Frequency of Colonization of Corn Kernels by Atoxigenic *Aspergillus flavus* Applied as a Biocontrol Agent

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ABSTRACT

Aflatoxin contamination of corn is a chronic problem among Southern states. Others have demonstrated the efficacy of using atoxigenic isolates of this fungus to lessen aflatoxin contamination of cotton by "competitive exclusion" of toxigenic strains from cotton field soils. This decreases the inoculum potential of toxigenic strains, presumably allowing the atoxigenic strains to out compete the toxigenic strains at the infection court. To successfully use this approach with corn, it is necessary to demonstrate that the biocontrol isolate can colonize corn kernels. A proprietary *A. flavus* isolate (Circle One Global, Inc., Shellman, Ga), fixed to barley seed, was distributed atop soil between 16 corn rows at the rate of 20 lbs/A on June 4, 2003. The plot was combine-harvested on September 4, and kernels fom each row were bagged separately. Randomly collected sub samples (200 kernels/row) were plated on AFPA selective medium. *A. flavus* was recovered from approximately 50% of the sampled kernels. Single NIT mutants of the isolates were selected and paired with *cnx* and *nirA* mutants of the biocontrol fungus which is VCG 24, one not previously recovered in Louisiana. Approximately 60% of the kernels colonized by *A. flavus*, or 30% of the kernels sampled, were identified as VCG 24 and presumed to be the biocontrol isolate originally applied between the rows.

INTRODUCTION

The use of atoxigenic *A. flavus* for biocontrol of aflatoxin contamination in cotton was developed during the 1990's by Peter Cotty for the Arizona agro-ecosystem (3).

Robert Brown, also of the USDA-ARS-SRRC in New Orleans, performed pre-harvest co-inoculation experiments with atoxigenic and toxigenic isolates directly into corn ears. Results indicated significant reduction in aflatoxin contamination (2)

Interest in using the same approach on peanuts in Georgia resulted in selection of an atoxigenic *A. flavus* NRRL 21882 by Joe Dorner and associates at the National Peanut Lab in Dawson, GA. They showed that the isolate was effective in reducing aflatoxin contamination on peanuts and corn when the biocontrol isolate was applied to soil on fungal infested rice over a four year period (5).

The present work was intended to determine the ability of the biocontrol agent to colonize developing corn kernels during and after the critical window of infection, which is approximately 20 days after mid-silk.

MATERIALS AND METHODS

Syngenta corn hybrid N 83-N5 was planted April 1, 2003, at the LSU AgCenter Ben Hur Research Station, Baton Rouge. The biocontrol agent known as Afla-Guard (http://www.circleoneglobal.com) was applied between the rows with a calibrated walk behind seed planter just prior to silking. Post-harvest kernel samples from each 200 foot row were surface sterilized and plated on AFPA selective medium (1,7). Single isolates were obtained from individual kernels and single Nit mutants were obtained for each isolate using the protocol of Ken Papa (6). The biocontrol isolate (VCG 24) was also subjected to Nit selection and *nirA* and *cnx* mutants were characterized. The sample isolate mutants were paired with the *nirA* and *cnx* biocontrol mutants on starch medium (4) to determine homology. The rationale was that the preponderance of mutants would be *niaD* which is known to be most frequent. Those isolates not VCG24 were tested for aflatoxin production using the plate assay of Saito & Machida (8).

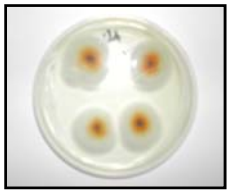

Figure 1. AFPA selective medium for *Aspergillus* isolation from corn kernels showing the positive orange coloration. Some isolates of *Aspergillus* from corn fail to give this reaction, however they can be identified by their wrinkled white growth when viewed from the bottom of the plate.

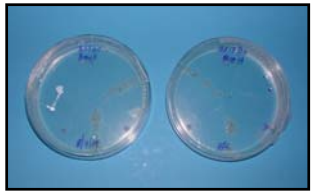

Figure 2. Nit mutant plates for determination of VCG 24. VCG 24 *cnx* and *nirA* mutants at the bottom of the plate showing positive control complementation. The unknown field isolate mutant is at the top. Complementation with either of the bottom mutants confirms the unknown is VCG 24 and the type of nit mutant which it is.

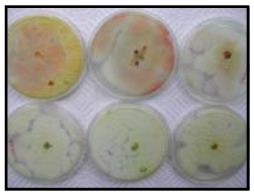

Figure 3. Ammonia vapor red-pink reaction of aflatoxin producing isolates in the top row. Yellow-brown lack of reaction in the bottom row of non-toxin producing isolates.

Table 1. Experiment Summary

Total kernels plated: 3200	
Aspergillus isolated: 1626/3200, 50.8%	
Typical: 1290/1626, 79.3%;	
Wrinkled white: 336/1626, 20.7%	
Isolates lost: 298/1626, 18.3%	
Isolates remaining: 1328	
Number VCG 24: 824/1328, 62%	
Number not VCG 24: 504/1328, 38%	
Number toxigenic: 87/504, 17.3%	

RESULTS

The selective AFPA medium produces an orange color specific to *A. flavus* or *A. parasiticus* when viewed from the reverse side of the plate (Fig. 1). This medium does not support sporulation. It was noted that many of the kernels produced white mycelium characteristic of *Fusarium verticilliodes*, an endophyte of corn, and these did not show color. However, some of the white mycelial growth appeared wrinkled when viewed in reverse. When these wrinkled white areas were plated on PDA containing hygromycin, typical *A. flavus* colonies developed. *A. flavus* is insensitive to hygromycin while *Fusarium* is inhibited. Thus it appeared that AFPA can produce false negatives when corn kernels contain mixed cultures of *A. flavus* with other fungi.

Figure 2 depicts a homologous reaction of a sample isolate nit mutant with the VCG 24 *cnx* and *nirA* tester isolates.

Figure 3 depicts the positive (red-pink) and negative reaction (yellow-brown) characteristic of the Saito & Machida ammonia vapor test for aflatoxin production.

A previous study in our lab indicated the validity of the ammonia test by comparing 53 *A. flavus* isolates from growers fields in a number of aflatoxin assays including TLC, HPLC, and cyclodextrin enhanced UV-fluorescence plate assays. In that study 9/53 (17%) were toxigenic.

The frequencies of colonization, VCG 24 identity and toxigenicity of non VCG 24 isolates from the biocontrol plot are indicated in Table 1.

CONCLUSIONS

The AFPA medium is subject to false negatives.

Approximately 50% of kernels in the test were colonized by *A. flavus*.

Approximately 60% of the colonized kernels were VCG 24, the same as the atoxigenic biocontrol agent applied between the rows.

Those kernels colonized by non VCG 24 isolates were predominantly atoxigenic as well, 82.7%. This corresponds with results from growers fields in which only 17% of the isolates were toxigenic. It appears that nature is already performing atoxigenic biocontrol.

LITERATURE

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