EVALUATION OF ATOXIGENIC STRAINS OF *ASPERGILLUS FLAVUS* FOR AFLATOXIN CONTROL IN CORN ON COMMERCIAL FARMS IN TEXAS - 2015

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Summary

Afla-Guard, a commercial product labeled for aflatoxin control on corn, was evaluated for its effectiveness to reduce aflatoxin contamination in corn in three replicated, randomized experiments on non-irrigated farms in different corn production areas in Texas. Like AF-36, the other commercial product available for aflatoxin control in Texas, Afla-Guard consists of a strain of *Aspergillus flavus* that does not produce aflatoxin (i.e. atoxigenic strain) and they prevent aflatoxin production by out-competing native, toxin-producing strains for space to colonize in corn kernels. The experimental replicates on farms (6, 9 or 12 rows by 100 feet long) were small enough to allow precise application of the atoxigenic strains by hand, but large enough to harvest with the grower's combine, and were separated by a distance of 100 feet. All locations received above-average rainfall early in the season and some rain after the application of the atoxigenic strain. The average aflatoxin levels for all treatments at the three locations ranged from 0 to 12 parts per billion (ppb), which is below the action threshold of 20 ppb for food or dairy feed. Average levels of fumonisin at these locations ranged from 0.3 to 6.9 parts per million (ppm), which was below or slightly above the action threshold of 5 ppm. The levels of aflatoxin contamination at these three locations were too low to receive a benefit from the application of an atoxigenic strain.

Objective

The objective of these experiments was to evaluate Afla-Guard to control aflatoxin in corn in replicated, randomized experiments in commercial fields in different corn production areas of Texas (Fig. 1).

Fig. 1. Locations of the experiments (counties shown in green).

At the Hill county location, an additional objective was to compare a V5 to V6 application with the recommended, later application timing, R1.

Materials and Methods

Experimental Design: Each treatment was replicated four times in a randomized complete block design. The replicates consisted of 12, 9 and 6 rows at the Hill county, Jackson county and Fort Bend county locations, respectively and replicate rows were 100 feet long. Replicates were separated from each other by a distance of 100 ft. In all experiments, the atoxigenic strains were applied at 10 lb/A by hand to the tops of rows. Details of the locations are given in Table 1.

Table 1. Experimental details for the three locations.

Location	Hybrid	Row spacing (inches)	Afla-Guard Applied	Harvest date
Hill county	DK 62-08	30	5/20/15 (V5-V6) or 6/6/15 (R1)	8/13/15
Fort Bend county		40	5/19/14 (V8-VT)	8/1/15
Jackson county	Pioneer 1395	40	5/1/15 (V10)	7/13/15

The replicates were harvested with the grower's combine. Samples were obtained by holding a bucket over the auger that moves the corn from the concave to the combine's grain bin (Fig.2).

Fig. 2. Sampling corn for aflatoxin analysis in the experiments. The bucket is held under the auger as the combine moves through the plot so that only 10-11 lb. from a plot is sampled.

To reduce the possibility of cross-contamination, incoming grain was not collected for the first 30 seconds. Thereafter, only a portion of the harvest was continuously collected, allowing for sampling of the whole replicate (i.e. stream sampling). The amount of corn collected per plot ranged from 10-11 lb. Prior to grinding with a Romer mill, the samples were split in half with a Boerner divider. Total aflatoxin was quantified from 50-g subsamples using the Vicam Aflatest USDA FGIS procedure. Total fumonisin was quantified using the Envirologix QuickScan system.

With sub-samples of the harvest, the proportion of intact corn kernels colonized by *A. flavus* was determined as follows. Kernels were surface-disinfested in 10% bleach for two min, rinsed twice with sterile water and incubated 4 days on moist, sterile paper towels in 8 in.× 8 in. aluminum trays sealed in Zip-loc plastic bags. One hundred kernels were evaluated for each replicate. Toxigenicity of *A. flavus* isolates growing on kernels was determined by inoculating them onto ½ strength potato dextrose agar (PDA) plates, incubating plates for 11 days, and adding 10 ml 100% methanol to plates. After an overnight incubation, the methanol was poured into a small cup, an additional 5 ml 100% methanol was added, plus 20 ml distilled water, and 1 ml of this mixture was placed on an

Aflatest column and the Vicam Aflatest USDA FGIS procedure was followed from this point. Plates of a toxigenic strain (NRRL 3357) and two atoxigenic strains were used as controls.

Results

The average levels of aflatoxin at all three locations were below the action threshold level of 20 ppb. The highest levels of aflatoxin were detected at the Hill county location, in two replicates of the early (V5 to V6) Afla-Guard treatment (Table 2). The control and the later application timing had no or trace aflatoxin. There was no or trace aflatoxin in Fort Bend county treatments (Table 3) and no aflatoxin in the Jackson county treatments (Table 4). Fumonisin was also at the highest levels in Hill county (Table 2), but these levels were either below or slightly above the action threshold of 5 ppm. Fumonisin was very low in Fort Bend county (Table 3) and Jackson county (Table 4).

Colonization of intact kernels by *A. flavus* in the Afla-Guard treatments ranged from 1 to 7.5% at the locations and were usually higher than the controls, which ranged from 1.6 to 2% (Tables 2,3,4). The frequency of toxigenic isolates in the Afla-Guard treatments was zero, while toxigenic isolates were detected in the controls, although at low levels, ranging from 0 to 25% (Tables 2,3,4).

At Fort Bend county, on August 1, 50 ears were sampled from each plot and evaluated for visible ear rot caused by A. flavus and isolations from these areas were made onto PDA. After two months, the isolates on the plates were evaluated for toxigenicity as described above. Visible ear rot in the Afla-Guard treated plots ranged from 4% to 14% (average: 9%). Only one of the four control plots had ear rot (2% incidence). None of the ear isolates were toxigenic.

Table 2. Effect of Afla-Guard applied at different times on aflatoxin and fumonisin, Schronk Farm, Hill County, TX.

Treatment	Aflatoxin (PPB)*	Range of Aflatoxin (PPB)	Fumonisin (PPM)*	Intact kernels colonized by *A. flavus* (%)*	Kernel incidence of toxigenic *A. flavus* (%)*
Afla-Guard on 5/20/15 (V5-V6)	12	0 - 33	4.2 (±0.9)	5	0 (*n*=23)
Afla-Guard on 6/6/15 (R1)	0.25	0 - 1	4.2 (±1.3)	5.3	0 (*n*=34)
Control	0	0	6.9 (±1.8)	2	11 (*n*=9)

*Mean of four replicates. Standard deviations, ±, or number of isolates tested, *n*, in parentheses.

Table 3. Effect of Afla-Guard on aflatoxin and fumonisin, Poehls Farm, Fort Bend County, TX.

Treatment	Aflatoxin (PPB)*	Range of Aflatoxin (PPB)	Fumonisin (PPM)*	Intact kernels colonized by *A. flavus* (%)*	Kernel incidence of toxigenic *A. flavus* (%)*
Afla-Guard on 5/19/14 (V8-VT)	0	0	0.3	1	0 (*n*=6)
Control	0.25	0-1	0.6	1.6	0 (*n*=4)

*Mean of four replicates. Number of isolates tested, *n*, in parentheses.

Table 4. Effect of Afla-Guard on aflatoxin and fumonisin, Stuhrenberg Farm, Jackson county, TX.

	Aflatoxin in (PPM)*		Intact kernels colonized by *A. flavus* (%)**	Kernel incidence of toxigenic *A. flavus* (%)*
Afla-Guard on 5/1/15 (V10)	0	1.1	7.5	0 (*n*=30)
Control	0	1.6	2	25 (*n*=8)

*Mean of four replicates. Number of isolates tested, *n*, in parentheses.

Discussion

The low levels of aflatoxin in the controls at all three locations suggest that environmental conditions were not conducive to aflatoxin development during the 2015 season. Thus, these replicated experiments conducted on non-irrigated farms did not demonstrate any benefit of applying an atoxigenic strain. In 2011, a drought year in Texas, using the same experimental approach, an economical reduction in aflatoxin was seen in one out of four fields with the application of an atoxigenic strain.

There were higher levels of colonization by *A. flavus* in harvested, non-symptomatic corn kernels from Afla-Guard-treated plots in Jackson county, as compared with the control, and all of the isolates from the Afla-Guard-treated plots were atoxigenic. In a 2009 study, there was a higher incidence of visible *A. flavus* on ears of drought-stressed corn treated with an atoxigenic strain and most of these isolates were atoxigenic (T. Isakeit *et al*., Can. J. Plant Pathol., 32:407-408, 2010, Abstract). Monitoring *A. flavus* colonization of harvested kernels can provide additional information on the effectiveness of atoxigenic strain treatment, as well as its movement within the field.

This research shows that it is possible to measure the effects of atoxigenic strains using plot sizes that are large enough to harvest with the grower's combine, but small enough to treat by hand. Treating by hand allows for precise placement of the atoxigenic formulations. The 100-ft separation of replicates is large enough to minimize cross-contamination, although, based on the finding of atoxigenic isolates in the controls, this should be verified in future experiments. Previous studies have shown a gradient of movement which is negligible at 30-42 ft. from a point source (Olanya *et al*., Plant Disease 81:576, 1997; B. Hassett, unpublished). Yet, the separation of plots is small enough to have replicates close enough to minimize variability in aflatoxin indirectly affected by variations in soil type, fertility, or drainage. With our experimental approach, it is possible to evaluate timing and dosage of atoxigenic strains in experimental designs that will take into account the variation of aflatoxin levels that occur naturally within fields. With experiments done over several years, we anticipate generating information that will allow growers in different areas of Texas to have an understanding of when they will benefit from an atoxigenic treatment.

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