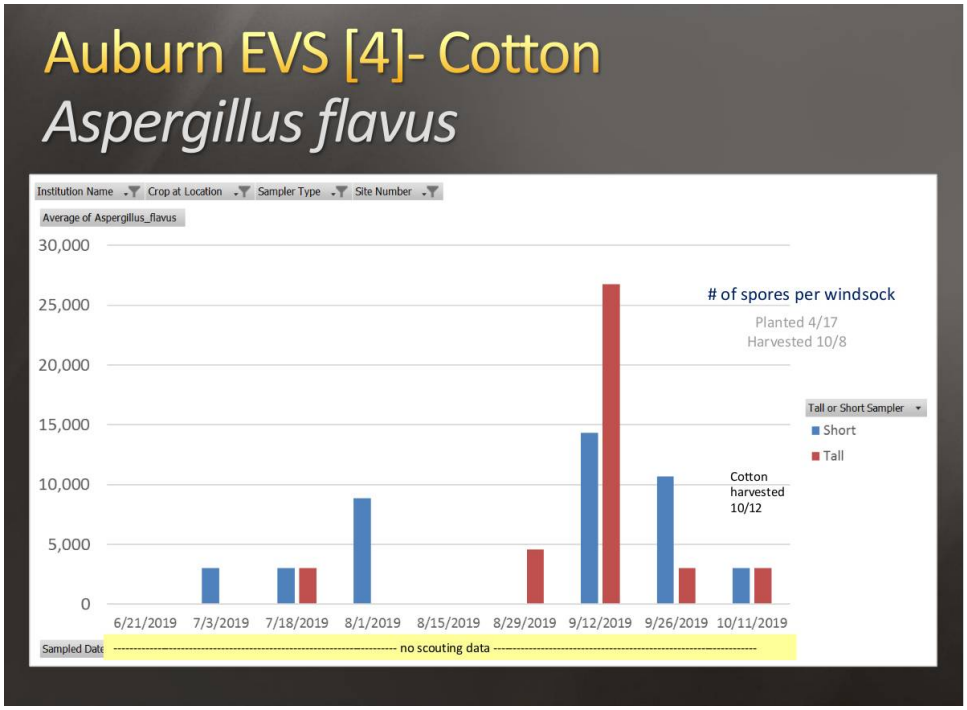
EVS Lawrence Site

Google Maps satellite view from the mesur.io dashboard shows the location of the spore collector. This is a Cotton field managed by Auburn University.

Satellite view of EVS Lawrence Site. Red dot shows collector location at site.



Environmental Suitability for Sporulation of A. flavus using Battilani sporulation model.



Actual Spore Counts. Date under bar is date of removal of capture cassette

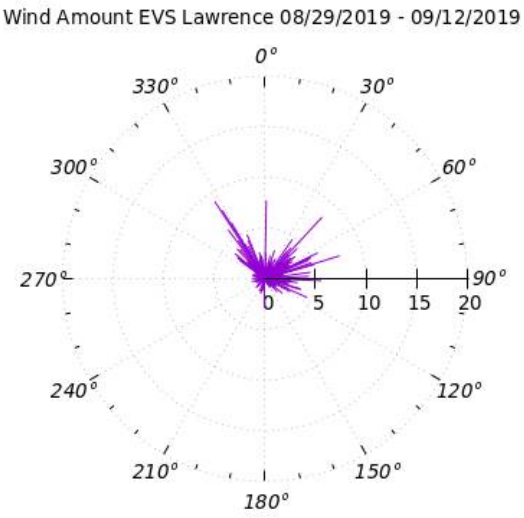
The actual spore capture data for late June through August may have been due to local sporulation since the model predicts high environmental suitability for sporulation during this period. The short collector captured more spores than the tall collector, suggesting local spore dispersal was the larger process.

Note the peak in *A. flavus* spore capture in September. This capture period runs from 8/29/2019 - 09/12/2019. During this period environmental suitability for sporulation decreased dramatically. Note the large peak at the tall collector. This suggests that the spores captured were brought in by the wind from somewhere else rather than being dispersed from a local *A. flavus* population.

According to USDA, harvest time for Corn in Alabama is Aug 11 – Sep 20, with an end date of Oct 15 . The harvest time for peanuts is Sept 22 – Oct 22.[2]

The correlation between corn harvest and *A. flavus* capture suggests that a major source of spores may be corn harvest.

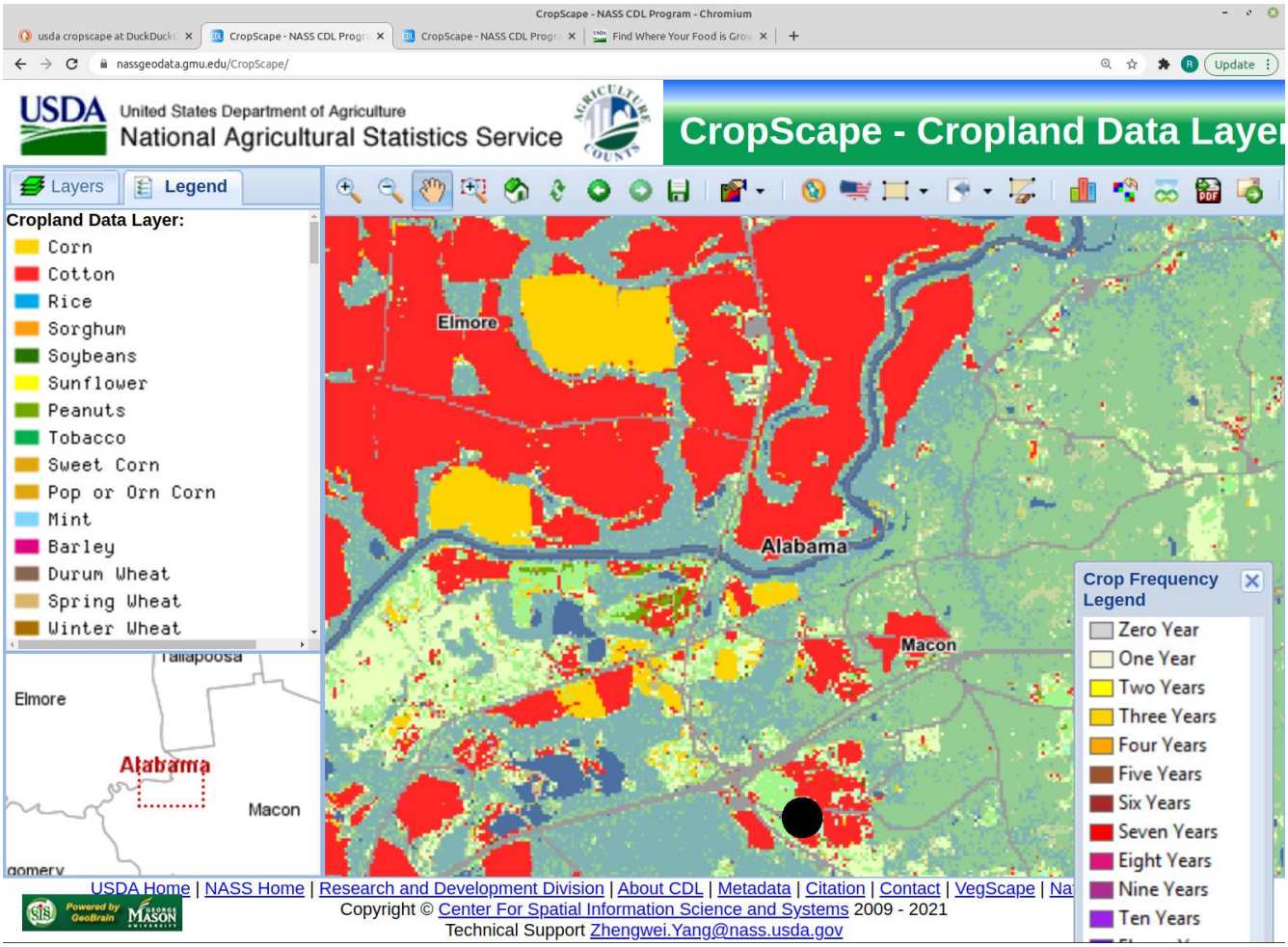
The wind data for the period of 08/29/2019 – 09/12/2019 was used to construct a wind rose that shows the amount of wind passing over the site vs. the wind direction.



Wind Rose for EVS Lawrence site

From the wind rose, most of the wind comes from the NorthWest to the NorthEast. This suggests that the origin of external spores is likely to be from a Northerly direction.

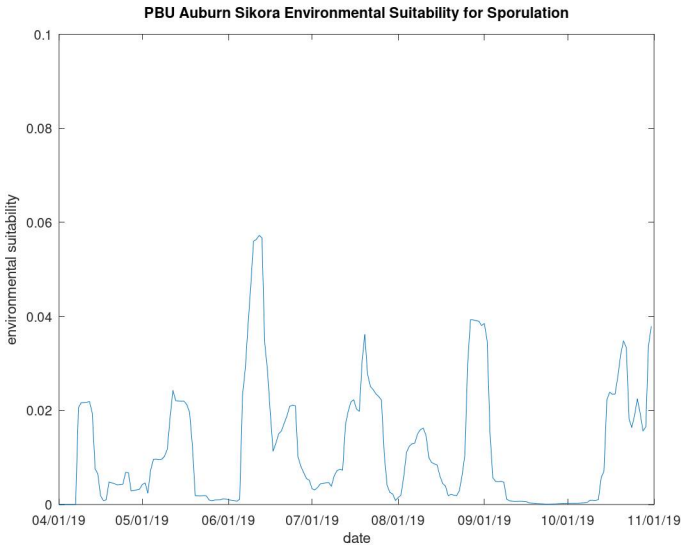
The USDA CropScape [4] map, below, for the area surrounding the Cotton field in question is shown below. There are corn fields less than half a mile way, to the NorthWest of the Cotton field. It is possible that these corn fields are the source of *A. flavus* spores collected at this Cotton site.



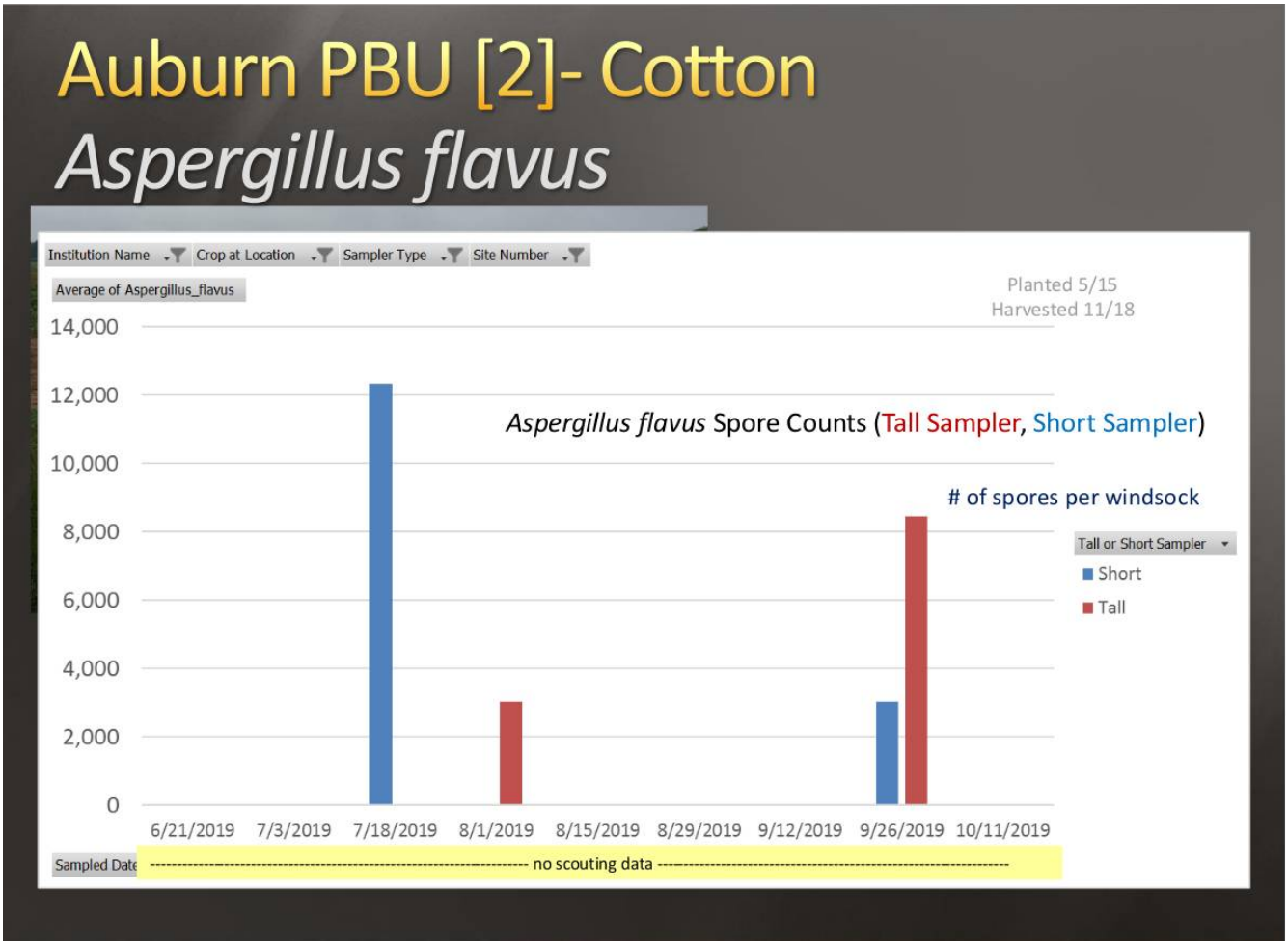
USDA CropScape Map. Black Dot is field location. Orange fields to the North - NorthWest are corn. Distance is about 3.6 miles.

Auburn PBU [2]

Satellite view of Auburn PBU Lawrence Site. Dot shows site.



Environmental Suitability for Sporulation of A. flavus as computed using Battilani sporulation model.

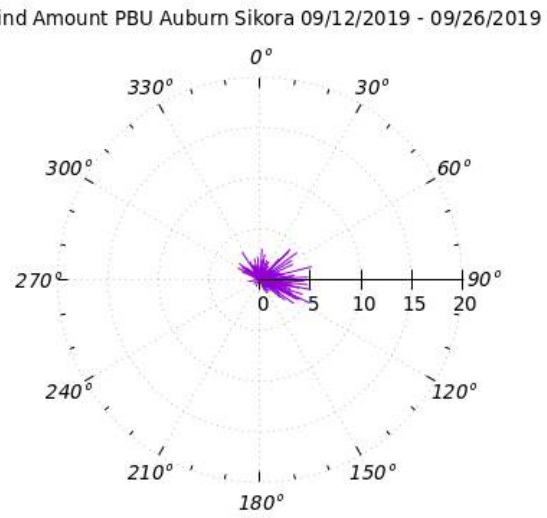


Spore Capture Data

The collection peak in early July corresponds to the time of tropical storm Barry and is probably not related to a local sporulation event. The smaller collection amount in late July may be a result of some kind of post storm activity.

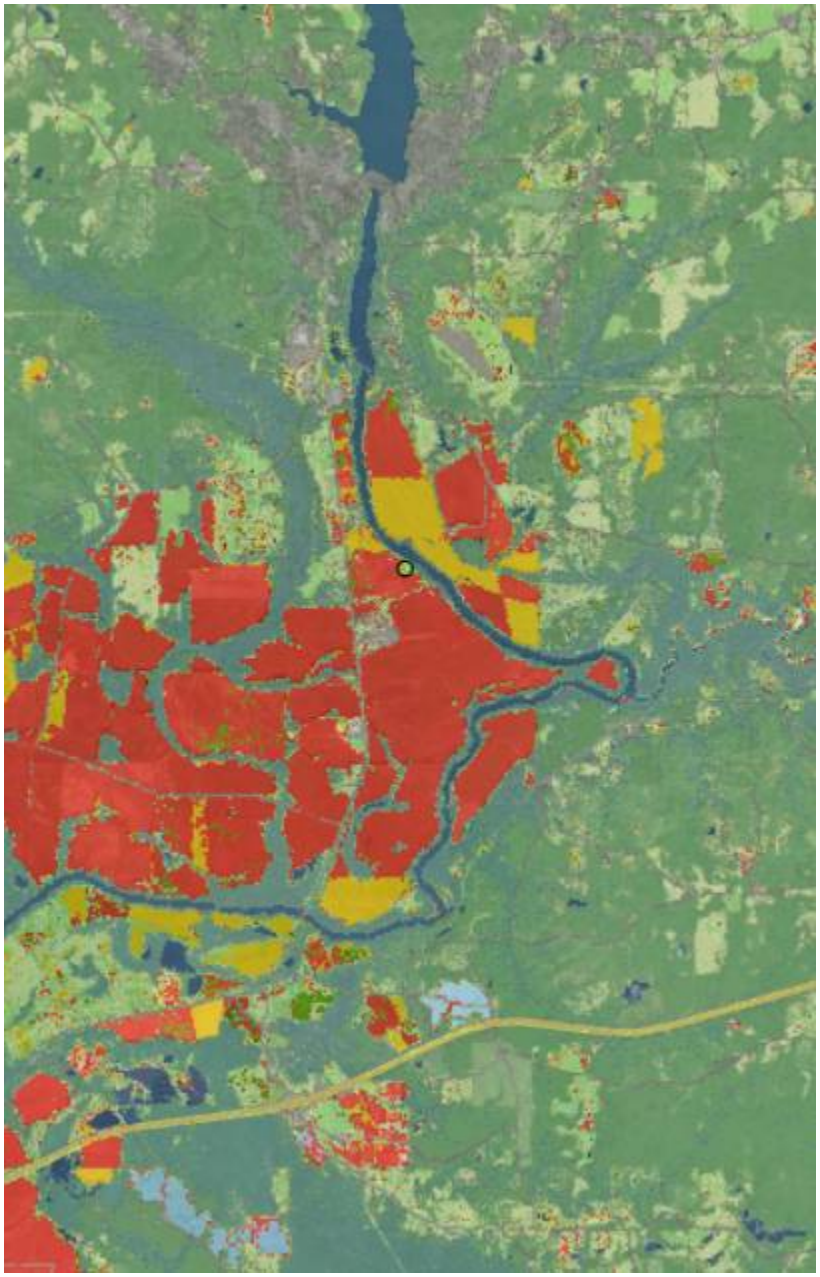
Note the peak in September. According to USDA, harvest time for Corn in Alabama is Aug 11 – Sept 20, with an end date of Oct 15. Harvest time for peanuts is Sept 22 – Oct 22.

The collection peak ending 9/26/19 occurs during corn harvest and peanut harvest time. This peak occurs during a time when the model suggests no sporulation or dispersal of *A flavus*. The tall collector had a higher spore count, suggesting that at least some of the spores were blown in from another location.



Wind Rose for Auburn PBU Lawrence Site

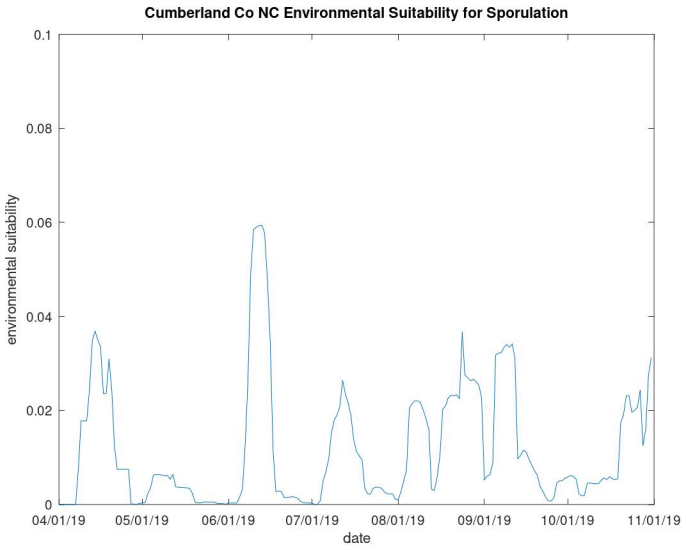
The CropScape view of the site shows a corn field to the North of the site. This is a potential source of the A. flavus detected.



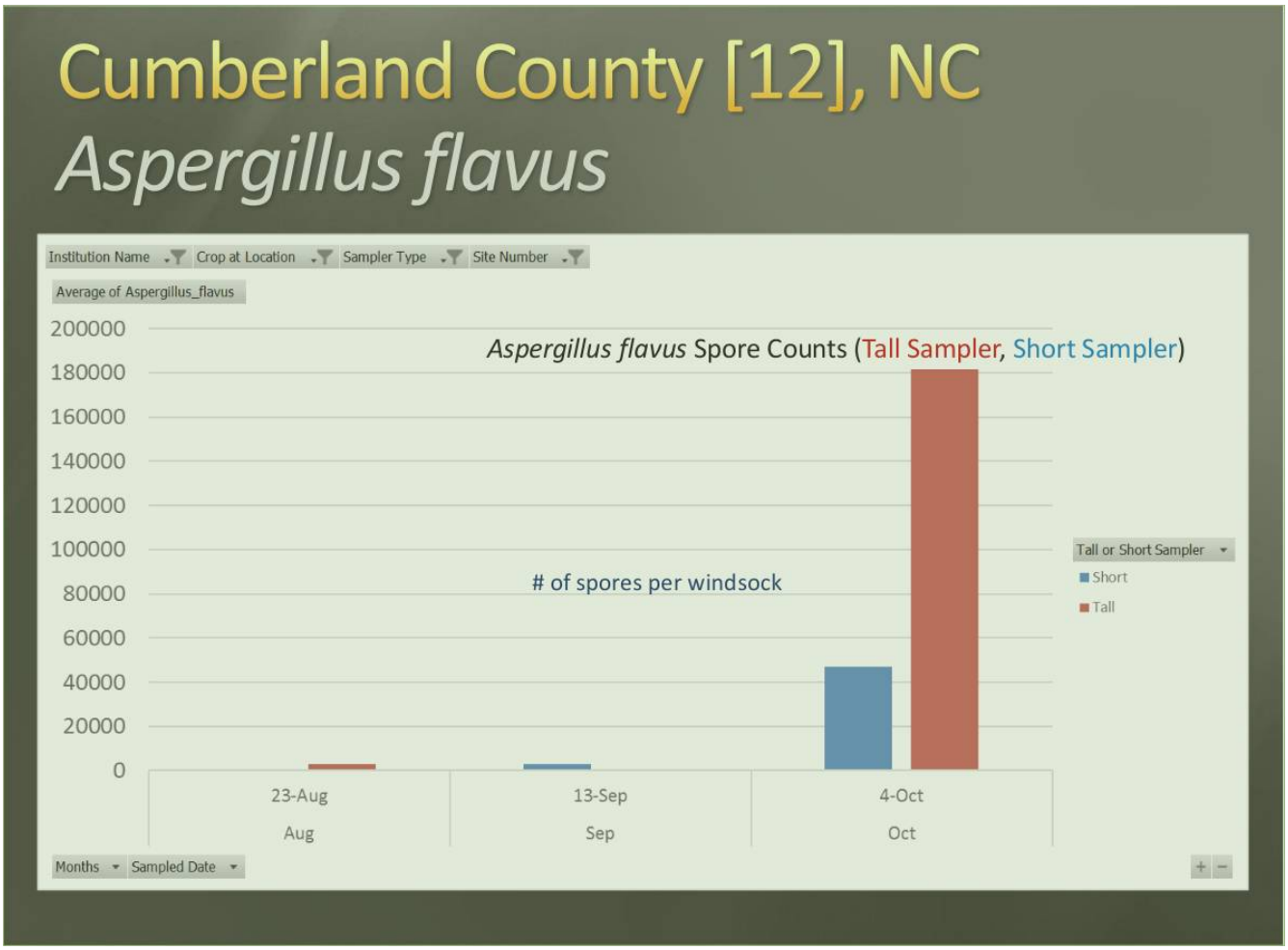
USDA CropScape Map. Dot is field location. Orange fields are corn.

Cumberland County [12]

Satellite view of Cumberland County Site



Environmental Suitability for Sporulation of A. flavus
as computed using Battilani sporulation model.



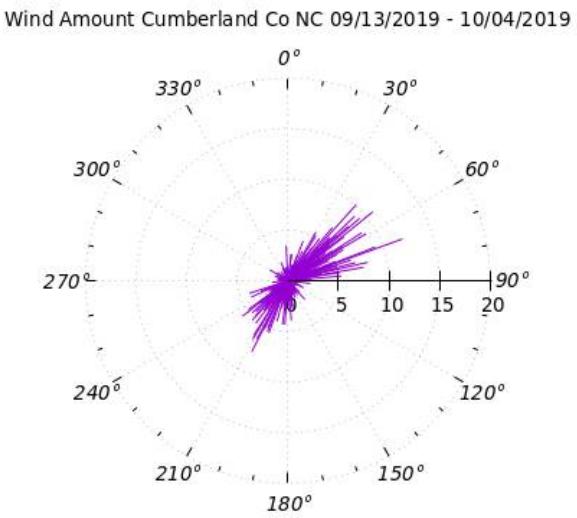
Spore Capture Data

Note the *A. flavus* peak in the period 13 Sept - 04 October 2019. The Battilani model of sporulation did not show any significant sporulation during the time of peak spore collection.

Peaks shown in the model do not correspond to peaks of detection by our spore traps.

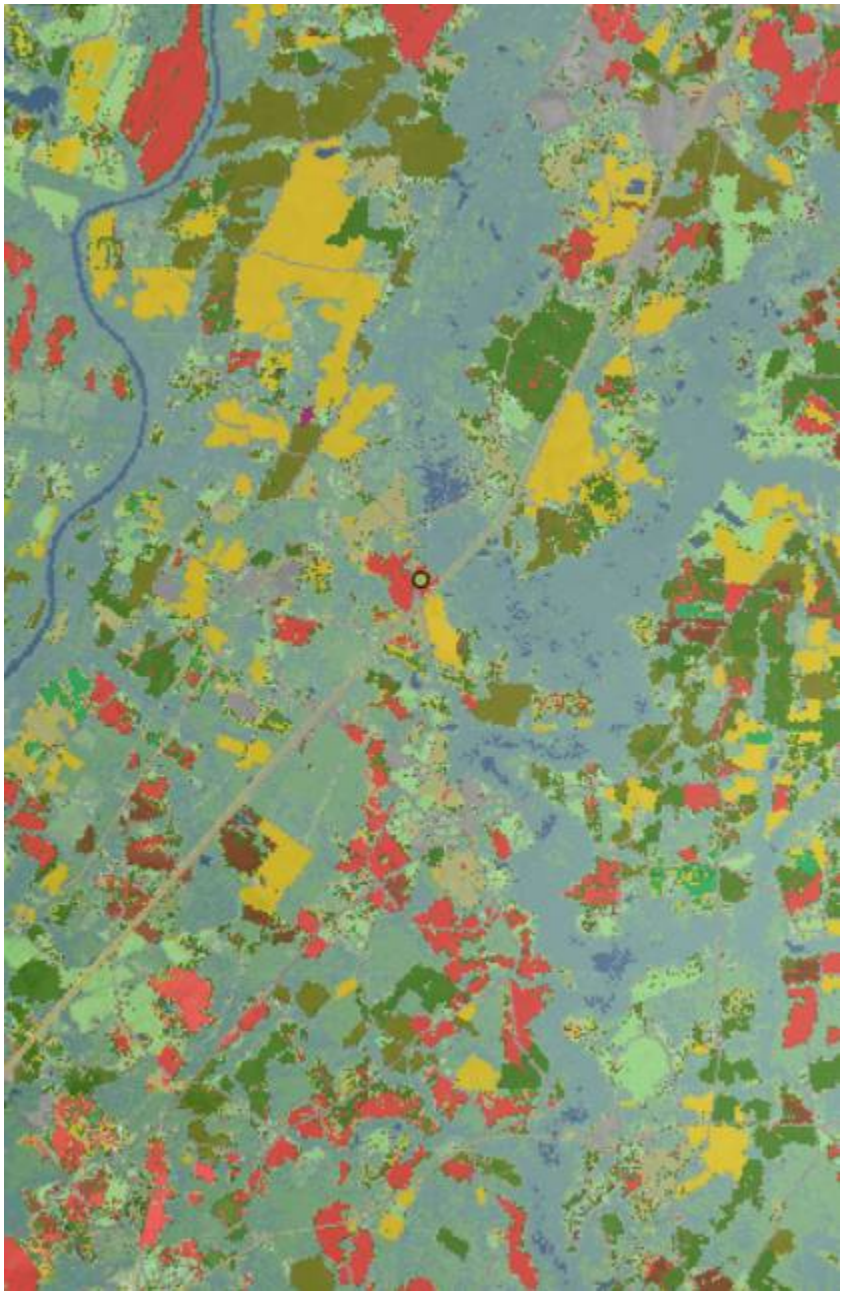
According to USDA, harvest time for Corn in North Carolina is September 10 – Oct 10. Peanut harvest time is Oct 10 - Oct 30. The peak in October coincides with corn harvest more than peanut harvest.

The very small capture amounts in August and early September may be the result of local sporulation and dispersal.



Wind Rose for Cumberland County Site

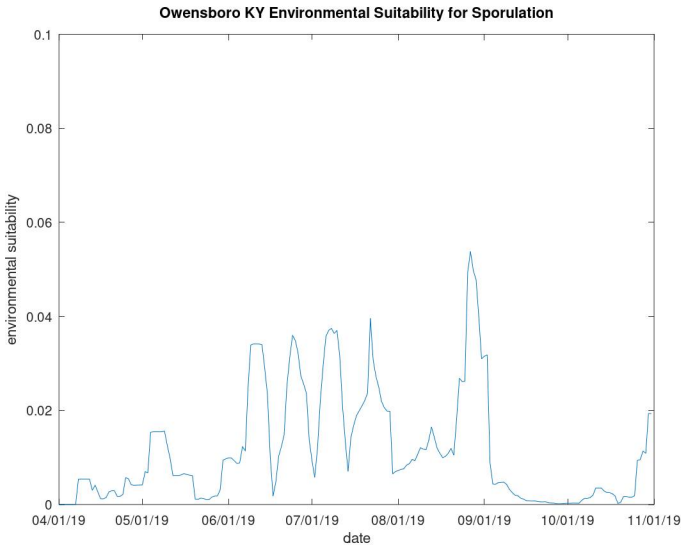
CropScape shows that there were several corn fields around this site.



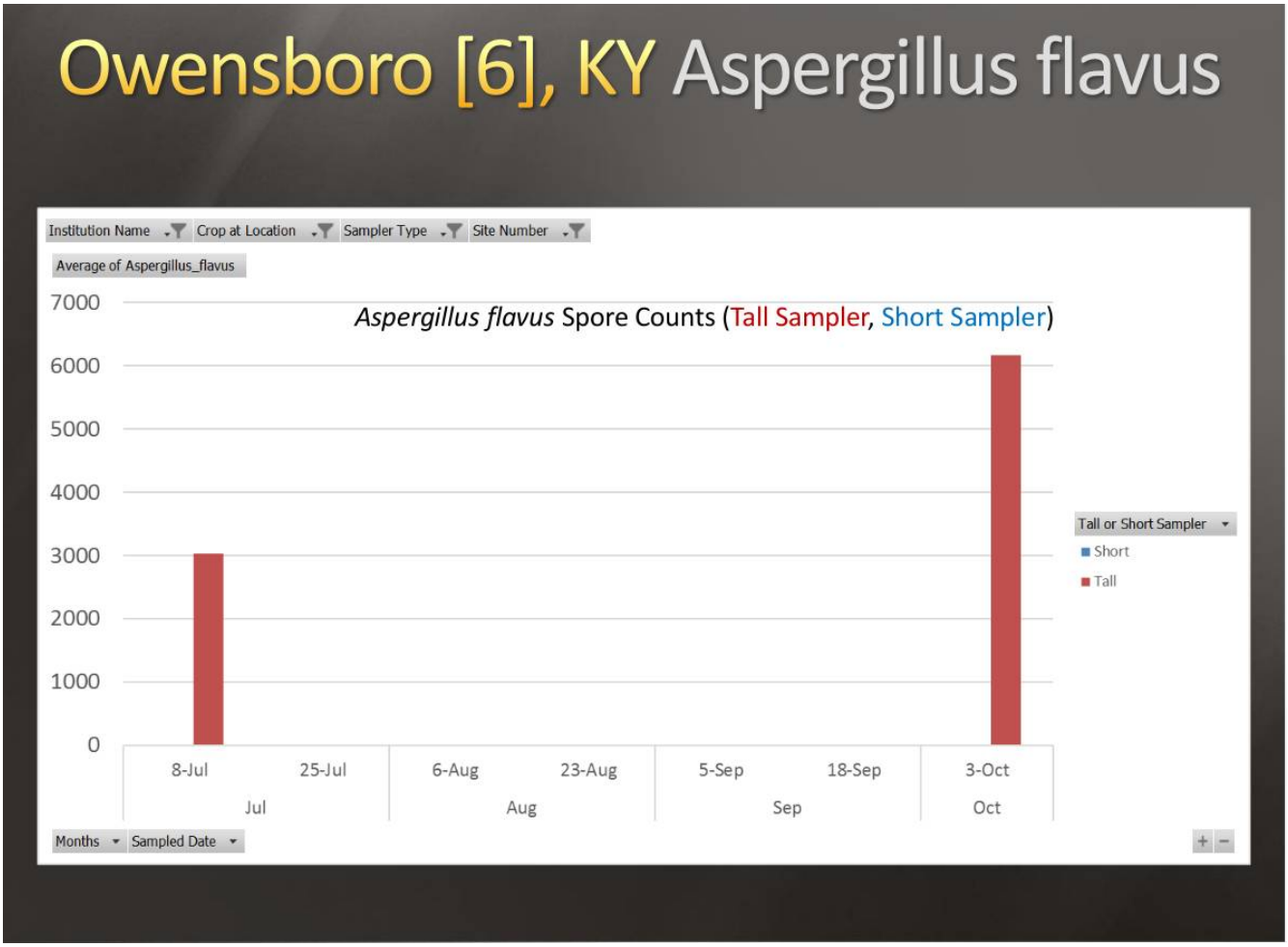
USDA CropScape Map. Dot is field location. Orange fields are corn.

Owensboro [6]

Satellite view of Owensboro Site



Environmental Suitability for Sporulation of A. flavus as computed using Battilani sporulation model.



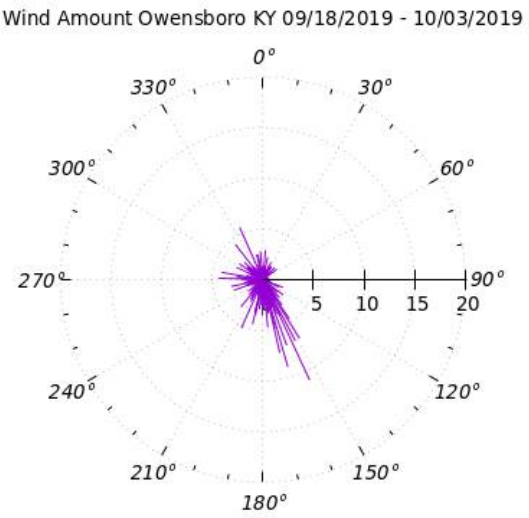
Spore Capture Data

The collection peak in early July occurs before tropical storm Barry. The cause of this peak is not known.

Note *A. flavus* peak in period 18 Sep – 03 Oct. The Battilani model of sporulation did not show any significant sporulation during this peak of spore detection.

This is a soybean site. Soybean harvest in KY is Oct 10 – Nov 14. This peak is probably not associated with soybean harvest.

This peak corresponds with corn harvest dates in KY: Sep 9 - Oct 24. Harvest dates for peanuts in KY not shown.



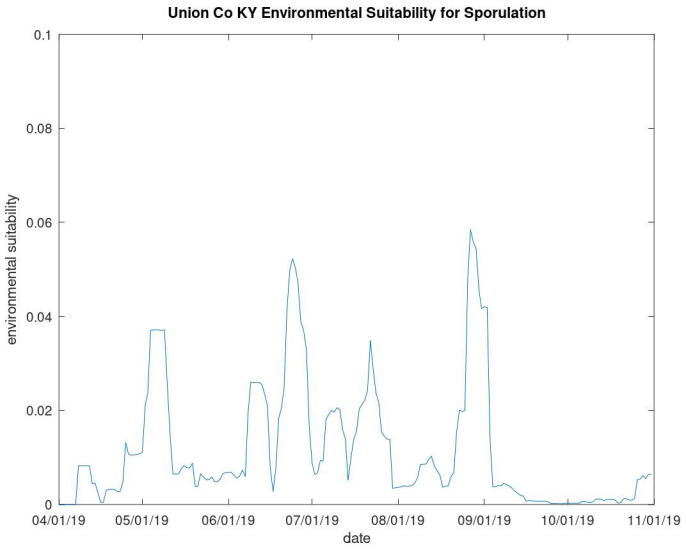
Wind Rose for Owensboro Site

The CropScape Map shows several corn fields downwind of the site which could have contributed to the *A. flavus* spore count.

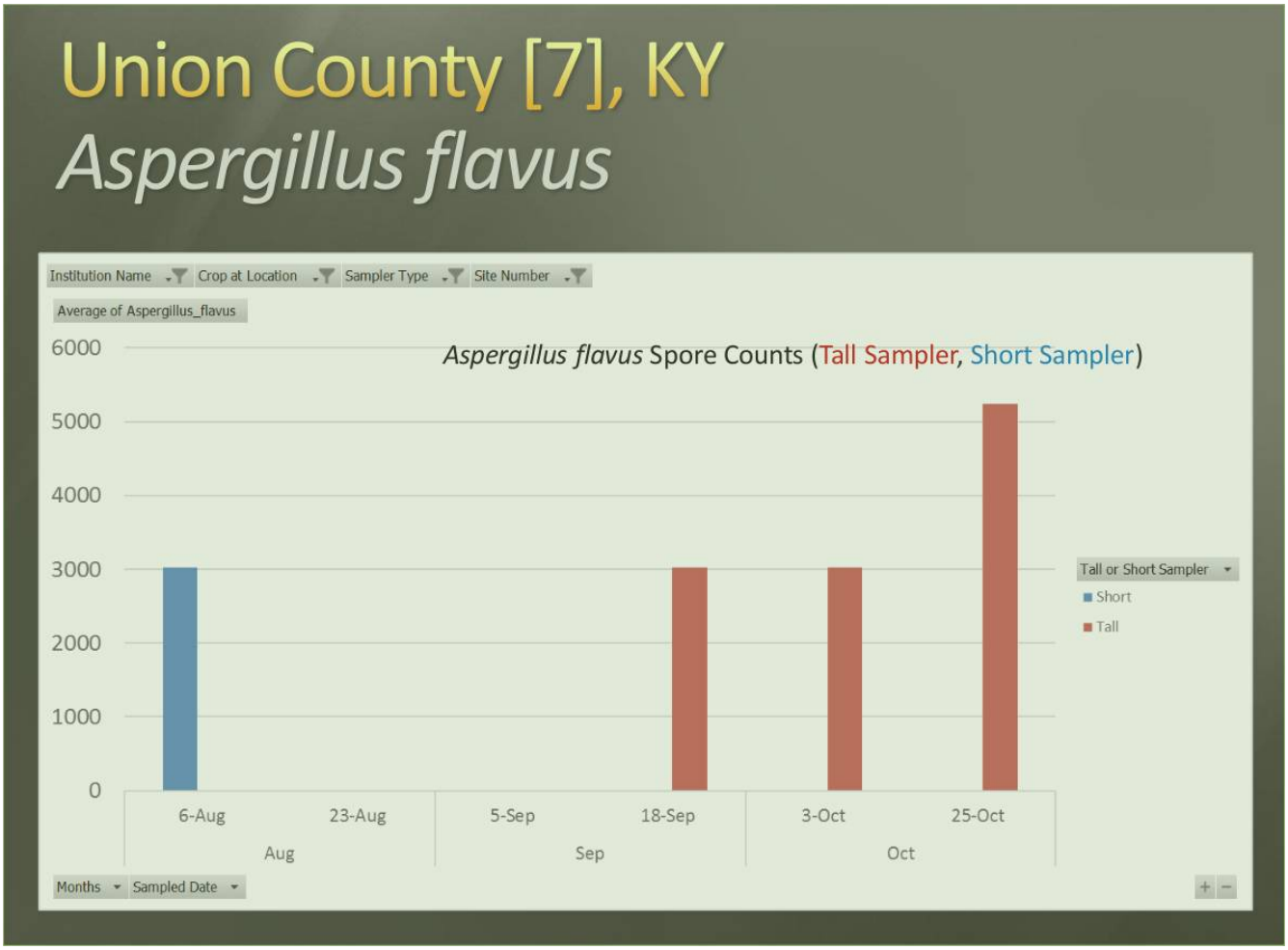
USDA CropScape Map. Dot is field location. Orange fields are corn.

Union County [7] Ky

Satellite view of Union County Site



Environmental Suitability for Sporulation of A. flavus
as computed using Battilani sporulation model.



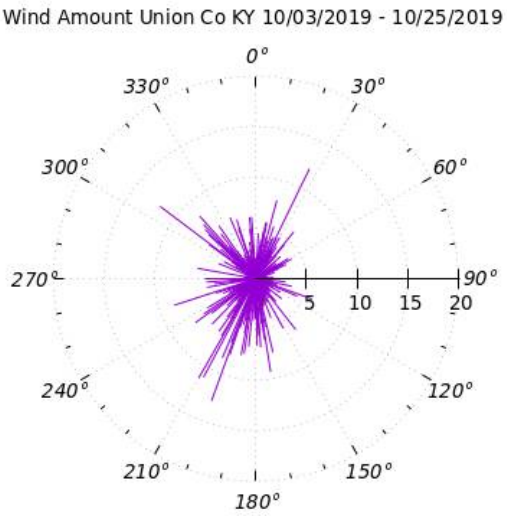
Spore Capture Data

The 6 August spore capture may correspond to a large dispersal peak shown in the model as occurring in July. The timing is not that close, but having the peak on the Short collector suggests that it may be a local event. Another possibility is the influence of tropical storm Barry.

The 05 Sept - 25 Oct collection peaks occur at a time when the model does not predict any sporulation or dispersal event. The spores were captured in the Tall collector, not the Short collector.

This is a soybean site. Soybean harvest in KY is Oct 10 – Nov 14. Corn harvest dates in KY are Sep 22 – Oct 22. No peanut harvest dates shown for KY.

Tall sampler results suggest that spores have been transported in from somewhere else. It is possible that corn harvest may have contributed to the spore capture at this site.



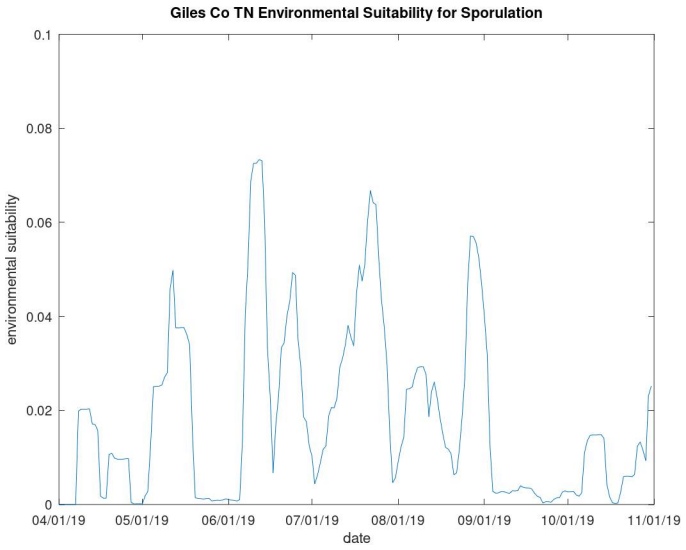
Wind Rose for Union County Site

USDA CropScape shows that corn was grown in areas around the site, which could have been the source for spores collected at the site.

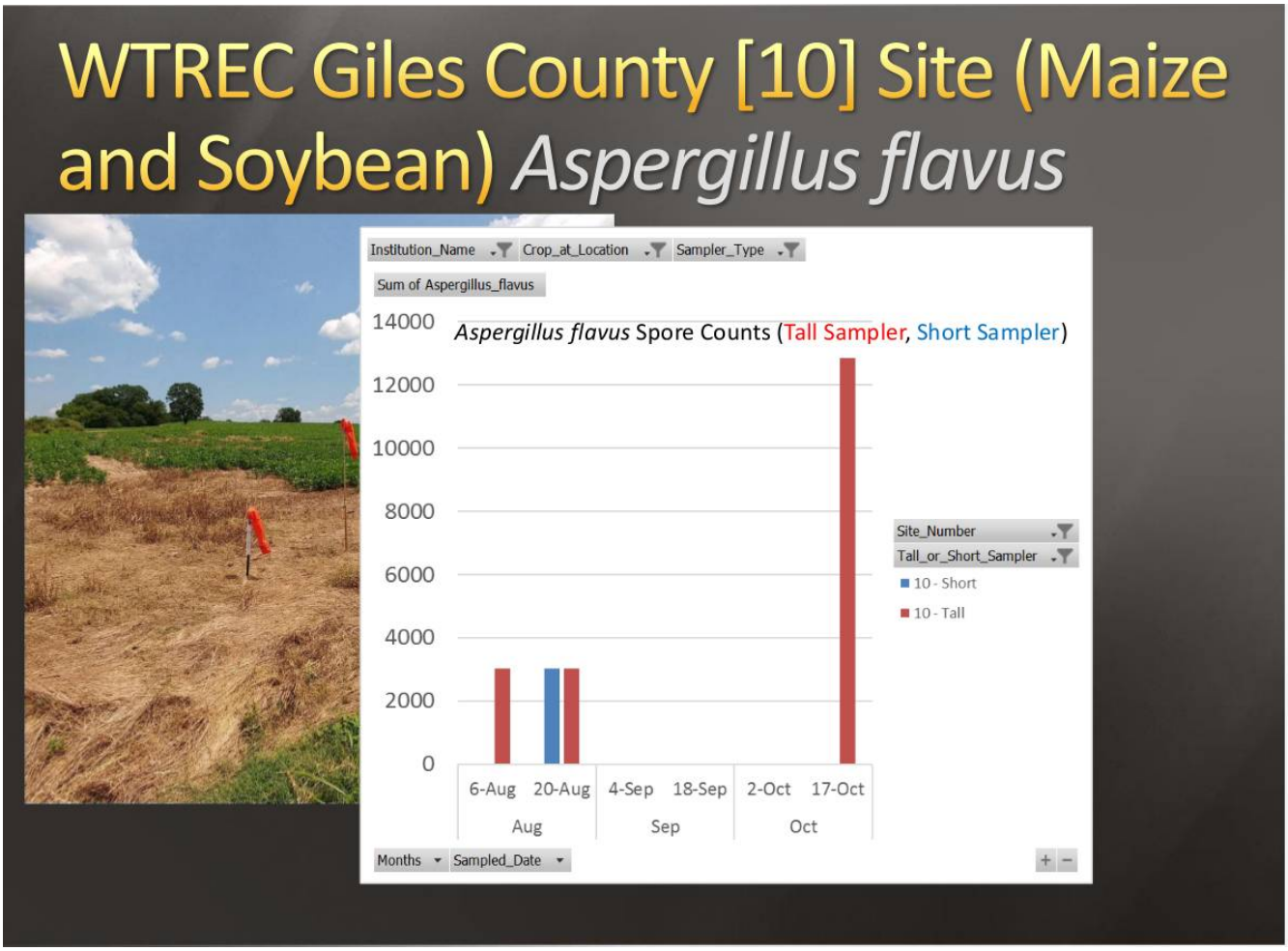
USDA CropScape Map. Dot is field location. Orange fields are corn.

WTREC Giles County [10]. TN

Satellite view of WTREC Giles County



Environmental Suitability for Sporulation of A. flavus
as computed using Battilani sporulation model.



Spore Capture Data

Corn and soybean are grown at this site.

The collection peaks in August may be the result of a local dispersal event. The model suggests that local sporulation and dispersal may be the cause of these peaks.

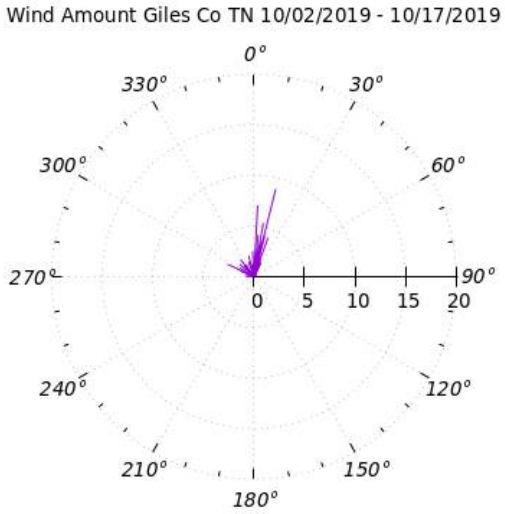
The peaks in periods 23 July – 20 August correspond to the period when corn silking occurs. According to USDA silking in Tennessee in 2019 was 89% complete by July.

This suggests that A. flavus spores were present at the time of corn silking.

Corn Harvest dates are Sep 1 – Oct 10. Soybean harvest dates are Oct 05 – Nov 20.

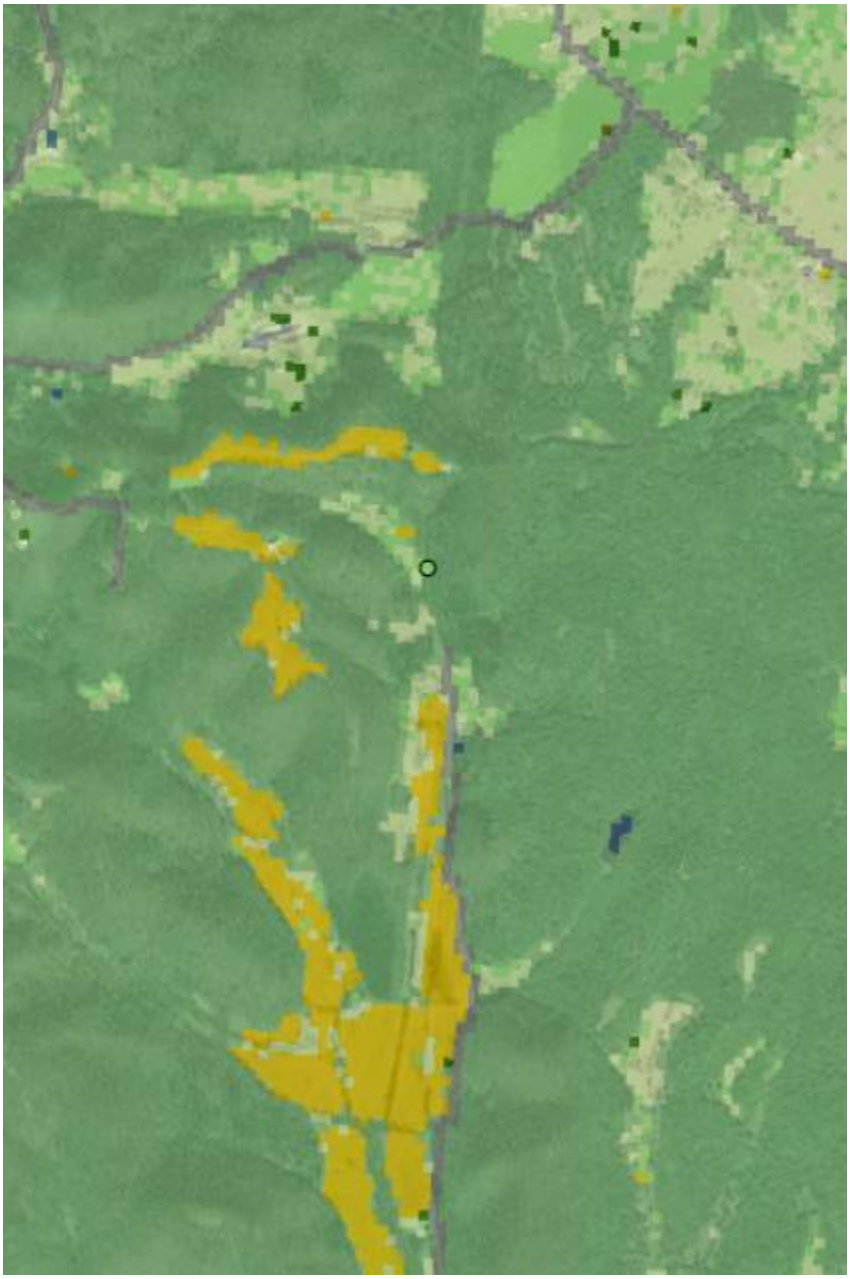
The *A. flavus* peak in period 02 October – 17 October corresponds to low environmental suitability of the sporulation model. The model suggests a relatively small amount of spores for that time and the quantity of spores captured was very large.

The harvest operation for corn on site and near by may be the source of the *A. flavus* spores captured.



Wind Rose for WTREC Giles County

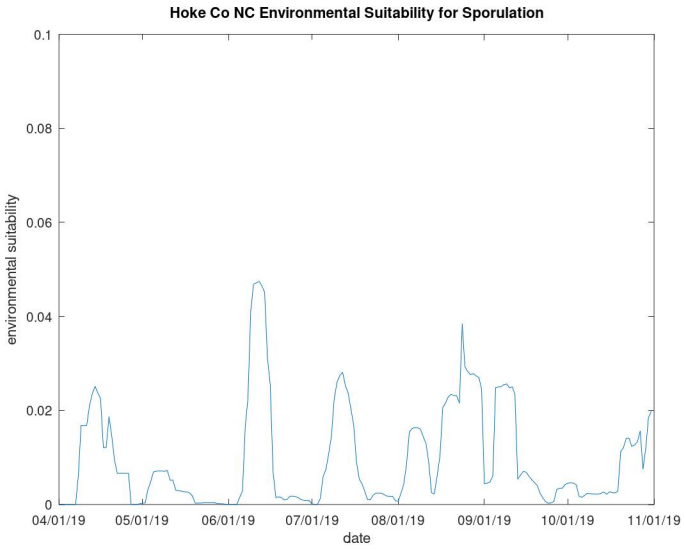
CropScape shows that there is a corn field to the North of the site which could be a source of spores.



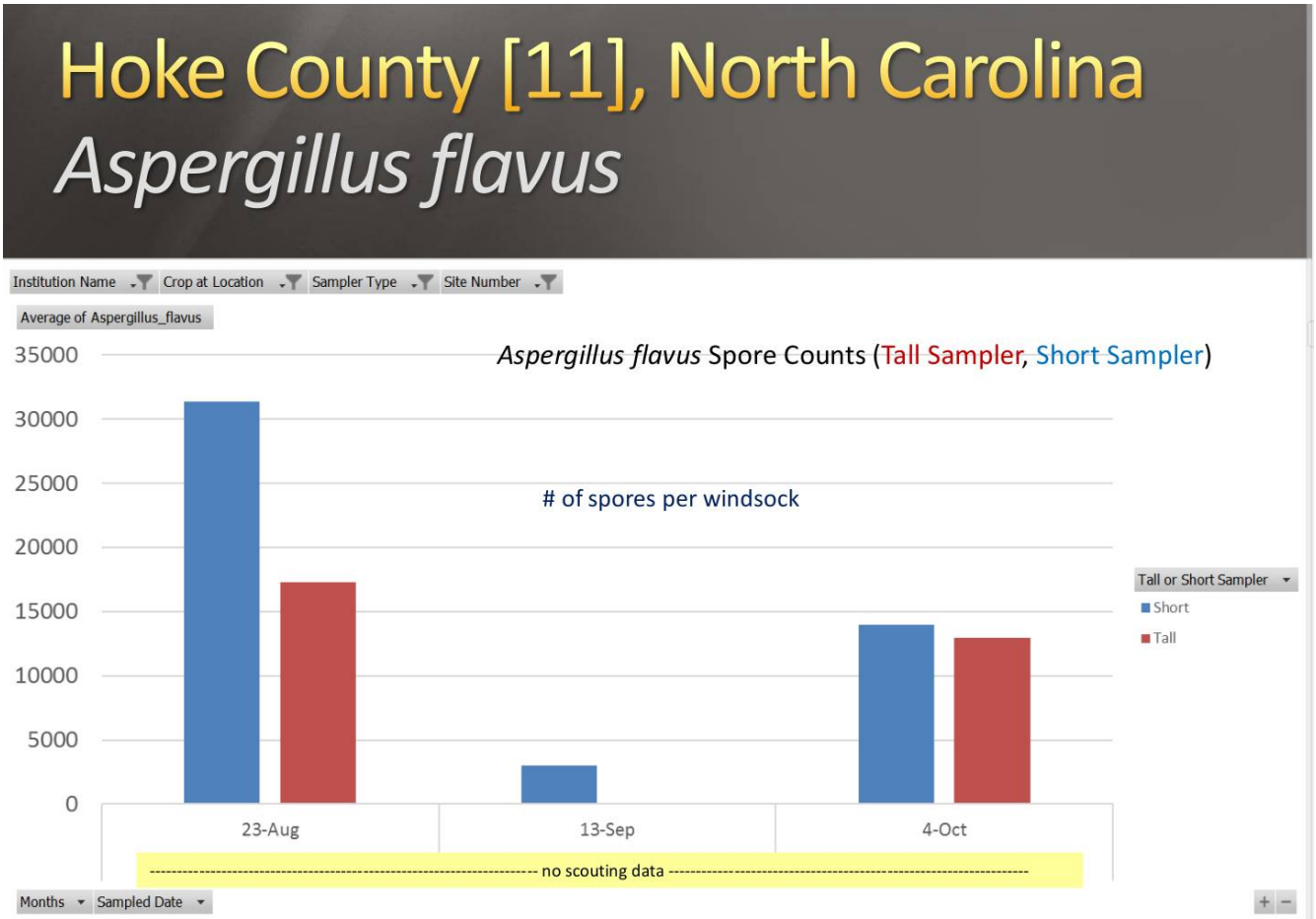
USDA CropScape Map. Dot is field location. Orange fields are corn.

Hoke_Co_NC

Satellite view of Hoke_Co_NC Site



Environmental Suitability for Sporulation of A. flavus
as computed using Battilani sporulation model.



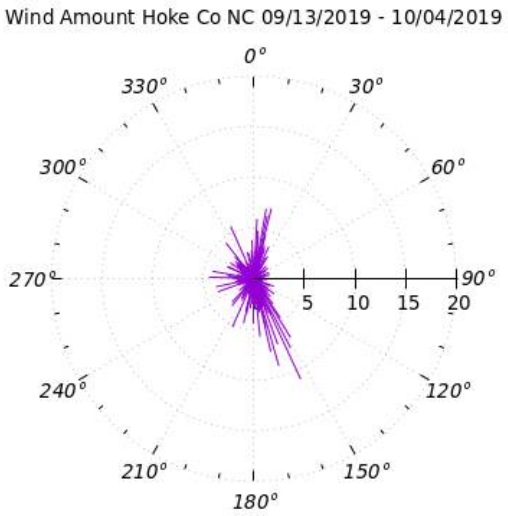
Spore Capture Data

August sampler results correlate reasonably well with model. Greater spore numbers in short sampler suggests that this may be a local dispersal event. This could also be a weather driven effect.

For 13 September - 04 October collection period, the model predicts low environmental suitability for sporulation.

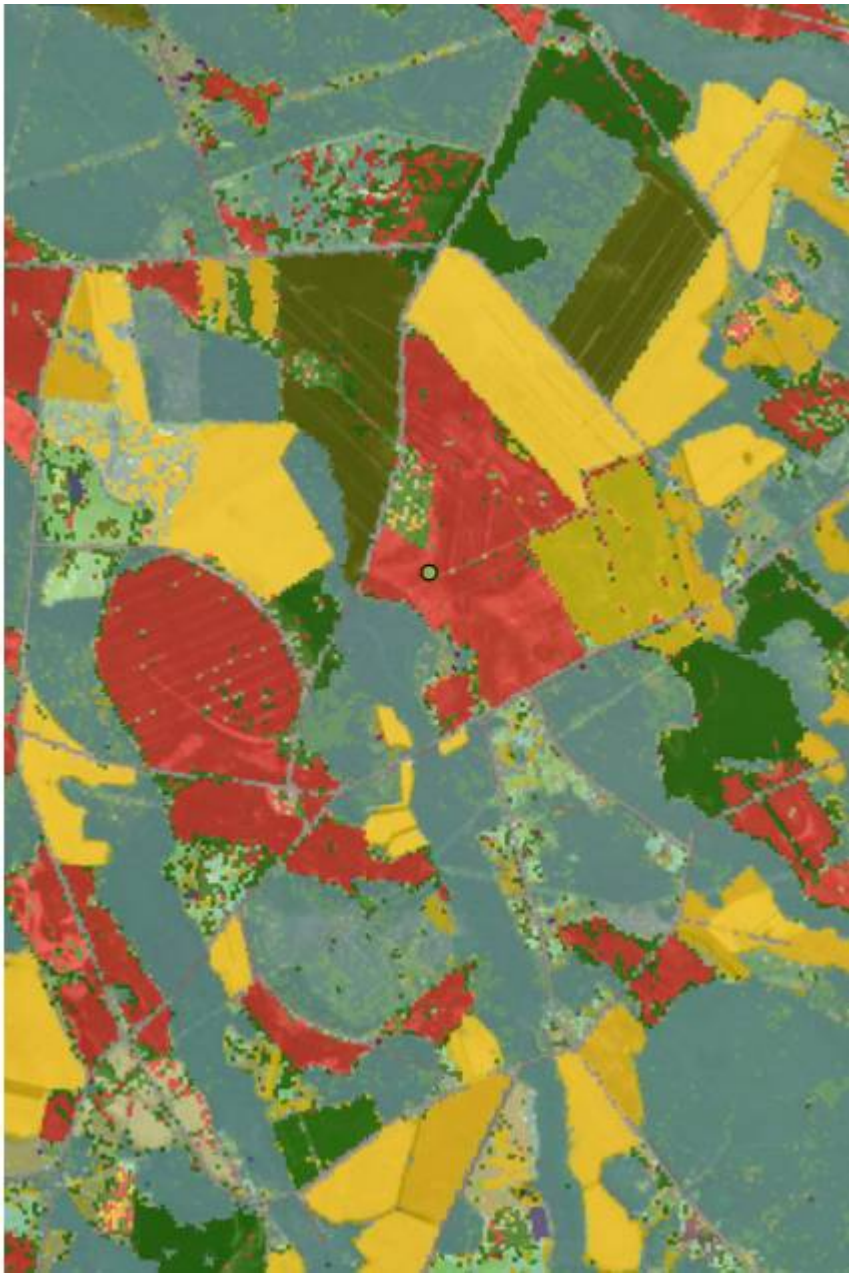
Corn harvest dates are Sept 10 – Oct 10 with an end Nov 1. Soybean harvest dates are Nov 10 – Dec 05. Peanut harvest dates are Oct 10 - Oct 30

The peak corresponds with corn harvest dates. Since both Tall and Short Samplers have collected spores, this peak may be due, in part, to local corn harvest.



Wind Rose for Hoke_Co_NC Site

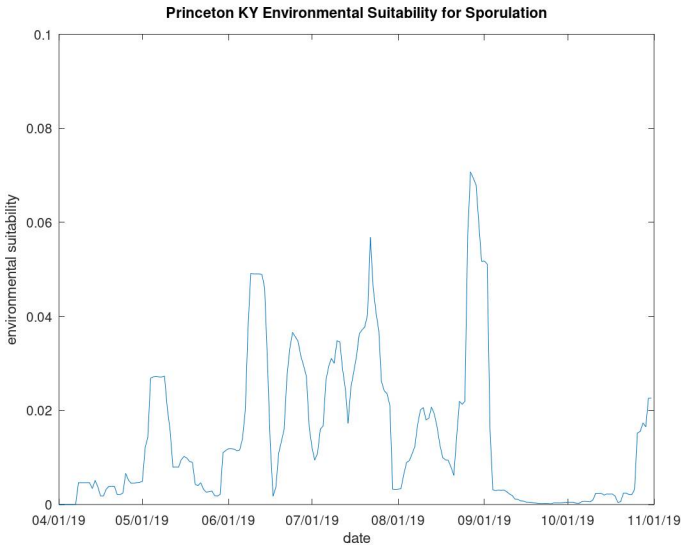
CropScape shows corn fields surrounding this site, which could be the source of spores.



USDA CropScape Map. Dot is field location. Orange fields are corn.

Princeton_KY

Satellite view of Princeton_KY Site



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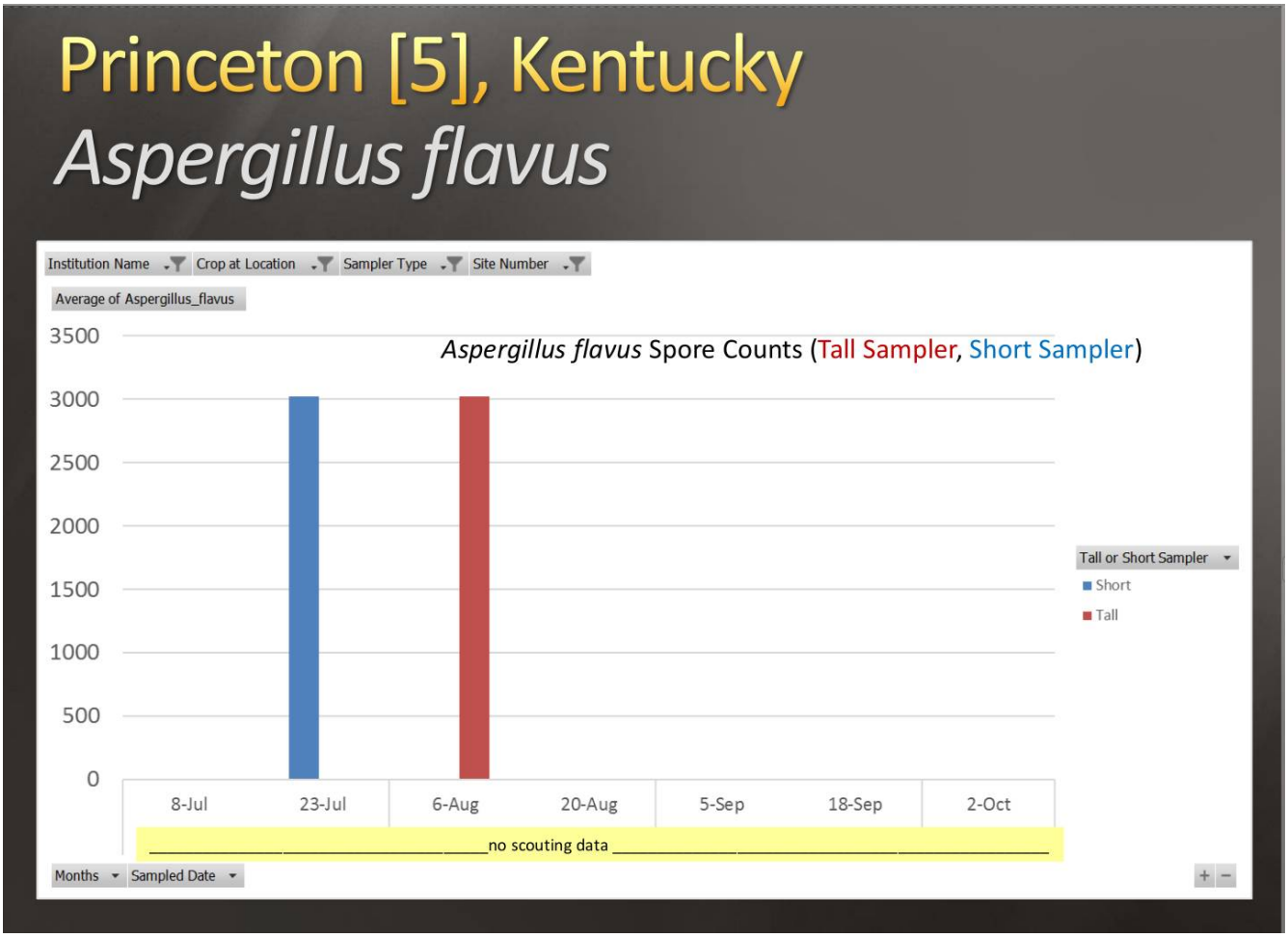
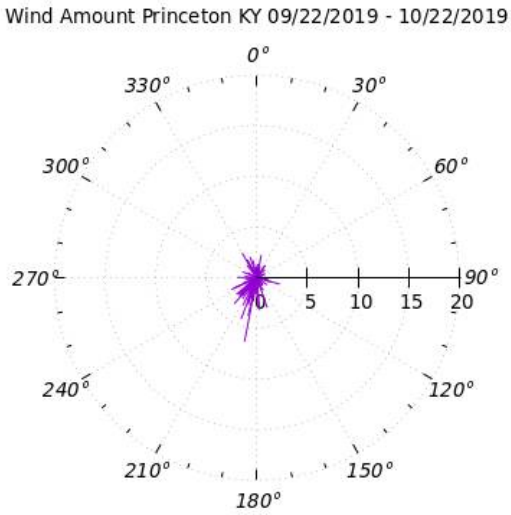


Spore Capture Data

The 23 July short collector peak corresponds with model peak. Because this peak is at the short sampler, it may be a local event.

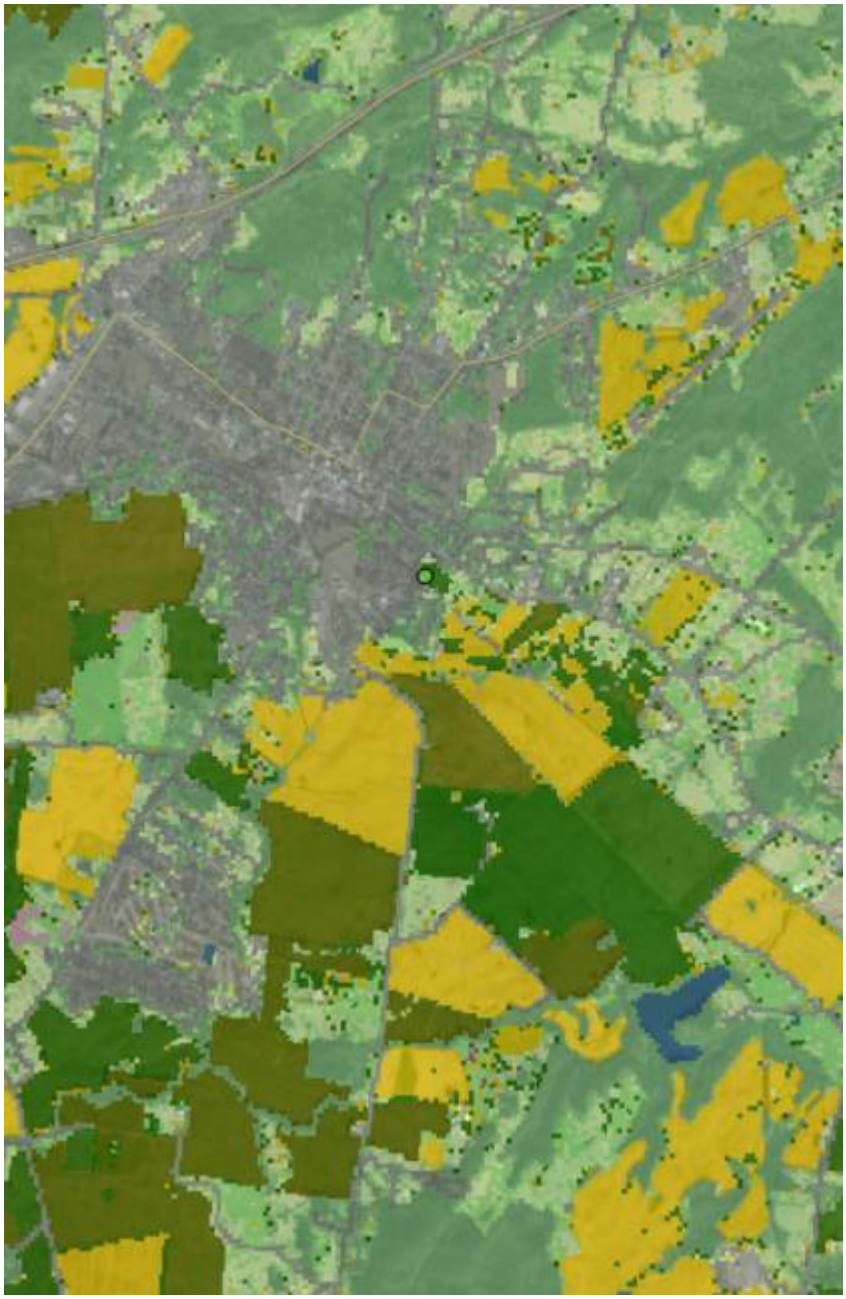
06 August tall collector peak corresponds with model peak. The peak occurs in the tall sampler and not in the short sampler. This suggests that the spores may have been transported in from another location by the wind. No source for this spore peak has been identified.

During corn or soybean harvest time no dispersal event was predicted by the model, and there is no collection peak during that time. This suggests that there was no source of A. flavus upwind from spore collector during the sampling period.



Wind Rose for Princeton_KY Site

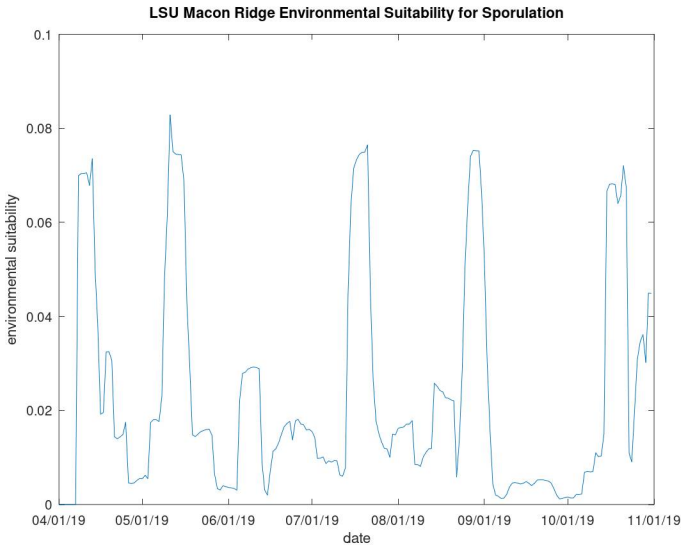
CropScape shows little corn near to the West of the site. This suggests that no corn harvest downwind coincides with no spore capture during corn harvest dates. It is also possible that local corn fields did not have a significant *A. flavus* infection.



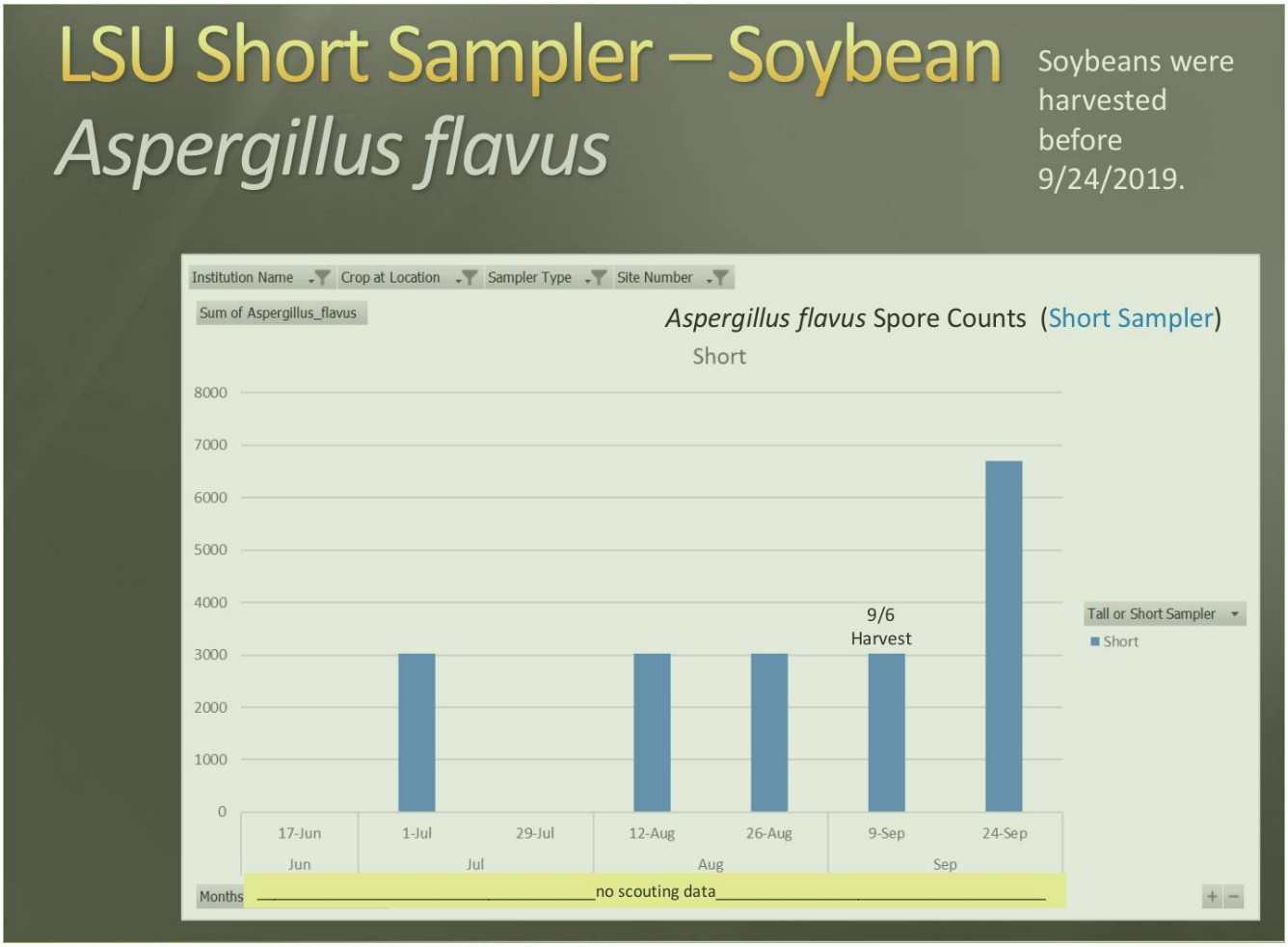
USDA CropScape Map. Dot is field location. Orange fields are corn. Note that there are no corn fields directly upwind from this site.

LSU Macon Ridge

Satellite view of LSU Macon Ridge Site



Environmental Suitability for Sporulation of A. flavus as computed using Battilani sporulation model.

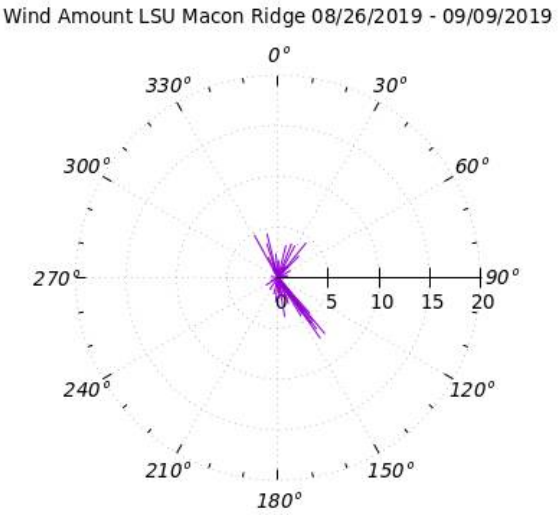


Spore Capture Data

Early collection peaks have some relation to model. However, the 09 Sep and 24 Sep captures occur at a time when environmental suitability for sporulation is predicted by the model to be low.

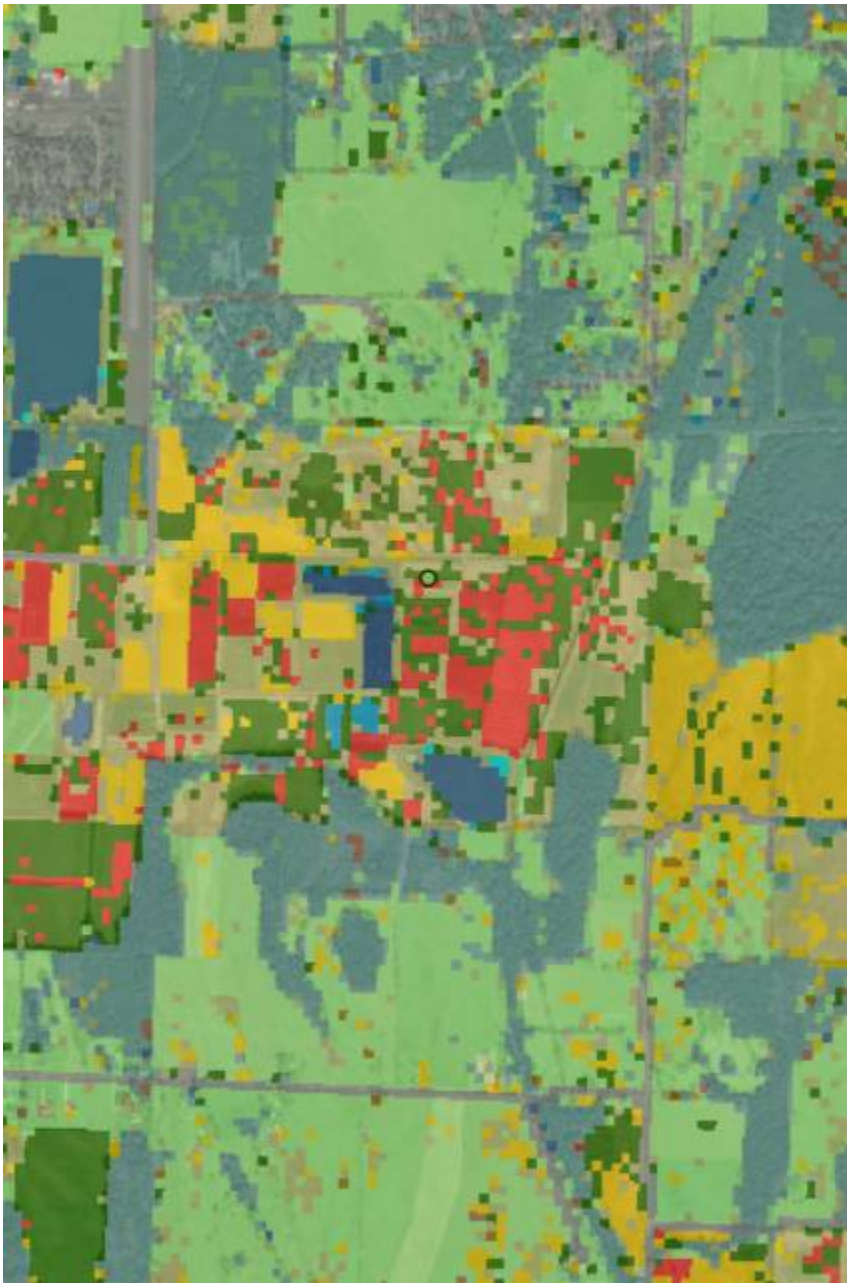
Corn harvest dates are Aug 11 – Sep 20 with latest Oct 15. Peanut harvest dates are Sep 22 - Oct 22. Soybean harvest at this site occurred 06 Sep.

The peak at 24 Sep is not explained by sporulation and dispersal model, by peanut harvest, or by corn harvest operations. This suggests that most of the spores captured are the result of local spore dispersal.



Wind Rose for LSU Macon Ridge Site

CropScape shows that the site was surrounded by Cotton and other crops, some of which may be peanut.

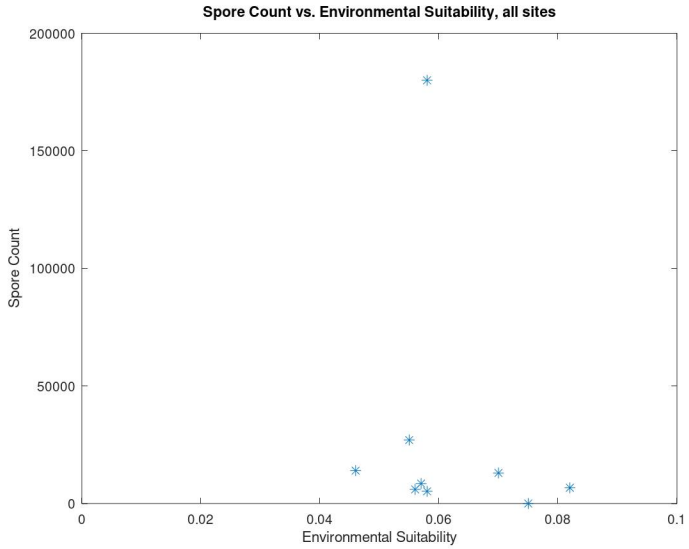


USDA CropScape Map. Dot is field location.

Discussion

At most sites, significant levels of *A. flavus* spores were collected during a time period that does not correspond to a time of high environmental suitability for the sporulation of *A. flavus*.

The spore counts were compared to the Environmental Suitability for sporulation of *A. flavus* and no pattern was found. The figure below shows Spore Count vs Environmental Suitability for all sites. This suggests that the spore counts were not related to sporulation and dispersal, but rather to some other kind of event.

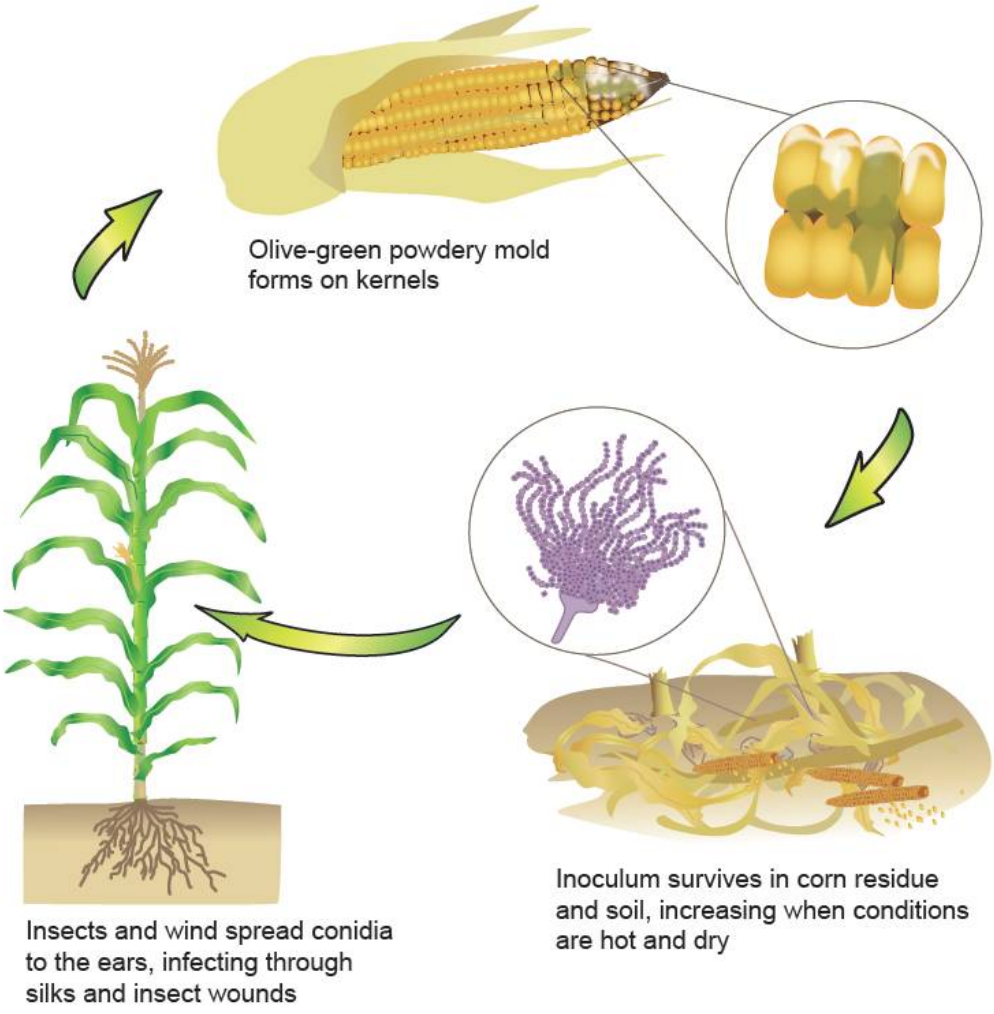


Spore Count vs Environmental Suitability

One explanation for this is that corn harvest upwind of the spore traps is creating the large quantities of spores of *A. flavus* captured by the collectors. The spore count for this period would be related to the level of A. flavus infection of upwind corn and the size of fields harvested.

In order to validate the assumption that corn harvest was the source of the airborne *A. flavus* spores that were collected, the life cycle of *A. flavus* was examined.

The Scientific Life Cycle of *A. flavus* [13] is shown below. In this life cycle model, spores infect ears of corn and grow on the kernels. The infected ears fall to the ground and the inoculum survives through the winter. What is not shown in this cycle is the Harvest operation and the subsequent no-till agriculture.



A. flavus Life Cycle [13]

Corn Harvest

Below is a photo of an ear of corn that has an *A. flavus* infection. When an ear of corn having *A. flavus* infection passes through the harvest machine it reaches the threshing drum, where husks are removed from the ear and then the kernels are removed from the cob. As kernels are separated from the cob, the fan blows the kernels and fungi away from the cobs and corn stalks. All combine harvesters have fans that blow out whatever fungal spores come into the harvester.

The *A. flavus* life cycle is a system that includes the plant, the harvester, and no till Agriculture.
The corn harvest operation releases spores into the atmosphere . Those spores can be carried by the wind for long distances.

Left: Ear of corn with *A. flavus* infection[3], Right: Harvest Operation

To put it simply, the *A. flavus* life cycle in commercial agriculture goes through the harvester. What comes in the front, goes out the back or side. For the harvest of corn that has *A. flavus* infection, some spores are mixed with the corn stalks and some spores are blown out of the back of the harvester, into the atmosphere, and further dispersed by the wind. Some spores go out with the corn kernels. Some spores go out with the chopped stalk and husk.

The contents of corn harvest dust was investigated in 1984 by Robert A Hill, et al. “Viable fungi in corn dust” [7].

From “Viable fungi in corn dust”, Abstract:

“Numbers of viable fungal propagules in corn dusts in southern Georgia were estimated during various farm and grain elevator operations in 1979, 1980, and 1982. A six-stage Andersen sampler for viable microbial particles was used to sample the dusts with various agar media. The most abundant fungi in corn dusts were species of yeasts: Aspergillus, Penicillium, Cladosporium, Alternaria, Helminthosporium, and Fusarium. However, the relative abundance of these fungi differed between years. There was a greater incidence of the Aspergillus flavus group in the hot, dry year of 1980 compared with the cooler, wetter years of 1979 and 1982. Fungi in the corn dusts sampled numbered between 10^4 and 10^9 viable propagules per m^3 of air. By contrast, outdoor air often contained fewer than 10^4 viable fungal propagules per m^3. Most A. flavus propagules were deposited at stages three and four of the Andersen sampler, which correspond to the trachea, primary bronchi, and secondary bronchi in the human respiratory system. In an assessment of the air spores by exposing sterile petri dishes, more large-spored fungi, like Alternaria tenuis, and fewer small spored fungi, such as A. flavus, were detected when compared with colony counts from petri dishes exposed to air in the Andersen sampler.” [7]

From “Viable fungi in corn dust”, Discussion:

“The viable fungal component of combine harvester dusts, containing between 10^5 and 10^7 viable fungal propagules per m^3 of air in 1979, was dominated by yeasts too numerous to count reliably even with the shortest exposure time (30 s). Yeasts were also abundant in 1982 (Table 1), but fewer were found in the hot, dry season of 1980. The A. flavus group was a major component of combine harvester dusts in all years (Table 2). As many as 1.2×10^6 viable propagules of the A. flavus group were found per m^3 of air in 1980 (Table 2), and these comprised 60 to 80% of the total number of colonies counted. By contrast, less than 20% of the total number of colonies isolated from combine harvester dusts in 1979 and 1982 were the A.flavus group (Table 1). In those years, there were fewer than 10^5 viable

propagules per m^3 of air. Penicillium species were a major component of the viable fungi in those dusts but were more numerous in 1979 and 1982 (Table 1) than in 1980 (Table 2)."

TABLE 1. Viable fungal component of corn dusts in southern Georgia in 1980 and 1982[a]

Fungus	Corn dust at grain elevator during unloading from trucks (1980)		Corn dust behind combine harvester (1982)		Corn dust from loading truck (1982)
	Range	Mean	Mean a.m. samples	Mean p.m. samples	Mean
Aspergillus flavus group	0.01–0.55	0.15	0.03	0.02	0.04
Cladosporium spp.	<0.01–0.01	<0.01	0.04	0.21	0.02
Fusarium spp.	0.0–<0.01	<0.01	<0.01	0.0	<0.01
Penicillium spp.	0.002–0.01	0.01	0.08	0.17	0.01
Yeasts	0.0–0.01	0.01	0.04	0.06	0.02
Other fungi	0.002–0.02	0.01	0.01	0.03	0.01
Total fungi	0.033–0.49	0.18	0.20	0.49	0.10

[a] Spore density was estimated in millions per cubic meter of air with an Andersen sampler.

TABLE 2. Viable fungal component of corn dusts in southern Georgia in 1979 and 1980[a]

Fungus	1979 Corn dusts from trucks unloading at grain elevator		1980 Corn dusts behind combine harvesters		1980 Corn dusts from trucks unloading at grain elevator	
	Range	Mean	Range	Mean	Range	Mean
Aspergillus flavus group	0.01–0.83	0.33	0.7–1.2	0.94	0.4–1.1	0.80
Total aspergilli	0.02–0.83	0.33	0.8–1.2	1.0	0.4–1.1	0.81
Penicillium spp.	0.05–0.52	0.33	0.1–0.2	0.14	0.01–0.2	0.10
Total fungi	0.01–1.21	0.78	1.1–1.6	1.36	0.4–1.3	0.94

[a] Spore density (millions per cubic meter of air) estimated with an Andersen sampler.

Table 1 and Table 2 from "Viable fungi in corn dust": [7]

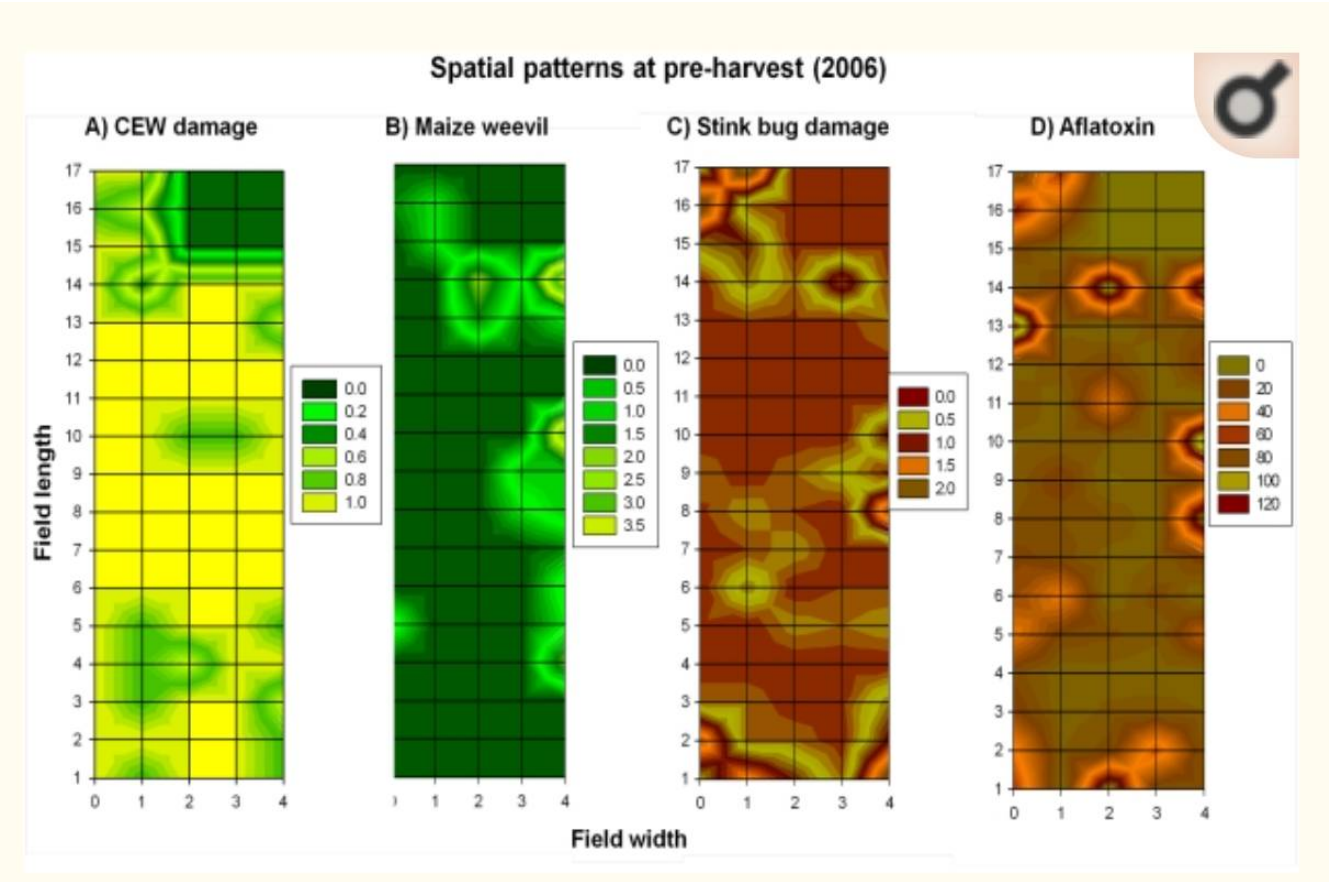
Agroecosystems and their A. flavus population

From Mehl, et al.[12]:

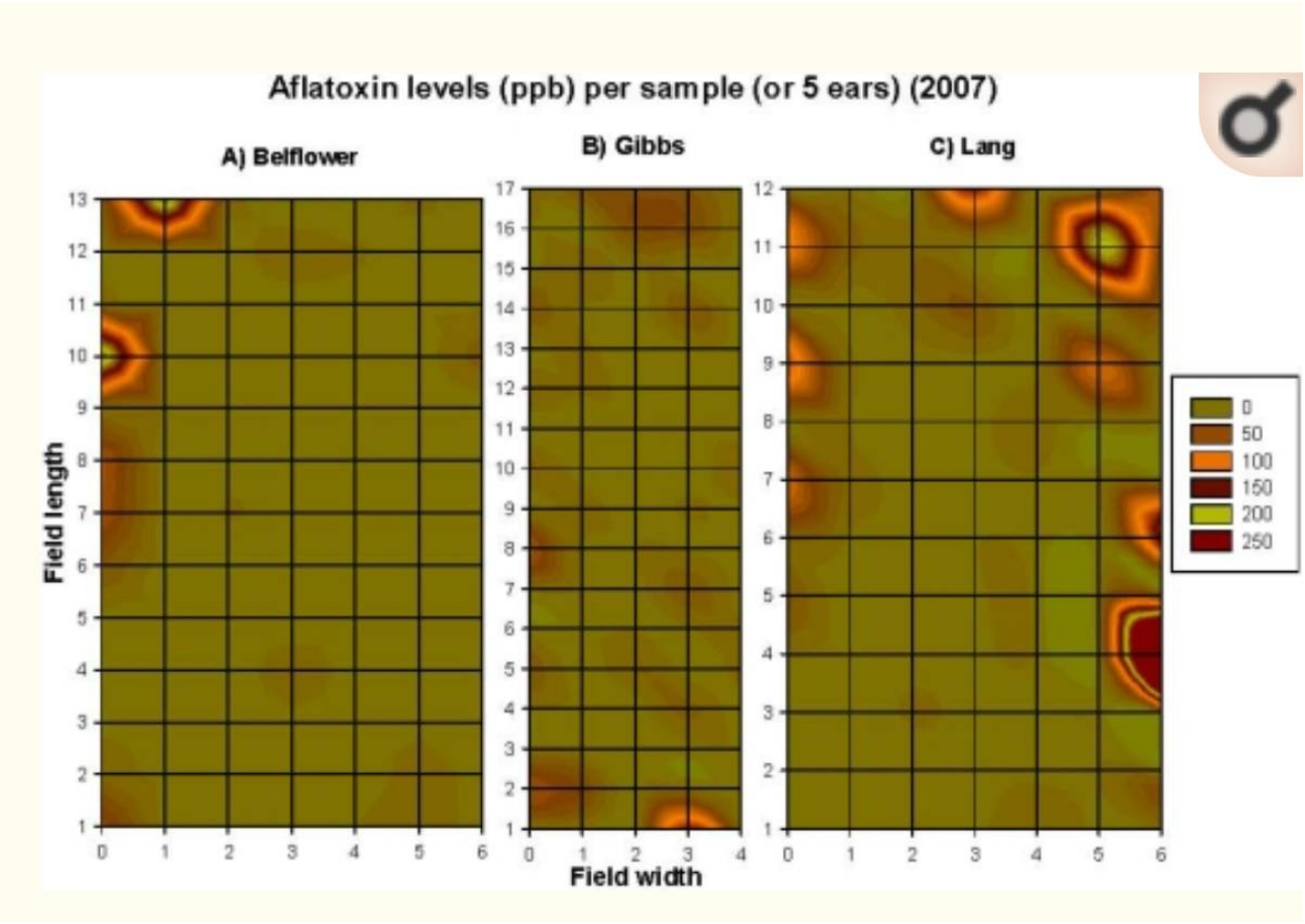
"Agronomic practices influence structures and average aflatoxin-producing potentials of A. flavus populations and, as a result, incidences and severities of crop contamination."

"Vegetative compatibility analyses provide insight into population genetic diversity as well as changes in compositions of crop-associated A. flavus associated with various events.[54,67–71] A. flavus populations are composed of many VCGs, and multiple VCGs may occur within a single crop component or aliquot of soil.[70] Some VCGs are common in the environment whereas others are rarely isolated,[67,70,71] and relative frequencies of morphotypes and VCGs vary among crops, fields, regions, seasons, and years.[15,22,44,67,72–75] Thus, each agroecosystem has its own unique, continuously fluctuating assemblage of genetically diverse A. flavus that must be managed to minimize crop contamination." [references in publication]

The pre-harvest distribution of aflatoxin contamination (ppb) in the corn samples on three University of Georgia and USDA-ARS research farms located at Tifton, GA were examined to assess the patterns of ear-feeding insect infestations, associated insect damage, and aflatoxin contamination at pre-harvest. [6] The results for the years 2006 and 2007 are shown below.



Results of 2006 Survey [6] - At a single site, corn ear worm damage, number of maize weevils, stink bug-discolored kernels, and aflatoxin concentration varied throughout the corn field at pre-harvest.



Results of 2007 Survey [6] - At three sites, aflatoxin concentration varied throughout the corn fields at pre-harvest. (not drawn to scale, all field squares are same size)

In this survey, Aflatoxin concentration varied widely over the area of the fields. This suggests the possibility of "smart" harvest operations, where low aflatoxin areas would be harvested and high aflatoxin areas would be dealt with in some other manner.

Conclusion

The work described here is the result of one year of data at nine sites, and must be regarded as preliminary.

In most of the spore collection sites under consideration, spores were captured at a time when the environmental suitability for sporulation is low. A mechanism for the airborne dispersal of spores is proposed that involves the harvest of corn using combine harvesters.

Plausible causal links from spore collection at cotton fields back to corn harvest have been demonstrated.

The synthesis of actual spore count data with weather data, modeled environmental suitability, wind, and crop data, can lead to a greater understanding of the *A. flavus* lifecycle in commercial agriculture.

The life cycle of *A. flavus* in commercial agriculture is a multi-crop, multi-field, multi-year phenomenon. Both a pathogen-centric approach and a crop-centric approach are required to understand and validate spore capture data and plant infection areas within a commercial agricultural setting.

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