



CHAPTER THREE

IMPACT OF INDIGENOUS STORAGE SYSTEMS AND INSECT INFESTATION ON THE CONTAMINATION OF MAIZE WITH FUMONISINS

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ABSTRACT

Four storage systems of maize commonly used by farmers in Benin, West Africa, were tested to determine their impact on infection of maize with *Fusarium* fungi and subsequent contamination with fumonisins. The study showed that *F. verticillioides* was the predominant *Fusarium* species found in all maize samples. *Fusarium* incidence was significantly higher when maize was stored on a cemented floor in a house (40.3 ± 17.4 %) than in the other systems. The lowest *Fusarium* incidence was recorded when maize was stored in a bamboo granary (25.5 ± 13.5 %) ($p < 0.05$). This suggests that storage systems used by farmers may affect infection of maize with *Fusarium*, if these systems create conditions favourable to fungal growth. The storage systems did not have a significant effect on contamination by fumonisin, total fumonisin level being not significantly different from one system to another ($p > 0.05$). This indicates that a high *Fusarium* infection level in maize in a storage structure during the storage period may not necessarily result in a high level of contamination by fumonisin. Fumonisin content significantly decreased over the storage period depending on storage systems. A 35 % decrease in fumonisin level over the storage period was observed in maize stored on the cemented floor in a house, 41 % in that stored on a platform, 57 % in maize stored in a mud silo, and 76 % in maize stored in a bamboo granary. Damage by lepidopterous pests was significantly and positively correlated with both infection of maize with *Fusarium* ($r = 0.8$, $p < 0.01$) and contamination by fumonisin ($r = 0.9$, $p < 0.01$). In general, the number and damage of coleopterous insects were significantly and negatively correlated with infection of maize with *Fusarium* and contamination by fumonisin, but one of them, *Sitophilus* spp., was found positively but not significantly related to *Fusarium* during the first month of storage ($r = 0.2$, $p > 0.05$).

Key words: Benin, maize, *Fusarium*, fumonisins, insect infestation and storage systems,

INTRODUCTION

In Benin like in most Sub-Sahara African countries, maize is generally harvested late and is stored in cob form in wooden granaries, under the roofs of the farmers' houses, or on cemented floors in houses (Fiagan 1994, Hell *et al.* 2000a). Maize is also stored in grain form in clay containers, mud silos, or in bags. Most of these systems create inadequate storage conditions unfavourable for good drying of maize, particularly in humid and semi-humid zones. They consequently promote the development of fungi and subsequent production of mycotoxins. There are two important mycotoxigenic fungi mostly found associated with stored maize. These are *Aspergillus flavus* that produces aflatoxins (Hell *et al.* 1995, Udoh *et al.* 2000) and *Fusarium verticillioides*, which produces fumonisins (Marasas *et al.* 1979).

Fumonisin is a recently discovered mycotoxin (Gelderblom *et al.* 1988). They cause fatal diseases in horses and swine, possess cancer-promoting activity in rats, and are associated with porcine pulmonary oedema (Nelson *et al.* 1994). Oesophageal cancer in humans has been related to consumption of maize with high concentrations of fumonisins (Marasas 1995, Wang *et al.* 2000). The International Agency for Research on Cancer (IARC) has evaluated fumonisin B₁ (FB₁) as possibly carcinogenic to humans, belonging to the group 2B carcinogens (IARC 2002). The US Food and Drug Administration (FDA) had recommended that the fumonisin levels in human foods not exceed 4 mg kg⁻¹ (FDA 2000), whereas Switzerland proposed 1 mg kg⁻¹ as tolerance level for fumonisins in dry maize products intended for human consumption (Marasas *et al.* 2001).

It is likely that storage systems of maize with components such as time of harvest, type of storage structure, hygiene and insect infestation, interact and influence fungal infection and mycotoxin contamination. Hell *et al.* (2000a) for instance, found higher aflatoxin levels when maize was stored under or on top of the roof of farmers' houses, than in ventilated granaries. In Nigeria, Udoh *et al.* (2000) showed that insect infestation in maize stores was correlated with aflatoxins and that higher levels were found in maize stored in mud silos. Insects present in maize stores can act as wounding or spreading agents, providing an opportunity for the fungus to circumvent the natural protection of the integument and establish infection sites in vulnerable interior (Bilgrami and Choudhary 1998, Dowd 1998).

Regarding the infection with *Fusarium* and the contamination by fumonisin, a survey conducted in 2000 in Benin showed high levels of fumonisins in villages where maize cobs were stored under the roofs of farmers' houses and on the cemented floors in houses. Storage conditions were suspected to be linked to these elevated fumonisin levels (Gnonlonfin 2000).

The present study was undertaken to further consider the influence of different storage systems used in Benin on the natural occurrence of *Fusarium* and fumonisin contamination in maize. In addition, the effect of insect damage on maize infection by *Fusarium* and contamination by fumonisins was evaluated.

MATERIAL AND METHODS

Research site

The experiment was conducted from August 2001 to April 2002 in Benin, West Africa, precisely in Yè, a village where a high fumonisin level (12 mg kg^{-1}) was recorded during the national survey of 2000 (Gnonlonfin 2000). This village is situated in the Southern Guinean Savannah, a region that has been reported to have high aflatoxin contamination (Setamou *et al.* 1997, Hell *et al.* 2000b, Udoh *et al.* 2000). The annual rainfall pattern of this region is bimodal with precipitation averaging 1100 – 1500 mm, allowing for two maize growing seasons. The first and main growing season runs from April to July, whereas the second and shorter season is from September to November. Maximum temperatures range from 26 to 35°C and annual relative humidity averages 85 – 90 %.

Maize cultivar

The 90-day cultivar DMR-ESR-W, an improved IITA variety, was used. It is the improved variety most commonly recommended to farmers in Benin. DMR is resistant to downy mildew (*Peronosclerospora sorghi*) and to maize streak virus (Schulthess *et al.* 2002). Maize cobs were harvested in August according to the farmers' practice of harvesting late in order to obtain grain moisture content less than 20 %.

Experimental design

The experiment compared the development of *Fusarium* in maize during storage and contamination by fumonisins in improved and traditional storage systems. Four storage systems were assayed and there were three replicates for each system, arranged in a randomised block design. The storage systems included:

- Maize cobs with husks, harvested late (about one month after maturity), were stored for 8 months on the cemented floor in a house. This is the traditional system commonly used in the experimental area.
- Maize cobs with husks were stored for 8 months in an aerated woven bamboo granary (Fig 1).
- Maize cobs with husks were stored for 8 months on a platform (Fig 2).
- Maize cobs with husks were stored for 4 months on a platform, shelled, and grains stored, after hand sorting, in a mud silo for 4 months (Fig 3).

The latter three systems are indigenous storage systems improved by research and recommended to farmers. The storage structures (bamboo granary, platform and mud silo) were installed outside, each covered with a thatched roof. There was no insect control, but the structures were not accessible to rodents. Each wooden pole supporting the bamboo granary and platform had a simple but effective device to exclude rodents (Fig 1 and 2).

Sampling method

Fifty cobs were randomly sampled at 0, 1, 4, 5, 7 and 8 months after stocking in each storage system. The cobs were collected in each granary at top (10 cobs), in centre and on sides of the granary (30 cobs) and at bottom (10 cobs). The cobs were dehusked and manually shelled. Thereafter, a sub-sample of 1 kg of grains was taken from each sample. This sub-sample was then equally divided into two lots of 500 g. The first, unground, was intended for moisture, insect and fungal evaluations, whereas the second, ground, was intended for fumonisin analyses (Fig 4).

Laboratory analyses

Grain moisture content was determined on-farm just after sampling using an electronic moisture meter (model HOH-EXPRESS HE 50, PFEUFFER, Germany). Determination of grain moisture content was replicated three times. For insect evaluations, samples were sieved. Insects present in each sample were counted and identified in the laboratory using keys from NRI (1991) and Weidner and Rack (1984). Damage caused to grains by

lepidopterous and coleopterous insects were separately assessed on the basis of a 1000-grain sub-sample (Setamou *et al.* 1998), using the Pantenius formula as follows (Pantenius 1988):

$$\% \text{ of insect damage} = \frac{B}{A} \times 100$$

A = total number of grains in a sub-sample. In the present experiment, A = 1000

B = number of damaged grains by insects in the sub-sample

Twenty-five grains from each sample in four replicates (100 grains) were surface disinfested in a 10 % sodium hypochlorite solution for 2 min and rinsed twice in distilled water. The grains were plated in Petri dishes containing Potato Dextrose Agar (PDA) with five grains per Petri dish. The Petri dishes with grains were incubated at 25 °C with a 12:12-hour light and dark regime. After seven days of incubation, fungi were identified. *Fusarium* were isolated, transferred onto Carnation Leaf Agar (CLA) and incubated at 25 °C for seven days with a 12:12-hour light and dark regime. *Fusarium* species were identified using keys from Nelson *et al.* (1983) and Pitt and Hocking (1999). Total fumonisin content was determined at 0, 4 and 8 months after stocking using the VICAM method (VICAM 1998).

Statistical analyses

Statistical analyses were performed using SPSS for Window version 10.0 (SPSS Inc., Chicago, Illinois). Analysis of variance (ANOVA) and Tukey's HSD test were used to compare the means of fungal incidence and total fumonisins detected in each storage system and throughout the storage period. Pearson correlation test was used to assess relationships among *Fusarium* incidence, fumonisin level and damage by lepidopterous and coleopterous insects. The same test was used to assess the relationship between *Aspergillus* incidence and insect damage. Before analyses, insect numbers were transformed to log (x+ 1), but the data are presented untransformed.

RESULTS

Fungal incidence, grain moisture content and fumonisin levels in the different storage systems

The incidence of fungi in maize subjected to the different storage systems is presented in Table 1. The major genera found were *Fusarium* spp. (32 %), *Aspergillus* spp. (34 %) and *Penicillium* spp. (27 %). *F. verticillioides* was found in all samples. Total fungal incidence was significantly higher in maize stored on the cemented floor in a house than in the other storage systems ($p < 0.05$). *Fusarium* incidence was also significantly higher when maize was stored on the cemented floor in a house (40.3 ± 17.4 %) and lower in maize stored in the bamboo granary (25.5 ± 13.5 %) ($p < 0.05$). There was a significant decrease in *Fusarium* incidence throughout the storage period, from 48.2 % at harvest to 11.7 ± 3.4 % after eight months of storage ($p < 0.01$) (Fig 5). However, this trend was much lower in maize stored on the cemented floor in a house, than in the other storage systems. When maize was stored on the floor in a house, a noticeable increase of *Fusarium* incidence was observed during the first month of storage, whereas in the other systems, it steadily decreased (Fig 6). In contrast to *Fusarium*, *Aspergillus* incidence increased from 5.3 % at harvest to 45.7 ± 3.4 % after eight months of storage ($p < 0.01$), and this trend was markedly greater in maize stored on the floor in a house (Fig 7).

In maize stored in a mud silo following four months on a platform, there was an apparent decrease of *Fusarium* incidence from the fourth to the fifth month after storage, from 32.1 ± 3.1 % to 27.0 ± 0.6 % (Fig 6). This decrease, probably due to sorting after shelling the maize cobs at 4 months of storage, was not statistically significant ($p > 0.05$).

There was no significant difference in maize grain moisture content (Fig 8) measured for the different storage systems ($p > 0.05$). It was, however, found that at eight months of storage, overall mean of grain moisture content was about 18% in the maize stored on the cemented floor in a house and 15 % in the other storage systems. On the other hand, grain moisture content significantly decreased during the storage period in the different storage systems. However, it remained numerically but not significantly higher in maize stored on the floor in a house (Fig 8). Grain moisture content increased in all the storage systems from the fourth month of storage (Fig 8), coinciding with the beginning of the rainy season in the

experimental area. It is likely that the increase of the relative humidity during this rainy period induced the increase of the grain moisture content.

All maize samples from the different storage systems contained fumonisins with levels ranging from 0.6 to 2.3 mg kg⁻¹, and all means higher than 0.1 mg kg⁻¹ (Table 2). There were no significant differences between the contamination levels in the storage systems ($p > 0.05$), although the total fumonisin level was slightly higher when maize was stored on the floor in a house (1.9 ± 0.3 mg kg⁻¹) and lower in maize stored in a bamboo granary (1.2 ± 0.8 mg kg⁻¹) (Table 2). Overall, total fumonisin level was the highest in freshly harvested maize (2.3 mg kg⁻¹), and significantly decreased during the storage period ($p < 0.01$) (Fig 9). This decrease was, however, lower in maize stored on the cemented floor in a house than in the other storage systems (Fig 9). There was a 35 % decrease in fumonisin level in maize stored on the cemented floor in a house, 41 % in that stored on a platform, 57 % in maize stored in a mud silo, and 76 % in maize stored in a bamboo granary. A significant difference was found among the storage systems with respect to the percentage of decrease ($p < 0.01$).

Influence of insect infestation on Fusarium infection and fumonisin contamination

The borer *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) and to a lesser extent the cob feeder *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) were the lepidopterous insects encountered on maize cobs in all storage systems. They were not very numerous but were most frequently found at harvest and during the first month of storage (Table 3). For coleoptera, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), *Cathartus quadricollis* Guerin (Coleoptera: Cucujidae), *Tribolium* sp. and *Carpophilus* sp. were the species mostly found. They were predominant from the fourth month of storage (Table 3). *P. truncatus* was most common in the bamboo granary (318.1) and in maize stored on the platform (54.5) (data not shown).

Significant positive correlations were found between the number of lepidopterous insects and *Fusarium* infection (Table 4). *M. nigrivenella* ($r = 0.7$, $p < 0.01$) and *C. leucotreta* ($r = 0.4$, $p < 0.01$) were significantly related to *Fusarium* infection. Moreover, they were positively and significantly correlated with fumonisin contamination ($r = 0.8$, $p < 0.01$) and ($r = 0.6$, $p < 0.01$) for *M. nigrivenella* and *C. leucotreta*, respectively. There was also a significant and positive relationship between the lepidopterous insect damage and both *Fusarium* infection ($r = 0.8$, $p < 0.01$) and fumonisin contamination ($r = 0.9$, $p < 0.01$) (Table

4). Regarding the coleopterous insects, their number and damage was significantly and negatively correlated with *Fusarium* infection and fumonisin contamination (Table 4). Conversely, the lepidopterous insects *M. nigrivenella* ($r = - 0.8, p < 0.01$) and *C. leucotreta* ($r = - 0.6, p < 0.01$) were significantly and negatively related to *Aspergillus* infection. As for the coleopterous insects, with exception to *P. truncatus*, a positive and significant relationship existed between them and *Aspergillus* infection (Table 4).

DISCUSSION

The results have shown that storing maize on a cemented floor in a house is not an appropriate practice. This system appears to be more favourable for fungal development, compared to the other tested systems. Grain moisture content was high (18 %) and *Fusarium* incidence was also somewhat higher under these conditions. Generally, storage conditions in farmers' houses are confined and maize cobs are usually stored in a maizeer of the house, which is not always well ventilated. Throughout the storage period, grain moisture content, therefore, diminishes slowly but remains high enough to promote fungal development. In a recent study in Benin, Hell *et al.* (2000a) found that storing maize in non-ventilated conditions such as under the roof of the farmer's house has a higher risk of aflatoxin development. The authors related these findings to the fact that this storage system is used in the humid zones of Benin, where the rainfall pattern is bimodal with averages ranging from 1200 mm to 1500 mm, the mean relative humidity of air mostly around 90 %, and part of the harvest occurring during a rainy period. Pitt and Höcking (1999) reported that high humidities are likely to create suitable conditions for fungal development. This is linked to the fact that during storage, *Fusarium* requires a substrate with high moisture content ranging from 18 to 23 % for optimal growth (Christensen and Kaufmann 1974, Bacon and Nelson 1994, Orsi *et al.* 2000).

The woven bamboo granary and platform are typically ventilated storage structures. They are potentially less favourable for fungal growth and results obtained in the present study show them to be less conducive to *Fusarium* development during maize storage. Hell *et al.* (2000a) also found bamboo granaries to be associated with lower aflatoxin levels in the humid regions. It is thought that this structure allows maize grains to dry more rapidly from 20 % of moisture content at harvest to 14 % after three months of storage, provided that its diameter is not more than 2 m (FAO 1992).

Mud silos are durable storage structures better adapted to dry regions (Fandohan 2000). They are commonly used in the northern parts of West-African countries, where

environmental conditions are less humid. Mud silos are closed and non-ventilated granaries. Therefore, they are considered as favourable for fungal development, if maize grains moisture content is above 15 % or if maize storage occurs during the rainy season, where the relative humidity of air is higher than 90 % (Fandohan 2000). Prasad *et al.* (1987) found mud silos in India associated with high aflatoxin contamination. Hell *et al.* (2000a) postulated that humidity build-up might occur through convection, permitting *Aspergillus* spores to persist for a long time in a granary leading to a high risk of aflatoxin contamination. To overcome this risk, maize must be sufficiently dried (moisture content less than 15 %) to ensure unfavourable conditions for fungal development. In this study, the prestorage of maize on a platform before its final storage in a mud silo reduced grain moisture content from 22.5 % at harvest to less than 11 % after four months, hence creating unfavourable conditions for the development of fungi.

The overall changes observed in the incidence of both *Fusarium* (a significant decrease) and *Aspergillus* (a significant increase) with the storage time is probably due to storage conditions, unfavourable to *Fusarium* development and favourable to that of *Aspergillus*. Overall grain moisture content decreased during the storage period, falling below 18 % in all storage systems before the third month of storage. Marasas and Smalley (1972) found the same changes in the incidence of these fungi in mouldy maize meal, stored for three weeks. In contrast, Ngoko *et al.* (2001) found a significant increase of the incidence of both *Fusarium* spp. and *Aspergillus* spp. in maize samples collected from humid zones of Cameroon. This indicates that fungal development in stored maize depends on many factors including the storage conditions (grain moisture content, temperature during storage, insect infestation, type of storage structure used), and climatic conditions (relative humidity, temperature).

Although the storage systems tested in this study significantly influenced *Fusarium* development in stored maize, they did not have a significant effect on fumonisin contamination. Previous studies on aflatoxins, however, found that storage systems significantly influenced aflatoxin contamination in maize (Hell *et al.* 2000a, Udoh *et al.* 2000). Whereas aflatoxins are potentially serious problems during storage, fumonisin production primarily occurs in the field before harvest as *Fusarium* species do not grow in maize at less than 18 % moisture (Munkvold and Desjardins 1997, Doyle 1998, Riley and Norred 1999, Bolger *et al.* 2001). Consequently, Pittet (1998) found that the highest concentrations of aflatoxins are associated with the postharvest growth of *Aspergillus* moulds on poorly stored products. It is likely that in the current study, storage conditions in all systems were reasonably sound and that moisture content did not reach levels conducive for

the production of fumonisins. According to Munkvold and Desjardins (1997), there is strong evidence that proper storage systems with grain moisture content maintained at 13 – 14 % would normally prevent the production of fumonisins in stored maize. But, in tropical regions, which are known for their high mean temperatures and high relative humidities in many regions, ideal storage conditions are almost impossible to achieve (Vanek and Hoberg 1992).

The decrease in fumonisin content in maize observed during storage is reassuring with respect to the stability of the toxin in contaminated stored grains. In a recent 3-year survey study in Benin, similar results were found (Chapter 2). Instability of fumonisins was also found in previous studies in naturally or artificially contaminated food products over time. Scott *et al.* (1999) found fumonisins unstable in naturally contaminated ground rough rice, maize starch and maize meal over storage time. Orsi *et al.* (2000), also found an overall decrease of fumonisin content in stored maize after 140 days of a one year-storage period in Brazil. More recently, Kim *et al.* (2002) observed FB₁ and FB₂ to disappear completely in artificially contaminated Thai white rice flour after ten hours. These authors found up to 75 % and 90 % of decrease respectively in maize meal and in the flour of another type of white rice produced in the United Kingdom, after two months of storage. About 30 % decrease of total fumonisin B was also observed in maize cultures of *F. verticillioides* kept at 4 °C over 13 - 20 years (Gelderblom *et al.* PROMEC, Medical Research Council, Tygerberg, South Africa, 2002, unpublished data). In contrast, Ngoko *et al.* (2001) found FB₁ to increase with storage time in maize collected in different zones of Cameroon.

No explanation has yet been found as to the fate of fumonisins in naturally contaminated food products over the storage time. However, some factors including environmental conditions, intrinsic characteristics of stored products and chemical reactions are suspected. Munkvold and Desjardins (1997) argued against the view that fumonisin concentration increases in maize stores during storage, as long as conditions of grain moisture content and temperature are maintained at recommended levels. It is also suggested that fumonisin molecules might bind with the starch of the product during storage to form a complex, which is not detectable (Kim *et al.* 2002). Scott *et al.* (1999) reported that reaction of FB₁ with reducing sugars such as D-glucose is likely to explain the rapid fumonisin loss observed in maize starch. Kim *et al.* (2002) suspected the moisture content of the product, its texture and metal ions present in the product to influence fumonisin loss. In the case of the present study, environmental conditions during storage are likely to have affected fumonisin content in maize. Dry season and rainy season alternated during the eight months of storage.

Moreover the type of storage system used might also play a role and the decrease in fumonisin content was higher in one than in another system.

It was always believed that fumonisins are stable molecules and that keeping contaminated products at very low temperatures or submitting them to γ -irradiation before storage preserves stability of the toxin in the products for a long time. Thus, Gelderblom *et al.* (2002) found that analytical standards of fumonisin FB₁ remained stable (94 % of recovery) when kept in airtight conditions at 4 °C during a ten-year storage period. Likewise, Visconti *et al.* (1996) found fumonisins to be stable in γ -irradiated maize for at least six months at 25 °C.

In this study, insects played an important role in *Fusarium* development. Miller (1995) reported that a strong relationship exists between insect damage and *Fusarium* grain rot. Indeed, injuries caused by insects are generally sites of fungal infection of maize (Munkvold and Desjardins 1997). Results of the present study show that damage caused by lepidopterous insects was significantly correlated with both *Fusarium* development and fumonisin contamination in maize (Table 4). Schulthess *et al.* (2002) reported similar results considering the effect of *F. verticillioides* on the infestation of maize by various insects. Depending on the feeding habits or preferences of the larvae, they can attack maize stems, cobs, silks or grains, spreading fungal inoculum within the plant during their movement and feeding (Dowd 1998). Studies showed that in Indiana, lepidopterous insect-damaged grains were colonised by *Fusarium* spp. up to 82 %, but only 0.03 % by *A. flavus* (Rambo 1974), and that cobworms (lepidopterous species not specified) collected from maize in Missouri, were infected by *Fusarium* spp. (63 %) and *A. flavus* (37 %) (Fennell *et al.* 1975). Dowd (1998) reported that holes created by lepidopterous larvae have the highest levels of colonisation by *Fusarium* spp.

Mussidia nigrivenella was the lepidopterous pest most often found on maize in the present study. Ako *et al.* (2003) found that many insects were associated with *Fusarium* in West Africa, including *M. nigrivenella*, which attacks maize, cotton and beans and causes maize yield losses ranging from 5 to 15 % (Setamou *et al.* 2000). According to Setamou *et al.* (1998), *M. nigrivenella* usually attacks maize cobs and damages grain from the tip of the cob. By boring a channel, the insect breaks the testa of grains, which constitutes a natural barrier for fungal growth, promoting easy spread of fungi. *M. nigrivenella* damage also predisposes maize to pre- and postharvest infestation with storage coleopterous insects. The latter preferentially enter the holes produced by the *M. nigrivenella* larvae, thus further enhancing in the dissemination of fungal inoculum (Sanford and Luckmann 1963, Setamou *et al.* 1998).

It would look surprising that in this study, whereas there was no significant correlation between *Fusarium* infection and fumonisin contamination, a positive correlation was observed between lepidopterous insect damage and fumonisin contamination. These two correlations would match if this study was conducted only in field. Fumonisin is more likely to be produced in field subsequently to *Fusarium* infection if the environmental conditions are favourable. Damage of lepidopterous insects also more often occurs in field, not during the storage period. In this work, *Fusarium* infection, fumonisin contamination as well as damage of lepidopterous insect were all evaluated after harvesting i.e. during the storage period. The positive correlation observed between damage of lepidopterous insects and fumonisin contamination is probably due to the fact both insect damage and fumonisin level were found to decrease throughout the storage period. This positive correlation would not be observed in other circumstances as fumonisin production is dependant of several factors including environmental conditions.

In the present study, the abundance of coleopterous insects increased only after four months of storage. Results indicated that these insects are less likely to be implicated in the infection process of maize with *Fusarium*. There is, however, ample evidence of their involvement in fungal infection in the field, and the sap beetles, *Carpophilus* spp. are the insects best known to spread *Fusarium* in maize (Dowd 1998). Fennell *et al.* (1975) found that amongst sap beetles collected in maize fields in Missouri, 7 % were contaminated with *A. flavus* and 60 % with *Fusarium* spp. According to Dowd (1998), the implication of the sap beetles in vectoring *Fusarium* spp., as is the case with the other insects, may vary from one area to another within a country. This also depends on many agronomic factors including husk coverage of maize cobs, silk channel, grain pericarp thickness, the abundance of beetles, maize variety and weather conditions (Dowd 1998). Moreover, because of their association with the damage caused by the lepidopterous pests, the presence of sap beetles may not be recognised when evaluated at harvest, unless great care is taken to look for characteristic feeding marks and the presence of frass (Dowd 1998). All this may explain the negative correlation found between *Carpophilus* spp. and *Fusarium* development in the present study.

Sitophilus spp. and *Cathartus* spp. were negatively and significantly correlated with *Fusarium* incidence throughout the storage period, but there was a non-significant positive relationship between *Sitophilus* spp. and *Fusarium* incidence during the first month of storage ($r = 0.2$, $p > 0.05$) (data not shown). In contrast, Cardwell *et al.* (2000) in a recent field study on artificially infected maize with *F. verticillioides* found significant and positive correlations between the number of these insects and the fungus. The authors artificially infected maize

cobs in the field whereas in the present study, maize cobs were left to be naturally infected and evaluated during the eight-month storage period.

Unlike with *Fusarium*, both number and damage by all coleopterous insects found in maize samples, with exception to *P. truncatus*, was positively and significantly correlated with *Aspergillus* incidence during the storage period. The coleopterous insects were predominant, their number and damage increased in the granaries during storage. Their activity is likely to promote *Aspergillus* development, which markedly increased during the storage period, in contrast to that of *Fusarium*. Riley and Norred (1999) reported that growth of *Aspergillus* species and subsequent aflatoxin production during storage are favoured, among other factors, by insect and rodent activity. Moreover, it has been found that coleopterous insects, such as the nitidulid beetles (*Carpophilus* for instance), vector *Aspergillus* spp. and easily feed on maize contaminated by *Aspergillus*, whereas the lepidopterous insects cannot. The former insects are resistant to aflatoxin, possessing enzymes capable of degrading aflatoxin, whereas the latter insects are sensitive to aflatoxin (Dowd 1992).

It can be concluded that maize storage systems used by farmers may influence *Fusarium* development and fumonisin contamination. This suggests that any system creating conditions favourable for fungal growth and fumonisin production is not recommended. High levels of *Fusarium* infection in maize in a granary may not necessarily result in fumonisin contamination. Fumonisin level decreases during storage, more in some storage systems. Insect damage, mainly that caused by lepidopterous insects occurring on maize in the field before harvest, is likely to facilitate infection of maize with *Fusarium*. This suggests that any action undertaken to reduce insect infestation before harvest and during storage could help to reduce *Fusarium* infection and subsequent fumonisin contamination. Some recommendations to reduce insect damage and resultant fungal contamination include using maize cultivars less susceptible to lepidopterous insects, harvesting without delay to avoid insect infestation in the field, sorting out damaged cobs or grains at harvest, where possible insect control, and choice of storage structure to ensure good drying.

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Table 1: Mean fungal incidence in maize in the different storage systems over all storage period

Storage systems	No. of samples	Infected maize grains (%)		
		<i>Fusarium</i>	<i>Aspergillus</i>	<i>Penicillium</i>
Storage in bamboo granary	18	25.5 ± 13.5 a	24.4 ± 11.0 a	19.9 ± 8.0 a
Storage on platform	18	31.1 ± 15.7 ab	31.6 ± 15.1 a	24.5 ± 10.1 a
Prestorage on platform + storage in mud silo	18	30.2 ± 15.8 ab	33.5 ± 16.3 ab	23.3 ± 9.7 a
Storage on cemented floor in house	18	40.5 ± 17.4 b	46.8 ± 23.6 b	40.5 ± 18.2 b
Total	72	31.8 ± 16.2	34.1 ± 18.6	27.1 ± 14.4

No. of samples: number of samples collected from each storage system

Values shown are the mean (\pm Standard Deviation) percentage of maize grains infected by the different fungi.

Means within a column followed by the same letter are not significantly different ($p > 0.05$)

Table 2: Mean total fumonisin level in maize samples in different storage systems over all storage period

Storage systems	No. of samples	Total fumonisin level (mg kg ⁻¹)	
		<i>Range</i>	<i>Mean</i>
Storage in bamboo granary	9	0.6 – 2.3	1.2 ± 0.8
Storage on platform	9	1 – 2.3	1.5 ± 0.6
Prestorage on platform + storage in mud silo	9	1 – 2.3	1.5 ± 0.6
Storage on cemented floor in a house	9	1.5 – 2.3	1.9 ± 0.3
Total	36	0.6 – 2.3	1.6 ± 0.6

No. of samples: number of samples collected from each storage system

Values shown are the range and the mean (± Standard Deviation) total fumonisin level



Table 3: Mean number of insects in maize (per 1 000 grains sample) during the storage period

Months after stocking	<i>Mussidia</i>	<i>Cryptophlebia</i>	<i>Sitophilus</i>	<i>Prostephanus</i>	<i>Tribolium</i>	<i>Carpophilus</i>	<i>Cathartus</i>
0	12.7	2.0	4.0	0	0.7	5.3	8.7
1	2.5	0.4	18.6	0	0.3	5.7	46.8
4	0	0	295.8	32.5	33.1	21.3	285.7
5	0	0	671.8	143.3	107.6	18.3	473.0
7	0	0	641.1	253.8	110.7	16.4	710.4
8	0	0	570.7	134.2	100.7	23.9	633.4
Overall	2.5	0.4	367.0	94.0	58.8	15.2	359.7

Table 4: Correlation among insect infestation, fungal incidences and fumonisin contamination in maize

	Lepidopterous insects			Coleopterous insects					Grain damage (%)
	No <i>M. nigrivenella</i>	No <i>C. leucotreta</i>	Grain damage (%)	No <i>Sitophilus</i>	No <i>P. truncatus</i>	No <i>Tribolium</i>	No <i>Cathartus</i>	No <i>Carpophilus</i>	
<i>Fusarium</i> incidence	+ 0.653 **	+ 0.438 **	+ 0.802 **	- 0.611 **	- 0.642 **	- 0.573 **	- 0.339 **	- 0.463 **	- 0.645 **
Fumonisin level in maize	+ 0.834 **	+ 0.631 **	+ 0.852 **	- 0.792 **	- 0.671 **	- 0.696 **	- 0.505 **	- 0.641 **	- 0.581 **
<i>Aspergillus</i> incidence	- 0.823 **	- 0.594 **	- 0.517 **	+ 0.754 **	+ 0.095	+ 0.701 **	+ 0.533 **	+ 0.349 **	+ 0.557 **

** Correlation is significant at the 0.01 level (2-tailed)



Figure 1: Bamboo granary used in South-Benin for storing maize cobs



Figure 2: Platform used in Benin for storing maize cobs



Figure 3: Mud silo used in Benin for storing maize grains

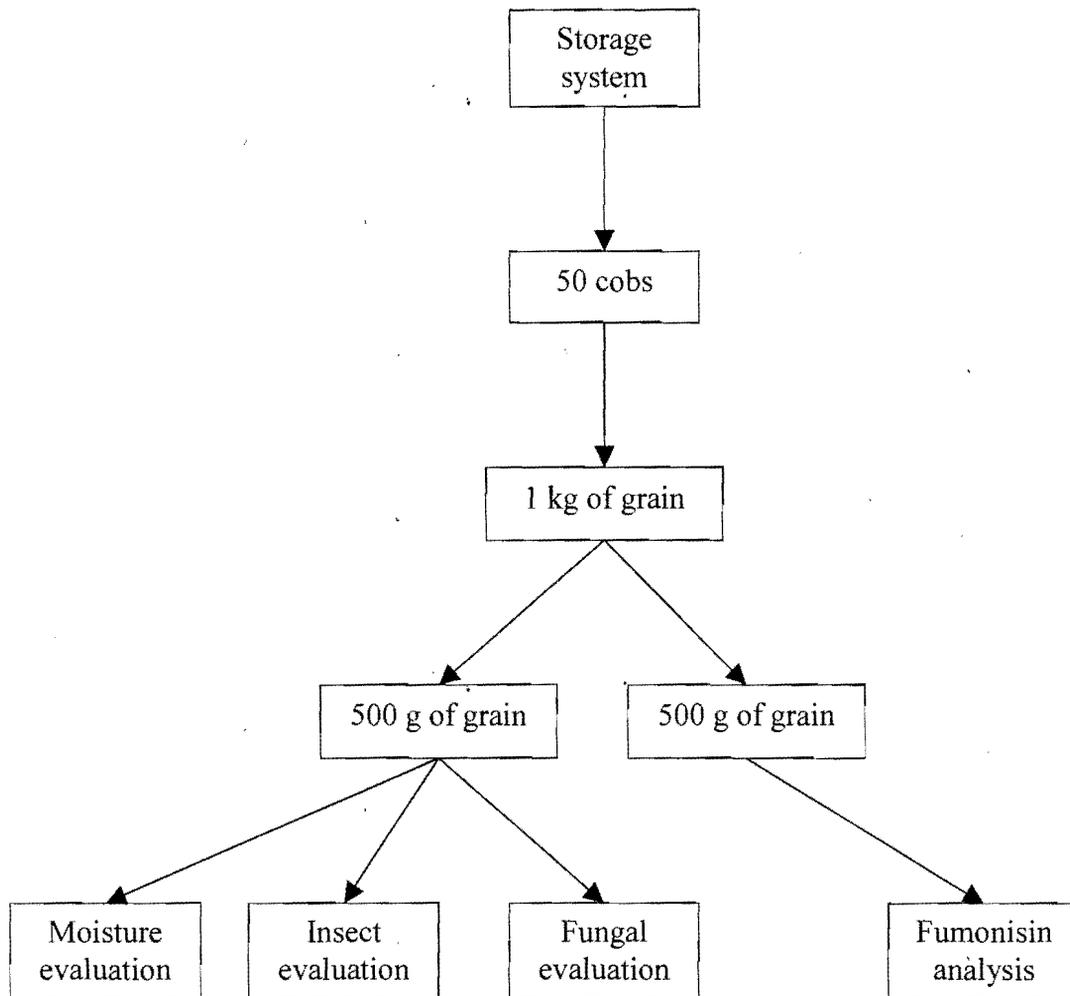


Figure 4: Diagram summarising the sampling method used during this study

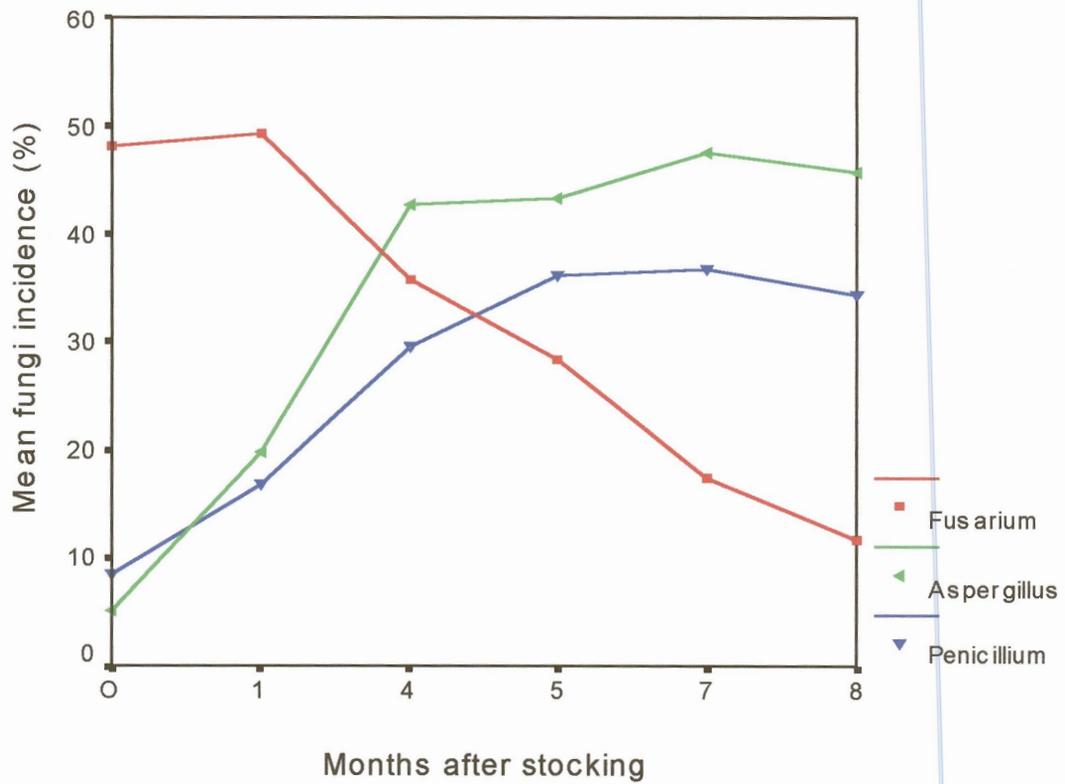


Figure 5: Changes in the mean incidences of fungi in maize during the storage period

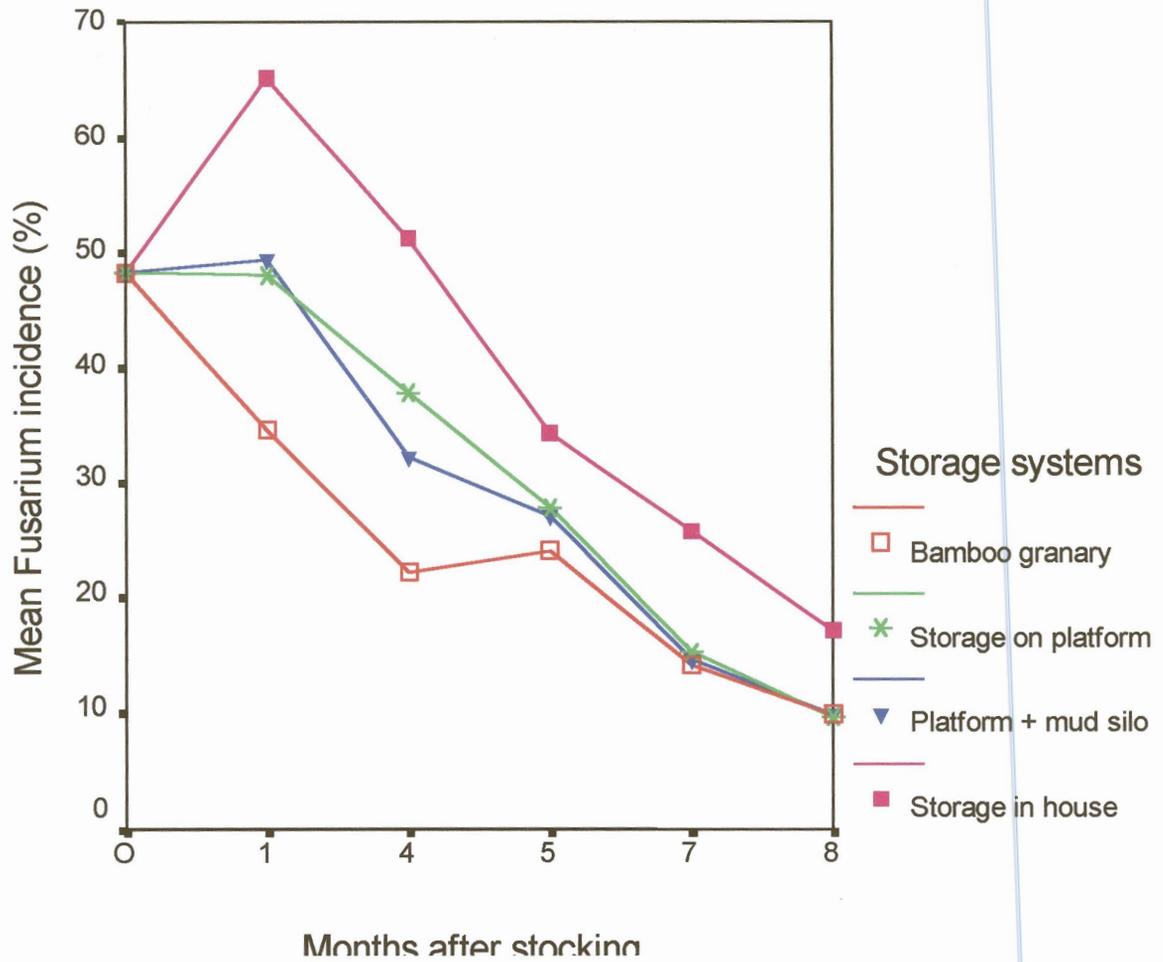


Figure 6: Changes in *Fusarium* incidences in different storage systems during the storage period

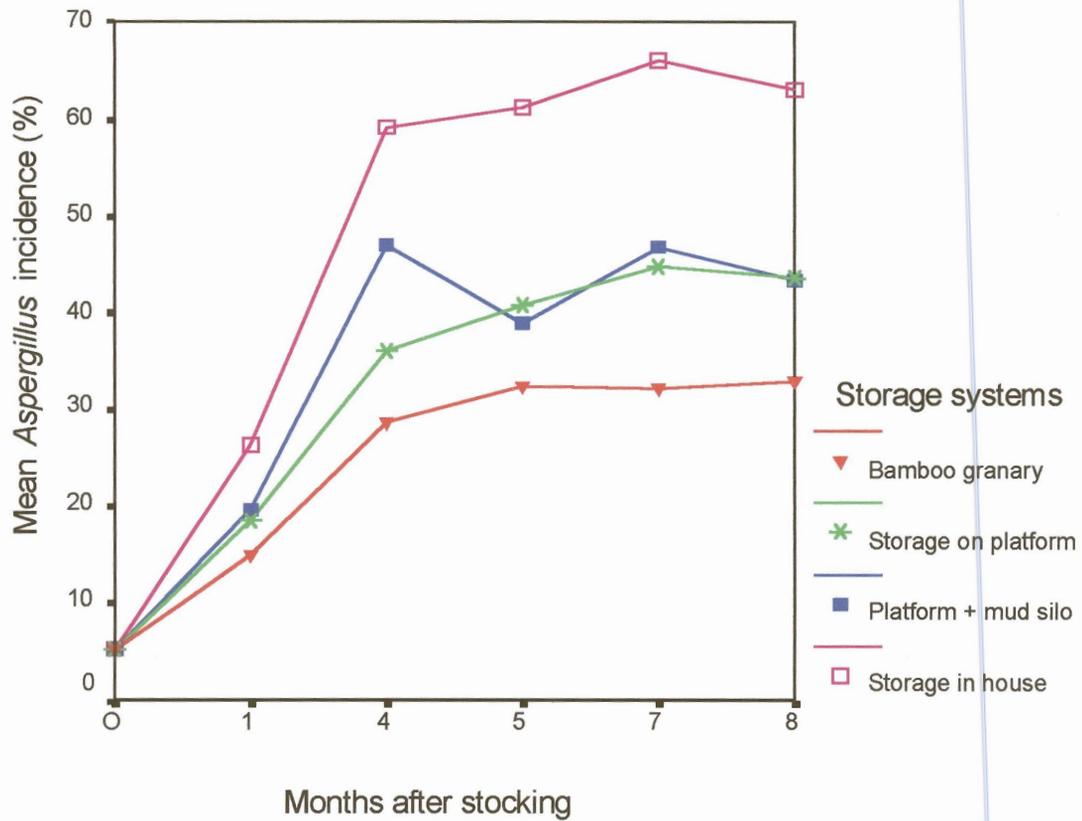


Figure 7: Changes in *Aspergillus* incidences in different storage systems during the storage period

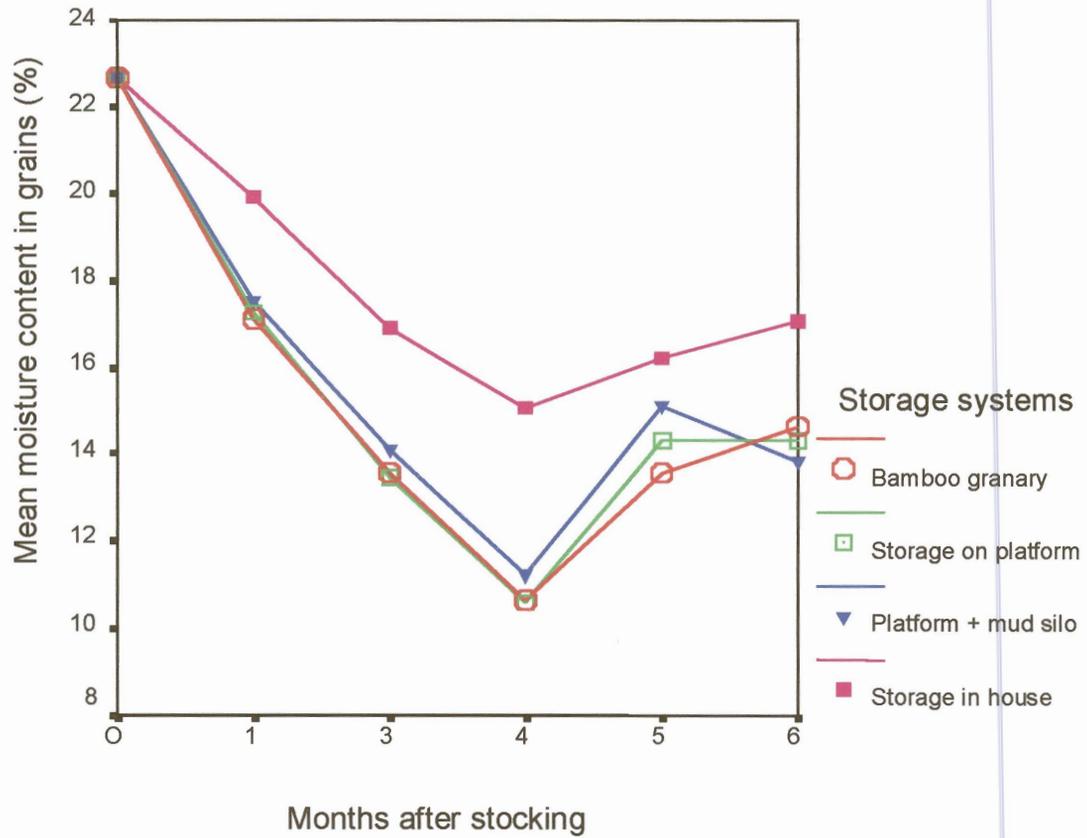


Figure 8: Changes in grain moisture content in different storage systems during the storage period

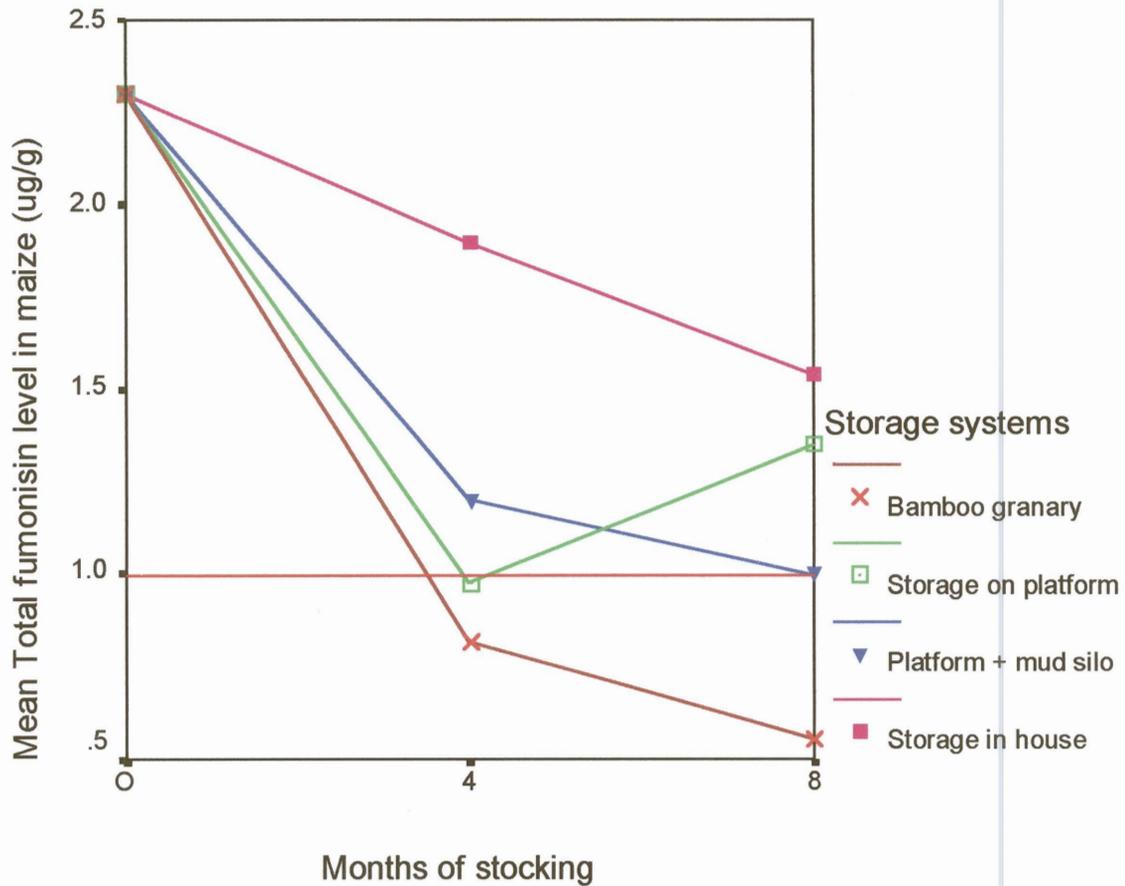


Figure 9: Changes in total fumonisin level in maize in different storage systems during the storage period

Line at 1.0 mg kg^{-1} is the maximum tolerated fumonisin level accepted in Switzerland.