A Spatio–Temporal Investigation of Risk Factors for Aflatoxin Contamination of Corn in Southern Georgia, USA using Geostatistical Methods

Ruth Kerry[1], B.V. Ortiz[2], B. R. Ingram[3], B.T. Scully[4]

[1] Department of Geography, Brigham Young University, UT, USA

[2] Crop, Soil, and Environmental Sciences Department Auburn University, AL, USA

[3] Facultad de Ingeniería, Universidad de Talca, Curicó, Chile

[4] USDA-ARS, U.S. Horticultural Research Laboratory, Ft. Pierce, FL, USA.

ABSTRACT

Aflatoxin is a mycotoxin produced by the *Aspergillus flavus* fungi that can severely contaminate corn grain. The U.S. Food and Drug Administration (FDA) have set a limit of 20 ppb, total aflatoxin, for interstate commerce of food and feed as it can induce liver cancer in humans and animals. Contamination is exacerbated by high temperatures, drought conditions and light-textured soil which are all common in Georgia (GA). Lack of irrigation infrastructure can further amplify drought stress and aflatoxin contamination. Accurate aflatoxin assessment requires the collection of multiple corn samples, is expensive and conducted at harvest which does not allow for the use of in-season mitigation strategies to reduce the risk. Given the expense of measurement and the consequences of crop loss, an important goal for agricultural extension services is the prediction and identification of years and counties at higher risk of aflatoxin contamination. This would allow growers to deploy management tactics to reduce risk and to reduce unnecessary expense on aflatoxin testing. In this research, aflatoxin levels were analysed by Poisson kriging and used to validate a strategy for identifying high risk years and counties. It is based on mapping risk factors (Maximum June temperatures, June rainfall, % corn planted area and % soil drainage types) that are above key thresholds. The aflatoxin data used were county level, collected unevenly in space and time from 1977 to 2004 in 53 counties in southern GA. Averaging and typical geostatistical methods were unreliable for producing a temporal summary of the spatial patterns because aflatoxin data were highly skewed and approached a Poisson distribution, and averages for counties based on fewer observations are less reliable. Poisson kriging down-weights the influence of these in variogram computation and the estimation process. Comparison tests confirmed significant differences in aflatoxin levels between counties and years that were identified as having different levels of risk using the risk factors approach. Sensitivity analysis for Poisson kriged aflatoxin risk showed that the more years of data are clearly better for this analysis, but fewer than 15 years of data were not advisable.

Keywords: Aflatoxin, Corn, June Maximum Temperature, June Rainfall, Southern Georgia, Geostatistics, Poisson Kriging, Soil type, Soil drainage

1. Introduction

Aflatoxin is a mycotoxin produced by fungi (*Aspergillus flavus* or *Aspergillus parasiticus*) which can contaminate several staple crops such as peanut, (Brenneman et al. 1993), millet (Wang et al. 2010, Wilson et al. 1993), rice (Abbas et al 2005), sorghum (Adegoke et al. 1994), wheat (Patriarca et al. 2014) and corn (Payne, 1992). Aflatoxin can cause liver cancer in humans and animals (Barrett, 2005 and FDA, 2012). The Food and Drug administration office (FDA) of the USA have set a limit of 20 ppb, total aflatoxin, to restrict use of corn, peanut products, cottonseed meal, and other animal feeds and feed ingredients intended for dairy animals, for animal species or intended for immature animals. There is also a limit of 100 ppb restricting use of corn and peanut products intended for breeding beef cattle, swine, or mature poultry (FDA, 2015). Infection of corn with *A. flavus* or *A. parasiticus* is exacerbated by high temperatures, drought and high net evaporation (Guo et al. 2008, Horn et al. 2014 and Payne, 1992) associated with particular climatic areas (Abbas et al. 2007, Patriarca et al. 2014), agro-ecological zones (Setamou et al. 1997) and soil types (Palumbo et al. 2010). Statistically, there are 16-31 times more deaths from liver cancer in less developed countries due at least in part to aflatoxin contamination of food (Liu and Wu, 2010) and many of these countries are predominantly hot and often drought prone (Wu and Klangwiset, 2010). Several studies have examined possible increased contamination rates under climate change scenarios (Medina et al. 2014 and Medina et al. 2015) and suggest that aflatoxin contamination will increase in many areas as temperatures rise.

In Georgia (GA) and throughout the southern USA, corn is planted as a summer crop and is highly susceptible to aflatoxin contamination (Widstrom et al. 1996). Rainfall variability and high temperatures in this region during summer, along with light textured soils that exacerbate drought or water stress, all influence contamination. Also, lack of irrigation infrastructure in some areas can further aggravate water stress (Brenneman et al. 1993). Salvacion et al. (2011) found that June maximum temperatures and precipitation were key predictors of aflatoxin contamination in southern Georgia (GA), USA. Damianidis et al. (2015) found that the risk of aflatoxin contamination changes specifically with corn hybrid planted, soil type and the weather conditions before and after the mid-silk growth stage, which usually occurs in June across the Southeast US. Using the drought index, ARID, as an aflatoxin risk predictor, they also found that a 0.1 increase of in-field drought, as quantified by ARID, during key weeks before and after mid-silk, increased the probability of aflatoxin contamination over the FDA threshold of 20 ppb.

Accurate aflatoxin assessment usually requires the collection of multiple grain samples. There are several methods available but most are time-consuming and expensive (Papadoyanis, 1990) and conducted at harvest which does not allow implementation of in-season management practices to reduce risk. Given the expense of aflatoxin measurement, an important goal for agricultural extension services and crop consultants would be the ability to identify those years and counties most at risk of contamination to reduce unnecessary expense on testing in years and areas when there is little risk of contamination. Identification and prediction of years and counties at risk would allow the implementation of management strategies such as irrigation in season to reduce contamination risk and the use of resistant varieties (Chen et al. 2002, Chen et al. 2006, Guo et al. 2011 and Menkir et al. 2008). Another goal of agricultural extension services could be to provide an easy to use, computationally efficient, online decision support tool to assess aflatoxin contamination risk that could work for large datasets and crop consultants would require a simple approach to determining risk that could be executed in commercially available, user-friendly software.

The purpose of this research was to apply geostatistical methods to develop a predictive tool using a risk factors approach for identifying problematic years and counties with a longer term view to being able to implement the tool as part of an online decision support system. To validate the risk factors approach, a space-time summary of aflatoxin risk is needed. Similar to soil contaminants, aflatoxin data, as a crop toxin can be expected to be highly skewed. In soil contamination studies, indicator kriging (Goovaerts, 2009) has been used to map the risk of exceeding a particular contamination threshold (Goovaerts et al. 1997). Indicator kriging, however, requires sufficient data to compute a reliable variogram for each year and would result in a risk map for each year with no practical way to produce a space-time risk summary. Aflatoxin data collected from regional sampling is often skewed and approaches a Poisson distribution. Practitioners often analyze data that has been collected by third parties, but do not consider potential geostatistical investigations. Many times, such data have also been collected irregularly in space and time. The 27 year Georgia aflatoxin survey appears to fit these criteria, and these data are perhaps better understood using Poisson kriging. Poisson kriging was first developed by Monestiez et al. (2006) to investigate rare whale sightings, which tend to have a Poisson distribution, and had been observed irregularly in space and time. Poisson kriging has been further adapted for use with sightings of other rare animals (Kerry et al., 2013), used in studies of mortality rates from rare diseases (Goovaerts 2005, $2006_{a,b}$) and the investigation of crime rates (Kerry et al., 2010). Poisson (Goovaerts $2006_{a,b}$) and Binomial kriging (Oliver et al. 1998) have been used interchangeably in the literature for mapping rates of rare disease and although superficially different, often lead to similar results (Flanders and Kleinbaum., 1995) . Even though Binomial kriging may be more theoretically appropriate in certain cases where the characteristics of the data are known *a priori*, it adds an extra layer of complexity requiring an additional parameter to computations. Indeed, as the number of trials increases, the Binomial distribution approaches the Poisson distribution (Haining et al. 2010) and its use can be justified here since assumptions about the prevalence of aflatoxin are avoided. Furthermore implementations are not available in user-friendly commercially available software packages. Spatially irregular observations or the analysis of rate or proportion data can suffer from the ‘small number problem” (Haining et al., 2010) and be unreliable in areas that have received less sampling effort or are sparsely populated. For example, if a given county was only sampled in a particularly high risk year but other counties were sampled over several years, the county with just one measurement would seem to have very high aflatoxin levels. Binomial or Poisson kriging can be used to give a space-time summary of aflatoxin contamination data collected over a 27 year period (1977-2004) in 53 counties in southern GA that takes account of the “small number problem”, but here the latter will be used due to computational simplicity with a view to the eventual implementation in an online decision support tool or use by agricultural consultants using commercially available software. Due to irregular sampling in space and time there are insufficient data to employ other geostatistical methods for individual years. The space-time summary of aflatoxin contamination in southern GA produced by Poisson kriging will be used to assess the viability of a risk factors approach for identifying the counties and years at greatest risk of aflatoxin contamination. Based on existing literature, several key risk factors namely Maximum June temperatures (June TMax) (Salvacion et al., 2011), June Rainfall (June RF) (Salvacion et al., 2011; Damianidis et al., 2015; Windham et al., 2009)), amount of corn grown and proportion of droughty soil types (Damianidis et al., 2015) are examined and key thresholds related to aflatoxin contamination identified. Another secondary aim of this research is to conduct a sensitivity analysis of the number of years of data used to create a Poisson kriged space-time summary of risk.

2. Methods

2.1. Data Collection

Between 1977 and 2004, corn grain samples were collected at harvest to measure within county aflatoxin content. Samples were collected using a grab sampling technique where 10 ears were collected for each sampling and there was an average of 3 replications per county. The study area was 53 counties in southern GA (Fig. 1A). Aflatoxin levels in ppb were measured by the USDA-ARS Crop Protection and Management Research Unit and the University of Georgia, Natural Products Laboratory in Tifton, GA. Aflatoxin levels were not measured in every county in every year. Data were collected for a maximum of 45 counties in 1978 (Fig. 1C) and a minimum of 23 counties in 1990 (Fig. 1D) with an average of 37 counties sampled each year that measurements were made. Fig. 1A shows that aflatoxin was measured in the fewest years in the north eastern counties of southern GA. Measurements were not made in any counties in 1979-1980 and 1986-1989. For all years combined there was a total of 705 measurements and these data approached a Poisson distribution (Fig. 2).

Monthly weather data were obtained for each year 1977-2004 from the Georgia Weather Network (http://georgiaweather.net), with weather stations delineated as black points in Fig. 1A. All counties do not have a weather station, some have more than one and the weather stations are not located at the center of the county. There are 82 for the state as a whole, but the installation date of stations varies so data are not available for all stations in all years. Monthly maximum temperatures for June (June Tmax, °C) and June rainfall data (June RF, mm) (Salvacion et al., 2011), were collected and summarized from the recording stations.

The area planted with corn per county was determined using The CropScape - Cropland data layer produced by the National Agricultural Statistics Service (NASS, http://nassgeodata.gmu.edu/CropScape/). Unfortunately this information was not available for

1977-2004 so the proportion of land in each county planted as corn had to be determined from the 2008-2009 growing season which was the first growing season with full coverage in southern GA. This assumes that the areas growing corn in southern GA have not changed markedly in the study period. Non-spatial data relating to the area of corn grown in each county by year were available using the quick stats tool of USDA-NASS (https://www.nass.usda.gov/Statistics_by_State/Georgia/Publications/County_Estimates/2016/GACorn14_15.pdf) for correlation analysis. These showed strong, positive and significant (p=0.05) correlations between the area of corn in each county for all years in the study and 2008. This means that the highest corn producing counties are quite consistent in time and that our assumption above about the CropScape data is reasonable. However, the larger the gap between two years, the lower the correlation coefficient was showing that there will be most uncertainty in the corn data for this study for 1977. This will also have an effect on the uncertainty of the soil data mentioned below.

A geo-corrected 1:250,000 map of soil associations (NRCS, 2006) was simplified and used to generate a map with 3 drainage classes: excessively, well and poorly drained soil. The percentage of land areas with soil in each drainage class in the 2008/2009 corn growing area (as identified using CropScape above) was calculated for each county.

2.2. Statistical Methods

As weather data and aflatoxin data were not available for every county each year, geostatistical methods were applied to estimate missing values and fill these data gaps.

The first step in geostatistical analysis was to compute an experimental variogram using the standard method of moments estimator (Matheron, 1965, Equation (1)) to characterize the spatial structure of the variation. The formula estimates the semi-variances, γ, at a given lag distance, $\mathbf{h}$:

$$\hat{\gamma}(\mathbf{h}) = \frac{1}{2m(\mathbf{h})} \sum_{i=1}^{m(\mathbf{h})} \{z(\mathbf{x}_i) - z(\mathbf{x}_i + \mathbf{h})\}^2 \,, \tag{1}$$

where $z(\mathbf{x}_i)$ and $z(\mathbf{x}_i + \mathbf{h})$ are the observed values of z at $\mathbf{x}_i$ and $\mathbf{x}_i + \mathbf{h}$ and $m(\mathbf{h})$ is the number of pairs of data points separated by the lag $\mathbf{h}$ (Webster and Oliver, 2007). In other words, counties that are located close together show less variation between them than counties that are separated by larger distances.

A continuous model is then fitted to the experimental variogram using weighted least squares and then parameters of the variogram model are used in kriging, the geostatistical interpolation process. As the standard method of moments variogram estimator is based on variances (Equation 1) it is sensitive to large outliers or tails in a distribution (Kerry and Oliver, $2007_{a,b}$). A typical variogram where the semi-variance increases as the separating distance between points increases, up to a sill where semi-variances stay the same with increasing separation distance, indicates that the data no longer show spatial autocorrelation once they are a certain distance apart (Fig. 1B). Webster and Oliver (1992, 1993) found that variograms based on fewer than 50 observations are of little worth and recommended that at least 100 data points are needed to compute a reliable variogram for kriging. The number of samples needed to compute a reliable variogram does however, depend on the form of variation (Webster and Oliver, 1992) present, spatial configuration of the samples and the probability distribution of the data (Kerry and Oliver, $2007_{a,b}$). Weather data are derived from well-established, carefully located stations and variation often displays quite predictable patterns in relation to latitude, proximity to coasts and elevation. Variograms for June TMax and June RF in Georgia showed good spatial structure (Fig. 5A,B) with markedly less than 100 data values for each year. Because the data were approximately normally distributed, the variograms were used for Ordinary Kriging of June TMax and June RF for each year to the centroid of each county in southern GA to fill in data gaps for some counties and also to a 1 km grid. The kriging variance maps for weather data in 1977 and 2001 (Fig. 1E,F) show that the spatial uncertainty in the kriged weather data varies according to the number and location of weather stations with data for a given year. Kriging data to a 1 km grid was used to see if there were areas smaller than the county level that were at the greatest risk of contamination. Ordinary kriging, a well-documented geostatistical interpolation method (Goovaerts, 1997; Isaaks and Srivastava, 1989; and Webster and Oliver, 2007), was also used to estimate the % corn planted area and each soil drainage class for the cells of the 1 km grid. Kriging of county level risk factors and aflatoxin data (areal support) to a 1 km grid (point support) represents a change in support. When there is good reason to believe that the size and shape of areal units (here counties) are somehow related to the phenomena of interest, then the size and shape of the areal units should be taken into account during variogram computation and kriging (Kerry et al. 2016). This could be achieved using Area-to-Point Poisson kriging (Goovaerts 2006_{b}; Kerry et al. 2010) and Area-to-Point ordinary kriging (Kyriakidis, 2004) for the aflatoxin and risk factor data, respectively. While natural features like rivers sometimes partially delineate county boundaries, they are largely administrative and not related to environmental phenomena that might influence aflatoxin contamination or the risk factors investigated. Also Area-to-Point kriging is more computationally intensive, an undesirable feature for developing an efficient online decision support tool, so the change in support was not taken into account here when producing the 1 km grid data.

Analysis and interpretation of the GA corn aflatoxin data with geostatistical methods is problematic. A variogram of aflatoxin contamination for each year for all counties in southern GA must be computed with an average of 37 data points and as few as 23 (Fig. 1D). Variograms for individual years were unreliable and showed little spatial structure. This is typical of highly skewed (Kerry and Oliver, $2007_{a,b}$) and sparse data (Webster and Oliver, 1992). The aflatoxin data were highly positively skewed and approached a Poisson distribution (Fig. 2). Poisson kriging (Monestiez et al. 2006) is ideal for data with a Poisson distribution, which have been irregularly observed in space or time as these data have been. The proportion of years a county had aflatoxin levels > 20ppb and > 100 ppb were Poisson kriged to county centroids and also to a 1 km grid. Ratios were calculated where, the numerator was the number of times aflatoxin levels were above one of the thresholds in a given county and the denominator was the number of years aflatoxin was measured in that county. The influence of ratios for counties with fewer observations was down-weighted in variogram computation and kriging. The influence of these unreliable proportions on the variogram was reduced by using the following weighted estimator:

$$\hat{\gamma}_{Rv}(\mathbf{h}) = \frac{1}{2\sum_{\alpha,\beta}^{N(\mathbf{h})} \frac{d(v_\alpha)d(v_\beta)}{d(v_\alpha)+d(v_\beta)}} \sum_{\alpha,\beta}^{N(\mathbf{h})} \left\{ \frac{d(v_\alpha)d(v_\beta)}{d(v_\alpha)+d(v_\beta)} \left[r(v_\alpha)-r(v_\beta)\right]^2 - m* \right\} \qquad (2)$$

where $N(\mathbf{h})$ is the number of pairs of counties ($v\alpha, v\beta$) whose centroids are weighted by the number of years with observations to homogenize their variance (Goovaerts, 2006_b) are separated by the vector **h,** and $m*$ is the denominator-weighted mean (weighted by the number of years with observations) of the N area ratios. The usual squared differences, $[r(v_\alpha) - r(v_\beta)]^2$, are weighted by a function of their respective denominator sizes, $d(v_\alpha)d(v_\beta)/[d(v_\alpha)+d(v_\beta)]$, which gives more importance to more reliable data pairs based on larger denominators (Monestiez *et al*. 2006, Goovaerts, 2005, $2006_{a,b}$). Poisson kriging is a form of kriging with non-systematic errors and is parametric, modelling the noise attached to each observation with a Poisson distribution. Observations with small denominators receive less weight in kriging, the estimation process, by adding an error variance term to the diagonal of the kriging system. For more details see Monestiez et al. 2006 and Goovaerts, 2005, $2006_{a,\ b}$. Geostatistical methods were carried out using SpaceStat (Jacquez et al. 2014).

2.3. Risk Factors Approach

By applying kriging, risk factor (June TMax, June RF, % Corn planted area and Soil drainage type) data were generated for each county and each node in a grid with 1 km spacing. These data were then converted to indicators (0/1) depending on whether each variable exceeded certain thresholds or not. Table 1 shows the thresholds chosen for each variable. The thresholds for June TMax and June RF were selected on the basis of 30-year normals in southern GA; values assigned a (1) show hotter or drier years than normal. The indicator thresholds for other risk factors were determined based on examination of histograms of these variables in association with natural marked breaks in the distribution or values associated with the tails of a normal distribution. In each case the condition that would be expected to increase aflatoxin contamination was assigned '1' and the condition that would constitute less risk of contamination a '0'. An additive approach was then used with the indicator data to determine the number of risk factors above/below the specified threshold for each county and year. The relationship between these additive indicator data and the Poisson kriged aflatoxin data was assessed. This suggested broad groupings of years and counties with different levels of aflatoxin contamination risk. These broad groupings were used to define grouping variables (Table 2) based on risk factors for Mann-Whitney U and Kruskal-Wallis H comparison tests (VSN International, 2015) to determine if there were significant differences in aflatoxin levels based on these thresholds identified by the risk factors approach.

2.4. Sensitivity Analysis

To determine the effect of the number of years of data used to create a space-time summary a Poisson kriging sensitivity analysis was performed. All years with available aflatoxin data (21 years) were sub-sampled to produce 10 random sub-samples with 5, 10 and 15 different years of aflatoxin data. Each sub-sample was Poisson kriged and the patterns for individual sub-samples of years and the mean patterns for a given sample size of years were compared visually with the Poisson kriged data generated using all available years of measurements. The Poisson kriged values for individual sub-samples and the mean for a given sample size of years were also compared statistically using Mann-Whitney U tests based on high (≥3 risk factors) and low (<3 risk factors) risk counties.

3. Results and Discussion

3.1. Summary Statistics of Aflatoxin Data

To summarize the risk of aflatoxin contamination in space and time, and to verify if the risk factor approach is useful, summary statistics for the data were calculated. The summary statistics.showed that the mean, and to a lesser extent the median, are influenced by the maximum value and this is particularly pronounced for the years where a smaller proportion of the counties were observed (e.g. 1977, 1985, 1990, 1991 and 2004) (Fig. 3). This suggests that 'the small number problem' (when proportions for a year are unreliable because they are based on measurements in fewer counties) affects summaries of aflatoxin by county (Fig. 4). Counties with the smallest number of sampling years (e.g. Candler, Clinch, Emmanuel, Laurens, Montgomery, Pulaski and Treutlen) are some of those with the largest and smallest mean aflatoxin levels. This indicates that when examined by county, the 'small number problem' is an issue that should be addressed. Analysis and summary of the spatial and temporal variability of mean aflatoxin levels by year and county does not provide a reliable indication of the years and counties at greatest risk of contamination. The correlation coefficient between the mean and median aflatoxin levels for years was r=0.89 (p<0.001) and was r=0.35 (p=0.015) for counties. This clearly indicates that the 'small number problem' is a greater complication for spatial analysis than for temporal analysis.

3.2. Poisson kriging of Aflatoxin Data

Two examples of variograms for aflatoxin data corresponding to individual years, 1978 and 1991, are shown in Fig. 5C,D. Both have a very erratic structure compared to a typical variogram (Fig. 1B) due to the small sample size for individual years (23-45 counties, Webster and Oliver, 1992) and the highly skewed distribution of the data (Kerry and Oliver, 2007), which approaches a Poisson distribution (Fig. 2). Small sample size and skewed data cause the variograms to have an erratic form or cause it to appear as if there is no spatial structure (ie variogram is pure nugget, essentially a horizontal line indicating no spatial autocorrelation). Variograms were computed for all years jointly by calculating a summary variable such as the mean or median aflatoxin for 1977-2004 for each county (Fig. 5E,F) and there were more data (705). Although these variograms are a little less like pure nugget variograms than the individual years (Fig. 5C,D), they are erratic in form and not suitable for kriging. This was due to the mean and median data exhibiting high skew values: 2.71 and 3.46, respectively, and the histograms (not shown) approaching a Poisson distribution. In contrast, when the proportions of aflatoxin values exceeding two critical thresholds (20 ppb and 100 ppb) were examined using the Poisson variogram (Fig. 5G,H), variograms showed good spatial structure with approximate ranges of 54 km and 33 km, respectively. These ranges show that the areas with > 100 ppb aflatoxin are on average smaller than those with >20 ppb.

The Poisson variograms (Fig. 5G,H)were used to Poisson krige the proportion of years that aflatoxin exceeded 20 ppb and 100 ppb and the maps produced for a 1 km grid are show in Fig. 9J and K, respectively and can be used to verify the risk factors approach for identifying the highest risk counties outlined below.

3.3. Analysis of Risk Data

3.3.1. Temporal Patterns

Analysis of temporal patterns for the risk of aflatoxin contamination is primarily centered on analyzing the risk associated with weather variables, specifically June TMax and June RF

(Salvacion et al. 2011, Damianidis et al., 2015), which help identify the specific years at greatest risk of contamination. Weather data for June Tmax and June RF in 2001, were ordinary kriged to county centroids and displayed on a county basis (Fig. 6A,B). Fig. 1F shows the kriging variance associated with these data and where they are likely to be most reliable. Applying the thresholds of June Tmax > 33°C and June RF < 50 mm (Table 1), which are based on 30 year normals for the region, it was observed that in 2001 most counties were not hotter than normal (Fig. 6C) and about one third of counties were drier than normal (Fig. 6D). A combined analysis of these two weather factors, indicated that no counties are at a high (2) risk of aflatoxin contamination and about one third of counties have a medium risk (1) (Fig. 6E). Fig. 6F shows the measured aflatoxin values for 2001 and the majority of values are below 20 ppb showing this was a low risk year for aflatoxin (Mean = 8.5 ppb and Median = 3 ppb, Fig. 3A,B). In contrast, Fig. 7A,B show the Ordinary kriged June Tmax and June RF for 1977 on a1 km grid. Fig. 1E shows the kriging variance associated with these data and where they are likely to be most reliable and that there is generally greater uncertainty in the 1977 weather data than that for 2001 (Fig. 1F) as data were available at fewer weather stations. Fig. 7A,B indicate how transition zones between high and low values may look and affect values within counties. Using the same thresholds (Table 1) as for 2001, Fig. 7C shows that in 1977 all counties were hotter than normal (> 33°C) and Fig. 7D shows that the majority of counties were drier (<50 mm) than normal. Fig. 7E shows that when the indicators for the two weather factors are combined the majority of southern GA is at high risk (2) of aflatoxin contamination in 1977. This agrees with the majority of measured aflatoxin levels being over 20 ppb and many being over 200 ppb (Fig. 7F) as well as the mean and median being well over this threshold, 470 and 192 ppb, respectively (Fig. 3A,B).

The proportion of counties with aflatoxin levels > 20 ppb was calculated for each year and correlated with the proportion of counties with a combined weather risk of two for each year using a scattergraph (Fig. 8). This produced a correlation with a coefficient of r=0.80 which is significant at $p<0.001$. Three distinct groups of years were identified in the scatter plot by visual empirical grouping, but statistically-based clustering methods could be used in the future. The groups identified years that had high (1977, 1981, 1998), medium (1978, 1990, 1992, 1993, 1995, 1996, 2000, 2002) and low (1991, 1994, 1997, 1999, 2001, 2003, 2004) risk of aflatoxin contamination. This grouping clearly suggests the influence of weather conditions on aflatoxin contamination.

3.3.2. Spatial Patterns

As with temporal patterns, analysis of spatial patterns of aflatoxin contamination to identify the counties at greatest risk requires the analysis of weather variables and their patterns of variability. However, patterns of the percentage of each county growing corn and the proportions of each county in corn production with well- and excessively-drained soil become relevant. These factors are relatively stable in time but vary considerable in space and should help determine the counties most at risk of aflatoxin contamination in corn. Figs. 9A-C show the patterns in percent corn, well- and excessively-drained soil and Figs. 9E-G show the indicators produced from these data using the thresholds in Table 1. Based on % corn planted area, there is greatest risk (1) in the south western part of the GA (Fig. 9E). For well-drained soil, risk is greatest in the west (Fig. 9F) and for excessively-drained soil the north is the highest risk area (Fig. 9G). Fig. 9D shows a map where the two weather risk factors (June TMax and June RF) used to identify the years most at risk of aflatoxin contamination have been combined and the proportion of years with two weather risk factors has been kriged to a 1km grid. This analysis provides a space-time summary of the areas most prone to drought, in southern GA. When converted to an indicator based on the threshold in Table 1, Fig. 9H exhibits the areas with a high (1, >30% of years) risk of drought. Fig. 9I displays the percentages of years with three or more risk factors for aflatoxin contamination where June Tmax and June RF are considered separate risk factors. The map has similarities to Figs 9G,H suggesting that weather and excessively drained soils are the greatest risk factors for aflatoxin contamination. Nevertheless, the crucial importance of both weather factors is shown by the striking similarity in the patterns shown in Fig. 9D,H which show drought summary and Fig. 9J,K which show the Poisson kriged summary of aflatoxin levels in all years. Fig. 9L presents the relationship between the proportion of years with 3 or more risk factors and proportion of years with aflatoxin levels >20ppb for each county. The correlation coefficient for this relationship was $r=0.59$, which is significant at $p<0.001$. Distinct groupings of counties are visible in the plot and have been delineated. Such groupings were not very well defined when just weather factors were considered and the correlation coefficient was lower, suggesting that the other risk factors (% corn planted area and soil drainage types) help to distinguish the spatial differences between counties in addition to weather patterns. The higher risk counties circled with solid black/red and dashed black/green lines (Fig. 9L) are the northern most counties in southern GA as well as those in the central area of the northern half of the state.

3.4. Confirmatory Analysis

A range of comparison tests (Mann-Whitney U and Kruskal-Wallis H) were performed to determine whether there were significant differences in aflatoxin levels based on the risk levels identified with the risk factor approach. First, aflatoxin for all years was compared based on whether a county was a high risk county as identified by the risk factors approach (ie ≥ 3 risk factors in more than 20% of years) (Table 2A). When a Mann-Whitney U test was performed using average aflatoxin levels for all years as the test variable, there was not a significant difference (p=0.569) in aflatoxin levels between the counties identified by the risk factors approach as high risk counties and those not. However, when the Poisson kriged summary data for % years with > 20 ppb and 100 ppb aflatoxin were used as the test variables, the difference between the counties identified as at risk and those not was significant, p=0.002 and p=0.012, respectively. The order of class ranks also demonstrates that the lowest ranks were obtained for counties with 0-20% years with >3 risk factors. This indicates that the average aflatoxin levels do not give a good summary of the counties most and least at risk of aflatoxin contamination while the Poisson kriged data which down-weight the influence of proportions based on low numbers of observations, provided an acceptable summary.

A comparison of the raw measured aflatoxin levels for each year based on the number of risk factors identified for each year (e.g. weather factors), county (based on soil drainage type and % corn planted area) and years and counties together is reported in Table 2B. These results indicate that the differences between years based on weather data is significant at p<0.001, however, the differences between counties based on other risk factors is not significant (p=0.241), but does not show the expected order of class ranks. When the risk from weather and other factors is combined there is a significant difference (p<0.001) in the raw aflatoxin values and the order of class ranks shows that aflatoxin levels for each class increase as the number of risk factors identified increases as expected. This clearly demonstrates the importance of weather in an individual season for determining if a county is at risk of aflatoxin contamination. The temporal summaries of spatial patterns, further confirm the importance of weather in determining risk. Fig. 9B,C show the very small proportions of well and excessively drained soils in southern, particularly southeastern counties of the study area, yet there are high proportions of years with > 100ppb aflatoxin in south central areas (Fig. 9K) which coincide with high proportions of years with two weather risk factors.

Finally, Table 2C compares aflatoxin levels based on the proportion of years there were ≥3 risk factors per county split into the four grouping identified in Fig. 9L. When average aflatoxin levels were used, a significant difference was found between counties that had different proportions of years with ≥3 risk factors (p=0.043), however the order of class ranks was not as expected because average aflatoxin levels were not greater for counties with a greater percentage of years with aflatoxin levels > 20 or 100 ppb. When the comparison based on these groupings was performed with the Poisson kriged data (proportion of years aflatoxin levels were >20 and 100 ppb) as the test variables, the difference between the groups was highly significant (p<0.001) and showed the expected order of class ranks namely, the higher the proportion of years with ≥3 risk factors, the larger the proportion of years with > 20 and 100 ppb.

3.5. Sensitivity analysis

Maps of the Poisson kriged proportion of years exceeding 20 and 100 ppb aflatoxin indicate the average patterns based on 10 random sub-samples of 5, 10 and 15 years of data (Fig.

10A-C, E-G) and those are plotted to the same scale as the Poisson kriged map for all years (Fig. 10D,H). It is clear from these maps that the proportion of years with aflatoxin over threshold levels is underestimated for all sub-sample sizes. This degree of underestimation is also greatest for the smallest sample of years (5, Fig. 10A,E). When the same data used in Fig. 10A-Cand E-G were plotted using quantiles (not shown) they showed similar patterns to one another in terms of the highest and lowest risk counties. However, the largest percentages were found in the central and western counties in slight contrast to the maps for all years (Fig. 10D,H). This shows locational bias relating to which counties were sampled in more years (Fig. 1A) and were therefore more likely to be included in more of the random samples of years. There appears to be slightly less underestimation in the proportion of years that thresholds are exceeded and less locational bias for the 100 ppb threshold (Fig. 10E-H) than for the 20 ppb threshold (Fig. 10A-D). This is probably due to the fact that 100 ppb is a rarer occurrence than 20 ppb and Poisson kriging was specifically designed for kriging rare occurrences.

In contrast to Table 2A, Mann Whitney U tests showed no significant difference (p>0.05) between high (≥ 3 risk factors) and low risk counties (< 3 risk factors) for the mean PK > 20 ppb and > 100 ppb values for all sub-sample sizes and 88% of the individual sub-samples (predominantly sample size 5 and 10). This suggests that a sample size of at least 20 years of aflatoxin data is needed to get a reliable space-time summary of aflatoxin risk.

4. Conclusions

This research demonstrates that when data have been irregularly sampled in space and time and also approach a Poisson distribution, Poisson kriging is a useful way to generate a temporal summary of spatial patterns. Simple averages were shown to be unreliable where fewer observations were made and standard geostatistical methods do not work well when data have a Poisson distribution or have few data for individual years. Comparison tests showed that counties and years identified as having the greatest risk levels using the risk factors approach did have significantly higher proportions of years with aflatoxin levels over threshold levels. Average aflatoxin, however, was not significantly different between counties identified with different risk levels due to unreliable averages because of irregular sampling. Despite the significant differences between counties with different risk levels and the similarities in patterns, sensitivity analysis suggested that at least 20 years of data are desirable to generate reliable space-time summaries of aflatoxin risk. Sensitivity analysis also showed that Poisson kriged patterns are more appropriate with smaller sample sizes when the threshold is a rarer occurrence (eg 100 ppb).

Identification and monitoring the weather conditions and counties associated with the highest contamination risk will allow for in-season adaptation strategies such as irrigation to avoid drought as temperatures and rainfall in June are carefully monitored in the key weeks around the mid-silk period with respect to 30 year normals. Also, testing can be focused in the highest risk counties and very little expensive aflatoxin testing will be needed in low risk years. Also the highest risk counties should consider growing more drought resistant hybrids.

Future work could investigate developing an approach which takes into account the spatial variation in uncertainty associated with weather data availability. Also including new variables in the risk factors approach and fine tuning of the thresholds of the existing non-weather risk factors which were less reliable/important in distinguishing between aflatoxin levels should be considered. Fine-tuning of threshold values might improve the identification of the counties at greatest risk. The data kriged to a 1 km grid shows the potential for defining the smallest possible high risk areas which could reduce sampling cost but the potential value of using Area-to-Point kriging to produce these data should be assessed. Thresholding the risk factor data before kriging should also be investigated as a possible approach for pin-pointing the smallest at risk areas. The next major step in analysis is to include in season corn NDVI and thermal IR data in the risk factors approach to indicate in-season drought stress. This should allow areas smaller than a county that are at high risk to be identified within a particular season so that in-season adaptation strategies can be developed. An online interactive aflatoxin risk assessment tool that uses the risk factors approach outlined here is currently being developed and will include NDVI data. There is the potential that such an online tool could be adapted to other crops, states and even farms so that aflatoxin levels may be better managed.

Acknowledgements

Funding for this work was provided by NOAA RISA grant NA10OAR4310215 and the USDA-NIFA grant 2011-67003-30347.Thanks to Drs. David Wilson, (University of Georgia), Neil Widsrom (USDA-ARS) and Billy Wiseman (USDA-ARS). Thanks to Kaleb Kreamer for his support with data collection. Thanks go to Dr EunHye Yoo, University at Buffalo, SUNY for useful comments, feedback and ideas relating to this work.

References

Abbas, H.K., Weaver, M.A., Zablotowicz, R.M., Horn, B.W., Shier, W.T. 2005.

Relationships between aflatoxin production and sclerotia formation among isolates of *Aspergillus* section Flavi from the Mississippi Delta, Eur. J. Plant Pathol. 112, 3, 283-287.

Abbas, H.K., Shier, W.T, Cartwright, R.D. 2007. Effect of temperature, rainfall and planting date on aflatoxin and fumonisin contamination, in commercial Bt and non-Bt-corn hybrids in Arkansas, Phytoprotection 88, 2,41-50.

Adegoke, G.O., Otumu, E.J., Akanni, A.O. 1994. Influence of grain quality, heat, and processing time on the reduction of aflatoxin b-1 levels in tuwo and ogi - 2 cereal-based products, Plant Food. Hum. Nutr. 45, 2, 113-117.

Barrett, J.R. (2005) Liver Cancer and Aflatoxin: New Information from the Kenyan Outbreak. Environ. Health Persp. 113, 12, A837–A838.

Brenneman, T.B., Wilson, D.M., Beaver, R.W. 1993. Effects of diniconazole on *Aspergillus* populations and aflatoxin formation in peanut under irrigated and non-irrigated conditions, Plant Dis. 77, 6, 608-612.

Chen, Z.Y., Brown, R.L., Damann, K.E., Cleveland, T.E. 2002. Identification of unique or elevated levels of kernel proteins in aflatoxin-resistant maize genotypes through proteome analysis, Phytopathology 92, 10, 1084-1094.

Chen, Z.Y., Brown, R.L., Rajasekaran, K., Damann, K.E., Cleveland, T. E. 2006. Identification of a maize kernel pathogenesis-related protein and evidence for its involvement in resistance to *Aspergillus flavus* infection and aflatoxin production, Phytopathology 96, 1, 87-95.

Damianidis, D., Ortiz, B. V., Windham, G., Scully,B., Woli, P B. V.. 2015.

Predicting pre-harvest aflatoxin corn contamination with a drought index. In: J.V. Stafford (Ed.), Precision Agriculture 2015 – Proceedings of the 10th European Conference on Precision Agriculture (10 ECPA), Tel Aviv, Israel.

Flanders, W. D., Kleinbaum, D. G., 1995. Basic models for disease occurrence in epidemiology. Int. J. Epidemiol., 24, 1, 1-7.

Food and Drug Administration (FDA). 2012. Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins, Second Edition. Food and Drug Administration, Laurel, Maryland: Available online: http://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM297627.pdf

FDA 2015. http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074703.htm accessed August 2016.

Goovaerts, P. 1997. Geostatistics for Natural Resources Evaluation. Oxford University Press, New York, 483 pp.

Goovaerts, P. 2005. Geostatistical Analysis of Disease Data: Estimation of Cancer Mortality Risk from Empirical Frequencies Using Poisson Kriging. Int. J. Health Geogr. 4, 31.

Goovaerts, P. 2006$_a$. Geostatistical Analysis of Disease Data: Visualization and Propagation of Spatial Uncertainty in Cancer Mortality Risk Using Poisson Kriging and p-Field Simulation, Int. J. Health Geogr. 5, 7.

Goovaerts, P. 2006$_b$. Geostatistical Analysis of Disease Data: Accounting for Spatial Support and Population Density in the Isopleth Mapping of Cancer Mortality Risk Using Area to Point Poisson Kriging. Int. J. Health Geogr. 5, 52.

Goovaerts, P. 2009. AUTO-IK: a 2D indicator kriging program for the automated non parametric modeling of local uncertainty in earth sciences. Comput. Geosci. 35, 1255–1270.

Goovaerts, P., Webster, R., Dubois, J.P. 1997. Assessing the risk of soil contamination in the Swiss Jura using indicator geostatistics. Environ. Ecol. Stat. 4, 31–48.

Guo, B., Chen, Z., Lee, R.D., Scully, B.T. 2008. Drought stress and preharvest aflatoxin contamination in agricultural commodity: Genetics, genomics and proteomics, J. Integr. Plant Biol. 50, 10, 1281-1291.

Guo, B.Z., Krakowsky, M.D., Ni, X., Scully, B.T., Lee, R.D., Coy, A.E., Widstrom, N.W. 2011. Registration of Maize Inbred Line GT603, J. Plant Registr. 5, 2, 211-214.

Haining, R. P., Kerry, R., Oliver, M. A. 2010. Geography, Spatial Data Analysis and Geostatistics: An Overview. Geogr. Anal. 42:1, 7-31.

Horn, B.W., Sorensen, R.B., Lamb, M.C., Sobolev, V.S., Olarte, R. A., Worthington, C.J., Carbone, I. 2014. Sexual Reproduction in *Aspergillus flavus* Sclerotia Naturally Produced in Corn, Phytopathology 104, 1, 75-85.

Isaaks, E. H., Srivastava, R. M. 1989. Applied Geostatistics. Oxford University Press, New York, 561 pp.

Jacquez, G.M., Goovaerts, P., Kaufmann, A., Rommel, R., 2014. SpaceStat 4.0 User Manual: Software for the Space-Time Analysis of Dynamic Complex Systems, 04/2014; Edition: Fourth Edition, Publisher: BioMedware, Ann Arbor, USA.

Kerry, R., Goovaerts, P., Giménez, D., Oudemans, P., Muñiz, E. 2016. Investigating geostatistical methods to model within-field yield variability of cranberries for potential management zones. Precis. Agric. 17, 243-273.

Kerry, R., Goovaerts, P., Haining, R. P., Ceccato, V. 2010. Geostatistical Analysis of Car

Theft and Robbery in the Baltic States. Geogr. Anal. 42:1, 53-77.

Kerry, R., Goovaerts, P., Smit, I., Ingram, B. R. 2013. A comparison of multiple indicator kriging and area-to-point Poisson kriging for mapping patterns of herbivore species abundance in Kruger National Park, South Africa. Int. J. Geogr. Inf. Sci. 27, 1, 47-67.

Kerry, R., Oliver, M.A. 2007. Determining the Effect of Skewed Data on the Variogram. I. Underlying Asymmetry. Comput. Geosci. 33, 10, 1212-1232.

Kerry, R., Oliver, M.A. 2007. Determining the Effect of Skewed Data on the Variogram. II. Outliers. Comput. Geosci. 33, 10, 1233-1260.

Kyriakidis, P., 2004. A geostatistical framework for area-to-point spatial interpolation. Geogr. Anal. 36, 259–289.

Liu, Y., Wu, F. 2010. Global Burden of Aflatoxin-Induced Hepatocellular Carcinoma: A Risk Assessment, Environ. Health Persp. 118, 6, 818–824.

Oliver, M. A., Webster, R., Lajaunie, C., Muir, K. R., Parkes, S. E., Cameron, A. H., Stevens, M. C. G., Mann. J. R. 1998. Binomial Cokriging for Estimating and Mapping the Risk of Childhood Cancer. Math. Med. Biol. 15, 3, 279–97.

Papadoyannis, I. N. 1990. HPLC in Clinical Chemistry (Chromatographic Science Series) 1st Edition. Marcel Dekker, New York, pp. 504.

Matheron, G., 1965. Les Variables Régionalisées et leur Estimation: une Application de la Théorie de Fonctions Aléatoires aux Sciences de la Nature. Masson et Cie, Paris.

Medina, A., Rodriguez, A., Sultan, Y., Magan, N. 2015. Climate change factors and *Aspergillus flavus*: effects on gene expression, growth and aflatoxin production, World Mycotoxin J. 8, 2, 171-179.

Medina, A., Rodriguez, A., Magan, N. 2014 Effect of climate change on *Aspergillus flavus* and aflatoxin B1 production, Front. Microbiol., 5, 348 .

Menkir, A., Brown, R. L., Bandyopadhyay, R., Cleveland, T.E. 2008. Registration of Six Tropical Maize Germplasm Lines with Resistance to Aflatoxin Contamination, J. Plant Registr. 2, 3, 246-250.

Monestiez, P., Dubroca, L., Bonnin, E., Durbec, J. P., Guinet, C. 2006. Geostatistical Modelling of Spatial Distribution of *Balaenoptera physalus* in the Northwestern Mediterranean Sea from Sparse Count Data and Heterogeneous Observation Efforts. Ecol. Model.. 193, 615–28.

NRCS 2006. Digital General Soil Map of U.S., U.S. Department of Agriculture, Natural Resources Conservation Service, Fort Worth, Texas. Online_Linkage: URL:http://SoilDataMart.nrcs.usda.gov/

Palumbo, J.D., O'Keeffe, T.L., Kattan, A., Abbas, H. K., Johnson, B.J. 2010. Inhibition of *Aspergillus flavus* in Soil by Antagonistic Pseudomonas Strains Reduces the Potential for Airborne Spore Dispersal, Phytopathology 100, 6, 532-538.

Patriarca, A., Medina, A., Pinto, V. F., Magan, N. 2014. Temperature and water stress impacts on growth and production of altertoxin-II by strains of *Alternaria tenuissima* from Argentinean wheat, World Mycotoxin J. 7, 3, 329-334.

Payne, G.A. 1992. Aflatoxin in maize, Crit. Rev. Plant Sci. 10, 5, 423-440.

Salvacion, A., Ortiz, B. V., Scully, B., Wilson, D. M., Hoogenboom, G., Lee, D. 2011. Effect of Rainfall and Maximum Temperature on Corn Aflatoxin in the Southeastern U. S Coastal Plain. In: Proceedings of the Climate Information for Managing Risks, Orlando, Florida, May 24-27, 2011.

Setamou, M., Cardwell, K.F., Schulthes, F. Hell, K. 1997. *Aspergillus flavus* infection and aflatoxin contamination of preharvest maize in Benin, Plant Dis. 81, 11, 1323-1327.

VSN International 2015. Genstat Reference Manual (Release 18), Part 1 Summary. VSN International, Hemel Hempstead, UK.

Wang, T., Zhang, E., Chen, X., Li, L., Liang, X. 2010. Identification of seed proteins associated with resistance to pre-harvested aflatoxin contamination in peanut (*Arachis hypogaea*), Plant Biol. 10, 267.

Webster, R., Oliver, M.A. 1992. Sample adequately to estimate variograms of soil properties. J. Soil Sci. 43, 177–192.

Webster, R., Oliver, M. A. 1993. How large a sample is needed to estimate the regional variogram adequately? In: Soares, A. (Eds.) Geostatistics Tróia ’92. Springer, Netherlands, p. 155-166.

Webster, R., Oliver, M.A. 2007. Geostatistics for Environmental Scientists, 2nd Ed. Wiley, Chichester.

Widstrom, N.W., Forster, M.J., Martin, W.K., Wilson, D.M. 1996. Agronomic performance in the southeastern United States of maize hybrids containing tropical germplasm. Maydica 41, 1, 59-63.

Wilson, J.P., Hanna, W.W., Wilson, D.M., Beaver, R.W., Casper, H.H. 1993. Fungal and Mycotoxin Contamination of Pearl-Millet Grain in Response to Environmental-Conditions in Georgia, Plant Dis. 77, 2, 121-124.

Windham G.L., Williams W.P., Hawkins L.K., Brooks T.D. 2009. Effect of *Aspergillus flavus* inoculation methods and environmental conditions on aflatoxin accumulation in corn hybrids. Toxin Rev. 28,70-78. .

Wu, F., Khlangwiset, P. 2010. Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: Case studies in biocontrol and postharvest interventions, Food Addit. Contam. A. 27, 4, 496–509.

Table 1. Threshold values used for Risk Factor Indicators

Risk Factor	**Threshold for Indicator (1/0)**
June monthly maximum temperature (°C)*	>33°C
June monthly Rainfall (mm)*	<50 mm
Percent of county area growing corn (%)	>1.75%
Percent of county with well-drained soils (classes 1-4) (%)	>90%
Percent of county with excessively drained soils (classes 1-2.5)	>2.5%
Percent of years with 2 weather risk factors	>30 %

*Thresholds for June Tmax and June RF were chosen with respect to 30-year normal Tmax and RF in the area to show hotter and drier years than normal

Table 2. Summary of comparison tests (Mann-Whitney U and Kruskal-Wallis H) for aflatoxin risk

Test variable	Grouping variable	Classes in grouping variable	Order of class ranks (lowest to highest)	*p*-value
(A) Average aflatoxin for all years	Identified as a low/high risk county	1: 0-20% years ≥3 risk factors 2: >20% years ≥3 risk factors	1, 2	0.569
PK % years aflatoxin >20ppb	Identified as a low/high risk county	1: 0-20% years ≥3 risk factors 2: >20% years ≥3 risk factors	1, 2	0.002
PK % years aflatoxin >100ppb	Identified as a low/high risk county	1: 0-20% years ≥3 risk factors 2: >20% years ≥3 risk factors	1, 2	0.012
(B) Aflatoxin measured for individual years	Combined risk (year and county)	Number of risk factors 1: 0, 2: 1, 3: 2, 4: 3, 5: >4	1, 2, 3, 4, 5	<0.001
Aflatoxin measured for individual years	County risk	Number of risk factors 1: 0, 2: 1, 3: > 2	2, 1, 3	0.241
Aflatoxin measured for individual years	Year risk	Number of risk factors 1: 0, 2: 1, 3: > 2	1, 2, 3	<0.001
(C) Average aflatoxin for all years	% years ≥3 risk factors	1: 0-20%, 2: 20-50%, 3: 50-85%, 4: >85%	3, 4, 2, 1	0.043
PK % years aflatoxin >20ppb	% years ≥3 risk factors	1: 0-20%, 2: 20-50%, 3: 50-85%, 4: >85%	1, 2, 3, 4	<0.001
PK % years aflatoxin >100ppb	% years ≥3 risk factors	1: 0-20%, 2: 20-50%, 3: 50-85%, 4: >85%	1, 2, 3, 4	<0.001

PK – Poisson kriged

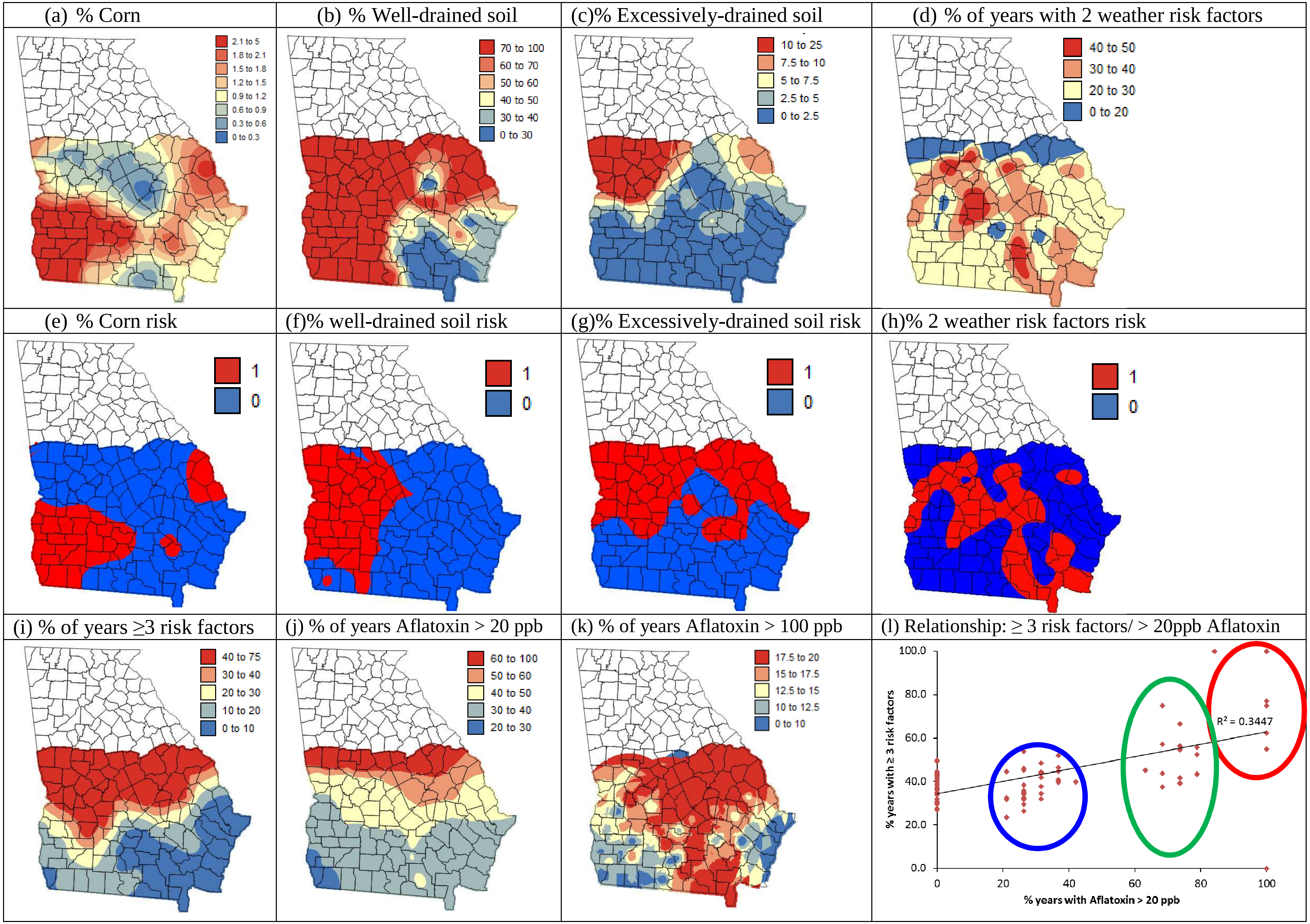
(a) % Corn
2.1 to 5
1.8 to 2.1
1.5 to 1.8
1.2 to 1.5
0.9 to 1.2
0.6 to 0.9
0.3 to 0.6
0 to 0.3
(b) % Well-drained soil
70 to 100
60 to 70
50 to 60
40 to 50
30 to 40
0 to 30
(c)% Excessively-drained soil
10 to 25
7.5 to 10
5 to 7.5
2.5 to 5
0 to 2.5
(d) % of years with 2 weather risk factors
40 to 50
30 to 40
20 to 30
0 to 20
(e) % Corn risk
1
0
(f)% well-drained soil risk
1
0
(g)% Excessively-drained soil risk
1
0
(h)% 2 weather risk factors risk
1
0
(i) % of years ≥3 risk factors
40 to 75
30 to 40
20 to 30
10 to 20
0 to 10
(j) % of years Aflatoxin > 20 ppb
60 to 100
50 to 60
40 to 50
30 to 40
20 to 30
(k) % of years Aflatoxin > 100 ppb
17.5 to 20
15 to 17.5
12.5 to 15
10 to 12.5
0 to 10
(l) Relationship: ≥ 3 risk factors/ > 20ppb Aflatoxin
100.0
80.0
60.0
40.0
20.0
0.0
% years with ≥ 3 risk factors
R^2 = 0.3447
0
20
40
60
80
100
% years with Aflatoxin > 20 ppb

Fig. 9. Risk factors and their associated indicators plotted for each county kriged to a 1 km grid (a, e) percent of county area growing corn, (b, f) percent of county area with well-drained soils, (c, g) percent of county area with excessively drained soils and (d, h) percent of years each county has 2 weather risk factors and (i) percent of years with ≥ 3 risk factors, (j) percent of years with Aflatoxin > 20 ppb and (k) percent of years with aflatoxin > 100 ppb for each county.
(l) Relationship between percent of years with ≥ 3 risk factors and percent of years with > 20 ppb Aflatoxin for each county. Ellipses show visual empirical groupings of years with low (blue/dotted), medium (green/dashed) and high risk (red/solid) of aflatoxin contamination

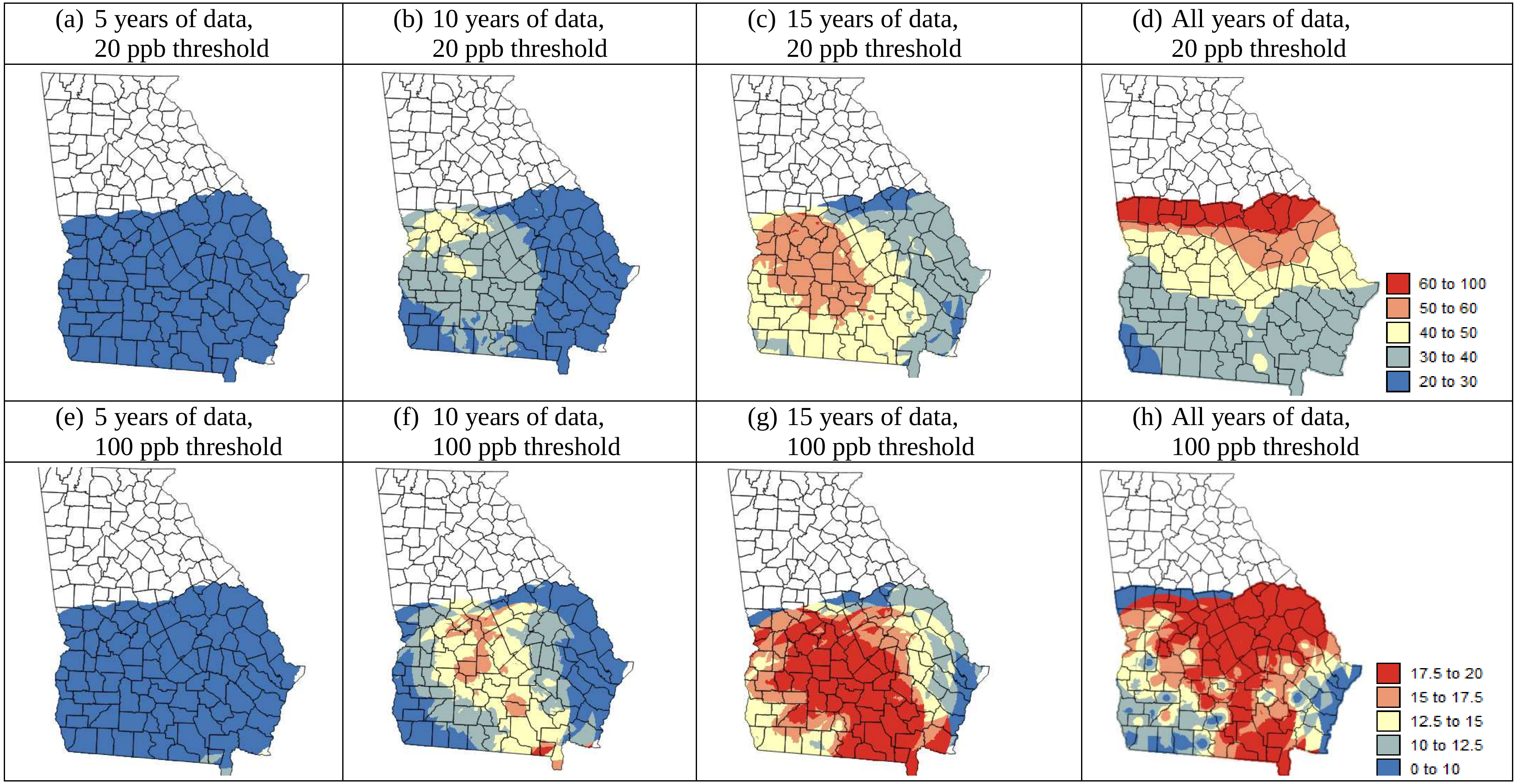

Figure 10. Poisson kriged maps of the proportion of years aflatoxin > 20ppb and 100ppb using (a, e) 5, (b, f) 10, (c, g) 15 and (d, h) all years of available data.

Poisson kriging gives a useful spatio-temporal summary of aflatoxin contamination risk

Risk factors approach identifies distinct groups of counties with different risk

More than 15 years data needed for a good Poisson kriged space-time summary