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The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, west Africa

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Abstract

Aflatoxin level in 300 farmers' stores in four agro-ecological zones in Benin, a west African coastal country, were determined over a period of 2 years. At sampling a questionnaire was used to evaluate maize storage practices. Farmers were asked what storage structure they used, their storage form, storage period, pest problems in storage and what was done against them. Beninese farmers often changed their storage structures during the storage period, transfering the maize from a drying or temporary store to a more durable one. Most of the farmers complained about insects damaging stored maize. Often, storage or cotton insecticides were utilized against these pests. Regression analysis identified those factors that were associated with increased or reduced aflatoxin.

Maize samples in the southern Guinea and Sudan savannas were associated with higher aflatoxin levels and the forest/savanna mosaic was related to lower toxin levels. Factors associated with higher aflatoxin were: storage for 3–5 months, insect damage and use of *Khaya senegalensis*-bark or other local plants as storage protectants. Depending on the agroecological zone, storage structures that had a higher risk of aflatoxin development were the "Ago", the "Secco", the "Zingo" or storing under or on top of the roof of the house. Lower aflatoxin levels were related to the use of storage or cotton insecticides, mechanical means or smoke to protect against pests or cleaning of stores before loading them with the new harvest. Fewer aflatoxins were found when maize was stored in the "Ago" made from bamboo or when bags were used as secondary storage containers.

Keywords: Aflatoxin; West Africa; Maize; Storage systems; Pests; Storage structures

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1. Introduction

Maize is the most important cereal grown in the Republic of Benin. In 1996/1997, maize was produced on 517,985 hectares, with a total production of 504,506 tons for the same year (ONASA, 1997). In a study of 295 persons in Benin, 61% ate maize every day of the week and a further 23% consumed maize five to six times a week (Lutz, 1994).

Maize can be contaminated with storage fungi, some of which may develop as by-products mycotoxins that can be harmful to animal and human health. Mycotoxins that develop from *Aspergillus flavus*, a common postharvest fungus in maize, are called aflatoxins.

Aflatoxin content may vary with season and storage time (Lillehoj and Zuber, 1988). In Vietnam it was observed that toxin contamination was higher during the rainy season and increased with storage time (Le Van et al., 1995). Ahmad (1993) reported that aflatoxin contamination in storage was dependent on the storage system. In India, aflatoxin contamination was highest in the "kothi", made out of mud and rice husk, as compared to the "mora", made from paddy hay ropes wound into a container, to gunny bags or to iron bins (Prasad et al., 1987).

The maize storage practices in Benin vary with agro-ecological region and ethnic group. Storage structures in the south of Benin which has a bimodal rainfall pattern differ from the stores used in northern Benin, where the rainfall is unimodal (Fiagan, 1994). In the south, stores are constructed out of plant materials, whereas in northern Benin a high percentage of stores are built from clay (Fiagan, 1994). Fiagan (1994) observed that the storage of maize in an intermediary structure may lead to the contamination of maize with pests and pathogens. Often farmers will leave their maize on the floor in a corner of the room or in the courtyard, with the maize cobs in immediate contact with the floor, increasing the risk of *Aspergillus* development (Smith, 1991).

Only one study has previously looked at aflatoxin distribution in stored maize in Benin. Bouraima et al. (1993) recorded fast fungal development, especially in the southern regions of Benin, so that stored products were not edible after storage. The objectives of this study were to identify storage practices that were associated with higher or lower aflatoxin levels in stored maize in Benin, so that recommendations on aflatoxin-reducing practices can be given to extension services.

2. Material and methods

2.1. Agro-ecological zones, survey questionnaire and sampling

Four different agroecological zones of Benin were surveyed in 1993 to 1995 to assess farmers' maize storage practices and aflatoxin levels in maize samples collected from stores in each zone.

The agroecological zones in Benin lie in vertical bands from south to north. The southernmost is the forest/savanna mosaic (FSM) with two maize growing seasons, average rainfall between 1300 and 1500 mm and temperatures normally ranging between 25 and 35°C. The southern Guinea savanna (SGS) just north of the FSM, has the same growing seasons, rainfall averaging 1200–1300 mm and temperatures of 26–38°C. The northern Guinea savanna (NGS) has only one maize growing season from April to September, rainfalls between 1000 and 1100 mm and temperatures from 28 to 40°C. In the far north, the Sudan savanna (SS) has one growing season, low rainfall of less than 900 mm and average temperatures from 28 to 50°C.

Surveys were conducted in 30 villages in Benin and ten farmers were interviewed per village. The questionnaire was adapted from a similar study on the economic evaluation of insect damage on maize by Albert (1991) in Togo. Questions were asked about the storage practices, storage structures, pest problems in storage, and the farmers' solutions to these problems (Table 1). Answers were used to evaluate storage techniques, and all procedures after harvest and during storage. In 1993/94 maize was sampled from 300 farmers' stores at the beginning of storage. For the second sampling, six months later, the number of stores per village was reduced by half to give a total of 150 samples. Samples were collected from the same 150 farmers in the 1994/95 season at the same times. Those farming practices that could change

Table 1
Questionnaire concerning maize storage practices administered to 300 farmers in Benin Republic in 1993 and 1994

Storage practices

When is the harvested maize stored? Directly after harvest_____ Pre-storage_____

Why do you pre-store?______

Where do you pre-store? Field_____ In the house_____

For how many months do you store?_____

What storage method do you use? List_____

Where is your storage structure located? Field_____In the house_____Courtyard_____

What construction material did you use? Wood_____Clay_____ Metal_____

For how many seasons have you used the store?_____

Do you store maize in the store every season? No, why?_____Yes_____

Do you store other products in the store, together with maize? No_____Yes, list?_____

Where is your storage structure located?

Storage problems

Do you have storage problems? Yes____ No____

Which storage problem is the most important? Insects_____ Rodents_____Birds_____Mould_____ Others_____

When did you observe this problem?

At the beginning of storage

After a few months

At the end of storage

What did you do to solve this problem? List_____

Does the grain germinate in storage? Yes____ No____

Do you clean the storehouse before storage? Yes____ No____

Do you remove old grains? Yes____ No____

What else did you do to clean the store before storage? List_____

If you treated the storehouse before storage, what methods did you use? Ash_____Sand_____
Insecticides(specify)_____Smoke_____Manure_____Neem_____Others (specify)

How did you store your maize? As grain_____In the husk_____Dehusked_____Other_____

Did you use pesticides during storage? If yes, give name_____

Did you take any other precautions? List_____ from year to year were re-evaluated, i.e. storage structure, storage form, storage treatment and length of storage.

2.2. Aflatoxin analysis

Extraction was performed by the method of Singh et al. (1991). The samples were ground using a Romer® Mill, extracted with methanol/water (60:40, v/v) and then purified with chloroform. The extract was preserved in the refrigerator (5°C). The aflatoxin extract was spotted on silica-gel pre-coated plates (Sigma Chemical) and developed unidimensionally in a TLC tank in a chloroform/acetone (96:4, v/v) solvent system. Under longwave UV-light (365 nm) the fluorescence of the sample spot was compared to the fluorescence of the aflatoxin standard. The aflatoxin concentration was calculated according to Singh et al. (1991).

2.3. Statistical analysis

Stepwise linear regression was used to identify the factors that affected aflatoxin development ($p \leqslant 0.05$). Aflatoxin results were $\log(x + 1)$-transformed to normalize data before analysis (Zar, 1974). The answers to "yes or no" answers were entered as binomial values. Answers to categorical questions were entered as numbers. The statistical package used was SPSS (Norusis and SPSS, 1993).

3. Results

3.1. Maize storage practices in Benin

3.1.1. Pre-storage

Across agroecological zones, 70% of the farmers stored maize directly after harvest. If maize was not stored immediately this was due to lack of manpower, other priorities at that time of the year, or because storage structures were not ready. The pre-storage of maize in heaps in the field was common in the NGS (16.3%), whereas pre-storage in a room was prevalent in the FMS (23.8%) and the SS (33.3%). Pre-storage in the courtyard also occurred in the FMS (7.5%) and SS (3.3%).

3.1.2. Storage structures

Traditional storage structures in Benin varied with zone. The improved storage "Crib", as promoted by the FAO (Fiagan, 1994), was rarely accepted with only 6.3% of the farmers in the SGS using this structure. In the south of Benin, the "Ago", a giant basket made from woven raffia palms, tree branches or from bamboo was used by 71.2% in the FMS and 18.8% in the SGS. In the FMS maize is often stored on the "Ava" (8.7%), platforms that are between 30 and 50 cm off the ground on which maize cobs are arranged in layers. In the SGS 27.3% of the farmers stored maize under the roof of their house. The "Zingo", a granary with a wooden conical base posed on a stone and a thatched roof, was used by 30% of the farmers in the SGS and 31% in the NGS. Maize storage platforms that are raised 80–130 cm off the ground, sometimes higher when placed over the kitchen fire, were used by 7.5% (SGS) and 3.7% (NGS). Maize grains were stored in bags by 3.7% of the farmers in the NGS and 6.7% in the SS. In the NGS (46.3%) and SS (30%) maize was stored in the "Secco", a giant basket made from *Hypparhenia diplandra*. Otherwise in the SS maize was stored in clay stores (45%) or on top of the roof (6.7%).

In Benin many farmers used two stores during the storage season, with the initial store built in the field. Field stores were taken down in the dry season from February to April when bush fires and theft, because of depleting food stocks, might endanger the stored maize. In the SGS the "Zingo" and then the clay granary (22.5%) or bags (7.5%) were used. In the NGS farmers used the "Secco" in the field and then the clay granary near the house (8.8%), other farmers used the "Zingo" then bags (31.3%) or stored maize first in a room on the floor then in bags (6.3%). In the SS farmers used the "Secco" and then bags (3.3%), stored maize in a room on the floor and then in bags (5%) or the "Zingo" and then bags (1.7%).

Usually maize was stored alone except in the SS zone where farmers used clay granaries with three or four compartments in which maize was put with sorghum and groundnut (10%), or with cassava chips (10%). In the SGS, maize was stored with groundnut (10%), cowpea (12.5%) or cowpea and groundnuts (15%).

3.1.3. Length of storage

The time of storage varied between the ecozones (Table 2). Storage for 5–12 months was common in the FMS and SGS. In the FMS, 13.7% stored maize for more than 12 months, so that maize is stored after the new harvest comes in. In this area the size of maize stores is used to assess the wealth and social prestige of their owners and maize can be stored for up to three years. Maize was usually stored between 3 and 8 months in the NGS. In the SS a storage period of 7–12 months was practiced.

3.1.4. Storage form

Maize was stored with the husk, without the husk or as grains, depending on regional preference. In the FMS 91% and in the SGS 39% stored maize with the husk. In the NGS

Table 2
Farmers' responses concerning the length of their storage period (%)

Storage period	FMS[a]	SGS[b]	NGS[c]	SS[d]
3–5 months	11.2	12.5	36.2	10.0
6–7 months	23.8	17.5	31.2	20.0
8–10 months	27.5	51.2	23.8	30.0
11–12 months	23.8	18.8	8.8	36.7
> 12 months	13.7	0.0	0.0	3.3

[a] FSM = Forest/Savanna Mosaic.
[b] SGS = Southern Guinea Savanna.
[c] NGS = Northern Guinea Savanna.
[d] SS = Sudan Savanna.

45% of the farmers stored with the husk and 39% without the husk, while in the SS 57% stored without husk and 30% stored maize as loose grain. Another storage practice was to store with husk and then degrain: 24% in the SGS, 7.5% in the NGS, 12% in the SS used this storage form. Storage form was chosen by farmers with the aim of preventing insect damage, improve drying and/or prolong storage time.

3.1.5. Storage problems

More than 80% of the farmers across the ecoregion complained about storage problems. Farmers noticed primarily insects and rats at the beginning of storage (Table 3). Six months later the percentage of farmers who complained about insects damaging their stored maize increased to 87% (FMS), 95% (SGS), 90% (NGS) and 73% (SS) respectively. In the NGS 20% and in the SS 13% complained about lepidopterous pests. In the driest area, the SS 17% had problems with fungi, while 6.7% of the farmers reported fungi in the humid zone (FMS).

3.1.6. Storage treatments

The farmers reaction to storage problems varied. Approximately 50% of the questioned farmers did not do anything to counter storage problems. Otherwise, the storage treatment varied from farmer to farmer and within regions (Table 4). Those who treated their maize used commercial insecticides, either a specific formulation for stored grains such as Sofagrain® (pirimiphos-methyl and permethrin), Actellic® (pirimiphos-methyl) or Percal M® (permethrin and malathion) or insecticides commonly used against cotton pests. Few Beninese farmers used rodenticides. Another method of resolving storage problems was to sell the maize. Farmers also used traditional storage protectants like neem leaves (*Azadirachta indica*), pepper (*Piper guineense*), ash, ash mixed with sand, kerosene, smoke or manure. In the FMS 51% and in the SGS 49% were satisfied with the efficacy of their storage treatment. In the NGS only 27.5% and in the SS 30% felt that their control method resolved their storage problems. Farmers generally cleaned their stores and removed old stocks before loading them with new maize. Under certain conditions, e.g. heavy rains or bad roofing, maize in Benin could germinate

Table 3
Farmers' responses concerning storage problems at the beginning of storage (%)

Storage problems	FMS[a]	SGS[b]	NGS[c]	SS[d]
No problem	17.5	17.5	6.2	13.3
Molds	3.8	3.8	6.3	3.3
Insects	38.7	18.8	43.7	43.3
Rats	17.5	18.7	15.0	26.7
Rats + termites	0.0	2.5	1.3	1.7
Rats + insects	22.5	38.7	27.5	11.7

[a] FSM = Forest/Savanna Mosaic.
[b] SGS = Southern Guinea Savanna.
[c] NGS = Northern Guinea Savanna.
[d] SS = Sudan Savanna.

Table 4
Farmers' responses concerning storage treatment (%, multiple answers were possible)

Storage treatment	FMS[a]	SGS[b]	NGS[c]	SS[d]
No treatment	50.0	63.8	43.8	45.0
Storage insecticides	18.7	26.3	16.3	10.0
Cotton insecticides	17.5	18.3	1.3	6.7
Rodenticides	7.5	2.5	2.5	8.3
Traditional means	11.3	18.8	11.3	15.0
Sale	5.0	0.0	21.3	16.7
Sorting	2.5	0.0	5.3	6.7
Smoke	3.8	0.0	11.3	3.3
Mechanical means	0.8	6.3	6.3	5.0
Redo storage structure	0.0	1.3	1.3	0.0

[a] FSM = Forest/Savanna Mosaic.
[b] SGS = Southern Guinea Savanna.
[c] NGS = Northern Guinea Savanna.
[d] SS = Sudan Savanna.

while in store. To avoid this farmers redid the floor of their storage structure or changed the roofing branches.

3.2. Storage factors that influence aflatoxin level in stored maize in Benin

3.2.1. Aflatoxin levels

During the survey more than 750 maize samples were evaluated for aflatoxin levels over the 2 year period. Most of the samples across agroecozones and across sampling occasions had less than 5 ppb aflatoxin (Tables 5 and 6). At the beginning of storage between 2.2 and 5.8% of the samples showed toxin levels of more than 100 ppb, 6 months later the percentage of samples with such high levels was between 7.5 and 24% (Table 6). Comparing aflatoxin levels at the beginning of storage to those after 6 months of storage, there was an increase in the toxin levels in samples from the NGS and SS. The percentage of samples exceeding the 20 ppb

Table 5
Percentage of samples in aflatoxin classes per agroecological zone at the beginning of storage ($n = 443$)

Zone	< 5 ppb	5–10 ppb	10–20 ppb	20–50 ppb	50–100 ppb	> 100 ppb
FMS[a]	86.6	0.9	3.6	1.8	2.7	4.5
SGS[b]	67.8	5.0	5.8	8.3	7.4	5.8
NGS[c]	80.8	2.5	6.7	3.6	4.5	2.5
SS[d]	90.1	–	1.1	2.2	4.4	2.2

[a] FMS = Forest Mosaic Savanna.
[b] SGS = Southern Guinea Savanna.
[c] NGS = Northern Guinea Savanna.
[d] SS = Sudan Savanna.

Table 6
Percentage of samples in aflatoxin classes per agroecological zone after 6 months of storage (n = 301)

Zone	< 5 ppb	5–10 ppb	10–20 ppb	20–50 ppb	50–100 ppb	> 100 ppb
FMS[a]	85.0	3.8	2.5	1.3	–	7.5
SGS[b]	68.4	1.3	7.6	7.6	7.6	7.6
NGS[c]	71.3	5.0	11.3	–	5.0	8.8
SS[d]	67.8	1.6	4.8	1.6	–	24.2

[a] FMS = Forest Mosaic Savanna.
[b] SGS = Southern Guinea Savanna.
[c] NGS = Northern Guinea Savanna.
[d] SS = Sudan Savanna.

level at the beginning of storage, was 8.9% in the FMS, 21.5% in the SGS, 10.6% in the NGS and 8.8% in the SS. Six months later this limit was exceeded by 8.8% of the samples in the FMS, 22.8% in the SGS, 13.8% in the NGS and 25.8% in the SS.

3.2.2. Surveys in the 1993/94 season

Maize from stores in the SGS of Benin had a significantly higher risk of containing aflatoxin at the beginning of storage than that from the other zones (Table 7). Across all ecozones aflatoxin development was negatively related to the use of storage insecticides. High aflatoxin levels were associated with short storage periods of 3–5 months, and levels were lower when

Table 7
Storage factors that are significant[a] when regressed against aflatoxin levels (Y) at the beginning of storage (93–94) across and within agro-ecological zones[b,c]

Agro-ecological zone	Regression analysis	R^2	n	F-value
Across zones	$Y = 0.82 + 0.66x^1 - 0.37x^2 - 0.57x^3$	0.10	300	10.42**
FMS	$Y = 0.78 - 0.78x^4$	0.07	80	4.07*
SGS	$Y = 0.78 + 1.89x^5$	0.12	80	10.97**
NGS	$Y = 0.26 + 1.73x^6$	0.14	80	12.43**
SS	$Y = 0.16 + 2.24x^7$	0.10	60	6.51*
x^1 Sample from the SGS		$t = 3.57$**		
x^2 Maize stored for 8–10 months		$t = 2.14$*		
x^3 Storage insecticides used		$t = 3.50$**		
x^4 Storage insecticides used		$t = 2.23$*		
x^5 Maize stored for 3–5 months		$t = 3.31$**		
x^6 Maize stored in the "Ago"		$t = 3.53$**		
x^7 Maize stored in the "Crib"		$t = 2.55$*		

[a] F and t-tests.
[b] Agro-ecological zones: FSM = Forest/Savanna Mosaic SGS = Southern Guinea Savanna NGS = Northern Guinea Savanna SS = Sudan Savanna.
[c] *significant at < 0.05; **significant at < 0.01.

Table 8
Storage factors that are significant[a] when regressed against aflatoxin levels (Y) after six months of storage (93–94) across and within agro-ecological zones[b,c]

Agro-ecological zone	Regression analysis	R^2	n	F-value
Across zones	$Y = 0.96+0.69x^1-0.35x^2$	0.11	150	10.00**
FMS	no variable entered			
SGS	$Y = 1.59-1.54x^3+2.17x^4+0.66x^5$	0.45	40	9.89**
NGS	no variable entered			
SS	$Y = 1.22-1.25x^6+0.92x^7-0.86x^8$	0.45	40	7.09**
x^1 Sample from the SS		$t = 3.66$**		
x^2 Farmers were aware of storage problems		$t = 2.04$*		
x^3 Storage structure cleaned prior to use		$t = 3.09$**		
x^4 Maize stored with sorghum		$t = 3.15$**		
x^5 Maize stored as shelled grain		$t = 2.99$*		
x^6 Farmers notice maize germination		$t = 2.55$*		
x^7 Maize damaged by storage insects		$t = 2.79$**		
x^8 Maize stored as shelled grain		$t = 2.44$*		

[a] F and t-tests.
[b] Agro-ecological zones: FSM = Forest/Savanna Mosaic SGS = Southern Guinea Savanna NGS = Northern Guinea Savanna SS = Sudan Savanna.
[c] *significant at <0.05; **significant at <0.01.

maize was stored for 8–10 months. Maize stored in the NGS in the "Ago" and in the SS in the "Crib" had a higher risk of aflatoxin development.

The country-wide regression analysis of data from sampling 6 months after harvest showed a significant positive relationship between the SS zone and aflatoxin levels in the maize (Table 8). Those farmers that were aware of their storage problems were less likely to have aflatoxin development in their maize. In the SGS maize that was stored in the same store as sorghum or storage as grain maize was associated with higher aflatoxin levels. Cleaning of the store before storing the new harvest was associated with reduced aflatoxin levels in the SGS. In the SS reduced aflatoxin development was observed when grain maize was stored or maize germinated in the store. Insect damage observed on maize stored in the SS was related to high aflatoxin levels.

3.2.3. Surveys in the 1994/95 season

Across the country, the presence of aflatoxin in stored maize at the beginning of the 1994/95 season was negatively related to the use of cotton insecticides, storing maize as grains, storing maize over smoke or storing it in the "Ago" made from bamboo (Table 9). Aflatoxin risk increased when maize was stored for a period of 3–5 months or under the roof of the house. Likewise in the SGS, there was an increased risk of aflatoxin development if maize was stored under the roof. When maize in the NGS was stored in the "Secco" or the "Zingo" higher levels of aflatoxin were found in the grain. Storing maize in bags, after initial storage in another container, was associated with less aflatoxin contamination. In the same region *Khaya senegalensis* bark, used to protect maize against insects, increased the risk of aflatoxin development. In the SS, farmers that were aware of their post-harvest problems had less aflatoxin, while those that stored maize on top of the roof had more.

Across agro-ecological zones, the aflatoxin risk in samples stored for 6 months increased when maize was stored as grains, when the store was more than 5 years old and when fresh plant materials were used as storage protectants (Table 10). Higher aflatoxin levels were found in the SGS. In the FMS, aflatoxin risk was reduced when farmers noticed that grains germinated in storage. In the SGS, high aflatoxin levels were positively related to maize being stored with cowpea. A reduction of aflatoxin contamination was achieved through the use of mechanical means, such as rodent traps, rat guards or sorting to safeguard against storage pests. In the NGS, storage of maize for 6–7 months was associated with reduced aflatoxin contamination, whereas the use of traditional plants to protect the store against pests or storage of maize for 3–5 months increased the risk of aflatoxin contamination. In the SS, aflatoxin levels were positively related to maize being protected with local plant materials, storing it on top of the roof or storing in containers that were more than 5 years old. Unlike the 1993/94 sampling, in this period of storing maize, the “improved” crib was associated with lower aflatoxin contamination.

Table 9
Storage factors that are significant[a] when regressed against aflatoxin levels (Y) at the beginning of storage (94–95) across and within agro-ecological zones[b,c]

Agro-ecological zone	Regression analysis	R^2	n	F-value
Across zones	$Y = 0.71 + 0.39x^1 - 0.74x^2 - 0.54x^3 - 0.64x^4 - 0.46x^5 + 0.50x^6$	0.21	150	6.62**
FMS	no variable entered			
SGS	$Y = 0.60 + 0.75x^7$	0.12	40	5.03**
NGS	$Y = 0.32 + 2.13x^8 - 0.55x^9 + 0.74x^{10} + 1.07x^{11}$	0.46	40	10.70**
SS	$Y = 1.44 - 65x^{12} + 1.28x^{13}$	0.27	30	8.14**
x^1 Maize stored for 3–5 months		$t = 2.21$*		
x^2 Farmers use smoke to protect stored maize		$t = 2.12$*		
x^3 Farmers use cotton insecticide as storage protectant		$t = 3.22$**		
x^4 Maize store as shelled grain		$t = 2.70$*		
x^5 Maize stored in “Ago” of bamboo		$t = 2.12$*		
x^6 Maize stored under roof of house		$t = 2.40$*		
x^7 Maize stored under roof of house		$t = 2.24$*		
x^8 Use of *Khaya senegalensis* as storage protectant		$t = 3.73$**		
x^9 Use of bags as secondary storage		$t = 2.74$*		
x^{10} Maize stored in “Secco”		$t = 3.18$**		
x^{11} Maize stored in conical stores		$t = 4.16$**		
x^{12} Farmers aware of insect damage in store		$t = 2.51$**		
x^{13} Maize stored on the roof of the house		$t = 2.85$**		

[a] F and t-tests.
[b] Agro-ecological zones: FSM = Forest/Savanna Mosaic SGS = Southern Guinea Savanna NGS = Northern Guinea Savanna SS = Sudan Savanna.
[c] *significant at <0.05; **significant at <0.01.

Table 10
Storage factors that are significant[a] when regressed against aflatoxin levels (Y) after six months of storage (94–95) across and within agro-ecological zones[b,c]

Agro-ecological zone	Regression analysis	R^2	n	F-value
Across zones	$Y = 0.22 + 1.64x^1 - 0.26x^2 - 0.74x^3 - 0.26x^4$	0.22	150	10.14**
FMS	$Y = 0.14 + 1.11x^5 + 0.63x^6 - 0.18x^7$	0.41	40	9.12**
SGS	$Y = 0.62 + 0.61x^8 - 0.80x^9$	0.23	40	5.53**
NGS	$Y = 0.66 - 0.50x^{10} + 1.25x^{11} + 1.22x^{12}$	0.41	40	8.27**
SS	$Y = 0.16 + 2.18x^{13} + 2.01x^{14} - 2.16x^{15} + 0.70x^{16}$	0.85	30	36.55**
x^1 Use of traditional plants as storage protectants		$t = 3.88$**		
x^2 Maize stored as shelled grain		$t = 2.61$*		
x^3 Storage container > 5 years old		$t = 2.78$**		
x^4 Sample from SGS		$t = 2.40$*		
x^5 Storage containers > 5 years old		$t = 3.33$**		
x^6 Use of traditional plants as storage protectants		$t = 3.47$**		
x^7 Farmers notice germination of maize		$t = 2.08$*		
x^8 Maize stored with cowpea		$t = 2.38$*		
x^9 Use of mechanical storage protectant		$t = 2.74$**		
x^{10} Maize stored for 6–7 months		$t = 3.02$**		
x^{11} Use of traditional plants as storage protectants		$t = 2.43$*		
x^{12} Maize stored for 3–5 months		$t = 2.37$*		
x^{13} Use of traditional plants as storage protectants		$t = 7.36$**		
x^{14} Storage containers > 5 years old		$t = 9.39$**		
x^{15} Maize stored in "Crib"		$t = 6.09$**		
x^{16} Maize stored on roof of the house		$t = 2.66$**		

[a] F and t-tests.
[b] Agro-ecological zones: FSM = Forest/Savanna Mosaic SGS = Southern Guinea Savanna NGS = Northern Guinea Savanna SS = Sudan Savanna.
[c] *significant at < 0.05; **significant at < 0.01.

4. Discussion

There was an increase in the percentage of samples showing high aflatoxin levels from the beginning of storage to 6 months later. The means observed in these aflatoxin positive samples at the beginning of storage were between 22 and 190 ppb and 6 months later between 31 and 221 ppb (Hell, 1997). Thus, the risk of chronic exposure to aflatoxin in Benin is high, since most of the maize is produced for human consumption and Beninese are known to consume maize-based meals up to three times per day (Lutz, 1994).

The data collected during this study support the results from previous authors showing how *A. flavus* interacts with storage factors. It was previously observed that aflatoxin was related to storage structure (Ahmad, 1993; Prasad et al., 1987), length of storage time (Lillehoj and Zuber, 1988) and storage pests (Sinha and Sinha, 1991, 1992).

4.1. Storage structure

Maize in Benin that was stored under the roof of the house was associated with higher aflatoxin levels. This storage method was only found in the southern zones, FMS and SGS, where, due to the bimodal rainfall distribution, at least part of the harvest may occur during a rainy period. *A. flavus* does not exhibit extended growth below the a_w of 0.85 (Sauer and Burroughs, 1980) or 17% grain moisture (Kawasugi et al., 1988), thus bringing partially dried maize ears into a dark space, away from any sunshine or air movement would not be a good practice. Another predisposing factor affecting maize below a metal sheeting would be high temperatures which are known to be advantageous to *A. flavus* and aflatoxin production (Gonzales et al., 1988; Cotty et al., 1994).

Another storage structure associated with aflatoxin development was the "Ago" in the NGS. It seems that this storage system was not adapted to the climatic conditions of the northern savannas as, most of the farmers who used these stores had migrated to this area importing their traditional stores from the southern climatic zone. The aeration of these structures was poor when they were filled to the maximum, and maize stored with the husk or when not well dried resulted in increased fungal development (Smith, 1991). The "Crib" in the SS gave mixed results in the regression against aflatoxin levels. The positive relation of aflatoxin in the 1993/94 storage period with maize that was stored in the "Crib" may have been due to an outlier that had a very high toxin level. The following year, in the same zone, the "Crib" was associated with lower aflatoxin. In a similar study in Nigeria, Udoh (1997) also showed reduced risk of aflatoxin contamination in the "Crib" stores which were used by 8% of the farmers in the NGS of Nigeria.

The "Ago" made out of bamboo, was associated with lower aflatoxin when used in the SGS. These results are confirmed by the FAO, that showed that an introduced improved bamboo storage container in the same form as the "Ago", dried stored maize from 20% grain moisture at harvest to 14% after 3 months of storage (FAO, 1992). Usage of bags as a second storage structure later in the storage season was associated with lower toxin levels. The change in storage structure in Benin is usually accompanied by maize processing that involves either dehusking or degraining. Most farmers also sort maize at this time: discolored grains that might have been infected with fungi are sorted out and the potential for aflatoxin development is consequently reduced.

With increasing age of the storage structure, the risk of aflatoxin contamination increased. Most of the storage structures were used for 1–3 years before major parts of the storage structure had to be replaced. Traditional stores that are utilized for more than 5 years are those made from clay or wood. Prasad et al. (1987) reported in surveys of traditional storage structures in India, that the amount of aflatoxin contaminating the stored cereal samples was between 430 and 2830 ppb, and the greatest amount was detected in cereals stored in the "Kothi" made out of clay. In clay stores, humidity build-up might occur through convection, and this could explain why *Aspergillus* spores could persist for a longer time in these type of stores, increasing the risk of aflatoxin contamination. In rare cases farmers light fires under the storage structures to reduce the humidity and control insects inside the store. Smoking of maize in Benin was associated with lower aflatoxin contamination. Smoking was a very effective mean of protecting maize against storage insects and compared well with chemical insecticides like Actellic® (pirimiphos-methyl) (Daramola, 1986). Udoh (1997) reported that between 3.6 and 12% of the farmers in the different agro-ecological zones of Nigeria used smoke to protect their maize and aflatoxin levels decreased when smoke was used to protect maize.

4.2. Storage time

In this study it was noticed that regional and ethnic differences existed in the length of storage. The "Mina" ethnic groups in southern regions stored their maize for long periods apparently for sociological reasons, since large and multiple maize stores bring social esteem for their owners (Smith, 1991). In general, however, storage periods in the south were shorter, because farmers were not forced to conserve their maize for long, as the second season crop was harvested in December. Farmers in the north cultivated higher acreages of maize and usually used improved varieties, and as a consequence produced more maize per hectare that would last for longer periods.

The influence of storage time on aflatoxin content was only noticed for a storage period of 3–5 months, which generally resulted in a higher aflatoxin content of the stored maize samples. It seems that farmers who stored maize for a short period, do not take as many precautions nor care as much as those that store maize for a long period. The former consumed the maize as quickly as possible, while farmers who intended to sell the maize after a longer storage period were more likely to dry carefully, sort out damaged cobs, and use insecticides.

4.3. Storage form

In the southern zones of Benin, a high percentage of farmers stored maize with the husk. Farmers also selected cobs for good huskcover before storing them. Good huskcover serves as a barrier against insect attack and water seepage. In India, maize with good huskcover had lower aflatoxin levels (Bilgrami et al., 1991). The same conclusion was drawn by McMillian et al. (1987) who found lower aflatoxin contamination in maize varieties that were judged to have a tight huskcover in 2 out of 3 years. The influence of storage with or without husk on aflatoxin development in Benin was zone specific and depended on the prevalence and type of insect pest. There are reports of higher development rates of insects on maize stored as loose grains (Kossou et al., 1992; Vowotor et al., 1995) which would have an aflatoxin-increasing effect (Sinha and Sinha 1991, 1992; Wright, 1992). In Benin, storing maize with the husk was common in the southern agroecological zones for protection against Coleoptera, but farmers in the north, where lepidopteran ear borers would continue to feed inside the husk and sun drying was possible, preferred removing the husk.

4.4. Storage pests

In a study by the National Plant Protection Service in Benin, the majority of farmers complained about storage insects affecting their maize, with rats also being a constant problem (SPV/GTZ, 1992). This is supported by this study where, in all zones except the SGS, more than 40% of the farmers cited insects as the principal pest observed in storage, and around 20% cited rats and insects. The pressure of insects on stored maize under West-African conditions can be very high, with grain losses of up to 30% recorded in Togo for a storage season from 6 to 9 months (Pantenius, 1988). There was a negative relationship between farmers that complained about storage problems and aflatoxins. It seems that farmers who noticed when their maize was damaged by pests or fungi, took measures to reduce these problems. The same was observed for those farmers who noticed that their maize germinated in store, which was negatively related to aflatoxin content. The cleaning of grain stores of the remains of the previous harvest is one of the basic hygiene measures to combat storage problems (Smith, 1991). The regression analysis in this study revealed that aflatoxin levels were lower when farmers cleaned their grain stores before storing the new harvest.

In this study, use of insecticides reduced the risk of aflatoxin contamination. Wright (1992) remarked that *A. flavus* contamination was strongly correlated with high densities of weevils. Since the early 1960s, researchers have found that insect damage was highly associated with aflatoxin contamination (Sinha and Sinha, 1991). The same authors (1992) showed that *A. flavus* infection in insect-damaged grain was 87%, while in insect-free samples it was 25%. It is well documented that insects can move *Aspergillus* spores in the store (McMillian et al., 1987, 1990; Lynch and Wilson, 1991; Lynch et al., 1991; Gorman and Kang, 1991). Insects have also been shown to act as vectors of fungal spores (Dowd, 1991, 1994). Mutiro et al. (1992) evaluated insect damage and aflatoxin development on maize in traditional and improved storage structures in Zimbabwe. When pirimiphos-methyl was applied to stored maize, insect damage was significantly reduced and no aflatoxins were detected. Sétamou et al. (1998) showed significantly higher levels of aflatoxin in pre-harvest maize in Benin when the earborers *Mussidia nigrivinella* Ragonot and *Sesamia calamistis* Hampson were present in the ear. These borers continue to cause feeding damage for up to a month after the ear is harvested.

4.5. Storage treatment

One of the problems with pesticide use in Benin is the widespread use of cotton insecticides on stored grains (SPV/GTZ, 1992). In this study up to 18.3% of the farmers used cotton insecticides to protect their stored grains. Cotton pesticides in Benin are distributed on a credit basis through the state extension service and are more readily available than the recommended storage insecticides such as Actellic®, Sofagrain® or PercalM® (SPV/GTZ, 1992). Cotton insecticides have a higher toxicity and persistence so that they constitute a danger to the consumer especially when ingested soon after treatment. The abuse of highly toxic pesticides for the control of storage pests in developing countries is a recurring problem (Wasilewski, 1987; SPV/GTZ, 1992). Udoh (1997) in Nigeria also found that inappropriate pesticides such as Furadan®, Aldrex®, and Gammalin 20® were used on stored maize.

Damage by insects is often followed by moulds because insects produce a microclimate propitious for development of storage fungi (Tuite, 1984). In a study by El-Kady et al. (1993) it was observed that Actellic® did not have any direct effect on *A. flavus* development in maize grains, thus it was concluded that the aflatoxin-reducing effect of insecticides is a secondary effect through the reduction of insect infestation. Farmers who used insecticides to protect their stored maize usually had fewer problems with aflatoxins.

In this study, application of local plant substances to protect against storage insects increased aflatoxin concentration in the stored maize samples. This is contradictory to many studies in which plant substances were used in vitro to control growth of *Aspergillus* fungi

(Bhatnagar and McCormick, 1988; Dube et al. 1990; Cardwell and Dongo, 1994). It seems that the in vitro effect cannot necessarily be achieved by applying plant substances directly to the stored cobs. Many plant substances that were used in store are also used in traditional medicine. It has been reported that *A. flavus* can grow on medicinal plants and develop aflatoxins (Narita et al., 1988; Roy and Kumari, 1991). Thus the mixing of plant substances with stored cobs may actually increase the risk of aflatoxin development instead of controlling it. Also plant materials such as leaves may increase the r.h. inside the grain store. When farmers in Benin compared the efficacy of their traditional products with the commercially available products, they always rated the indigenous solutions as being less efficient, but they used these substances because they had no access to or could not afford chemical products.

4.6. Storage with other commodities

Aflatoxin contamination was higher in maize stored with cowpea. Cowpea may become infected with *A. flavus* in the field (Gill et al., 1983; Umechuruba, 1985), and aflatoxin development has been observed in cowpea seeds (Seenappa et al., 1983; Koehler et al., 1985). Increased aflatoxin contamination was also noticed when maize was stored with sorghum. Sorghum can also be infected with *A. flavus* (Usha et al., 1994) and aflatoxin development was observed in sorghum grains in the field (McMillian et al., 1983). No correlation between the storage of groundnuts together with maize and an increase in aflatoxin development was observed in Benin, even though groundnuts are easily infected with *A. flavus* in Nigeria (McDonald, 1964; McDonald and Harkness, 1967), and aflatoxin development has been described by many authors (Mehan et al., 1991).

5. Conclusions

Several storage factors that may help to reduce aflatoxin levels in stored maize in Benin were identified in the present study: control of storage insects through the sorting out of damaged cobs, the use of appropriate storage insecticides and "awareness" of the farmers of the risk that insects and aflatoxins present to their stored maize. Use of a storage container that is suitable for the agroecological region also helps to control toxin levels. Further tests are necessary to show how storage form influences aflatoxin levels in the different agro-ecological zones of Benin.

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