

# CHARACTERIZATION OF *ASPERGILLUS FLAVUS*, AFLATOXIN, FUMONISIN IN CORN: EFFECTS OF COVER CROP, ROTATION, AND GLYPHOSATE



K.N. Reddy\*, H.K. Abbas, C.H. Koger, R.M. Zablotowicz, and C.A. Abel  
USDA-ARS: SWSRU, CGPRU, and SIMRU, Stoneville, Mississippi



## INTRODUCTION

Corn and cotton seed are frequently contaminated with aflatoxins and fumonisins produced by *Aspergillus* and *Fusarium* fungi, making them unfit for human and animal consumption. Mycotoxin contamination is influenced by the level of infestation by toxigenic fungi and environmental factors that stress crop plants. In 1998, drought and high temperatures in Mississippi contributed to significant levels of both aflatoxin and fumonisin in corn. Because of food safety concerns, it is critical to investigate cultural practices and crop management techniques that maximize crop yields and minimize inoculum potential of the causal fungi and mycotoxin production. Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway. Roundup Ready corn and cotton created by stable integration of a transgene that code for insensitive EPSPS are resistant to glyphosate. Fungi possess a sensitive EPSPS and are susceptible to glyphosate. These field studies examined the effects of simple agronomic practices on *Apergillus flavus* in soil and levels of aflatoxin and fumonisin in corn and cotton seed.

## OBJECTIVE

To study the effects of crop rotation, cover crop, and selection of conventional and Roundup Ready cultivars on *Apergillus flavus* in soil and levels of aflatoxin and fumonisin in corn and cotton seed.

## MATERIALS AND METHODS

### GENERAL CONDITIONS:

Location: USDA-ARS Southern Weed Science Research farm, Stoneville, MS.

Soil: Dundee silt loam.

Years: 2000, 2001, 2002, 2003, 2004.

Tillage:

\*1<sup>st</sup> year: previous fall – subsoil, disking, and bedding.

\* Subsequent years: just re-bedding after harvest.

Row spacing: 102 cm, 8 rows/plot.

Crops irrigated as needed.

Data: 1. *A. flavus* populations in soil determined using modified dichloronitroaniline rose bengal agar.  
2. Aflatoxin and fumonisin in seed determined using ELISA.

### 1. CORN-COTTON ROTATION STUDY:

Production practices: Table 1; Treatments: Table 4.

Plot size: 45.7 m long and 8.1 m wide.

Design: Randomized Complete Block Design with 4 replications.

### 2. COVER CROP STUDY IN ROUNDUP READY CORN

Cover crop: Hairy vetch and none. Killed at planting corn.

Variety: AG RX 738RR in 2002; DKC69-72RR in 2003.

Planting date: April 5, 2002 & April 1, 2003.

Plot size: 15.2 m long and 8.1 m wide.

Herbicides:

PRE: None.

POST: Glyphosate at 0.84 kg ae/ha at 3 and 5 WAP in a 38-cm wide band, broadcast, and no herbicide.

Design: Split-plot arrangement in RCBD with 4 replications.

Harvest date: August 22, 2002 & August 18, 2003.

### 3. COVER CROP STUDY IN NON-ROUNDUP READY CORN

Similar to Roundup Ready corn in Study 2.

Variety: Pioneer 3223 in both years.

Herbicides:

PRE: Atrazine 1.8 kg/ha + Metolachlor 1.4 kg/ha.

POST: Atrazine 1.0 kg/ha + Metolachlor 0.7 kg/ha + Carfentrazone 9 g/ha at 5 WAP in a 38-cm wide band, broadcast, and no herbicide.

Table 1. Production practices for cotton and corn rotation in 2000-2004.

| Practices                           | Cotton                             |  |   |   |  | Corn   |  |   |                              |   |  |
|-------------------------------------|------------------------------------|--|---|---|--|--|--|---|------------------------------|---|--|
|                                     | 2000                               | 2001   | 2002  | 2003  | 2004   | 2000   | 2001   | 2002  | 2003                         | 2004  |  |
| Variety: Conventional Roundup Ready | Stoneville 474 DP 436 RR           | Stoneville 474 DP 436 RR   | Stoneville 474 DP 436 RR                      | Stoneville 474 DP 436 RR  | Sure-Grow 747 DP 444 BG/RR   | Pioneer 3223 AG RX 738 RR  | Pioneer 3223 AG RX 740 RR                          | Pioneer 3223 AG RX 738 RR   | Pioneer 3223 DKC69-72 RR     | Pioneer 3223 DKC69-71 RR2   |  |
| Planting date                       | 11 May                             | 18 April   | 1 May   | 22 April  | 22 April   | 7 April  | 22 March   | 5 April   | 31 March                     | 24 March  |  |
| Herbicides: PRE:                    | Conventional Var Roundup Ready Var | Fluometuron 1.1 to 1.7 kg/ha + Pendimethalin 1.1 kg/ha<br>Fluometuron 1.1 to 1.7 kg/ha + Pendimethalin 1.1 kg/ha |   |   |  |  | Atrazine 1.8 kg/ha + Metolachlor 1.4 kg/ha<br>None |   |                              |   |  |
| EPOST (3-4 WAP)                     | Conventional Var Roundup Ready Var | Pyriithiobac 105 g/ha<br>Glyphosate 1.1 kg/ha  | Pyriithiobac 105 g/ha<br>Glyphosate 1.1 kg/ha | Pyriithiobac 105 g/ha<br>Glyphosate 1.1 kg/ha                   | Fluazifop 210 g/ha + Pyriithiobac 105 g/ha<br>Glyphosate 1.1 kg/ha | Pyriithiobac 105 g/ha<br>Glyphosate 1.1 kg/ha                    | None<br>Glyphosate 1.1 kg/ha                       | None<br>Glyphosate 1.1 kg/ha  | None<br>Glyphosate 1.1 kg/ha | None<br>Glyphosate 1.1 kg/ha  |  |
| LPOST (5-9 WAP)                     | Conventional Var Roundup Ready Var | Sethoxydim 0.28 kg/ha<br>Sethoxydim 0.28 kg/ha   | None<br>None                                  | Fluometuron 0.9 kg/ha + MSMA 2.24 kg/ha<br>Glyphosate 1.1 kg/ha | Fluometuron 0.9 kg/ha + MSMA 2.24 kg/ha<br>Glyphosate 1.1 kg/ha    | Fluometuron 0.9 kg/ha + MSMA 2.24 kg/ha<br>Glyphosate 1.18 kg/ha | None<br>None                                       | Atrazine 1.0 kg/ha + Metolachlor 0.7 kg/ha + Carfentrazone 9 g/ha<br>Glyphosate 1.1 kg/ha | None<br>Glyphosate 1.1 kg/ha | Atrazine 1.0 kg/ha + Metolachlor 0.7 kg/ha + Basagran 0.8 kg/ha<br>Glyphosate 1.1 kg/ha |  |
| Cultivation (Conv. & RR)            | None                               | Once   | Once  | None  | None   | None   | None   | None  | None                         | None  |  |
| Harvest date                        | 22 September                       | 20 September   | 23 September                                  | 19 September  | 23 September   | 14 August  | 16 August  | 28 August   | 18 August                    | 10 August   |  |

## RESULTS

Table 2. Aflatoxin and fumonisin in CORN grain as affected by Roundup Ready and Non-Roundup Ready cotton-corn rotation in 2002-2004.

| Cultivar          | Rotation   | Aflatoxin       |      |       | Fumonisin       |      |      |
|-------------------|------------|-----------------|------|-------|-----------------|------|------|
|                   |            | 2002            | 2003 | 2004  | 2002            | 2003 | 2004 |
|                   |            | ----- ppb ----- |      |       | ----- ppm ----- |      |      |
| Non Roundup Ready | Continuous | 0 a             | 1 a  | 0 b   | 2 a             | 16 a | 4 a  |
|                   | Rotation   | 1 a             | 1 a  | 0 b   | 2 a             | 15 a | 4 a  |
| Roundup Ready     | Continuous | 0 a             | 1 a  | 34 a  | 2 a             | 17a  | 1 b  |
|                   | Rotation   | 1 a             | 1 a  | 20 ab | 4 a             | 13 a | 1 b  |

Note: Means within a column followed by the same letter are not significantly different at the 5% level using Fisher's protected LSD test.

Table 3. Aflatoxin and fumonisin in COTTON seed as affected by Roundup Ready and Non-Roundup Ready cotton-corn rotation in 2002-2004.

| Cultivar          | Rotation   | Aflatoxin       |      |      | Fumonisin       |      |      |
|-------------------|------------|-----------------|------|------|-----------------|------|------|
|                   |            | 2002            | 2003 | 2004 | 2002            | 2003 | 2004 |
|                   |            | ----- ppb ----- |      |      | ----- ppm ----- |      |      |
| Non Roundup Ready | Continuous | 1 a             | 1 a  | 0 a  | 0 a             | 0 a  | 0 a  |
|                   | Rotation   | 1 a             | 2 a  | 1 a  | 0 a             | 0 a  | 0 a  |
| Roundup Ready     | Continuous | 0 a             | 1 a  | 2 a  | 0 a             | 0 a  | 0 a  |
|                   | Rotation   | 1 a             | 2 a  | 3 a  | 0 a             | 0 a  | 0 a  |

Note: Means within a column followed by the same letter are not significantly different at the 5% level using Fisher's protected LSD test.

Table 4. *Aspergillus flavus* populations in surface 5-cm soil at planting and harvest as affected by Roundup Ready and Non-Roundup Ready cotton-corn rotation in 2002-2004.

| Crop rotation       | 2002   |         |        | 2003  |       | 2004  |       |
|---------------------|--|---------|--------|-------|-------|-------|-------|
|                     | April  | June    | Sept   | March | Sept  | March | Sept  |
|                     | ----- Log (10) colony forming units/g soil ----- |         |        |       |       |       |       |
| Non-Roundup Ready   |  |         |        |       |       |       |       |
| Cot-Cot-Cot-Cot     | 2.5 a  | 2.8 a   | 2.6 d  | 2.6 a | 2.6 a | 2.0 b | 2.3 a |
| Corn-Corn-Corn-Corn | 2.4 a  | 2.7 ab  | 2.6 d  | 2.6 a | 2.5 a | 2.3 b | 2.5 a |
| Cot-Corn-Cot-Corn   | 2.3 a  | 2.6 abc | 2.6 d  | 2.5 a | 2.5 a | 2.2 b | 2.6 a |
| Corn-Cot-Corn-Cot   | 2.5 a  | 2.7 ab  | 2.8 c  | 2.7 a | 2.6 a | 2.2 b | 2.6 a |
| Roundup Ready       |  |         |        |       |       |       |       |
| Cot-Cot-Cot-Cot     | na   | 2.8 a   | 3.2 a  | 2.6 a | 2.5 a | 2.0 b | 2.4 a |
| Corn-Corn-Corn-Corn | na   | 2.7 ab  | 3.0 b  | 2.5 a | 2.4 a | 2.8 a | 2.8 a |
| Cot-Corn-Cot-Corn   | na   | 2.4 c   | 3.2 a  | 2.5 a | 2.5 a | 2.3 b | 2.5 a |
| Corn-Cot-Corn-Cot   | na   | 2.5 bc  | 3.1 ab | 2.6 a | 2.5 a | 2.3 b | 2.5 a |

Note: Means within a column followed by the same letter are not significantly different at the 5% level using Fisher's protected LSD test.

## CONCLUSIONS

- A. flavus* populations in surface 5-cm soil before planting and harvest ranged from 2.0 to 3.2 log (10) cfu/g soil with no clear trend among eight rotation systems in 2002-2004, although *Aspergillus* populations were significantly greater in plots with Roundup Ready cultivars compared to conventional cultivars on several sampling dates.
- In cotton seed, aflatoxin and fumonisin levels were (<3 ppb) similar regardless of rotation and glyphosate.
- In corn grain, aflatoxin was above the regulatory level ( $\geq 20$  ppb) only in Roundup Ready cultivar in 2004. Fumonisin was higher in conventional cultivar (4 ppm) regardless of rotation in 2004. However, in 2002 and 2003, the mycotoxins levels were similar regardless of rotation and glyphosate.
- In corn grain from cover crop study, aflatoxin and fumonisin levels were very low and unaffected by either hairy vetch cover crop or glyphosate (data not shown).
- These results indicate the potential for increased aflatoxin levels (1 of 3 years) in corn and a stimulation of *A. flavus* populations in a Roundup Ready cropping system under climatic conditions encountered in Mississippi.

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