EVALUATION OF ATOXIGENIC STRAINS OF *ASPERGILLLUS FLAVUS* FOR AFLATOXIN CONTROL IN CORN ON COMMERCIAL FARMS IN TEXAS - 2011

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Summary

Two products, Afla-Guard and AF 36, are labeled for aflatoxin control on corn in Texas. Both consist of strains of *Aspergillus flavus* that do not produce aflatoxin (i.e. they are atoxigenic) and they prevent aflatoxin production by out-competing native, toxin-producing strains for space during the colonization of developing corn seed during the growing season. These products were evaluated for their effectiveness to reduce aflatoxin contamination in corn in four replicated, randomized experiments on non-irrigated farms in different corn production areas of Texas. The experimental replicates (8 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. Rainfall was substantially below normal during the growing season, providing sub-optimal conditions for activation of these products. At the Ellis county farm, Afla-Guard treatment significantly (P=0.05) reduced the average aflatoxin levels to 37% of the control, which was 340 parts per billion (ppb). At the Hill county farm, aflatoxin levels with the AF 36 and Afla-Guard treatments (including an Afla-Guard treatment at V5-V6) were 35-42% of the control, which was 161 ppb. However, this reduction was not uniform among replicates, nor was it statistically significant (P=0.05). On farms in Colorado and Nueces counties, the levels of aflatoxin in the untreated portions were probably not high enough (4 and 31 ppb, respectively) to economically justify treatment, particularly in the Nueces county field that yielded 40 bu/A. At the Nueces county farm, aflatoxin was significantly (P=0.05) reduced with Afla-Guard, but not AF 36. The proportions of harvested seed colonized by *A. flavus* following atoxigenic strain treatments in the experiments ranged from 1-13%, which were 2.5× to 4× higher than that of the controls. Our experimental approach can be used to evaluate timing of application of atoxigenic strains or other factors that can affect aflatoxin management.

Objective

The objective of these experiments was to evaluate two products, AF 36 and Afla-Guard, to control aflatoxin in corn in replicated, randomized experiments in commercial fields in different corn production areas of Texas (Fig. 2). The specific objectives were: (1) to compare an application earlier than V7 with the recommended application timing, V7 to R1; (2) to compare the effectiveness of AF 36 and Afla-Guard in the same field.

Materials and Methods

Experimental Design: Each treatment was replicated four times in a randomized complete block design and each replicate consisted of 8, 100-ft rows. Replicates were separated from each other by a distance of 100 ft. The specific treatments are listed in the sections for each county. In all experiments, the atoxigenic strains were applied at 10 lb/A by hand to the tops of rows.

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. 1). 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 11-18 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.

After 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. Two hundred kernels were evaluated for each replicate.

Fig. 1. 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 11-18 lb. of a plot is sampled.

Nueces county: The experiment consisted of the following treatments: AF 36 applied on Mar. 30, when corn was "knee high" (V4-V5); AF 36 applied on Apr. 20, when corn was at V10; Afla-Guard applied on Apr. 20; and a control.

The hybrid 'Pioneer 33F85' was planted Feb. 22 on a Victoria clay (fine, montmorillonitic, hyperthermic, Udic Pellusterts) at a population of 18,046 plants per acre, using a 38 in. row spacing. The fertilizer applied was 300 lb/A 25-5-0 and 1 qt/A Roundup was used for weed control. Corn was at VT on Apr. 25. Rain occurred in Feb. (0.02 in.), Mar. (0.59 in.), May (2.97 in.), and Jun. (0.56 in.). Details of weather conditions, from the second atoxigenic application to harvest, are shown in Fig. 3. On Jul. 8, the treatments were harvested, but only 6 of the 8 rows were harvested using the grower's 6-row combine. The grower's yield for this field was 45 bu/A with a level of 15 ppb aflatoxin.

Hill county: The experiment consisted of the following treatments: Afla-Guard applied on Apr. 26, when corn was "knee high" (V5-V6); Afla-Guard applied on May 10, when the corn was at V9-V10; AF 36 applied on May 10; and a control.

The hybrids ('DK 69-43' and 'DK 69-40') were planted Mar. 19 in Houston black clay (fine, montmorillonitic, thermic Udic Pellusterts) at a seeding rate of 22,900/A and a 30 in. row spacing. The fertilizers applied were 120 lb/A NH_3 and 7 gal/A 11-37-0. The herbicide used was Roundup. Rain occurred May 2 (0.8 in.), May 11 (1.4 in.), May 20 (0.06 in.), May 21 (trace), and Jun. 21 (0.5 in.). Details of weather conditions, from the second atoxigenic application to harvest, are shown in Fig. 4. On Jul. 20, the treatments were harvested. The grower's yield in this field was 30 bu/A.

Colorado county: The experiment consisted of the following treatments: AF 36 applied May 5, when corn was 50% was either at VT or R1; Afla-Guard applied May 5; and a control.

The hybrid 'DK66-05' was planted March 26 in Mohat loam (coarse-silty, mixed, superactive, calcareous, hyperthermic Typic Udifluvents), using a 36 in. row spacing. Details of weather conditions, from the application of the atoxigenics to harvest, are shown in Fig. 5. The field was harvested Jul. 22. The grower's yield in this field was 89 bu/A.

Ellis county: The experiment consisted of Afla-Guard applied on May 10, when corn was at V6-V9, and a control.

The hybrids ('DK69-40' and 'P1498 HR') were planted Mar. 10 in Burleson clay (Fine, montmorillonitic, thermic Udic Pellusterts), using a 36" row spacing. The treatments were harvested Jul. 29. The grower's yield was 40 bu/A.

Results

Nueces county: The average level of aflatoxin in the control plots was 31 ppb (range: 22-50 ppb) (Table 1). The average level of aflatoxin with the Afla-Guard treatment at V10 growth stage was 2 ppb (range: 0-4.5 ppb), which was significantly less (P=0.05) than that of the control. This is a reduction to 6% of the control. In contrast, neither of the AF 36 treatments significantly reduced aflatoxin levels in comparison with the control (Table 1).

Table 1. Comparison of aflatoxin among treatments, Mayo Farm, Nueces county, Robstown, TX.

Treatment	Aflatoxin (PPB)*	Range of Aflatoxin (PPB)	% Colonization of kernels by *A. flavus**
AF 36 on 3/30/11 (V4-V5)	27 a	5 - 67	7 a
AF 36 on 4/20/11 (V10)	30 a	1 - 65	10 a
Afla-Guard on 4/20/11 (V10)	2 b	0 - 4	7 a
Control	31 a	22 - 50	2 b

*Mean of four replicates. Log-transformed aflatoxin data was analyzed. Numbers within a column followed by different letters are significantly (P=0.05) different using Fisher's protected LSD.

The levels of harvested kernels colonized by *A. flavus* ranged from 7-10% with the atoxigenic strain treatments, which was significantly (P=0.05) greater than that of the control, 2% (Table 1).

Hill county: The mean aflatoxin levels were 35-42% of the control with AF 36 and Afla-Guard treatments (Table 2). However, because of the variability among replicates within treatments, these differences were not statistically significant (P=0.05) using an analysis of variance. Friedman's test, a nonparametric ranking test, also did not show any statistical difference (χ_r^2=3.3, 3 df).

The proportion of harvested kernels colonized by A. flavus ranged from 9-13% with the Afla-Guard strain treatments, which was significantly (P=0.05) greater than that of the control, 3% (Table 2). Kernels from the AF 36 treatment had a higher proportion of colonization (6%) than that of the control, but this difference was not statistically significant (*P*=0.05).

Table 2. Comparison of aflatoxin among treatments, Hejl Farm, Hill county, Hillsboro, TX.

Treatment	Aflatoxin (PPB)*	Range of Aflatoxin (PPB)	% Colonization of kernels by *A. flavus**
Afla-Guard on 4/26/11 (V5-V6)	60 a	7 - 140	13 a
Afla-Guard on 5/10/11 (V9-V10)	67 a	6 - 120	9 ab
AF 36 on 5/10/11 (V9-V10)	56 a	34 - 96	6 bc
Control	161 a	64 - 270	3 c

*Mean of four replicates. Log-transformed aflatoxin data was analyzed. Numbers within a column followed by different letters are significantly (*P*=0.05) different using Fisher's protected LSD.

Colorado county: The levels of aflatoxin in the treatments and the control were all very low; the highest level was 12 ppb in one replicate (Table 3). The proportion of harvested kernels colonized by A. flavus was low at this site compared with the other three sites in the study, but there was a significantly (*P*=0.05) higher level of *A. flavus* colonization with the Afla-Guard treatment than the AF 36 or control (Table 3).

Table 3. Comparison of aflatoxin among treatments, Mahalitc Farm, Colorado county, Eldridge, TX.

Treatment	Aflatoxin (PPB)*	Range of Aflatoxin (PPB)	% Colonization of kernels by *A. flavus**
Afla-Guard on 5/5/11 (VT-R1)	0 a	0	3 a
AF 36 on 5/5/11 (VT-R1)	0 a	0	1 b
Control	4 a	0 - 12	1 b

*Mean of four replicates. Numbers within a column followed by different letters are significantly (*P*=0.05) different using Fisher's protected LSD.

Ellis county: The Afla-Guard treatment applied at V6-V9 significantly (*P*=0.05) reduced aflatoxin to 126 ppb, which was 37% of the control, 340 ppb (Table 4). The proportion of harvested kernels colonized by *A. flavus* was significantly (*P*=0.05) higher with the Afla-Guard treatment, as compared with the control.

Table 4. Comparison of aflatoxin between treatments, Wilson Farm, Ellis county, Avalon, TX.

Treatment	Aflatoxin (PPB)*	Range of Aflatoxin (PPB)	% Colonization of kernels by *A. flavus**
Afla-Guard on 5/10/11 (V6-V9)	126 a	86 - 150	10 a
Control	340 b	180 - 630	4 a

*Mean of four replicates. Log-transformed aflatoxin data was analyzed. Numbers within a column followed by different letters are significantly (*P*=0.05) different using an analysis of variance.

Discussion

The replicated experiments conducted on non-irrigated farms showed that the benefits of applying atoxigenic strains under the conditions of the extreme drought of 2011 were not consistent. On two of the farms, in Nueces and Ellis counties, the application of Afla-Guard significantly (P=0.05) reduced aflatoxin contamination, in comparison with the controls. At the Nueces county farm, the level of aflatoxin in the control was relatively low and unless the corn was intended for food or dairy feed, it is questionable whether there would have been an economic benefit from application of an atoxigenic strain. At the Hill county location, there were reductions in aflatoxin with Afla-Guard and AF 36 treatments that were not statistically different from the control. At the Colorado county location, the level of aflatoxin the control was too low to warrant application of atoxigenic strains. So, out of the four experiments, just the one in Ellis county showed a clear benefit in applying an atoxigenic strain.

One of our hypotheses was that the two atoxigenic strains have similar activity. In the Nueces county experiment, there was a significant reduction in aflatoxin with Afla-Guard, but not AF 36. In contrast, in Hill county, the trend of reduction with AF 36 was similar to that of the Afla-Guard treatment. The reason for this discrepancy is not known. Based on preliminary experiments showing differences in sporulation of the two strains over different relative humidities (B. Hassett, unpublished), our hypothesis to explain this discrepancy is that the atoxigenic formulations may differ in their ability to sporulate under extremely dry conditions. The experiments done to date are insufficient to know whether the strains will have similar activity; more experiments are needed.

We also hypothesized that an early application (i.e. earlier than V9) would be advantageous in a drought year, as the material may have more opportunity to sporulate, especially following an early-season rain. For example, with the Hill county experiment, the Afla-Guard applied V5-V6 was exposed to one more rain shower than Afla-guard applied at V9-V10. In lab tests, both atoxigenic strains sporulate, but not profusely, between 84% and 100% relative humidity (B. Hassett, unpublished). Such conditions occurred for 5-10 hr on almost a daily basis at the Nueces county location (Fig. 3). A longer exposure to conditions favoring sporulation will allow for more spore production. Additional experiments are needed to determine optimal timing.

There were significantly higher levels of colonization by *A. flavus* in harvested, non-symptomatic corn kernels from atoxigenic-treated plots, as compared with the control. However, no further testing was done to determine toxigenicity of the *A. flavus* colonies. A 2009 study found 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.

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. 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 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.

Acknowledgements

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Fig. 2. Locations of the experiments. Counties indicated by first initial.

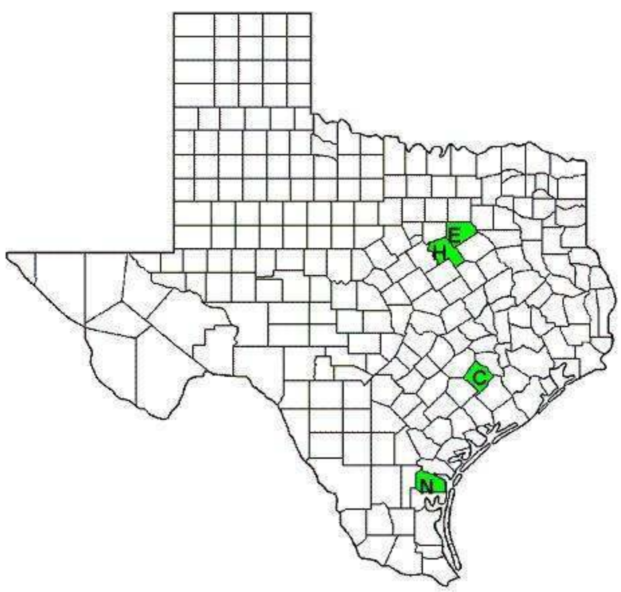


Fig. 3. Daily weather conditions during the experiment in Nueces county. Green bars indicate the number of hours per day that the relative humidity exceeds 84%.

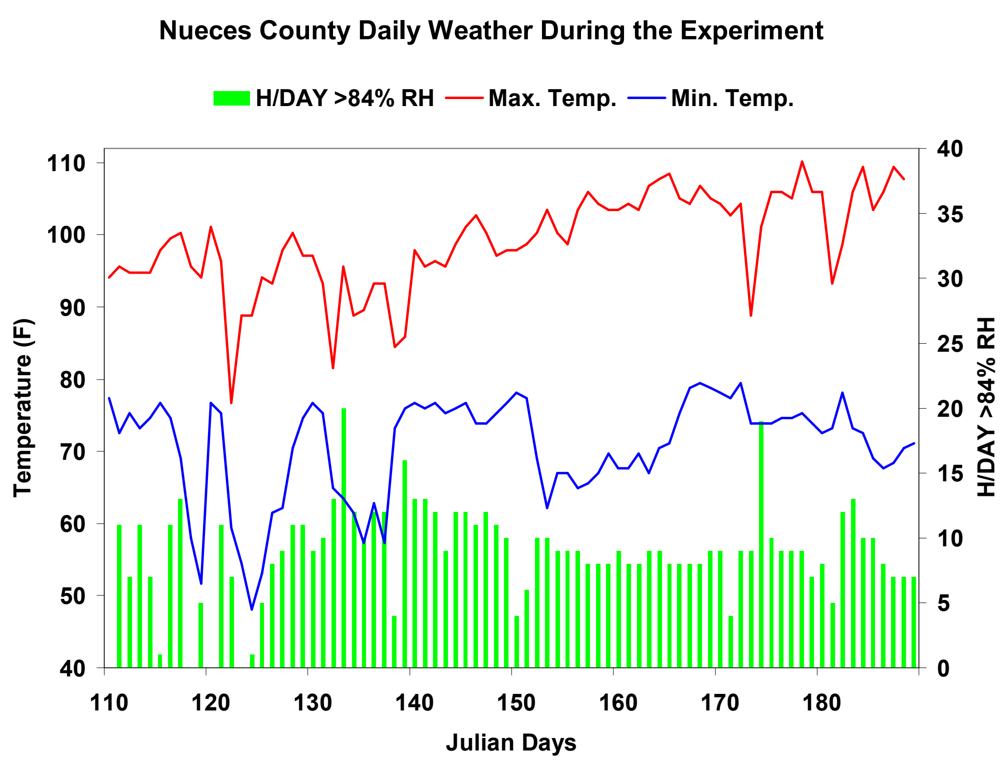


Fig. 4. Daily weather conditions during the experiment in Hill county. Green bars indicate the number of hours per day that leaf wetness exceeds 10, on a scale of 0-14.

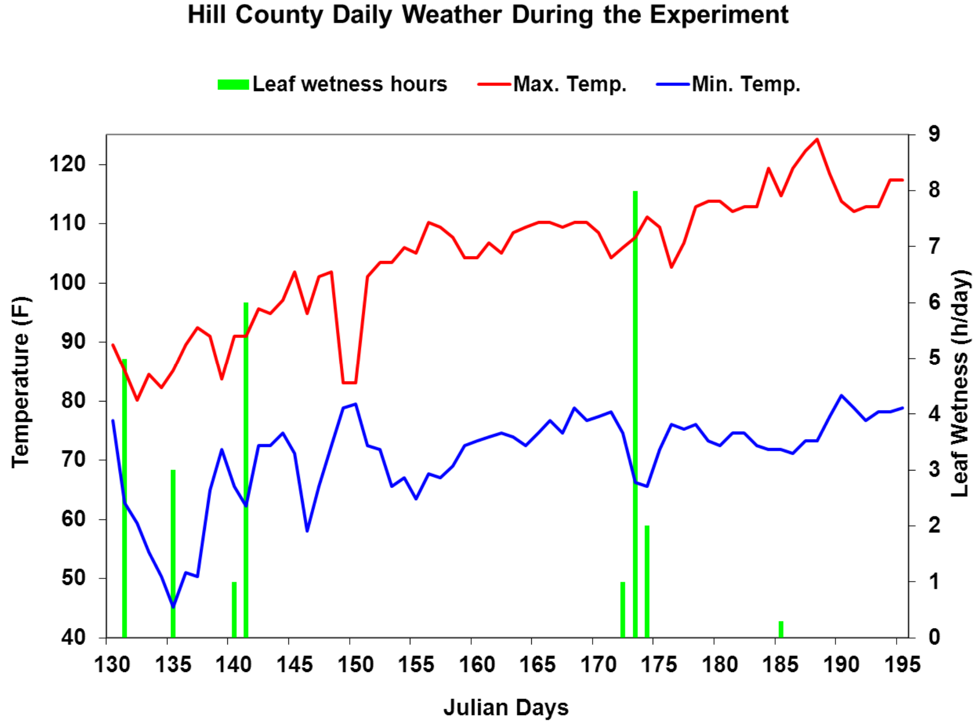
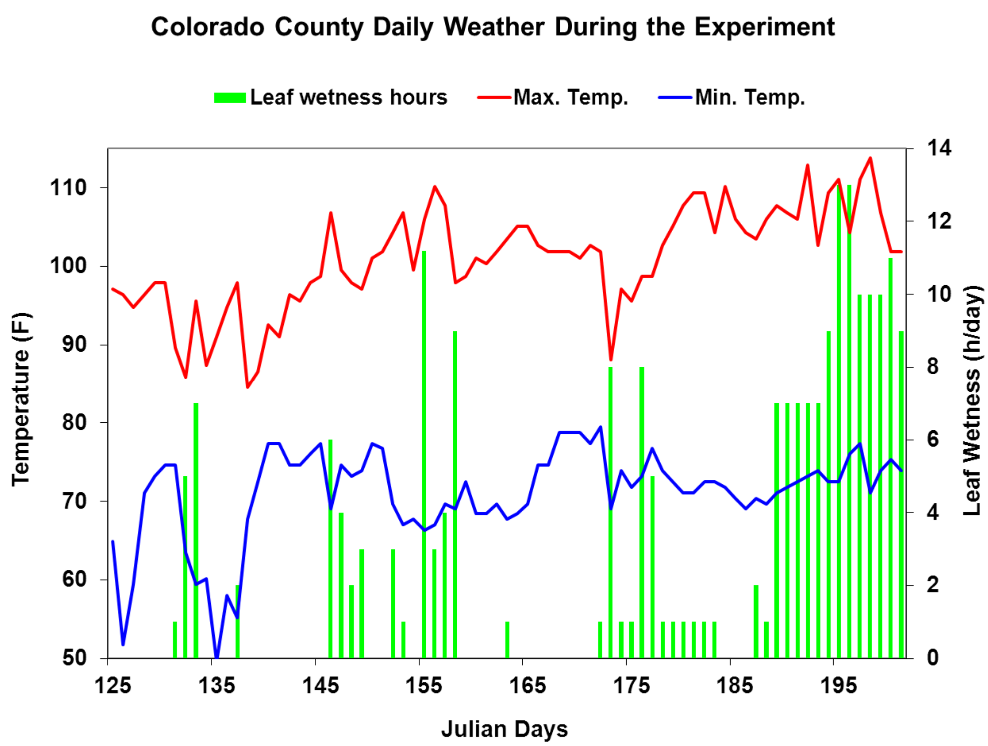


Fig. 5. Daily weather conditions during the experiment in Colorado county. Green bars indicate the number of hours per day that leaf wetness exceeds 10, on a scale of 0-14.



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