

## PLANT PATHOLOGY AND NEMATOTOLOGY

### Module Storage Time, Leaf Grade and Seed Moisture Influence Fiber Quality and Aflatoxin Contamination of Cotton in South Texas.

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#### ABSTRACT

**Cotton is the most important natural fiber used to produce apparel, home furnishings, and industrial products. Cotton fiber quality influences both manufacturing efficiency and quality of the finished products. The color, length, strength, and purity of cotton fibers all contribute to fiber quality. Cottonseed is used as food (primarily oil) and is a preferred feed for dairy cows, with dairies paying a premium for cottonseed free of aflatoxin. Modules (14,200) of seed cotton grown in South Texas from 2002 through 2008 were analyzed for fiber quality and seed aflatoxin content. Harvest date, gin date, leaf grade, and seed moisture were related with fiber quality and seed aflatoxin content. Module storage time from harvest to ginning also influenced aflatoxin contamination and fiber quality. Standard fiber quality measurements, including lint color and spot, were related with aflatoxin content and, thus, might be useful predictors of seed aflatoxin contamination. Results suggest reducing module storage time, leaf and stem impurities, and seed moisture can prevent fiber quality deterioration and reduce concentrations of aflatoxins in cottonseed.**

Cotton was produced on more than four million hectares in the U.S. during 2011. Cotton, the most common natural fiber used in apparel, home furnishings, and industrial products (Wakelyn et al., 2007), is grown mainly for fiber cells, components of the seed epidermis that are separated from the remainder of the seed after harvest during ginning. However, non-fiber portions of the seed are also

valuable. After the lint is removed from the seed cotton, the remaining white fuzzy seed can either be processed to yield both a high value food oil and other products or used whole as a feed for animals (Wakelyn et al., 2007). In cotton production, the seed accounts for 15 to 20% of crop value (Cotty, 2001).

There is a complex classification system used to grade cotton lint for commerce. Lint grades as defined by the U.S. Department of Agriculture, are accepted worldwide as fiber quality standards (Wakelyn et al., 2007). Color, fiber length, strength, fineness, and purity are graded. Color grade, a measurement of the degree of reflectance and yellowness, is an indicator of fiber deterioration, which is associated with undesirable processing and dyeing characteristics (Cotton Program, 2001; Wakelyn et al., 2007). Leaf grade, a purity measure, indicates the quantity of plant material in the lint. Leaf grade is affected by variety, harvesting method, and ginning practices (Cotton Program, 2001). Fiber length, average length of the longer one-half of fibers, is dictated by variety, cropping conditions, and ginning practices (Cotton Program, 2001). Strength reflects the force required to break the fiber, and micronaire is an indicator of air permeability, indicating both fiber fineness (linear density) and maturity (degree of cell-wall development) (Montalvo, 2005). Micronaire affects processing performance and quality of the end product and it is influenced during the growing season by environmental conditions (Cotton Program, 2001; Wakelyn et al., 2007). Fiber characteristics are used together by manufacturers to estimate fiber value based on expected performance during textile manufacturing (Cotton Program, 2001; Wakelyn et al., 2007).

Dairies and oil mills compete for cottonseed and largely determine the ultimate seed price (Wu et al., 2008). Cottonseed is a preferred feed for dairies, which pay a premium for seed containing less than 20 µg/kg aflatoxin (Cotty, 2001; Park and Troxell, 2002; Wedegaertner, 2010). Aflatoxins are toxic and carcinogenic secondary metabolites produced by fungi of the genus *Aspergillus* that frequently contaminate several crops and are limited in foods

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and feeds around the world by regulation (Park and Troxell, 2002; van Egmond, 2002). *Aspergillus flavus* Link is the primary agent causing aflatoxin contamination of cottonseed (Cotty, 1990; Diener, 1989). Because aflatoxins are known to pass from feed to milk (Allcroft and Carnaghan, 1963; Munksgaard et al., 1987; van Egmond, 1989), U.S. regulations prohibit dairies from feeding seed with over 20 µg/kg aflatoxins to prevent human exposure to aflatoxins. These regulations make aflatoxin content the most important factor dictating seed value (Cotty, 2001) in areas where aflatoxin contamination is common. Because Texas has the largest cotton acreage in the U.S. (Anonymous, 2011) and aflatoxin contamination frequently causes significant economic loss to Texas cotton crops (Jaime-Garcia and Cotty, 2003), aflatoxin contamination has been a concern to cotton growers in the state for decades.

The process through which aflatoxin contamination occurs has been divided into two phases (Cotty, 2001). The first phase is associated with damage to developing bolls and partial suture opening in the field (Cotty, 2001; Russell, 1982), generally during June and early July in South Texas. The second phase occurs when the mature crop is 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 late 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 during the second phase (Bock and Cotty, 1999; Cotty, 1991; Jaime-Garcia and Cotty, 2003). Effects of environmental conditions during module storage on aflatoxin contamination of cottonseed is not well understood (Batson Jr. et al., 1997; Russell and Lee, 1985).

Environmental conditions during cotton production, harvest, and pre-ginning storage impact both cotton lint grade (Cotton Program, 2001) and seed aflatoxin content (Cotty, 2001; Russell, 1982; Russell et al., 1976). However, little information exists on the relation of lint grade to cottonseed aflatoxin content. The current study examined influence of leaf grade, seed moisture, and module storage time on lint deterioration and cottonseed aflatoxin contamination. The potential utility of standard lint quality measurements in predicting the likelihood of unacceptable levels of aflatoxin in cottonseed lots is described.

## MATERIALS AND METHODS

Modules of seed cotton (14,200) processed in a gin in South Texas during 2002 to 2008 (Table 1) were analyzed for lint quality (Table 2) with official methods (Cotton Program, 2001) by the USDA-classing office in Corpus Christi, TX. Cotton modules are relatively compact units of seed cotton approximately 30 m X 4 m X 3 m and weighing up to 10 tons. After ginning, the resulting seed from one to eight modules, originating from the same field, was shipped by truck (3,145 truckloads total) to the Valley Co-op Oil Mill in Harlingen, TX (Table 1). The gin has an identity ticket system to ensure correspondence between fiber and seed samples. Cores of seed (3-5 kg each) were taken from six locations in each truck with a vacuum sampler with a 6.3-cm internal diameter intake tube. Samples were combined, immediately split into two subsamples, one for seed grade and moisture determination and the other for aflatoxin analysis. Seed for aflatoxin determination was cracked with a roller and sieved to separate kernels from hulls (approximately 250 g of kernels per sample were obtained). Kernels were mixed and a 40-g subsample was ground and analyzed for aflatoxin content (Jaime-Garcia and Cotty, 2003). Total aflatoxin concentration (aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>) was quantified with a commercial ELISA kit (Veratox®, Neogen Corporation, Food Safety Division, Lansing, MI) following the manufacturer's instructions.

**Table 1. Number of cotton modules and truckloads of cottonseed analyzed, and total precipitation and aflatoxin content in cottonseed originating from South Texas from 2002 through 2008**

| Year         | Cotton modules <sup>x</sup> | Seed truckloads <sup>x</sup> | Rain (mm) <sup>z</sup> | Aflatoxins <sup>y</sup> |
|--------------|-----------------------------|------------------------------|------------------------|-------------------------|
| 2002         | 1,805                       | 373                          | 31                     | 28                      |
| 2003         | 2,630                       | 573                          | 24                     | 34                      |
| 2004         | 2,739                       | 583                          | 11                     | 56                      |
| 2005         | 2,521                       | 533                          | 21                     | 48                      |
| 2006         | 1,490                       | 341                          | 31                     | 30                      |
| 2007         | 1,888                       | 471                          | 81                     | 177                     |
| 2008         | 1,127                       | 271                          | 19                     | 25                      |
| <b>Total</b> | <b>14,200</b>               | <b>3145</b>                  |                        |                         |

<sup>z</sup> = Average total precipitation (mm) occurring during the month of July in areas included in the current study.

<sup>y</sup> = Average aflatoxin content of all seed truckloads produced in the study area during the year.

<sup>x</sup> = Seed truckloads correspond to the seed from 1 to 8 modules originating from the same field.

**Table 2. Variables analyzed for cottonseed and cotton lint produced in South Texas from 2002 to 2008**

| Variable              | Name      | Description  |
|-----------------------|-----------|--|
| Day of harvest        | Harvest   | Day of year (Julian day) cotton was harvested  |
| Day ginned            | GinDay    | Day of year (Julian day) cotton was ginned   |
| Module storage time   | ModST     | The number of days from harvest to gin (days)  |
| Leaf grade            | Leaf      | Grade reflecting leaf fragments on the lint (Grades 1-7, 1 the least fragments and 7 the most)   |
| Seed moisture         | SeedMst   | Percent by weight of water content in the seed   |
| Color grade           | Color     | Degree of reflectance and yellowness of the lint (Grades 1-8, 1 good middling, 8 below grade)    |
| Spot grade            | Spot      | Measure of evenness of lint color (Grades 1-5, 1 no stain, 5 yellow stained)                     |
| Length                | Length    | The average length of the upper half of lint fibers (in 32 <sup>nds</sup> of an inch)            |
| Micronaire            | Micron    | Grade reflecting fineness and maturity of fiber (Poor < 35 and > 49; Optimum = 37 to 42)         |
| Strength              | Strength  | Force needed to break fibers in a defined bundle (g/tex, low indicates weak fiber, poor quality) |
| Seed grade            | SeedGrd   | Grade reflecting seed quality (higher, better quality)   |
| Aflatoxin content     | Aflatoxin | µg total aflatoxin per kg seed   |
| Natural log aflatoxin | Logafla   | The natural logarithm of aflatoxin content   |
| Percent over 20       | Per20     | Percent of truckloads with aflatoxin content over 20 µg/kg                                       |

Harvest and gin dates, module storage time, leaf grade, and seed moisture were subjected to regression analysis to determine effects on both lint quality and aflatoxin contamination.

**Module Storage Time.** Seed cotton was stored in compressed and tarped modules (Searcy et al., 2010) in the field from harvest to ginning. Module storage time was recorded for each module as the number of days elapsed from harvest to ginning. Lint quality measurements, seed grade, and aflatoxin content in the seed were averaged based on module storage over 5-d periods (0, 5, 10, 15, 20, 25 and 30). For example “0” included modules ginned on the harvest date, “5” included modules ginned one to five days after harvest, etc. Group 30 contained modules ginned 26 or more days after harvest.

**Leaf Grade.** Leaf grade is a visual estimate of the magnitude of non-lint plant material remaining in the lint after ginning. Leaf grades are identified by numbers 1 through 7, where 1 indicates lint with the least unwanted plant material residues and 7 the most (Cotton Program, 2001). All lint quality and seed variables were averaged by leaf grade from 1 through 7.

**Seed Moisture.** The percent (by weight) of the seed composed of water (seed moisture), was categorized into nine categories (7-15%). All variables under study were averaged for each year (2003- 2008) and seed moisture category for statistical analyses. This resulted in a total of 54 data points for each variable. Seed moisture values were not available for 2002.

**Data Analyses.** Correlation and multiple regression analyses were performed for all the lint and seed variables with SAS v. 9.1 (SAS Institute Inc., Cary, NC). Percentage of truckloads with aflatoxin  $\geq$  20 µg/kg (percent over 20) and the natural logarithm of aflatoxin content were calculated prior to statistical analyses. All standard fiber and seed quality measurements, harvest date, gin date, and module storage time were subjected to Pearson’s correlation analysis. Data averaged by either module storage time, seed moisture, and leaf grade were further subjected to linear and nonlinear regression analyses to evaluate overall influences on lint quality and aflatoxin content. Analysis with averaged data removes sample-to-sample variability and examines only the variance explained by the variable analyzed. Assessment of the potential utility of standard lint quality measurements as indicators of aflatoxin contamination was performed also by both linear and nonlinear regression analyses. Grapher v. 4.0 (Golden Software, Inc., Golden, CO) was used for model fitting and to generate figures.

## RESULTS

Lint and seed quality are influenced by the timing of both pre- and post-harvest activities (Tables 3 and 4). Pearson’s correlation analyses indicate that both harvest date and gin date affect both the quality of cotton lint and seed, and aflatoxin contamination in cottonseed. Positive correlations of color, spot, length, leaf grade, aflatoxin content, and percent over 20 with both harvest

and gin date indicate that both lint and seed quality deteriorate, and aflatoxin content increases as harvest or ginning is delayed (Table 3). This is indicated also by negative correlations between harvest date and both lint strength and seed grade. Likewise, color, spot, length, and aflatoxin, correlate positively and micronaire, strength, and seed grade correlate negatively with leaf grade (higher grades indicate more leaf residues in the lint) and suggest that lint and seed quality deteriorate and aflatoxin increases when leaf residues increase. Seed moisture was positively correlated with color, spot, and aflatoxin, whereas it was inversely correlated with lint strength and seed grade suggesting a deterioration of lint and seed quality and an increase in aflatoxin

contamination as seed moisture increased. Harvest date, gin date, leaf grade, and color had the highest positive correlation coefficients with aflatoxin. Seed grade and lint strength were negatively correlated with aflatoxin content, indicating that as both seed grade and lint strength deteriorate aflatoxin contamination increases (Table 3). The low correlation coefficients between some variables do not necessarily reflect a lack of relationship between the variables. Pearson's correlation analysis describes linear relationships and when relationships between variables are nonlinear, correlation coefficients might be low even when relationships are strong. In such cases, nonlinear regression models better describe relationships (Table 3).

**Table 3. Correlation coefficients and probabilities for relationships among lint and cottonseed variables for cotton grown in South Texas from 2002 through 2008<sup>z,y</sup>**

| Variables | Harvest | GinDay | ModST  | Leaf   | SeedMst | Color  | Spot   | Length | Micron | Strength | SeedGrd | Aflatoxin | Logafla | Per20 |
|-----------|---------|--------|--------|--------|---------|--------|--------|--------|--------|----------|---------|-----------|---------|-------|
| Harvest   | 1       |        |        |        |         |        |        |        |        |          |         |           |         |       |
| GinDay    | 0.943   | 1      |        |        |         |        |        |        |        |          |         |           |         |       |
|           | <.0001  |        |        |        |         |        |        |        |        |          |         |           |         |       |
| ModST     | 0.361   | 0.478  | 1      |        |         |        |        |        |        |          |         |           |         |       |
|           | <.0001  | <.0001 |        |        |         |        |        |        |        |          |         |           |         |       |
| Leaf      | 0.267   | 0.226  | 0.047  | 1      |         |        |        |        |        |          |         |           |         |       |
|           | <.0001  | <.0001 | <.0001 |        |         |        |        |        |        |          |         |           |         |       |
| SeedMst   | 0.213   | 0.209  | -0.052 | 0.163  | 1       |        |        |        |        |          |         |           |         |       |
|           | <.0001  | <.0001 | <.0001 | <.0001 |         |        |        |        |        |          |         |           |         |       |
| Color     | 0.411   | 0.346  | -0.180 | 0.622  | 0.273   | 1      |        |        |        |          |         |           |         |       |
|           | <.0001  | <.0001 | <.0001 | <.0001 | <.0001  |        |        |        |        |          |         |           |         |       |
| Spot      | 0.439   | 0.466  | 0.093  | 0.339  | 0.297   | 0.393  | 1      |        |        |          |         |           |         |       |
|           | <.0001  | <.0001 | <.0001 | <.0001 | <.0001  | <.0001 |        |        |        |          |         |           |         |       |
| Length    | 0.370   | 0.375  | 0.095  | 0.153  | 0.004   | 0.123  | 0.037  | 1      |        |          |         |           |         |       |
|           | <.0001  | <.0001 | <.0001 | <.0001 | 0.674   | <.0001 | <.0001 |        |        |          |         |           |         |       |
| Micron    | 0.097   | 0.064  | -0.050 | -0.280 | 0.098   | -0.057 | 0.072  | -0.070 | 1      |          |         |           |         |       |
|           | <.0001  | <.0001 | <.0001 | <.0001 | <.0001  | <.0001 | <.0001 | <.0001 |        |          |         |           |         |       |
| Strength  | -0.259  | -0.236 | 0.132  | -0.189 | -0.226  | -0.425 | -0.380 | 0.412  | -0.083 | 1        |         |           |         |       |
|           | <.0001  | <.0001 | <.0001 | <.0001 | <.0001  | <.0001 | <.0001 | <.0001 | <.0001 |          |         |           |         |       |
| SeedGrd   | -0.383  | -0.369 | 0.076  | -0.249 | -0.248  | -0.412 | -0.366 | -0.087 | 0.093  | 0.352    | 1       |           |         |       |
|           | <.0001  | <.0001 | <.0001 | <.0001 | <.0001  | <.0001 | <.0001 | <.0001 | <.0001 | <.0001   |         |           |         |       |
| Aflatoxin | 0.267   | 0.261  | 0.117  | 0.192  | 0.098   | 0.253  | 0.109  | 0.125  | -0.026 | -0.136   | -0.158  | 1         |         |       |
|           | <.0001  | <.0001 | <.0001 | <.0001 | <.0001  | <.0001 | <.0001 | <.0001 | <.0001 | <.0001   | <.0001  |           |         |       |
| Logafla   | 0.247   | 0.241  | 0.156  | 0.251  | 0.150   | 0.259  | 0.146  | 0.087  | -0.090 | -0.150   | -0.086  | 0.686     | 1       |       |
|           | <.0001  | <.0001 | <.0001 | <.0001 | <.0001  | <.0001 | <.0001 | <.0001 | <.0001 | <.0001   | <.0001  | <.0001    |         |       |
| Per20     | 0.276   | 0.273  | 0.159  | 0.255  | 0.176   | 0.279  | 0.172  | 0.109  | -0.089 | -0.153   | -0.155  | 0.522     | 0.811   | 1     |
|           | <.0001  | <.0001 | <.0001 | <.0001 | <.0001  | <.0001 | <.0001 | <.0001 | <.0001 | <.0001   | <.0001  | <.0001    | <.0001  |       |

<sup>z</sup> = Variables as defined on Table 2.

<sup>y</sup> = Top value, Pearson's correlation coefficient; bottom value, probability r = 0.

Multiple regression models for aflatoxin content included leaf grade, day of harvest and/or day of ginning, module storage time, and seed moisture as independent variables. These same variables were also included in multiple regression models for lint quality variables (color, spot, strength, micronaire) and seed grade (Table 4). Module storage time, leaf grade, and seed moisture were further analyzed individually by regression analysis using averaged data (Figs. 1- 3).

**Module Storage Time.** Aflatoxin concentrations increased with the length of module storage (Fig. 1). The rate of increase for the combined data (2002-2008) was low for seed ginned within 10 d, but increased exponentially for seed ginned between 10 and 20 d after harvest (Fig. 1f). Influences of module storage differed among individual years with most years fitting logistic models. In 2002 and 2004, exponential growth in the rate of increase started 15 d after module construction (Figs. 1a and 1c), whereas in 2007 exponential increases started after as little as 5 d of storage in modules (Fig. 1e). All models had highly significant relationships ( $P < 0.001$ ) with high coefficients of determination ( $R^2 > 0.80$ ).

**Leaf Grade.** Regression analyses indicated that leaf grade influenced both lint quality variables and aflatoxin content (Fig. 2). Color, spot, and aflatoxin increased exponentially with leaf grade (Figs. 2a, 2d, and 2f), and strength, micronaire, and seed grade fit negative models (Figs. 2b, 2c, and 2e). All models were highly significant ( $P < 0.0001$ ) and had high coefficients of determination ( $R^2 > 0.9$ ).

**Seed Moisture.** Most (more than 14,000) seed samples had moisture content between 7 and 15%, with a few samples below 7% (25) or above 15% (78); those below 7% were included in the 7% category and those above 15% in the 15% category. Most variables in this study changed exponentially with seed moisture (Fig. 3). Responses of aflatoxin content, spot, and micronaire to seed moisture fit positive exponential models (Figs. 3a, 3d, and 3e). However, lint strength and seed grade decreased exponentially with seed moisture (Figs. 3b and 3c). Lint color increased linearly with seed moisture (Fig. 3f). All the examined variables displayed highly significant relationships ( $P < 0.0001$ ) with high  $R^2 (> 0.90)$ , except for micronaire, which displayed a somewhat weaker relationship ( $P = 0.012$ ,  $R^2 = 0.62$ ).

**Lint Quality Measurements as Indicators of Aflatoxin Content.** Several lint quality measurements including color, spot, length, strength, and micronaire displayed potential predictive value for aflatoxin content (Table 3). Regression analyses for aflatoxin content as a function of each of the averaged cotton lint quality variables fit exponential models, except for fiber strength and micronaire, which best fit linear models (Fig. 4). All lint variables had significant relationships with aflatoxin ( $P < 0.001$ ) with high  $R^2 (> 0.84)$ . The 5 yr average seed grade also had a significant relationship with aflatoxin content fitting a quadratic linear model ( $P < 0.01$ ,  $R^2 = 0.901$ ) (Fig. 4c).

Table 4. Multiple regression models for cotton lint and seed variables from cotton grown in South Texas from 2002 to 2008<sup>2</sup>

| Variable (Y)  | Model equation <sup>3</sup>                      | R <sup>2</sup> | P>F     |
|---------------|--|----------------|---------|
| Lint color    | $Y = -5.1 + 0.01dh + 1.04lg - 0.03mst + 0.18psm$ | 0.844          | <0.0001 |
| Lint spot     | $Y = -2.8 + 0.01dh + 0.21lg - 0.02mst + 0.08psm$ | 0.767          | <0.0001 |
| Lint length   | $Y = 28.6 - 0.1dh + 0.14dg - 0.2psm$             | 0.759          | <0.0001 |
| Micronaire    | $Y = 40.8 - 4.7lg + 0.08dg$                      | 0.581          | <0.0001 |
| Lint strength | $Y = 46.9 - 0.02dh - 1.53lg + 0.09mst - 0.53psm$ | 0.619          | <0.0001 |
| Seed grade    | $Y = 293 - 0.52dh - 14.7lg + 1.13mst - 2.96psm$  | 0.813          | <0.0001 |
| Aflatoxin     | $Y = -413 + 61.8lg + 3.06mst + 19.2psm$          | 0.555          | <0.0001 |
| Log aflatoxin | $Y = -4.98 + 1.21lg + 0.06mst + 0.23psm$         | 0.579          | <0.0001 |
| Percent 20    | $Y = -1.87 + 0.3lg + 0.02mst + 0.1psm$           | 0.680          | <0.0001 |

<sup>2</sup> Multiple linear regression modeling was performed using the average value of all modules harvested on the same date.

<sup>3</sup> dh = day of harvest; dg = day ginned; mst = module storage time; lg = leaf grade; psm = percent seed moisture.

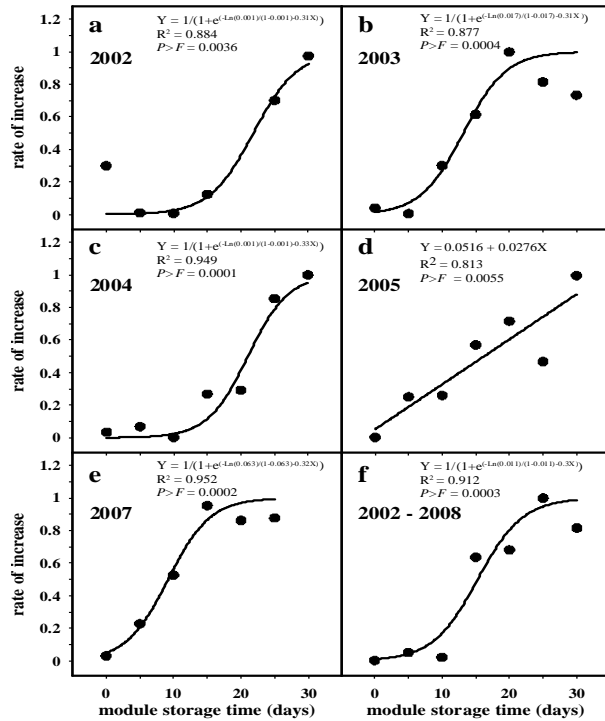


Figure 1. Effect of module storage time on the rate of increase of aflatoxin content (natural logarithm) in seed of cotton grown in South Texas for (a) 2002, (b) 2003, (c) 2004, (d) 2005, (e) 2007, and (f) combined 2002 to 2008. Note: In the 2006 and 2008 seasons no cotton was ginned more than 10 d after harvest, therefore 2006 and 2008 were not included.

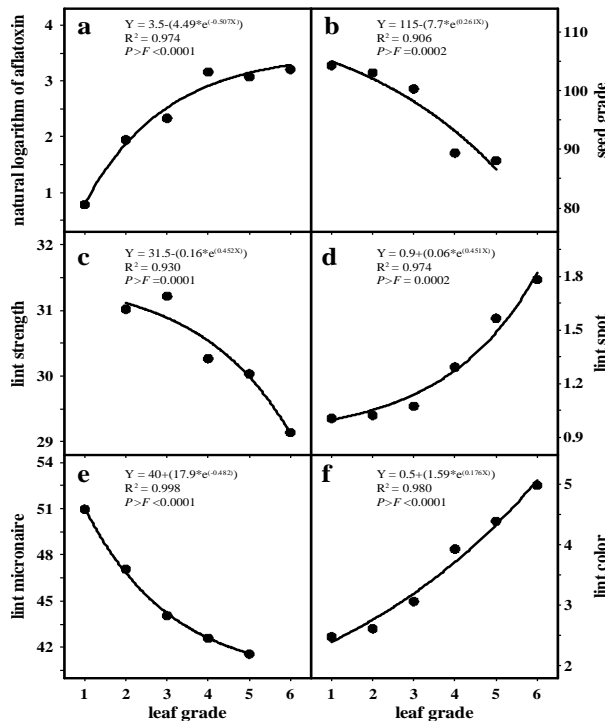


Figure 2. Effect of leaf grade on (a) natural logarithm of aflatoxin, (b) seed grade, (c) lint strength, (d) lint spot, (e) lint micronaire, and (f) lint color of cotton grown in the study region of South Texas during 2002 to 2008.

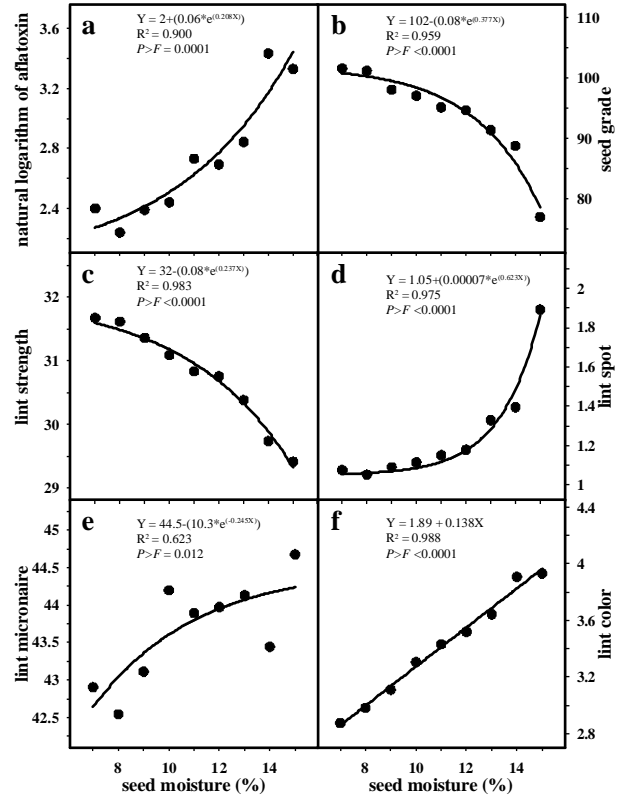


Figure 3. Effect of seed moisture on (a) natural logarithm of aflatoxin, (b) seed grade, (c) lint strength, (d) lint spot, (e) lint micronaire, and (f) lint color of cotton grown in the study region of South Texas during 2002 to 2008.

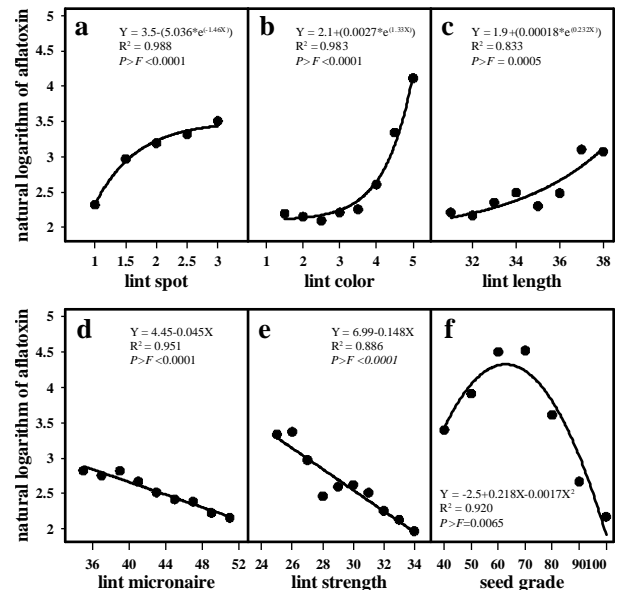


Figure 4. Relationship of aflatoxin content (natural logarithm) in seed to (a) lint color, (b) seed grade, (c) lint strength, (d) lint spot, (e) lint micronaire, and (f) lint length of cotton grown in the study region of South Texas during 2002 to 2008.

## DISCUSSION

Immediately following harvest, seed cotton is mechanically compressed into modules that are stored either along the sides of fields or in storage yards until the gin has capacity to separate the lint from the seed. During this initial storage period, the seed cotton remains associated with various unintended materials including leaves and twigs, and although modules are usually covered with tarps, the seed cotton is exposed to the environment and is vulnerable to deterioration. In the current study, the length of the module storage period is shown to have a positive relationship with cottonseed aflatoxin content. Thus, aflatoxin content increases as ginning is delayed. The influence of module storage time on aflatoxin is not linear (Fig. 1). The slow initial increases in aflatoxin concentrations might reflect periods required for fungal growth at the initiation of the second phase of aflatoxin contamination (Bock and Cotty, 1999; Cotty, 1991, 2001; Jaime-Garcia and Cotty, 2003; Russell, 1982; Russell et al., 1976). The second phase of contamination begins at crop maturation and continues until the seed is consumed. Rain on the mature crop is linked in South Texas to increased aflatoxin formation as a result of the second phase (Cotty and Jaime-Garcia, 2007; Jaime-Garcia and Cotty, 2003). In the current study, greater increases during module storage were seen during years with higher precipitation (e.g., 2007; Table 1, Fig. 1) confirming the importance of mature crop exposure to precipitation. Limiting cotton storage in modules to less than 1 wk is one way gins can minimize seed contamination. To increase the number of seed lots with acceptable aflatoxin content, seed from modules stored for greater than 1 wk should not be mixed with seed from recently harvested cotton. This is the first report of the influence of module storage time on aflatoxin contamination of cottonseed.

Influences of harvest date on cottonseed aflatoxin concentrations have been described for two areas with severe and frequent contamination, western Arizona and South Texas (Bock and Cotty, 1999; Cotty, 1991; Jaime-Garcia and Cotty, 2003). Results of the current study support association of both increased aflatoxin concentrations and fiber deterioration with delayed harvest. Prices of both cotton fiber and seed are dependent on quality (Wakelyn et al., 2007; Wu, 2004). Aflatoxin contamination limits both seed ac-

cess to markets and price (Cotty, 2001; Wu, 2004). Taken together, influences of delayed harvest on qualities of seed and lint can result in a crop with greatly reduce overall value. Complexities of interactions between yield and quality make it difficult to recommend precise harvest dates across large areas. Location and market specific cost benefit analyses will be necessary to determine optimal harvest dates for each production region with significant aflatoxin problems.

Variables examined in the current study account for only a portion of the variability in both fiber quality and aflatoxin content as shown by weak correlation coefficients (Table 3). Unexplained variability might be attributed to factors beyond the scope of the present study, including weather (rain, temperature, etc), field conditions, insects, diseases, cultivar (Cotton Program, 2001; Cotty and Jaime-Garcia, 2007; Jaime-Garcia and Cotty, 2003; Wakelyn et al., 2007), and, in the case of aflatoxin contamination, the structure of fungal populations (Jaime-Garcia and Cotty, 2006a, 2006b). However, regression analyses on averaged data (which account for only the variance attributed to the analyzed variable) indicate a clear influence of some of the variables including harvest date, gin date, module storage time, leaf grade, and seed moisture on both cotton quality variables and aflatoxin content (Table 4, Figs. 1- 3). Multiple regression analyses confirm previous reports indicating that timing of harvest influences aflatoxin contamination of cotton crops (Bock and Cotty, 1999; Jaime-Garcia and Cotty, 2003); thus harvest date was excluded from further analyses. Leaf grade and seed moisture are important components of cotton quality and affect the overall profit of the crop. In this study both measures were associated with both aflatoxin contamination and the quality of cotton fiber (Table 4). Leaf grade is directly correlated to the quantity of undesired plant material associated with seed cotton prior to ginning (Cotton Program, 2001; Wakelyn et al., 2007). Leaf grades reflect carryover of these crop contaminants through ginning and the associated cleaning. Pieces of leaves and stems increase the moisture content of seed cotton (Willcutt et al., 1997) and consequently might favor microbial growth and associated reductions in fiber quality and increases in aflatoxin content. Leaf and stem pieces also might provide nutrients for microbial activity and sources of *A. flavus* with increased inoculum potential. Seed grade and lint quality

variables, except for micronaire, deteriorate slowly in seed cotton with leaf grades up to 3 and more rapidly as leaf grades increase. Aflatoxin contamination increases rapidly as leaf grade increases up to grade 4, with slower increases at higher grades. This is the first report illustrating a relationship between leaf grade and aflatoxin content in cottonseed.

Individual regression analyses for seed moisture, leaf grade, and module storage time illustrate the importance of these variables in postharvest processes. Seed moisture affects both fiber quality and aflatoxin contamination (Fig. 3) with higher moisture associated with reduced fiber quality and increased aflatoxin contamination. All cotton fiber quality variables are correlated with seed moisture, but color deterioration, fiber strength, and seed grade are clearly the most influenced. Color is affected linearly and deteriorates gradually with increasing moisture, whereas spot and strength deteriorate slowly as moisture increases up to 12% and rapidly at moistures above 12%. Aflatoxin contamination follows a pattern similar to lint spot. This is consistent with previous observations that rain affects both fiber quality (Cotton Program, 2001) and aflatoxin concentration (Cotty and Jaime-Garcia, 2007; Jaime-Garcia and Cotty, 2003).

Regression analyses suggest that some fiber quality measurements might be useful indicators of seed lots with greater likelihood of being contaminated by aflatoxins. As such, surveys intended to detect contamination problems might increase efficiency through utilization of fiber quality measurements. Regression analyses indicate that all the fiber quality variables have some predictive value for aflatoxin content. However, color was the lint variable with the greatest predictive value as indicated by both model fit and the magnitude of aflatoxin increases in response to color change (Fig. 4). Fiber color can be affected by rainfall, freezes, exposure to sunlight, insects, and fungi, and by staining through contact with soil, grass, or cotton leaf fragments. Excessive moisture and temperature during storage both before and after ginning also can affect fiber color (Cotton Program, 2001; Curley et al., 1990; Wakelyn et al., 2007). Lint color grades do not reflect specific absorption spectra. Future research might provide the data necessary to quantitatively associate precise lint absorption spectra with aflatoxin contamination and, consequently increase the predictive value of lint color measurements.

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