Managing Aflatoxins

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Aflatoxins: Costly Contaminants in Many Crops

Aflatoxins are toxic chemicals produced by *Aspergillus flavus* and several closely related fungi. Aflatoxins have been shown to cause cancer at very low concentrations (1 ppb) in certain experimental animals. They have also been associated with human liver cancer. Governments around the world regulate the quantity of aflatoxins allowed in foods and feeds. In the U.S. the aflatoxin content of food must be below 20 ppb (parts per billion) and milk must have less than 0.5 ppb. When milk contains 0.5 ppb or more, it must be dumped and the dairy placed on quarantine. This is very costly and generally results in lawsuits. To be fed to dairy cattle, cottonseed must contain less than 20 ppb aflatoxin. Cottonseed and cottonseed meal containing 300 ppb or more cannot be used as feed for any animals.

Aflatoxins are not just a problem in cottonseed. They also contaminate corn, peanuts and several tree crops including almonds, pistachios and figs. Crops with even very low but detectable levels of aflatoxin often have a severe trading disadvantage in competitive markets worldwide.

ppb (parts per billion) means 1 part per billion (or microgram per liter) and corresponds to 1 second in 32 years, 1 penny in $10 million, 1 inch in 16-thousand miles, 1 pinch of salt in 10-tons of potato chips or 1 bad apple in 2-million barrels.
**AF36 Competitively Excludes Toxin Producing Fungi**

The species *Aspergillus flavus* exists throughout Arizona’s environment in agricultural and desert soils, in dust, in the air, on crops, and on native plants. *Aspergillus flavus* communities are composed of many genetically isolated groups or strains and these strains have different abilities to produce aflatoxins. Some strains, like the S strain, produce very high levels of aflatoxins. It is not unusual for an S strain isolate to produce over 1-million ppb in laboratory tests. Some strains produce much less aflatoxin, and atoxigenic strains, such as AF36, produce no aflatoxins. Strains often interact and compete during crop infection. This interaction influences the quantity of aflatoxins produced.

Not only does AF36 produce no aflatoxin, it interferes with aflatoxin production by other strains. AF36 reduces contamination in bolls in which it naturally occurs. However, only a small percent of the *Aspergillus flavus* in Arizona is AF36. Treatments seek to increase the proportion of *Aspergillus flavus* that is AF36.

The composition of strains existing in soils and crops in *Aspergillus flavus* communities differs between fields and areas in both strain composition and average aflatoxin producing potential. Proper application of AF36 allows this atoxigenic strain to competitively exclude aflatoxin producers. This changes the mixtures of fungi in treated fields so that the fungi have a lower potential to produce aflatoxins. The end result is less aflatoxin in crops grown in those fields.

The objective of AF36 treatment is to replace aflatoxin producers with AF36. This reduces the ability of fungi within treated areas to produce aflatoxins.
**Aflatoxin Contamination Occurs in Two Phases**

**Phase One.** The crop is first contaminated when *Aspergillus flavus* infects the developing bolls through wounds or cracks. Pink bollworm damage has often been associated with contamination. However, any wound to the developing boll may be sufficient to permit *Aspergillus flavus* infection.

**Phase Two.** The second phase of contamination is usually most important in Arizona. It occurs when mature seed (open bolls, seed cotton, or ginned seed) is exposed to high relative humidity (above 85%) or rewetting while temperatures are warm (above 80° F). Aflatoxin producing fungi are blown onto Arizona seed-cotton shortly after boll opening. If environmental conditions cause seed moisture to rise above 12%, these fungi will infect and contaminate the seed.

Aflatoxin increases during the second phase may occur both before and after harvest. Thus, while the crop is held in the field awaiting harvest or in modules waiting ginning, toxin contents may gradually increase. Increases also may occur in seed piles and in the hands of the end user. Rank cotton, dense canopies, dew, and late irrigation increase the severity of contamination in the second phase. Early harvest reduces the second phase of contamination.
How to Use AF36

AF36 is formulated on sterilized (the germ and undesirable microbes are killed) wheat seed. The wheat seed serves as both a carrier and as a food source for the fungus. To be effective, AF36 must grow on the wheat seed and produce spores. Growth initially appears white and fuzzy and then becomes greenish as the spores form and powdery green as the spores mature. The spores then spread throughout the field and compete with aflatoxin producers. For the fungus to grow there must be adequate humidity (generally supplied by irrigation) and temperature (above 70° F).

When crops are grown under conditions favorable for aflatoxin contamination, the quantity of *Aspergillus flavus* in the environment increases. In the warm Arizona cotton seasons, irrigation and periods of high humidity stimulate rapid fungal growth. AF36 is applied either just before or during the early stages of *Aspergillus flavus* increases. AF36 competes with aflatoxin producers and replaces them without increasing either the overall quantity of fungus in the field or the amount of the crop infected. This results in fewer aflatoxin producers being associated with the crop and, as a result, less aflatoxins. Furthermore, when AF36 infects a boll together with a toxin producer, it inhibits aflatoxin production. This is a second mechanism by which AF36 reduces contamination.

In theory, application of an atoxigenic *Aspergillus flavus* strain (i.e., AF36) at lay-by should give the atoxigenic strain preferential exposure to debris from the rapidly developing crop. This provides an advantage in the competition for crop resources during infection and during *Aspergillus flavus* population growth associated with crop production. However, the best time to treat may vary depending on soil type, planting date, and irrigation practices. Dry, sandy soils may require later treatment.
Use of AF36 changes the types of the fungi associated with the treated crop so that the atoxigenic strain (AF36) is very common and the incidence of aflatoxin producers is greatly reduced. However, influences of AF36 treatments extends beyond the treated crop. Treatments provide beneficial displacement even in fields adjacent to treated fields and over multiple years.

Just as AF36 disperses to neighboring fields, the aflatoxin producers from untreated fields can move into treated fields on dust, insects, and wind. Multi-year, area-wide approaches should allow reductions over entire areas and thus reduce the incidence of aflatoxin producers carried on wind and dust.
Fungi that make aflatoxins typically survive until the next season as spores and on crop debris. Similarly, some of the influences of AF36 applications remain between seasons. Thus, even one year after treatment, there is more AF36 and fewer aflatoxin producers in treated fields than prior to treatment. This suggests that multiple years of treatment will result in cumulative benefit and that the incidence of aflatoxin producers might be reduced to low levels over an entire production area through the use of AF36 treatments in an area-wide aflatoxin management program.

Aspergillus flavus typically becomes associated with crops in the field during crop development, and remains associated with the crop during harvest, storage and processing. Thus, crop vulnerability to aflatoxin contamination remains until the crop is ultimately used. Even aflatoxin that may form after cottonseed is marketed (i.e. at the dairy) can be attributed to the initial cottonseed supplier during litigation. Atoxigenic strains seeded into agricultural fields prior to crop development remain associated with the crop until use and may provide long-term postharvest protection from contamination.
Frequently Asked Questions

1. **WHAT ARE AFLATOXINS?**
   Aflatoxins are cancer-causing toxins produced by various strains of a common fungus (Aspergillus flavus). Cottonseed containing over 20 parts per billion of aflatoxin cannot be fed to dairy cows. This results in $20-$50 per acre annual revenue loss to Arizona growers.

2. **WHAT IS AF36 AND HOW DOES IT WORK?**
   AF36 is a strain of Aspergillus flavus that does not produce aflatoxins. When introduced into cotton fields at the proper time, AF36 outcompetes aflatoxin producing strains. This results in less contamination.

3. **WHEN AND HOW IS AF36 APPLIED?**
   AF36 is applied once a year on cotton crops near lay-by to be followed immediately by irrigation. Treatment rate is 10 pounds of colonized wheat seed per acre, applied by either air or ground.

4. **WHAT IS THE COST OF AF36?**
   The Arizona Cotton Research and Protection Council provides AF36 to producers for $5.00 per acre. Cost of application is additional but if Gandy or fertilizer boxes are located on tool bars behind cultivators, applications can be put on at the same time as cultivation.

5. **HOW QUICKLY WILL AF36 SHOW EFFECTS?**
   Treatments with AF36 will reduce aflatoxin levels in the initial year, often to below 20 ppb. However, the reduction may not be sufficient to lower aflatoxin content below 20 ppb if severe conditions for contamination occur in a given year.

6. **WHY SHOULD I TREAT LAND WHERE I HAVE SEED CONTRACTS?**
   Three main reasons: 1. If the seed doesn’t meet company requirements, you are left with seed to market. Clean seed can always find a home. 2. Treatments to the land will have carry-over benefit for subsequent years providing an improved starting point for next season. 3. Aspergillus flavus spreads between fields and across the area. Instead of spreading aflatoxin producers from an untreated field, you will be spreading the atoxigenic strain AF36 and reducing the aflatoxin producing potential of fungi in the area.
7. **DOES CLEAN VS DIRTY SEED PRICING WARRANT THE COST OF AN AF36 TREATMENT?**
   Yes. While seed prices vary from year to year, on average clean seed sells for $20 per ton or more than dirty seed. Additionally, as AF36 treatment program provides area-wide aflatoxin suppression over time the flexibility of moving clean Arizona seed to new markets both in and out of state will increase.

8. **SHOULD I TREAT PIMA COTTON WITH AF36?**
   Pima seed entering dairy markets must also be below 20 ppb aflatoxin. Pima produced in high aflatoxin areas can have economically damaging contamination problems. The same long-term and area-wide benefits accrued when upland cotton is treated exist when Pima is treated. Land planted to Pima may be adjacent to or rotated to upland cotton.

9. **IS AF36 A SILVER BULLET?**
   No, AF36 reliably reduces the quantity of aflatoxin producing fungi on the crop and in the environment and these reductions are associated with reductions in the quantities of aflatoxins in the crop. This has been shown on more than 60,000 acres of cotton treated over the past four years. However, if conditions favoring contamination are severe, reductions may not be sufficient to reduce contamination below the 20 ppb level in the first year. Multi-year and area-wide treatments allow for increased levels of reduction and reduced movement of aflatoxin producers into treated fields.

10. **WHY BOTHER TREATING IF MY SEED GETS MIXED WITH OTHER SEED AT THE GIN?**
    If clean seed is commingled with dirty seed, frequently the aflatoxin value for the pile ends up above 20 ppb. This results in considerable loss for the seed pool. A good aflatoxin management tool is to segregate seed by field. All first pick seed from the same field should be kept together. This is particularly important during periods when most seed lots are between 0 and 100 ppb. This practice can significantly increase the number of clean seed lots and thus profits. If all seed cannot be segregated on a field by field basis, the gin should at least keep all cottonseed from AF36-treated fields separate from cottonseed from untreated fields. Managing aflatoxin requires an organized effort and cooperation at the gin level. This is required even if no one at the gin chooses to use AF36. The alternative is a loss of income to the producer.
11. **HOW DOES THE AF36 PROGRAM BENEFIT ME IF I GIN AT A LINE GIN?**
It all begins with the gin’s willingness to separate and test AF36 treated seed. If you market your own seed the potential is obvious. If the gin markets the seed the increased value still leaves room for mutual financial benefits.

12. **IS SEPARATING SEED LOTS AT THE GIN WORTH THE EFFORT?**
Yes. The percent of seed lots that are clean can increase substantially at most gins and each clean lot yields the gin $1,000 to $5,000 in excess of dirty seed depending on the lot size and seed price.

13. **ISN’T IT SAFER TO AVOID RISKS AND ROUTINELY AMMONIATE SEED?**
Ammoniation is a useful aflatoxin management tool for Arizona. However, ammoniation has costs and requires repeated handling of seed. Furthermore, ammoniated seed cannot be marketed across state lines and frequently ammoniated seed sells at a discount. Ammoniation degrades seed quality and some users feel it is less palatable to dairy cows. This is particularly the case for highly contaminated seed that requires extensive ammoniation. Production of clean seed provides an opportunity to seek premium markets and premium prices. AF36 treatments provide the opportunity to increase the proportion of the crop that is acceptable for dairies while making the seed unacceptable for dairies more easily ammoniated.

14. **WHY SHOULD I TREAT LEASED LANDS?**
The first goal of AF36 treatments is to provide economically useful reductions in contamination during the first season applications are made. The second goal is to reduce the incidence of aflatoxin-producing fungi throughout a treatment area and provide long-term reductions in the vulnerabilities to aflatoxin contamination of all crops grown in the area. Treating a field helps reduce the quantity of aflatoxin producing fungi present the following season in treated fields and in neighboring fields. These effects can vary from small reductions (around 30%) to very large reductions 80% or more.

15. **MY SEED IS NORMALLY CLEAN. WHY SHOULD I TREAT?**
Aflatoxin levels vary considerably from one year to the next. Use of AF36 may be considered an inexpensive insurance program to ensure the maintenance of your clean seed and to keep levels low in your area. When used properly, AF36 should also provide some protection from formation of aflatoxins after the ginned seed is shipped.
16. **COULD AF36 INOCULATED WHEAT SPREAD KARNAL BUNT?**

No! The wheat used for AF36 is steam sterilized at 250°F for two hours before inoculation. All fungi associated with the wheat are killed before AF36 is grown on the wheat seed. This process also kills the germ.

17. **WHAT IF I HAVE A LONG-TERM (i.e. 3 YEAR) COTTON ROTATION?**

The primary goal of AF36 applications is to reduce aflatoxin contamination in the treated crop. In many cases, these initial aflatoxin reductions result in cottonseed with improved access to markets. However, the long-term goal is to gradually reduce the risk of contamination across our production areas by reducing the incidence of aflatoxin producers. Early studies in Yuma County demonstrated that AF36 persists at elevated levels in the soil for more than two years after application. While back to back AF36 applications may produce more dramatic results, treatment of cotton even in long rotations serves to gradually reduce the aflatoxin producing potential of resident fungi across the entire area. This is the long-term goal of AF36 use.

18. **WHY ARE GROUND APPLICATIONS PREFERRED?**

Use of Gandy or fertilizer boxes with drop tubes can position AF36 in the seed row where it has more shade and can avoid burial. Also ground treatments can reduce or eliminate application costs.

19. **IS THE COST OF AF36 WORTH IT IF ONLY SOME OF MY SEED IS CLEANED UP?**

Remember your concern should not only be for the effects on this season’s crop but also long-term influences on the incidence of aflatoxin throughout your production area. Also AF36 treatments reduce the quantity of aflatoxins even if those levels are not below 20 ppb. There can be benefit to having 150-ppb seed as opposed to 1,500 ppb seed. This difference represents a 90% reduction in the aflatoxin content.

20. **IF THE PRICE DIFFERENCE BETWEEN CLEAN AND DIRTY SEED IS MINIMAL, IS AF36 WORTH THE INVESTMENT?**

Markets vary from year to year. Building a reputation of clean seed will expand markets and should help to stabilize prices over time. Having a reputation for frequently producing seed with over 1,000 ppb does not help expand seed markets!
21. **SHOULD Gins PAY FOR THE AF36 TREATMENTS?**
Gins are logical centers for aflatoxin management programs because they generally have a role in sampling for aflatoxin, having analyses run, and marketing seed. Gins also greatly impact the quantity of seed lots contaminated by controlling the order in which modules are ginned and by managing seed pile composition. When gins select the areas to be treated and fund treatments, the widest participation is ensured and the number of producers that go in and out of the program year to year is reduced. This ensures the optimal benefits to the gin-average seed price.

22. **ARE OTHER CROPS BENEFITED BY AF36?**
Yes. In Arizona corn may benefit from a cotton/corn rotation; however additional studies are needed. There is also considerable interest in using AF36 on pistachios in both California and Arizona and on figs in California.

23. **HOW MANY TREATMENTS ARE NEEDED TO BE EFFECTIVE?**
One per year. Proper applications cause reductions in aflatoxin content with a single application. Greater reductions are achieved with multiple-year applications and when wider areas are treated.

24. **WHY SHOULD I PARTICIPATE IF MY NEIGHBOR DOES NOT?**
Treated fields show good results even if neighboring fields are not included in applications. However, you will help reduce the quantity of aflatoxin-producing fungi in your neighbor’s fields and your neighbor will contribute aflatoxin-producing fungi to your fields. It is hoped that once AF36 results are publicized, participation levels will increase.

25. **ARE THERE ANY NEGATIVE ENVIRONMENTAL EFFECTS ASSOCIATED WITH AF36?**
No. AF36 does not increase total levels of fungus in the environment; it simply displaces aflatoxin producers. Therefore the environment is progressively improved with respect to aflatoxin.
Improve the Aflatoxin Picture

- Participate in area-wide AF36 treatments.
- Harvest early, harvest dry. Tarp modules.
- Reduce irrigation after initial boll opening.
- Do not mix seed from spindle-picked cotton with seed from ground-gleaned cotton.
- After ginning, keep seed from different fields separated until after aflatoxin content is determined.

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