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Effect of Climate and Type of Storage Container on Aflatoxin Production in Corn and its Associated Risks to Wildlife Species

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ABSTRACT: The effects of grain storage containers on aflatoxin production, and the relationship between the level of aflatoxin and the number and weight of fluorescing kernels were determined in corn (*Zea maize*) stored in controlled climate regimes. Two hundred and forty 100-g samples were held up to 3 mos using four types of storage containers placed in four climates. Storage containers included corn placed in metal cans, paper bags, plastic bags, and paper bags placed in plastic bags. Climates were constant during the duration of the project and included a combination of temperatures and humidities. Temperatures were 29–32 C and 14–18 C; relative humidities were 85–88% and 35–40%. In addition, corn was exposed to environmental conditions conductive for aflatoxin production and 100 g samples were randomly collected, examined under ultraviolet light for fluorescence, and then quantified for aflatoxin levels. Corn samples tested negative for aflatoxin at the beginning of the project. Main (i.e., container, climate, and month) and interactive effects were not observed. Mean levels of aflatoxin ranged from 0 to 151 μg/kg. Aflatoxin was produced regardless of type of storage container, time of storage, and climatic conditions; however, only 8% of the samples produced aflatoxin levels that exceeded 50 μg/kg. Fluorescing corn ranged from 0 to 19 kernels per sample, while aflatoxin levels ranged from 0 to 1,375 μg/kg for the same samples. No relationships were found between the number and weight of fluorescing kernels of corn and aflatoxin levels. The black light test yielded a false negative rate of 23% when in fact the aflatoxin concentrations exceeded 50 μg/kg. Therefore, quantifying fluorescing grain under UV light should not be considered a feasible alternative for aflatoxin testing of grain intended for wildlife.

Key words: Aflatoxin, *Aspergillus flavus*, *Aspergillus parasiticus*, climate, fluorescence, grain storage.

Mycotoxins are toxic metabolites produced by fungi, which serve as a protective mechanism (Merck Veterinary Manual, 1986). Aflatoxin is one of the most widely occurring and dangerous mycotoxins (C.A.S.T., 1989). The term aflatoxin refers to a closely related group of metabolites produced by toxigenic strains of *Aspergillus flavus* and *A. parasiticus*. Aflatoxins are potent carcinogenic, mutagenic, teratogenic, and immunosuppressive agents, and their contamination of agricultural feed grains poses a serious threat to the health of animals and humans (Stoloff, 1980). Effects of aflatoxicosis include depressed feed efficiency and weight gain, abnormal liver chemistries, depressed immune function, carcinogenesis, and death (Pier, 1992). However, the effect that aflatoxins have on a given animal depends on the species of animal, physiological state, dosage, and length of exposure to the toxin (Pier, 1992).

Studies in the United States confirm widespread occurrence of aflatoxin in feed grains (C.A.S.T., 1989). Major crops affected by aflatoxin include corn, peanuts, and cotton, but any feed that is stored is vulnerable. Some factors contributing to aflatoxin production include the feedstuff, moisture level of feed, temperature, relative humidity, pH, plant stresses such as drought or insect infestation, and damaged or broken grain kernels (Jacques, 1988). Aflatoxin resulting from mold growth may be present at the time of harvest or may occur any time during processing, transport, or storage if conditions are favorable.

Because the U.S. Food and Drug Administration (Washington, D.C.) has imposed limits of <300 μg/kg aflatoxin in domestic animal feed (depending on feed and animal species; see Rustom, 1997), condemned grain potentially can be used to feed wildlife (Quist et al., 1997). Fischer et al. (1995) found >50% of bait piles for wildlife contaminated with aflatoxin, with

levels ranging from trace to 750 μg/kg. Baiting is a legal hunting practice in many states and shelled corn is commonly used as bait for white-tailed deer (*Odocoileus virginianus*; Fischer et al., 1995) and northern bobwhites (*Colinus virginianus*; pers. obs.). Aflatoxin concentrations of 800 μg/kg have been found to negatively impact white-tailed deer fawns (Quist et al., 1997) and aflatoxin-induced mortality was observed in northern bobwhites and ringneck pheasants (*Phasianus colchicus*) at aflatoxin concentrations of 125 μg/kg (Huff et al., 1992; Ruff et al., 1992). Although there are no restrictions concerning aflatoxin concentrations in feed intended for wildlife, the Texas State Feed and Fertilizer Control Service (Austin, Texas, USA) advises that corn with aflatoxin concentrations >100 μg/kg not be fed to wildlife (http://www.tpwd.state.tx.us/news/news/980907d.htm).

Most cases of animal poisoning by aflatoxins can be traced to the growth of fungi in stored feed grain (Jacques, 1988). Aflatoxin occurrence is significantly higher in warm humid climates although formation can occur in temperate climates, especially if on-farm feed storage is practiced (Smith, 1997). Feed grain intended for wildlife is typically purchased in bulk to make supplemental feeding programs economical (McBryde, 1995), and often is stored in bulk feed tanks or in their original packaging (either paper or plastic bags) within a non-thermal controlled building for several months prior to use. Therefore, storage conditions of purchased feed are sometimes less than adequate to prevent mold growth, and exposure of grain in feeders to conditions conducive to mold growth likely occurs. Consequently, feeding wildlife with highly contaminated grain can expose them to increased risk and potentially mitigate any realized benefit from supplemental feeding.

To circumvent problems of aflatoxin-contaminated grain, screening grain for aflatoxin has been suggested. Because the observation of fluorescence in grain indicates fungal growth that may have resulted in aflatoxin production (Gloria et al., 1998), viewing grain with a black light to determine the presence of aflatoxin has been suggested. Shotwell and Hesseltine (1980) suggested that the black light test was not appropriate for levels of aflatoxin <20 μg/kg. However, to our knowledge the efficacy of the black light test has not been determined for higher levels (i.e., 100–1,000 μg/kg) of aflatoxin. Therefore, our objectives were to determine the effects of four types of grain storage containers on aflatoxin production, and to determine if a linear relationship exists between the number and weight of fluorescing kernels and the level of aflatoxin.

Thirty kg of corn were thoroughly mixed, after which 275 100 g samples were collected. Thirty-five samples were randomly selected, ground in a Romer mill (Glen Mills, Inc., Clifton, New Jersey, USA), and quantified for levels of aflatoxin using the Aflatest® kit and fluorometer (Series 4, Vicam, Watertown, Massachusetts, USA) prior to the onset of the experiment. Detection limit for aflatoxin by this method is 1.0 μg/kg. The remaining 240 samples were held up to 90 days in four types of storage containers and within four climate regimes. Storage containers used in our study were selected to simulate the most common methods of obtaining and storing wildlife grain. Storage containers included corn placed in metal containers (i.e., coffee cans used to simulate wildlife feeders and bulk feed tanks), plastic zip-lock bags, paper bags, and paper bags placed inside plastic zip-lock bags (the latter three methods used to simulate the various types of grain sacks in the marketplace). Samples were placed in environmental chambers (Scientific Instrument Service, Pearland, Texas, USA) and exposed to a combination of temperatures and humidities. Range of temperatures were 29–32 C and 14–18 C; range of relative humidities were 85–88% and 35–40%. Climates were constant during the 90 day period. At 30-day intervals, five

samples within each type of storage container and climate regime were quantified for levels of aflatoxin as previously mentioned. The experimental design was a completely randomized factorial design and a general linear model's analysis of variance was used to test the effects of storage container, climate, and month on levels of aflatoxin (SAS Institute, Inc., 1989). Distributions of residual errors were tested for normality using the Shapiro-Wilk test (SAS Institute, Inc., 1989). Homogeneity of variances among treatments were evaluated with the Bartlett's test (Steel and Torrie, 1980). Statistical significance was inferred at $P \leq 0.05$. Descriptive statistics are presented as the mean ± 1 standard error.

In addition, 30 kg of corn were cracked and flattened with a baker's rolling pin, placed in open 75 L plastic containers, and maintained in a non-thermal-controlled storage shed for up to 6 months (December through May) in southern Texas. At random intervals during this storage period, 100 g samples were removed, coarsely ground (i.e., setting 0.5) in a Romer mill (Glen Mills, Inc., Clifton, New Jersey, USA) according to the procedures of Shotwell et al. (1974), and examined under ultraviolet light for fluorescence. Fluorescing kernels were counted and weighed to the nearest 0.1 g, and the entire sample was quantified for levels of aflatoxin as previously mentioned. The entire process was repeated until a minimum of 10 samples were obtained for each 100 μg/kg interval of aflatoxin within the range of 0–1,000 μg/kg. Relationships between levels of aflatoxin (μg/kg) and the number and weight of fluorescing kernels of corn were analyzed using least squares linear regression (PROC REG; SAS Institute, Inc., 1989). Statistical significance and descriptive statistics were inferred and presented as previously mentioned. Rate of false negative samples was defined as the proportion of samples that exhibited no fluorescent kernels but yielded aflatoxin concentrations >50 μg/kg. Aflatoxin concentration of 50 μg/kg was chosen because that was the minimum level in which morbidity in bobwhites was detected (Berthelot, 1994).

Average concentrations of aflatoxin during our study only exceeded the specified limit of 50 μg/kg on three occasions (Fig. 1). A discernable pattern as to the production of aflatoxin within type of storage container and climate was not apparent. Levels of aflatoxin in individual samples ranged from 0 μg/kg for corn stored in paper bags inside plastic bags within hot and dry conditions to 730 μg/kg for corn stored in metal containers within cool and humid conditions. However, main and interactive effects were not observed in the production of aflatoxin (Table 1); therefore, average aflatoxin concentrations were pooled across climates within storage containers and across storage containers within climates. Average aflatoxin levels in corn stored for 30, 60, and 90 days were ($\bar{x}$ ± SE) 41 ± 36, 11 ± 4, and 24 ± 10 μg/kg, respectively, for corn stored in metal containers, 3 ± 1, 49 ± 19, and 19 ± 3 μg/kg, respectively, for corn stored in paper bags, 3 ± 1, 5 ± 1, and 7 ± 2 μg/kg, respectively, for corn stored in plastic bags, and 2 ± 0.3, 4 ± 1, and 20 ± 11 μg/kg, respectively, for corn stored in paper bags inside plastic bags. Average aflatoxin levels of corn stored for 30, 60, and 90 days in

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FIGURE 1. Average aflatoxin production (μg/kg) in five 100 g samples of corn held for (A) 30, (B) 60, and (C) 90 days in four types of storage containers and within four climate regimes. Types of storage containers included corn stored in metal containers, plastic bags, paper bags, and paper bags placed inside plastic bags. Storage containers were selected to simulate commonly used storage containers for wildlife feed and packaging found in the marketplace. Hot climates had temperatures between 29–32 C, cool climates had temperatures between 14–18 C, humid climates ranged from 85–88% relative humidity, and dry climates ranged from 35–40% relative humidity.

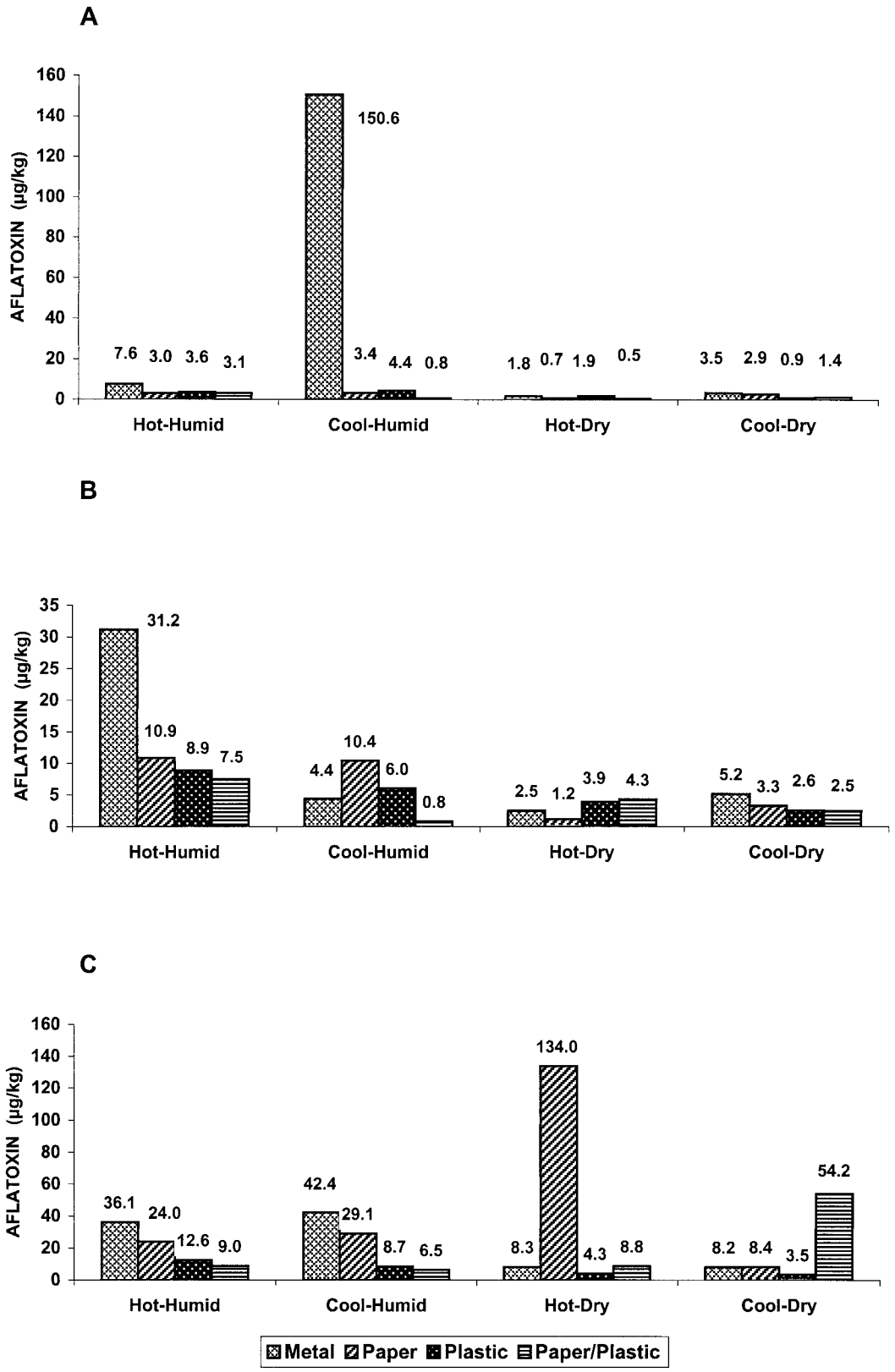

TABLE 1. Results of the ANOVA using a factorial experimental design for levels of aflatoxin produced in four climates and four types of storage containers at 30-day intervals for up to 90 days.

Source	df	ANOVA SS	MS	*F*-value	*Pr* > *F*
Climate[a]	3	10,828.1	3,609.7	1.40	0.243
Container[b]	3	14,429.3	4,809.8	1.87	0.136
Month[c]	2	4,546.5	2,273.2	0.88	0.415
Climate × Container	9	30,223.4	3,358.2	1.31	0.236
Month × Climate	6	13,866.8	2,311.1	0.90	0.496
Month × Container	6	11,478.7	1,913.1	0.74	0.614
Month × Climate × Container	18	43,412.1	2,411.8	0.94	0.533
Error	192	493,426.4	2,569.9		
Total	239	622,212.4			

[a] Climates included hot and humid (29–32 C and 85–88% RH), hot and dry (29–32 C and 35–40% RH), cool and humid (14–18 C and 85–88% RH), and cool and dry (14–18 C and 35–40% RH) conditions.
[b] Storage containers included corn placed in metal containers, paper bags, plastic bags, and paper bags placed inside plastic bags.
[c] Five samples of corn were removed from each climate and type of storage container at 30-day intervals.

hot and humid conditions were 4 ± 1, 15 ± 4, and 21 ± 8 μg/kg, respectively, 40 ± 36, 5 ± 1, and 22 ± 6 μg/kg, respectively, for corn stored in cool and humid conditions, 1 ± 0.3, 3 ± 1, and 39 ± 21 μg/kg, respectively, for corn stored in hot and dry conditions, and 2 ± 1, 3 ± 1, and 19 ± 11 μg/kg, respectively, for corn stored in cool and dry conditions. Levels of aflatoxin in individual samples tested as high as 730, 88, and 220 μg/kg for corn stored 30, 60, and 90 days, respectively. Proportion of individual samples that tested >50 μg/kg was 8% (Table 2). Linear relationships were not observed between levels of aflatoxin and the number ($F_{1, 158} = 5.9$; $P = 0.016$; $r^2 = 0.43$) or weight ($F_{1, 158} = 5.4$; $P > 0.021$; $r^2 = 0.37$) of fluorescent kernels of corn (Table 3). Fluorescing corn ranged from 0 to 19 kernels per sample while aflatoxin levels ranged from 0 to 1,375 μg/kg for the same samples. Overall, the black light test had a false negative rate of 23% when in fact the aflatoxin concentrations exceeded 50 μg/kg (Table 3). Only when aflatoxin levels exceeded 700 μg/kg was the black light test accurate in predicting the presence of aflatoxins (Table 3). There were no false positives.

Less than 8% of our samples produced aflatoxin levels that are considered potentially harmful to wildlife, in particular to wild birds. However even with this low rate of exposure, aflatoxin was produced in our samples regardless of type of storage container, length of storage, or climatic conditions in which the grain was subjected. Individuals who practice a supplemental feed or bait program for wildlife can decrease their risk of feeding aflatoxin-contaminated grain by first testing the grain at the time of purchase and should not store grain for longer than two months. Purchasers of grain should not rely on labels placed on bags of grain that state the level of aflatoxin because the label only pertains to the time the grain was tested. Corn used in our study tested negative for aflatoxin prior to the onset of the experiment. Also, of our samples that produced >50 μg/kg aflatoxin levels ($n = 19$), 74% became contaminated within the third month of storage. Our findings are consistent with previous studies that suggested an increased risk of aflatoxin production with prolonged storage (Jacques, 1988).

Our findings suggest that any of our types of grain storage containers would be acceptable for short-term storage. However caution should be used when interpreting the results of our study because the formation of aflatoxins can result from a variety of conditions. For example, storage of grain in plastic bags during our study resulted in the least number of samples that produced aflatoxin. However, in-

TABLE 2. Proportion of corn samples within each type of storage container, climate, and 30-day interval that had aflatoxin concentrations >50 μg/kg.

Storage container	Storage climate[a]				Total
	Hot-humid	Cool-humid	Hot-dry	Cool-dry	
Metal					
1 mo	0/5	2/5	0/5	0/5	2/20
2 mo	1/5	0/5	0/5	0/5	1/20
3 mo	2/5	2/5	0/5	0/5	4/20
Total	3/15	4/15	0/15	0/15	7/60
Paper					
1 mo	0/5	0/5	0/5	0/5	0/20
2 mo	1/5	0/5	0/5	0/5	1/20
3 mo	2/5	2/5	3/5	0/5	7/20
Total	3/15	2/15	3/15	0/15	8/60
Plastic					
1 mo	1/5	0/5	0/5	0/5	1/20
2 mo	0/5	0/5	0/5	0/5	0/20
3 mo	1/5	0/5	0/5	0/5	1/20
Total	2/15	0/15	0/15	0/15	2/60
Paper/plastic					
1 mo	0/5	0/5	0/5	0/5	0/20
2 mo	0/5	0/5	0/5	0/5	0/20
3 mo	0/5	0/5	0/5	2/5	2/20
Total	0/15	0/15	0/15	2/15	2/60

[a] Hot climates had temperatures between 29–32 C, cool climates had temperatures between 14–18 C, humid climates ranged from 85–88% relative humidity, and dry climates ranged from 35–40% relative humidity.

adequate post-harvest techniques such as drying practices can result in aflatoxin production (Smith, 1997). Grain with a high moisture content that is stored in plastic bags under warm conditions tends to result in a build up of moisture within the bags. Such conditions are conducive to mold growth and can facilitate aflatoxin formation. Also, corn maintained in the southeastern United States is considered

TABLE 3. Mean number and weight (g) of fluorescent kernels and mean aflatoxin concentration (μg/kg) in corn used to assess the efficacy of the blacklight test for feed grain intended for wildlife.

Aflatoxin range	n	Fluorescent kernels				Aflatoxin (μg/kg)		False negatives (%)[a]
		Number		Weight				
		$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	
0–99	23	2.3	1.7	0.6	0.4	24	5.5	34.8
100–199	17	3.1	2.7	1.4	1.2	149	8.0	47.0
200–299	12	2.7	2.4	1.3	1.2	224	4.5	41.7
300–399	15	3.1	3.1	2.3	1.5	364	4.9	33.3
400–499	15	3.3	2.6	2.5	1.7	453	6.3	46.7
500–599	18	2.7	1.6	2.4	1.7	548	6.2	16.7
600–699	10	9.8	6.1	4.4	2.7	661	7.1	10.0
700–799	10	10.6	4.3	3.4	1.4	735	9.1	0.0
800–899	10	7.8	4.3	2.5	2.1	836	8.3	0.0
900–999	11	6.1	5.6	2.6	2.4	942	9.7	0.0
1,000+	19	7.4	5.4	3.3	2.9	1,188	38.5	0.0

[a] Proportion of samples that exhibited no fluorescent kernels but had aflatoxin concentrations >50 μg/kg.

vulnerable to aflatoxin contamination because of the warm and humid climate of the region (Shotwell et al., 1974). Both *A. flavus* and *A. parasiticus* grow well and produce toxins when temperatures are >21 C (Wogan, 1975). However, climatic conditions are not the only factor that can affect aflatoxin production. Besides ambient temperature and moisture level of feed, fungal invasion is enhanced when plant stress occurs, such as in the case of drought or insect infestation, and by damaged or broken grain kernels (Jacques, 1988). Ranchers who store grain intended to be fed to wildlife should not assume that the grain is aflatoxin-free. Aflatoxin can be produced quickly in a wide range of climatic conditions and the tested storage practices did not limit aflatoxin production. Many styles of wildlife feeders allow grain to become moist enough to encourage mold growth and prolonged residence time of grain in the feeder can provide conditions conducive to mold growth, all of which increase the potential of exposure of wildlife to aflatoxins. Aflatoxin-contaminated corn used as bait was the suspected source for fatal aflatoxicosis of about 500 snow geese (*Chen caerulescens*) in Texas (Robinson et al., 1982).

Wildlife managers who feed grain and who are concerned about exposing wildlife to aflatoxin should test their grain periodically. Unfortunately, the black light test, which is inexpensive and quick, was not a reliable method for determining levels of aflatoxin up to 700 μg/kg. The inactivation of peroxidase during the artificial drying process used on commercial grain may have affected the efficacy of the black light test. Corn kernels that absorb water during storage may permit fungal growth and subsequent aflatoxin production, but fluorescence would not be detected due to the inactivation of the peroxidases (Fennell et al., 1973). Therefore, the more sensitive tests that quantify aflatoxins, although more costly, should be used.

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