BLACK (ULTRAVIOLET) LIGHT SCREENING OF CORN FOR PRESUMPTIVE DETECTION OF AFLATOXIN

This method was originally developed by: Odette L. Shotwell, Ph.D., Northern Regional Research Center ARS, USDA, 1815 North University, Peoria, Illinois 61604 U.S.A. It was approved as Official Method 45-15 by American Association of Cereal Chemists in 1997. This version is an update to include market experience in previous Iowa outbreaks. It is applicable to freshly harvested corn; its applicability to artificially high-temperature dried corn is not known.

Scope

The black light or bright greenish-yellow fluorescence (BGYF) test is based on the BGYF observed under ultraviolet (UV) light (366 nm) that can be seen on corn on which Aspergillus parasiticus and/or Aspergillus flavus is growing. The growth of this fungus may result in aflatoxin production. Aflatoxins themselves do not fluoresce bright greenish-yellow under long wave UV light (366 nm); BGYF is produced by the reaction of kojic acid formed by the fungus and a peroxidase enzyme also from the seed. The BGYF is also produced by the fungus on grain sorghum or cottonseed, but usually not on seed that has been killed by high temperature drying.

The BGYF test is the quickest method of detecting grain that could contain aflatoxin, but a positive test does not absolutely indicate aflatoxin is present. False negatives (aflatoxin without BGYF) are rare. The BGYF test requires very little equipment and can easily be done in the field.

Field of Application

The BGYF method is a presumptive test used to identify corn lots that may have aflatoxin in excess of acceptance levels.

Color Standards

Since various matter can fluorescence different colors under black light (366 nm), Tinopal bas (optical brightener made by BASF, sold by Seedburo, Inc., Chicago, IL), a green fluorescent crayola or a black-ray green fluorescent crayon (Ultra Violet Products, Inc., San Gabriel, California) should be used as a color standard to detect the presence of the fungus.

Apparatus

Goggles for ultraviolet protection that cut off at about 410 nm.

An ultraviolet lamp (366 nm). The lamp may be high intensity but the examination of corn should be done in a relatively dark place. The lamp can be battery operated for field use or a viewing cabinet with the lamp (366 nm) inside to protect viewer from ultraviolet light can be used.

A disc mill or similar grinder capable of rough grinding 5 lb samples. Grinding is not necessary, however, if the lamp is of high intensity. Grinding will affect the ability to use particle count as a criterion. Comparisons between inspections of whole-kernel corn samples in black light inspection apparatus and inspections under high-intensity lamp of corn from the same sample that was cracked in a disk mill showed no significant differences between grinding and not grinding.

Procedure

A 4.5 KG (5 lb) sample representative of the entire corn lot, best obtained by probing or continuously sampling a grain stream, should be examined under UV light of 366 nm. The operator should wear goggles that screen out. UV light, increase contrast, and protect eyes from possible damage unless a chromato-view chamber is used. Black light or UV examination should be done in a dark room or darkened chamber. A fluorescent standard with the same color as BGYF under UV light (366 nm) (see **Color Standards** above) should be used so that the BGYF is properly identified. If the whole-kernel sample is examined, a monolayer of corn should be inspected, making sure kernels are inspected from several angles.

The BGYF glows and is associated with the germ or starchy endosperm of the kernel. Whole kernels may have the glowing BGYF under the seed coat and can be broken with pliers to highlight the presence of BGYF. Cob tips may fluoresce yellow and tips of corn kernels and glumes (bees' wings) may fluoresce pale yellow. Soybeans without seed coats fluoresce a dull green yellow. However, false positives do not fluoresce with the BGYF color or they do not have a glowing fluorescence.

Confirmation

The BGYF material is water soluble; it will fluoresce in water.

Interpretation of Results

The BGYF test should be interpreted with judgment. The presence of one small dust speck of BGYF in 5 lbs of corn should not cause concern. Studies indicate the presence of one or more BGYF particles in a corn sample could be considered a potentially positive test. However data from 1983 and 1988 outbreaks indicates that particle count in unground samples can be used to increase the chances that accepted corn will average less than 20 ppb, even if some aflatoxin samples are kept and vice versa. Although a relationship exists between numbers or weights of BGYF particles or kernels and aflatoxin levels, the correlation is not high enough to encourage use of numbers or weights as an indication of actual aflatoxin levels. In fact, some corn lots with many fluorescing particles and kernels do not have detectable aflatoxin. Typically about one-third to one-half of the corn lots with a positive BGYF test (one particle per kg (2.2 lbs)) have less than 20 ppb aflatoxin, the United States Food and Drug Administration guideline.

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Experience in the 1983 and 1988 Iowa outbreaks led to the concept of using the count of glowing particles as a risk indicator for samples being over or under 20 ppb. The numbers below are derived from those years in Iowa. There is no assurance that the specific count figures will apply to future situations, but a procedure for validation and update is given.

Procedure Modification for Use of Particle Count

The test is performed as given above. The whole corn sample should be weighed before scanning. Count the number of glowing particles of whatever size. Divide the number of glowers by the weight in lbs. It is very important that the sample be 5 lbs or greater.

In 1983 and 1988 corn, 1.0 or more glowers per pound (2.2 glowers/kg) of sample gave much higher probability that the sample contained more than 20 ppb. The average level of those samples with fewer than 1.0 glowers per pound was less than 10 ppb, compared to more than 50 ppb for the samples with 1.0 glowers or more per pound.

Each operator should validate their cutoff by submitting 20 5-lb or heavier samples to an accurate lab. A company with multiple facilities might do the validation together, but use samples from several operators. Validation samples should have a wide and uniform distribution of glowing particles per pound. The same sample that the operator viewed under the black light, without further division or combination, should be submitted for analysis. The cutoff may have to be changed if the validation shows that 1 glower per pound is not stringent enough for the desired average of accepted corn. Continue validation tracking by using the black light on any samples (5 lb or greater) that are going to be sent for lab testing.

The goal of the black light screening is to make the average level of the accepted samples less than 20 ppb, preferably less than 10 pbb to account for the sampling error. In the 1983 and 1988 tests, the number of misclassified samples by the above method was reduced significantly if larger samples (than 5 lb) were used, and therefore the confidence in the average of accepted samples was increased.

This is a risk management method; there can be no guarantee of future test results on a screened lot or a blend of lots.

References

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