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Spatial Distribution of Aflatoxin in Growing Peanut

G. Vellidis¹, B. Ortiz¹, M. Renga², C. Perry¹, K. Rucker², F. Morari³

¹*Biological and Agricultural Engineering Department, University of Georgia, USA*

²*Cooperative Extension Service, University of Georgia, USA*

³*Dipartimento di Agronomia Ambientale e Produzioni Vegetali, Università di Padova, Italy.*

Corresponding Author: George Vellidis – yiorgos@uga.edu

Abstract

Aflatoxin is a carcinogenic toxin produced by the *Aspergillus flavus* and *Aspergillus parasiticus* fungi. This toxin is found in corn, cotton seeds, and peanuts and is consequently a major food safety issue. In this paper we report on a pilot study we conducted in Georgia, USA, to quantify the spatial distribution of aflatoxin in growing peanuts (*Arachis hypogaea* L.).

Keywords: peanuts, aflatoxin, *Aspergillus flavus*, spatial distribution

Introduction

Aflatoxin is a naturally occurring mycotoxin produced by the *Aspergillus flavus* and *Aspergillus parasiticus* fungi. The toxin is found in corn, cotton seeds, and peanuts. Because aflatoxin is a Group 1 carcinogen proven to cause liver cancer in humans, its concentration in food products is highly regulated in the USA, the European Union, Japan, and other countries. The Codex Alimentarius drawn up by the United Nation's World Health Organization and the Food and Agriculture Organization, and adopted by 165 member nations, has prescribed an upper limit of 15 ppb of aflatoxin in raw peanuts (*Arachis hypogaea* L.).

Peanuts are a major crop of several southeastern states of the USA including Georgia. A large percentage of the American peanut crop is processed to become peanut butter and candy bars— products consumed primarily by children. Consequently, the peanut industry in the USA expends about USD 1 billion a year to ensure that their food products are not contaminated by aflatoxin. Practically all of this money is spent after the peanuts are harvested. Yet, peanuts are mostly contaminated by aflatoxin while in the field.

Certain environmental conditions during the growing season promote the production of aflatoxin by *Aspergillus flavus* and *Aspergillus parasiticus* in peanut kernels. Many authors suggest that prolonged drought periods and elevated soil temperature at the geocarposphere during the last four weeks of the growing season are two key factors increasing the risk for aflatoxin contamination (Sanders et al., 1993; Sanders et al., 1985; Thai et al., 1990). These environmental conditions induce some physiological changes in the peanut plant which facilitate the colonization of the peanut kernels by the fungi and the subsequent production of aflatoxin. Dorner et al. (1989) found that drought conditions at the geocarposphere yielded a loss in kernel water activity which controls the phytoalexin production capacity. In the case of peanuts, phytoalexins inhibit spore germination and hyphal extension of the fungi. Consequently, when kernel water activity decreases as a result of prolonged drought, the capacity of the kernels to

produce phytoalexins decreases. The degree of maturity has also been related to aflatoxin contamination. However, this observation is strongly related to phytoalexin production as well because immature peanut kernels lose the capacity to produce phytoalexin under drought stress earlier than mature peanut kernels.

Little is known about the spatial distribution of fungal colonization of peanut kernels although it has been hypothesized that areas of a field more prone to drought are at higher risk for aflatoxin presence in peanuts. If there is a predictable distribution of aflatoxin risk within a field, site-specific management practices such as variable rate irrigation could be used to reduce the risk or high risk areas could be harvested separately to avoid mixing contaminated peanuts with uncontaminated peanuts.

The immediate objective of our pilot study was to test the hypothesis that the presence of aflatoxin in peanut kernels is spatially distributed. A more long-term objective is to develop techniques which can be used to delineate areas at high risk for aflatoxin contamination during the growing season.

Materials and methods

A 14 ha (34 ac) non-irrigated field in Tift County, Georgia, USA was used for the study. The field was planted to peanuts in early May and harvested in October 2007. Apparent soil electrical conductivity (soil EC_a) was measured in the field using a Veris® 3100 (Veris Technologies, Inc., Salina, KS). Soil EC_a has been broadly used as an indirect method to identify changes in soil texture (Kitchen et al., 2003; Sudduth et al., 2005) At the same time, a topographic map was created using a Trimble® RTK GPS system (Trimble Navigation Limited, Sunnyvale, CA, USA). Soil EC_a and elevation maps are shown in Figure 1. Using the soil EC_a data, the Management Zone Analyst software (Fridgen et al., 2004) was used to delineate management zones within the field. This software uses fuzzy clustering to delineate areas within the field with common properties and optimize the number of these areas. The analysis resulted in 4 management zones based on soil EC_a. After planting, 17 sampling locations were randomly selected within these 4 management zones (Figure 2).

The sampling locations were located within the rows of planted peanuts. In Georgia, peanuts are planted on slightly raised beds with 1.8 m centers. On either side of the bed is an alleyway. Within the bed, peanuts are planted in two parallel rows with approximately 0.45 m between them. Each row of peanuts actually consists of two closely spaced parallel rows of plants commonly referred to as a twin row. The twin rows are typically planted close to the edge of the bed. Our sampling locations were within the twin rows as shown in Figure 3.

In early July, Watermark® soil moisture sensors were installed at depths of 10, 15 and 30 cm below the soil surface at each sampling location. These sensors were read manually daily for the remainder of the growing season. In addition, a HOBO® datalogger with two temperature sensors was installed at each sampling location. One temperature sensor was installed at 10 cm to measure soil temperature while the second was installed in the lower canopy of the peanut plants to measure air temperature within the canopy. The dataloggers recorded temperature at 10 minute intervals.

Canopy reflectance data of the field were acquired twice during the growing season. The first was acquired in September with a Crop Circle sensor (Holland Scientific, Inc.,

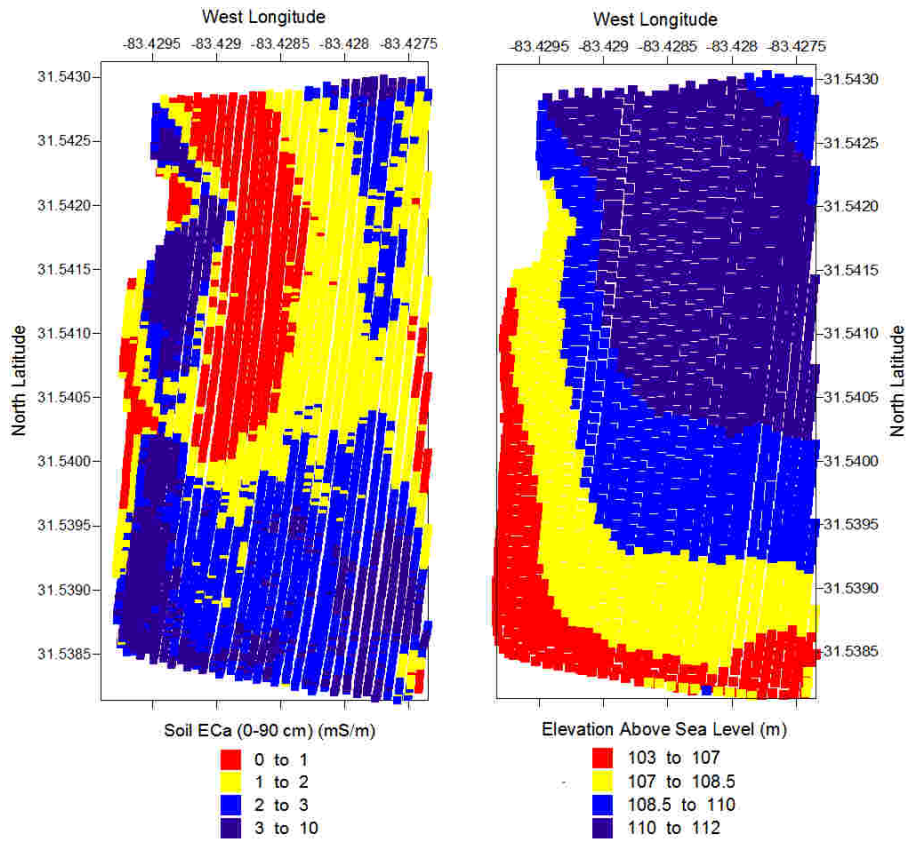


Figure 1. Soil electrical conductivity (0-90 cm) at left and elevation above sea level at right of the field used in the study.

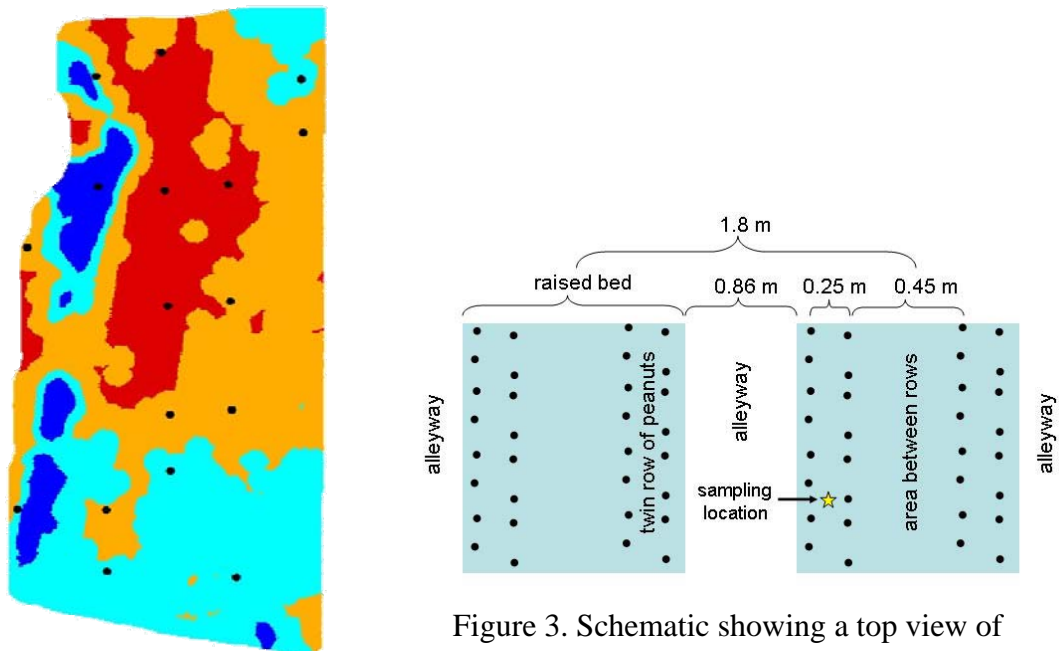


Figure 2. Management zones created from soil ECa and the location of the 17 sampling points.

Figure 3. Schematic showing a top view of the typical peanut production layout used in southern Georgia, USA. The small black circles represent peanut plants. The star indicates a sampling location.



Figure 4. Manuel Renga collects canopy reflectance data using the Crop Circle sensor.

Lincoln, NE, USA), a tractor mounted sensor which emits amber and near infrared (NIR) light, captures the percentage of this light reflected by the canopy, and reports a modified normalized difference vegetation index (Figure 4). The second image was also acquired in September from an airborne, 4-band (blue, green, red, NIR) multispectral camera.

Soils in the field are very sandy as indicated by the soil ECa data (Figure 1). Because June and July were extremely dry, the peanut plants experienced severe drought stress during these months. Several rain events occurred during August and September, but the sandiest areas of the field continued to experience drought stress.

Immediately prior to mechanical harvest of the field, all the peanuts from the 30 m² area surrounding the sampling locations were harvested for aflatoxin analysis. Because of the extreme yield variability in this field, the mass of peanuts harvested from each of these areas ranged from 3.6 to 11.2 kg. The peanuts were shelled and the kernels ground and analyzed for aflatoxin concentrations using the AflaTest® immunoassay (VICAM, Watertown, MA, USA). Two subsamples were separated from each batch of ground samples. Each subsample was analyzed in duplicate. A total of 4 aflatoxin concentrations were therefore available for each of the 17 sampling locations. These 4 values were averaged together and the average assigned as the aflatoxin concentration of the sampling location.

Results and discussion

The United States Department of Agriculture (USDA) has set acceptable limits for aflatoxin in shelled peanuts at 15 ppb. Peanuts are graded by the truck- or wagon-load when they arrive at a buying point using guidelines established by USDA. Several samples are collected from the wagons or trucks using a pneumatic sampler and among other things visually inspected for the presence of aflatoxin causing fungi. At some buying points, an immunoassay is performed on a subsample.

The results from the aflatoxin immunoassay performed on the peanuts collected from our study field are shown in Figure 5. In the figure, the numbers adjacent to the sampling point indicate the average measured aflatoxin concentration at each location. The concentration contour map was created by kriging the averaged concentrations. Averaged concentrations ranged from 50 to 445 ppb – considerably above the 15 ppb limits. Nevertheless, none of the 7 wagon-loads of peanuts harvested from this field were found to be at risk for aflatoxin contamination during the grading process. This is likely because the fungi typically infect only a few kernels. These individual kernels are likely to have a very high aflatoxin concentration but if the number of infected kernels per ton of harvested peanuts is small, the infected peanuts are very difficult to detect. Nevertheless, during storage and processing, the infected peanuts can cause the contamination of a large batch of peanuts with the aforementioned consequences.

The contour map (Figure 5) clearly shows that there are aflatoxin “hotspots” in the field and especially in the southwest corner of the field which confirms our hypothesis that the presence of aflatoxin in peanut kernels is spatially distributed. This field was harvested in a north-south direction (Figure 6) so that it is very likely that peanuts from at least one of the hotspots were included in several wagon-loads. Because the field was low-yielding, several passes of the harvester were needed to fill a wagon further ensuring the mixing of high-aflatoxin peanuts with lower-aflatoxin peanuts.

Time and financial constraints prevent growers from creating aflatoxin maps similar to Figure 5 immediately prior to harvest. However, if maps predicting the areas of the

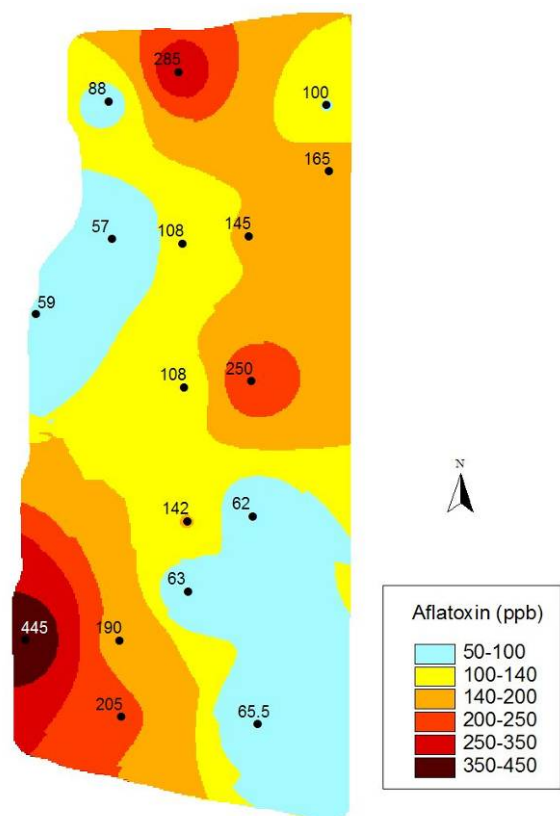


Figure 5. Aflatoxin concentrations in ppb as measured from peanut samples collected from a 30 m² area around each of the 17 sampling points.

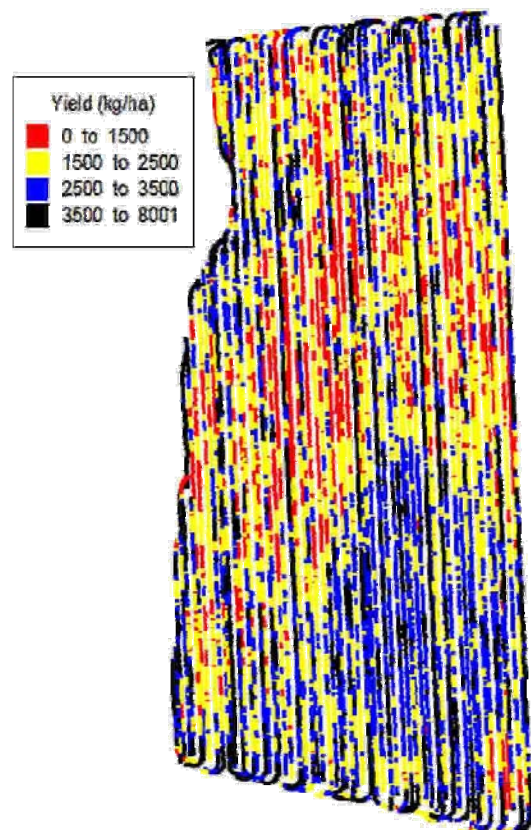


Figure 6. Peanut yield map of the field used in this study. Yields are reported in kg/ha.

field at high risk for aflatoxin contamination could be created from surrogate data, the areas identified as high-risk can be managed to reduce the risk or at the very least segregated and harvested and processed separately until sampling verifies presence or absence of aflatoxin.

We are currently evaluating the statistical relationship between aflatoxin concentration and soil ECa, soil temperature, soil moisture, and a number of vegetation indices created from the canopy reflectance data. Vegetation indices (VIs) have the most potential for capturing temporal crop response to environmental factors that induce aflatoxin production. Airborne multispectral images are the easiest forms of data to collect and may also be the most cost effective if many fields are captured with a single flight.

Conclusions

Aflatoxin is a naturally occurring highly carcinogenic mycotoxin which is found in corn, cotton seeds, and peanuts. The peanut industry in the USA expends about USD 1 billion a year to ensure that their food products are not contaminated by aflatoxin. Practically all of this money is spent after the peanuts are harvested. Yet, peanuts are mostly contaminated by aflatoxin while in the field. We conducted a pilot study to test the hypothesis that the presence of aflatoxin in peanut kernels is spatially distributed. Our results indicate that the aflatoxin contamination is spatially distributed in growing peanuts. Future work will focus on identifying techniques for identifying areas at high risk for aflatoxin during the growing season.

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