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AFLATOXIN IN SOUTH TEXAS COTTONSEED: GEOGRAPHIC DISTRIBUTION AND INFLUENCING FACTORS

R. Jaime-Garcia
Agricultural Research Service
United States Department of Agriculture
Tucson, AZ
P.J. Cotty
College of Agriculture
University of Arizona
Tucson, AZ

Abstract

Aflatoxins are toxic chemicals produced by the fungus *Aspergillus flavus*. Federal regulations limit uses of aflatoxin-contaminated cottonseed and cottonseed with 20 ppb or higher is not allowed into the dairy market. A total of 36,716 truck-loads of cottonseed from 35 gins received at the Valley Co-op Oil Mill in Harlingen, TX during the years of 1997 to 2001 were analyzed for aflatoxin content. South Texas was divided into five regions: Rio Grande Valley, South Coastal Bend, North Coastal Bend, Upper Coast and Winter Garden. The 1999 season had the highest levels of contamination with an average aflatoxin content of 112.3 ppb and 66% of the cottonseed truckloads exceeding 20 ppb. The lowest levels of aflatoxin contamination occurred in the years 1997 and 2000 with an average of 24 ppb, while the lowest incidence (15.5%) of the truckloads exceeding an aflatoxin content of 20 ppb occurred in 1997. In general, aflatoxin contamination increased as the ginning season progressed. Rainfall after boll opening correlated highly with aflatoxin content and rain during July had the highest influence. Geostatistical analyses revealed recurrent patterns of aflatoxin contamination. Greatest contamination occurred from the central Coastal Bend region through the southern Upper Coast region. The Rio Grande Valley region experienced the least contamination during the study period.

Introduction

Aflatoxins are toxic chemicals produced by fungi belonging to the genus *Aspergillus* (Cotty et al., 1994).The aflatoxin content of foods and feeds is limited by regulation throughout most of the world (Park et al., 1988; van Egmond, 2002). Aflatoxins in dairy-feed can be transferred to milk (Allcroft and Carnaghan, 1963; van Egmond, 1989). In the US, regulations prohibit aflatoxin concentrations over 0.5 µg/kg in milk (Park and Troxell, 2002). Milk exceeding that limit may be discarded and the producing dairy quarantined (Cotty, 2001). In order to prevent unacceptable aflatoxin levels in milk, regulations prohibit feeding dairy cows cottonseed with ≥ 20 ppb aflatoxin (Park and Troxell, 2002). Aflatoxin contamination of cottonseed causes significant economic loss annually. In the US, contamination is most severe in the desert production regions of Arizona, the Imperial Valley of California, and in South Texas (Cotty, 2001; Russell, 1982).

Aspergillus flavus contaminates cottonseed with aflatoxins in two phases (Cotty, 2001). The first phase occurs when developing bolls are damaged or have partial suture openings (Cotty, 2001; Russell, 1982). These generally occur during June and early July in South Texas. The second phase occurs when mature cottonseeds are exposed to high humidity and warm temperature either before or after harvest (Bock and Cotty, 1999; Cotty, 2001). Cotton boll opening in South Texas starts in late June. Contamination in the second phase can be increased by rain, late irrigation, and even dew or high relative humidity (Cotty, 1991; Russell et al., 1976). Delayed harvest predisposes cottonseed to aflatoxin contamination by increasing aflatoxin production in the second phase (Cotty, 1991).

Geostatistics and geographic information systems (GIS) allow the description, analysis, and display of spatial patterns of a wide variety of variables at any scale to aid in the solution of real world problems. These technologies have been applied to agricultural and plant pathological problems (Nelson et al., 1994; Jaime-Garcia et al., 2001; Orum et al., 1999).

The objectives of the present study were to assess seasonal patterns of aflatoxin contamination of cottonseed and identify both areas with highest risks of contamination and factors associated with contamination, in a spatial perspective in South Texas.

Materials and Methods

Sampling

A total of 36,715 truckloads of cottonseed received at the Valley Co-op Oil Mill in Harlingen, TX from gins in South Texas were analyzed for aflatoxin content from 1997 to 2001 (Table 1). Six samples of 3 to 5 kg of cottonseed were taken from different locations in each truck with the help of a vacuum sampler. The samples were mixed, split and subdivided and four single samples of 5 to 8 kg were obtained. Samples were cracked with a roller and sieved to separate kernels from hulls obtaining approximately 250 g of kernels per sample. The kernels were mixed and a subsample of 40 g was taken, ground, and analyzed for aflatoxin content. Aflatoxin content was obtained with either Aflatest (Vicam, 313 Pleasant St., Watertown, MA) or Veratox (Neogen Corporation, Food Safety Division, 620 Lesher Place, Lansing, MI) following the manufacturer's instructions. Aflatoxin data in ppb were organized by region and gin of origin, and date of receipt at the mill. Percentage of cottonseed (truckloads) with aflatoxin content equal to or higher than 20 ppb (Percent Over 20) was calculated for each date and gin of origin.

Data Analysis

The study area was over 450 km long by 100 km wide extending from the Rio Grande Valley in the south to Fort Bend County in the north in South Texas. The Winter Garden area, a separate area close to San Antonio, TX, also was included in the study. Each gin analyzed was geo-referenced in the Universal Transverse Mercator (UTM) projected coordinate system. The total area was divided into five geographic regions (Rio Grande Valley, South Coastal Bend, North Coastal Bend, Upper Coast, and Winter Garden) to determine if aflatoxin contamination is significantly influenced by spatial or geographic factors. Analysis of variance was used to assess differences among areas in aflatoxin content, and the Percent Over 20. Regression analyses for the Percent Over 20 as a function of time (Julian days) were performed for each region. Both linear and non-linear (exponential) models ($b_0 + b_1*X$ and $b_0 * e^{b1*X}$, respectively) were evaluated using SAS 8.0 (The SAS Institute Inc., Cary, NC). Multiple linear regression analyses with monthly average weather variables were performed for the annual averages of both aflatoxin content and Percent Over 20 using the stepwise procedure of SAS 8.0. For the weather variables the closest weather station to each gin was used. Monthly total rain and the monthly average of maximum, median, and minimum temperature were tested. Only gins (eleven) from the Coastal Bend and Upper Coast regions with a weather station both within 25 miles and having archive data for at least four years of the study were included in the analyses. This resulted in a total of 50 data points for each weather variable.

Spatial Analysis

Spatial analysis of aflatoxin contamination of cottonseed was performed using geostatistics (Nelson et al., 1999) to determine if geographic location significantly influences the extent to which cottonseed becomes contaminated with aflatoxins and to identify areas with greatest contamination problems. Geostatistical analyses were performed on both the annual average aflatoxin content and annual Percent Over 20 to obtain patterns of aflatoxin contamination throughout South Texas. The mean values for the entire season for each gin were used in these analyses. Model variograms were used in ordinary block kriging for estimation in unsampled areas. Block kriging uses point data and variogram models to estimate values in blocks or grids. ArcGIS, version 8.0 for Windows (ESRI, 380 New York ST., Redlands, CA) was used for block kriging. Interpolation of values for unsampled areas by block kriging was performed on a 2 x 2 km grid with a search neighborhood of 60,000 m and a maximum of 12 sample locations. Mean seasonal values for all gins were used for kriging procedures. ArcGIS v. 8.0 for Windows was used to create views and layouts of the kriged values with previously digitized features as background.

Results

In South Texas, aflatoxin contamination of cottonseed is a perennial problem with both temporal and spatial (regional) variation. Both, the annual average aflatoxin content and the percentage of cottonseed with aflatoxin content equal to or exceeding 20 ppb (Percent Over 20) differed significantly across crop seasons from 1997 to 2001 ranging from 24.0 to 112.3 ppb and 15.5% to 65.7%, respectively (Table 1). Highest levels of contamination occurred in 1999 with an average of 112.3 ppb of aflatoxin and an average of 65.7% of cottonseed truckloads with aflatoxin content equal to or exceeding 20 ppb. Lowest levels of contamination occurred in 1997 and 2000, with 24.0 and 24.4 ppb respectively, and 15.5% and 19.6% of cottonseed with aflatoxin content equal to or exceeding 20 ppb (Table 1). In general, the most severe aflatoxin contamination occurred from the northern portion of the Coastal Bend Region (North of Corpus Christi, TX) through the central portion of the Upper Coast Region (Port Lavaca, TX and Victoria, TX) (Table 1; Fig. 2 and 3). Statistically significant differences among regions were found for both the aflatoxin content and the Percent Over 20 (Table 1).

Seasonal Aflatoxin Contamination

In general, aflatoxin contamination increased as ginning season progressed (Fig. 2). Regression analyses of Percent Over 20 (Table 3) indicated that aflatoxin contamination has a low rate of increase early in the season, with much more rapid increase late in the season. Based on combined data from 1997 to 2001, seasonal progression of aflatoxin content equal to or higher that 20 ppb in South Texas best fit an exponential model with an r^2 equal to 0.89 and an e-base exponent of 0.0256. This gives an average rate of increase in the incidence of cottonseed with aflatoxin content equal to or higher that 20 ppb of 0.77% per day (Fig. 1).

Relationship of Contamination with Weather Variables

Multiple linear regression analyses by the stepwise method of SAS indicate that rain after boll opening and minimum temperature during early boll development are the most important variables influencing aflatoxin contamination in South Texas (Table 2). The variables included in both models were rain in June, rain in July, rain in August and temperature minimum in June (Table 2). The r^2 for these models were 0.70 for aflatoxin content and 0.73 for Percent Over 20. All variables included in the models were individually significant ($P \leq 0.05$). Rain during July is the variable with the greatest influence on both aflatoxin content and Percent Over 20, giving a partial r^2 of 0.52 and 0.53, respectively.

Spatial Patterns of Aflatoxin Contamination

Geostatistical analyses, variogram analyses and kriging interpolation of both aflatoxin contamination and Percent Over 20 indicated a patchy distribution which varied with season. Maps of both the estimated aflatoxin content and the estimated Percent Over 20 for the 1997 to 2001 seasons obtained by kriging show recurrent patterns of aflatoxin contamination (Figs. 2 and 3). Similar to the ANOVA results (Table 1), mapping showed increased incidence and severity of contamination in the southeast and all northern areas of the Coastal Bend region, and the south and central areas of the Upper Coast region, with variation in the patterns of contamination among seasons (Figs. 2 and 3). For 2000 and 2001, the highest contamination extended from the northern Coastal Bend areas through the Upper Coast. The Rio Grande Valley experienced the lowest contamination throughout the study period (Figs. 2 and 3).

Discussion

Aflatoxin contamination of commercial cottonseed in South Texas presents both temporal and spatial variation. Temporal variation occurs both, between and within seasons. Even though there is variation in aflatoxin contamination among seasons, levels of contamination observed during the seasons with least contamination still are of considerable economic importance. For instance, during 1997 and 2000 (when the lowest aflatoxin contamination occurred) the average aflatoxin content for both seasons was 24 ppb and the percentage of cottonseed exceeding 20 ppb was 15.5 and 19.6 for 1997 and 2000, respectively (Table 2). Although most seed produced in South Texas had relatively low aflatoxin content in these two seasons in some areas (North Coastal Bend) and (South Upper Coast) between 27 to 38% of cottonseed could not be sold in the lucrative dairy market due to unacceptable aflatoxin content (Figs. 2 and 3). Very high aflatoxin contamination occurred in 1999 when unacceptable aflatoxin content occurred in at least half of the cottonseed produced in all the South Texas areas, and over 70% of the cottonseed produced within the Coastal Bend and Upper Coast regions was unacceptable for dairy use (Table 1; Fig. 3). These frequencies of contamination suggest significant investment in technologies to limit contamination may be warranted.

Variation among seasons in aflatoxin content occurs in most areas and on most crops with aflatoxin problems (Wilson and Payne, 1994). This variation has been attributed to climatic factors, especially drought and high temperature, in corn and peanuts (Cole et al., 1982; Wilson and Payne, 1994) with increased contamination being associated with reduced rainfall. In South Texas, seasonal variation in aflatoxin content of cottonseed is also associated with variation in rainfall. However, increased contamination is associated with increased rain as suggested by the regression models in Table 2. In the desert valley production areas of Arizona, cotton is irrigated and the majority of cottonseed contamination has been associated with exposure of the mature crop (open bolls) to increased humidity and warm temperature (Bock and Cotty, 1999; Cotty, 2001). Irrigation practices vary in South Texas from supplemental to no irrigation and precipitation has an important influence on crop survival, yield, and on aflatoxin content. The quantity of rain that crops receive in South Texas varies geographically during the growing season and between seasons. Our data suggest that precipitation occurring when mature bolls are present, from June to August, is the environmental factor with greatest influence on aflatoxin contamination of cottonseed in South Texas (Table 2). Even a superficial examination supports this conclusion. For instance, the 1999 cotton season had the highest precipitation from June to August of the 5 years examined (368 mm) and the cottonseed produced that year had the highest levels of aflatoxin observed (Table 1, Figs. 2 and 3). On the other hand, 1997 and 2000 had the lowest precipitation (105 and 87 mm, respectively) and the lowest levels of contamination (Table 1, Figs. 2 and 3).

In general, aflatoxin contamination in South Texas increased as the ginning seasons progressed for all the areas (Fig. 1). The percentage of cottonseed with aflatoxin content above the 20 ppb threshold for dairy use (30) is very low in the beginning of the ginning season, but toward the end of the season over 50% of cottonseed contains greater than 20 ppb aflatoxins. Percent Over 20 increase in South Texas fits an exponential model reflecting rates of increase that escalate as the ginning season progresses. Thus, the greatest rates of increase occur towards the end of the season as the incidence of cottonseed lots exceeding 20 ppb approaches 100% (Fig. 1). In Arizona, delayed harvest is known to result in cottonseed with increased aflatoxin content due to increases in the second phase of the contamination process (Bock and Cotty, 1999). This phenomenon is the most likely cause of the observed increases in the levels of aflatoxin contamination during the ginning season in South Texas. These results suggest that throughout South Texas, earlier harvest will result in reduced contamination. However, compared with other regions in the US, South Texas already has a very early harvest and substantially earlier harvest may not be economically feasible.

Identification of recurrent patterns of areas with higher aflatoxin contamination provides a basis for selecting initial locations for intensive efforts to manage aflatoxins (i.e. through biocontrol with atoxigenic strains of *A. flavus*). Surface maps obtained by kriging of spatially autocorrelated variables can be compared over multiple seasons in order to identify recurrent patterns of agriculture problems at a regional scale (Jaime-Garcia et al., 2001; Nelson, et al., 1999). Surface maps of aflatoxin contamination in South Texas from 1997 to 2001 show some areas with either recurrent low or recurrent high aflatoxin problems

(Figs. 2 and 3). The Rio Grande Valley region had consistently low aflatoxin contamination during the study period (Figs. 2, and 3). On the other hand, aflatoxin contamination was highly prevalent in the southeast and northern areas of the Coastal Bend region, and the south area of the Upper Coast region (Figs. 2 and 3). The Rio Grande Valley region differs from the Coastal Bend and Upper Coast in several factors that influence aflatoxin contamination. For instance, the Coastal Bend and Upper Coast regions normally have higher precipitation levels than the Rio Grande Valley. Rainfall patterns may explain some of the changing spatial pattern of aflatoxin contamination across seasons. Additional variability could be explained by other factors that are spatially variable in South Texas including differing soil types, differing aflatoxin-producing potential of *A. flavus* communities (Cotty, 1997) and differences in harvest or ginning time. The percent of *A. flavus* strain S associated with commercial cottonseed from gins in the Coastal Bend to Upper Coast regions is significantly higher than those from the Rio Grande Valley (Cotty et al., 2001) and strain S isolates produce higher aflatoxin levels in cottonseed than typical or L strain isolates (Cotty, 1989; Cotty, 1997; Garber and Cotty, 1997). These two areas would be good candidates for initial efforts to develop aflatoxin management programs for South Texas similar to the biological control program used to manage aflatoxins in Arizona (Antilla and Cotty, 2000).

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Table 1. Aflatoxin content and percent of cottonseed with aflatoxin content over 20 ppb from gins in different regions of South Texas from 1997 to 2001.

Region	Years					
	1997	**1998**	**1999**	**2000**	**2001**	**Mean**
Aflatoxin						
Rio Grande Valley	10.2 B(b)	13.9 C(b)	86.2 BC(a)	12.7 B(b)	17.2 C(b)	33.6 D
South Coastal Bend	8.1 B(c)	39.4 B(b)	83.9 BC(a)	30.4 A(b)	14.9 C(c)	41.7 C
North Coastal Bend	39.9 A(c)	55.6 B(b)	156.3 A(a)	26.5 A(d)	37.6 AB(c)	67.7 A
Upper Coast	32.4 A(c)	45.4 B(b)	100.2 B(a)	31.8 A(c)	44.0 A (b)	55.6 B
Winter Garden	8.3 B(c)	105.4 A(a)	63.3 C(b)	28.5 A(bc)	30.5 B(bc)	46.6 C
South Texas	24.0 (c)	36.6 (b)	112.3 (a)	24.4 (c)	33.8 (b)	
Percent Over 20						
Rio Grande Valley	8.6 C(b)	11.3 B(b)	56.4 C(a)	8.5 C(b)	12.7 D(b)	22.5 C
South Coastal Bend	8.6 C(d)	34.5 A(b)	64.9 BC(a)	24.3 B(bc)	17.1 CD(cd)	33.3 B
North Coastal Bend	30.2 A(c)	37.3 A(b)	76.9 A(a)	22.8 B(d)	33.1 AB(bc)	42.6 A
Upper Coast	18.7 B(d)	27.9 A(c)	69.1 AB(a)	28.5 AB(c)	39.8 A(b)	40.1 A
Winter Garden	6.1 C(c)	28.6 A(b)	60.3 BC(a)	33.3 A(b)	25.9 BC(b)	29.6 B
South Texas	15.5 (d)	22.4 (c)	65.7 (a)	19.6 (c)	28.3 (b)	

[z] = Significance level (Tukey's HSD Test at $P = 0.05$) by areas (capital letters) and years (lower case in parenthesis)

Table 2. Regression models for aflatoxin content in cottonseed and Percent Over 20 as a function of weather variables in South Texas from 1997 to 2001.

Variable	Regression Model[a]	R^2	$P > F$
Aflatoxin content	y = -392.3+(6.8*jnr)+(13.55*jlr)+(4.15*agr)+(5.34*jntmn)	0.70	<0.001
Percent Over 20	y = -131.4+(3.61*jnr)+(6.22*jlr)+(2.63*agr)+(1.91*jntmn)	0.73	<0.001

[a] jnr = rain in June, jlr = rain in July, agr = rain in August, and jntmn = minimum temperature in June

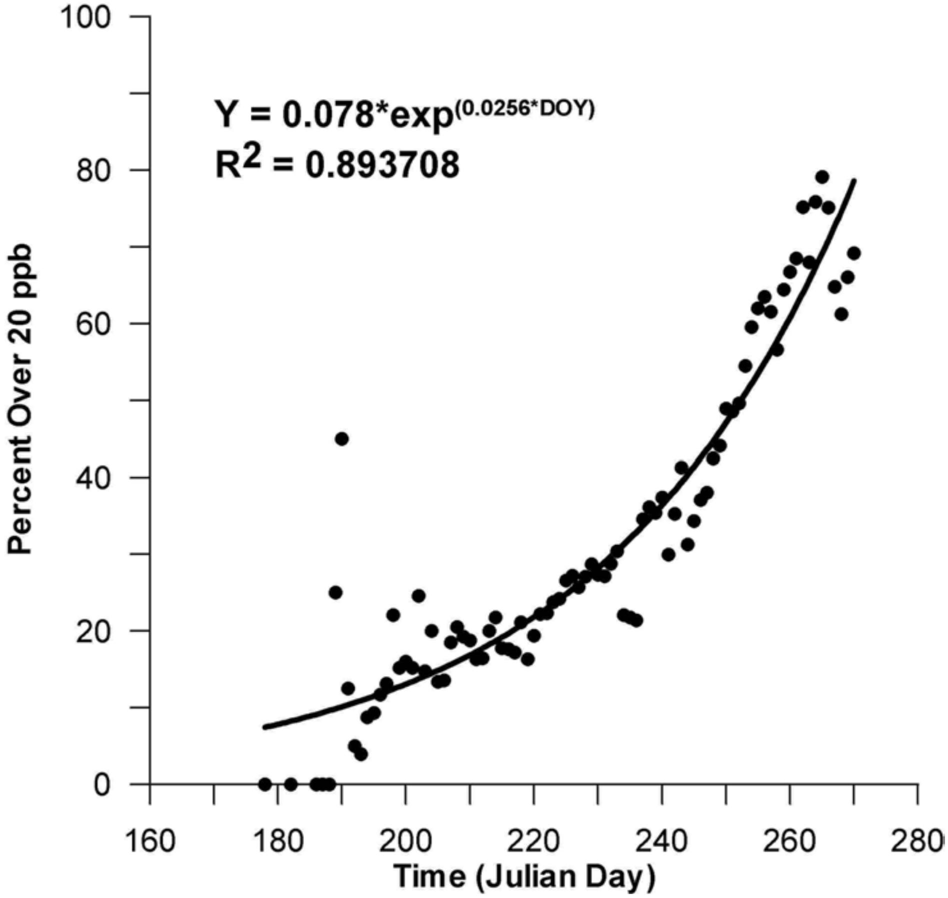

Figure 1. Percent of cottonseed truckloads with aflatoxin content equal to or exceeding 20 ppb (Percent Over 20) as a function of time (Julian day) in South Texas. Combined data for 1997 to 2001. The Percent Over 20 data were calculated for each date and gin of origin by dividing the number of cottonseed truckloads with aflatoxin content equal to or higher than 20 ppb by the total number of truckloads received and multiplying by 100.

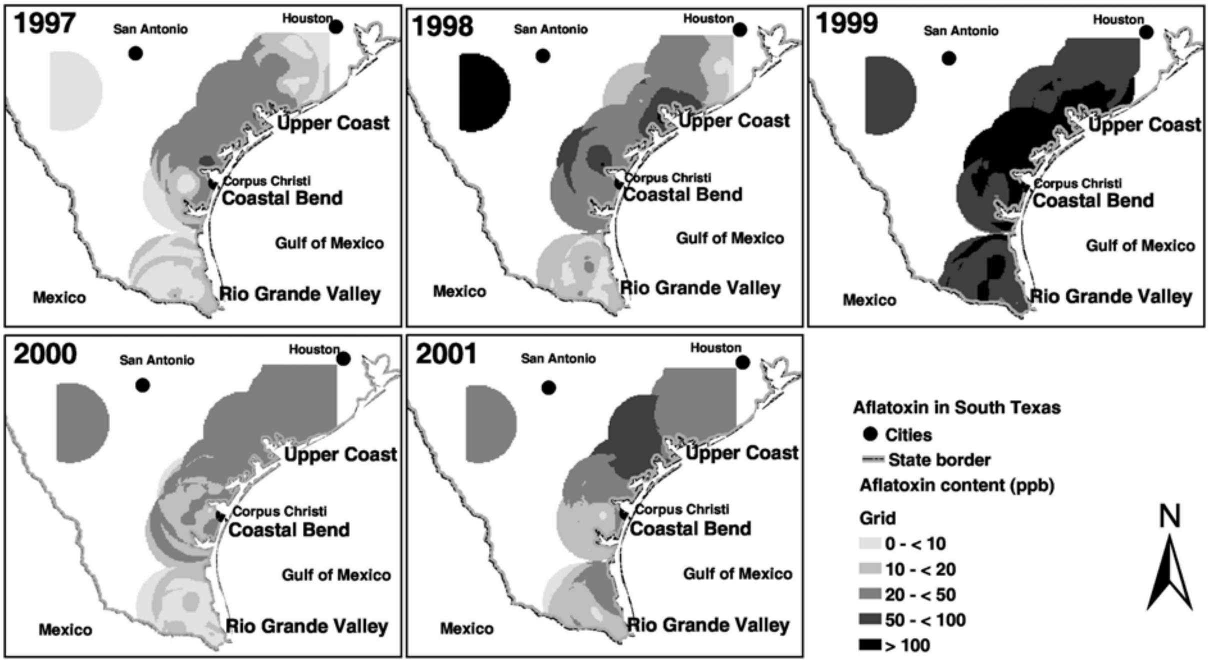

Figure 2. Estimated aflatoxin content, in ppb (parts per billion; ppb), in cottonseed ginned in South Texas during the seasons of 1997 to 2001 based on block kriging (2 x 2 km blocks) of the annual average aflatoxin content data from commercial cottonseed received at the Valley Co-op Oil Mill in Harlingen, TX from gins in South Texas. A search neighborhood radius of 60 km and a maximum of 12 sample points (gins) were used to generate the kriging estimates.

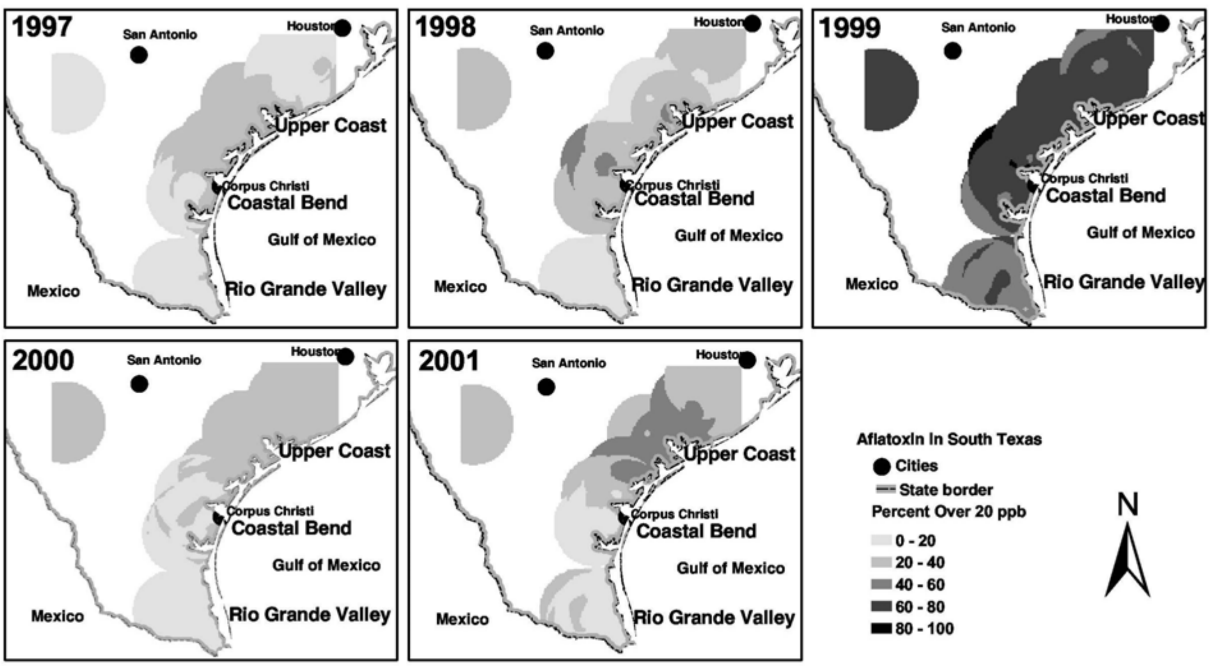

Figure 3. Estimated percent of cottonseed with aflatoxin content equal to or higher than 20 ppb (Percent Over 20) in South Texas during the seasons of 1997 to 2001 based on block kriging (2 x 2 km blocks) of the annual average Percent Over 20 data from commercial cottonseed received at the Valley Co-op Oil Mill in Harlingen, TX from gins in South Texas. A search neighborhood radius of 60 km and a maximum of 12 sample points (gins) were used to generate the kriging estimates.