Factors contributing to the distribution and incidence of aflatoxin producing fungi in stored maize in Benin

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Annex

List of Abbreviations:

a_w	Water activity is numerically equal to relative humidity, but expressed as a decimal value rather than a percentage
BGYF	Bright greenish yellow fluorescence
CARDER	Centre d'action rurale et developpement rurale
DAP	Double ammonium phosphate
DAPS/MDR	Direction agriculture et promotion sociale/Ministére de developpement rurale
FMS	Forest Mosaic Savanna
IITA	International Institute of Tropical Agriculture
INRAB	Institute National de la Recherche Agronomique du Benin
NGS	Northern Guinea Savanna
SGS	Southern Guinea Savanna
SONAPRA	Societé National sur la Promotion Agricole
SS	Sudan Savanna

Abstract (Deutsch)

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Key Words: Aflatoxine, Mykotoxine, Benin, West-Afrika, Lagermethoden.

Ziel dieser Studie war es, einerseits eine Übersicht über die Bedeutung von gelagertem Mais als Quelle der gesundheitlich äußerst relevanten Mycotoxinbelastung in der menschlichen Nahrung, insbesondere durch Aflatoxin, in Westafrika zu gewinnen. Andererseits sollten Erkenntnisse über Ursachen und mögliche Vermeidungsstrategien gewonnen werden. Über einen Zeitraum von zwei Jahren wurde der Aflatoxingehalt in 300 traditionellen Maislagern in den vier agroökologischen Zonen von Benin ermittelt. Parallel dazu wurde versucht, mittels einer Bauernbefragung, die Ernte- und Lagermethoden zu identifizieren, die einen Einfluß auf Aflatoxine in den einzelnen Ökozonen hatten.

Die Infektionsrate mit *Aspergillus flavus* in gelagerten Mais in 1993-94 waren vergleichsweise gering und betrugen kurz nach der Ernte zumeist nur 10 bis 20%. Im Verlauf von sechs Monaten Lagerungsdauer stieg der Befall allerdings erheblich an, so daß bis zu 55% der Körner infiziert waren. In 1994-95 lag der Prozentsatz der Körner die *A. flavus*-Befall aufwiesen zwischen 8 und 47%. In dem Beprobungszeitraum waren 25% der Proben Aflatoxin positiv, hiervon wiesen 60% wiederrum Gehalte von mehr als 20 ppb auf.

Verschiedene Faktoren beeinflußten den Aflatoxingehalt der Maisproben. Das Befallsrisiko wurde erhöht, durch den Anbau von Mais nach Mais und wenn Kulturen in die Fruchtfolge aufgenommen wurden, die das Wachstum von *A. flavus* begünstigten. Gleiche Effekte entstanden, bei Beschädigungen des Maises entweder im Feld, während der Ernte oder im Lager durch anthropogene Einflüsse oder Schädlinge. Insbesondere Schäden durch den Kornbohrer *Mussidia nigrivinella*, den Nitiduliden *Carpophilus spp.* und dem Maiskornkäfer *Sitophilus zeamais* waren mit erhöhten Aflatoxingehalten korreliert.

Indessen führte die Anwendung von Insektiziden oder Rauch im Lager zu reduzierten Pilzinfektionen. Mit niedrigeren Aflatoxingehalten waren folgende Maßnahmen assoziert: Ernte zum Reifezeitpunkt mit den Lieschblättern, Trocknung der Maiskolben außerhalb des Feldes ohne die Lieschblätter sowie Aussortierung der beschädigten und verdorbenen Kolben nach der Trocknung.

Bei Betrachtung der Lagerungsform, ergab die Lagerung als Körnermais die höchsten Aflatoxingehalte. Wesentliche Effekte gingen auch von den Lagerstrukturen aus. Es kam zu erhöhten Aflatoxingehalten, wenn Mais über dem Dachboden, auf dem Dach, im „Ago" in der nördlichen Guinea-Savanne oder in Lagerbehältern, die älter als 5 Jahre waren, gespeichert wurden. Hingegen waren Lagerstrukturen wie der „Ago" aus Bambus oder Lagerung in Jute oder Plastiksäcken mit niedrigen Aflatoxingehalten assoziert waren.

Abstract (English)

Kerstin Hell: Factors contributing to the distribution and incidence of aflatoxin producing fungi in stored maize in Benin

Key Words: Aflatoxin, mycotoxin, Benin, West-Africa, farming practices.

The aim of this study was to get an indication about the importance of stored maize as a source for the health threatening contamination with mycotoxins, especially aflatoxins in Benin, West-Africa. Information was also gathered about the possible cause of high contamination levels and strategies to reduce these adverse effects were evaluated. The aflatoxin incidence of 300 farmers' stores in four agroecological zones was evaluated over a two year period. At the time of sampling in the storage bins, a questionnaire was used to identify production, harvest and storage practices that had an effect on aflatoxin across agroecological zones.

In 1993-94 *A. flavus* development in stored maize shortly after harvest was comparatively low, with 10 to 20% of the grains contaminated. Six months later it increased to over 55%. In 1994-95 the percentage of grains that showed *A. flavus* presence was between 8 to 47%. Over the survey period 25% of all the samples were aflatoxin positive and out of these samples 60% had levels of more than 20 ppb.

There were several management practices that were positively related with aflatoxin contamination. Planting of maize in the same field consecutively, and in a crop rotation that incorporated crops that supported growth of *A. flavus*, increased the risk of contamination. Harvest practices associated with lower aflatoxin load were: harvest at maturity with the husk, drying outside the field without the husk, drying followed by sorting of damaged or spoilt cobs. Use of insecticides and smoking in storage reduced fungal contamination. Damage to maize, either biotic or man-made in the field, during harvest, or in storage had negative effects. Insects have long been implicated in the spread of *Aspergillus* spores and the development of aflatoxins. In this study a relationship was found between the presence of insects and aflatoxin.Damage due to the cob borer *Mussidia nigrivinella*, the nitidulid *Carpophilus* spp. and the maize weevil *Sitophilus zeamais* correlated with high aflatoxin incidence.

In a trial, the influence of storage form on aflatoxin contamination was evaluated. Maize that was stored as grains showed the highest aflatoxin content. Storage types that increased the risk of fungal development are storage on the ceiling, on roof tops, in the Ago (used in the Northern Guinea Savanna) and in storage containers that were more than 5 years old. Farmers' practices that were linked to lower aflatoxin contamination were: storage in either the Ago (made from bamboo) or use of jute or polyethylene bags as secondary stores.

CHAPTER 1   **Introduction**

1.1   Background of the research topic

Rapid population growth in the developing countries leads to an ever increasing demand for food. Because of rising demand, production has steadily increased over the past years. In Sub-Saharan Africa maize (*Zea mays*) production has virtually doubled in the last 30 years, rising at an average of 2.5% per year, of which 1.8% are through area expansion and 0.7% by yield increase (Gilbert 1995). In the production of food crops losses occur during the growth cycle in the field. Further losses occur during harvest, where up to 5% of grain weight can reduce the agricultural output (Compton *et al.* 1993). Further losses occur during storage, where Pantenius (1987) found average damages of 30% in stored maize after six months of storage in Togo.

Maize is the most important cereal grown in the Republic of Benin. In 1996/1997, maize was produced on 517,985 hectare, with total production of 504,506 tons for the same year (ONASA 1997). Rainfall is the decisive factor causing the instability of production in Benin. In the south there are two maize growing seasons whereas in the north there is only one (see section 1.3). The cultural zone of maize is expanding further every year towards the sahelian countries. The storage and preservation of the harvested maize is poorly managed by the average Beninese farmer, with losses measured of up to 30% (SPV 1992). Storage pests are the main cause of these losses, and under the tropical climatic conditions the development of storage fungi, especially species of the genera *Aspergillus* and *Penicillium*, is an unresolved problem. Under certain conditions these fungi can develop toxic metabolic by-products called mycotoxins. Toxins produced by some *Aspergillus* spp. are called aflatoxins. Aflatoxins were first discovered in Europe in animal feed. The outbreak of the Turkey X-Disease (Blount 1960) which killed thousands of turkeys in the 60's in Great Britain, led to the identification of the two causal fungi *Aspergillus flavus* (Link) and *Aspergillus parasiticus* (Speare). The aflatoxin derivatives associated with these two fungi are B_1, B_2, G_1 and G_2. These abbreviations are derived from the color these compounds fluoresce under ultraviolet light (365nm), with B standing for blue and G standing for green. These toxins are hazardous to animal and human health, and constitute a factor in economic losses in food production in the world (Lubulwa & Davis 1994). Effects on animal health are liver damage, decreased milk and egg production,

and immune suppression. In man the consumption of aflatoxigenic commodities can lead to acute aflatoxicosis. Ingestion of these grains is a synergistic factor in the development of several diseases, such as Reye's Syndrome, Liver cell cancer and Hepatitis B (CAST 1989). Studies in Kenya and Sudan showed higher levels of aflatoxins in Kwashiorkor children, as compared to normal or marasmic children (Hendrickse *et al.* 1982). Adhikari *et al.* (1994) suggested that aflatoxins reduce the levels of immunity and thereby the resistance to other sicknesses.

Maize is a good substrate for the development of *Aspergillus* spp. and resulting aflatoxins (CAST 1989). Among the factors that increase the risk of aflatoxin development and consumption in the tropics, as compared with the temperate regions are:

- higher temperature and humidity
- lower availability of capital for rapid grain drying
  and correct storage
- lower awareness of the problem
- higher insect pressure
- less reliable checks on the quality of food products
- human consumption of low quality grain
- prevalence of diseases that increase or accelerate
  the effects of mycotoxins
- the proportion of toxigenic to atoxigenic strains of
  A. flavus increases from temperate to
  tropical regions (Williams & McDonald 1983).

1.2   Objectives of this study

This work studied the distribution of *Aspergillus* species and related mycoflora in traditionally stored grains in the four agroecological zones of Benin. Aflatoxin contamination was measured, and related to accompanying fungi and grain moisture content. The different agroecological zones are defined by climatic parameters (see 1.3). In the first season (August-December 1993) a survey was conducted which touched 300 farmers in 30 villages. This number was reduced for the second sampling (March-April 1994) to 150 farmers in the same 30 villages, since results from the first season showed that the farmers practices were very uniform in each village, but heterogeneous from village to village. During the survey a questionnaire was administered with the aim of relating certain farming, harvest and storage practices to the resulting aflatoxin content of the samples taken from each farmer. Questions concerning the consumption of maize were asked to assess the risk of aflatoxin intake. The

insect presence on all the maize samples was recorded to evaluate the role of pre- and post-harvest insects on aflatoxin contamination. The questionnaire and the sampling was repeated in the 1994-95 season, with sampling of maize at the same time as in the first year. Certain answers that could possibly changed from year to year were reassessed. A factor that was closely evaluated in field trials was the storage form. Maize in Benin was stored either with the husk, without husk or as grains. The aim of this study was to find a solution to the aflatoxin problem which would be readily accessible to subsistence farmers.

1.3 Agroecological zones of Benin

This work was conducted in Benin, a West-African country with a surface area of 112 622 km^2. The population has reached about 5 million, with 63% of the population gaining their income through agriculture (Adam & Boko 1993). Agricultural production remains very traditional with rarely any mechanization. Maize is one of the staple foods of the Beninese, mainly consumed in the southern part of the country. It is transformed into a wide variety of local dishes like "akassa", "pate", "bouillie" which are part of the daily diet of the Beninese (FAO 1994).

Benin is divided into four agroecological zones (Figure 1.1):

Figure 1.1:   The four agroecological zones of Benin with the survey sites
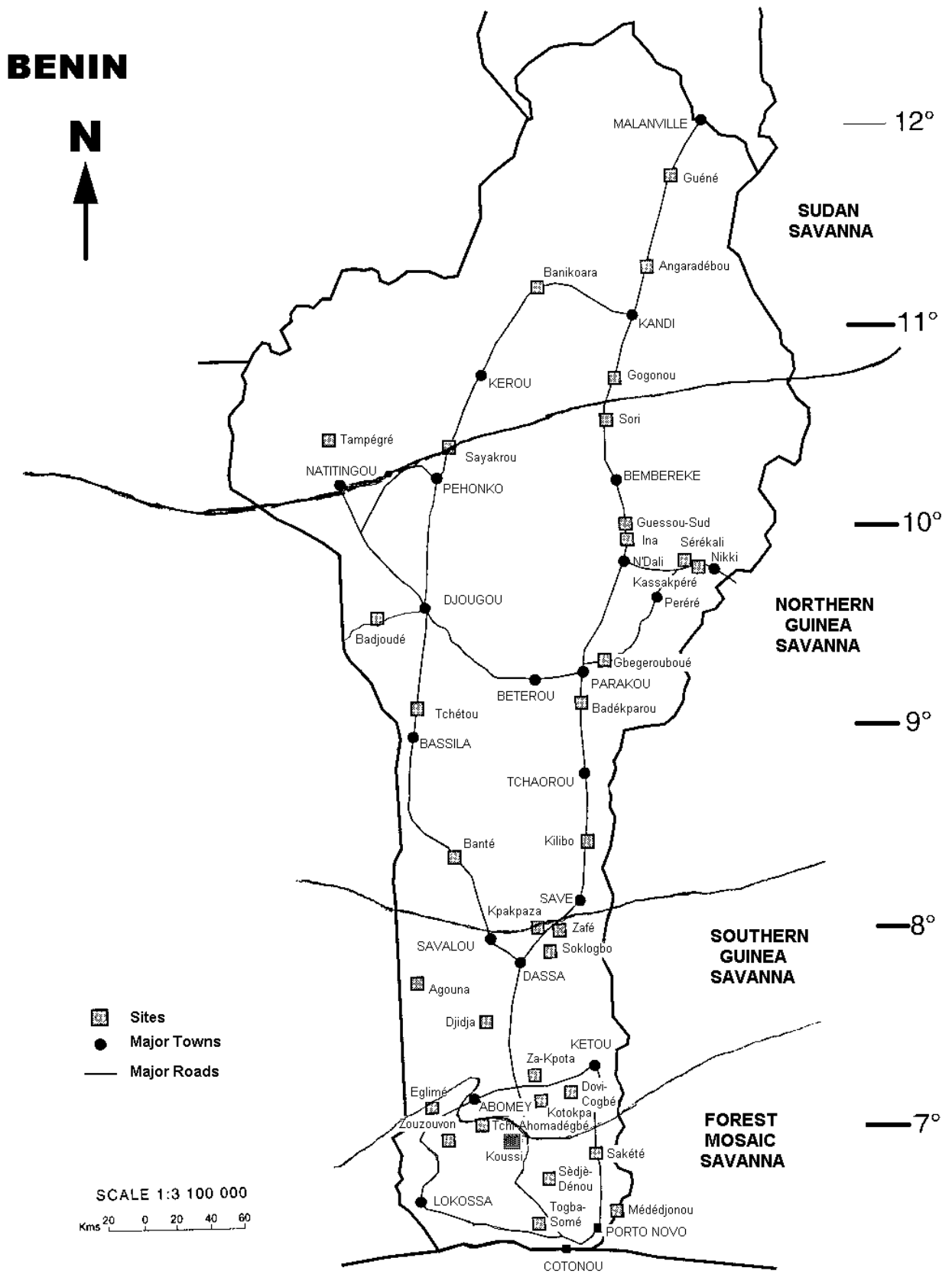
Forest Mosaic Savanna (FMS)

This zone can be typified by high relative humidity, mostly over 80% and a long wet season from April till July, a short dry season from July to August, and a short rainy season from September till November. Rainfall ranges between 1300 and 1500 mm. Maize is grown in this region during both wet seasons. Grain moisture of the first season harvest is high, usually between 16% to 18%, because the crop is collected in the middle of the rainy season and sufficient drying is hard to achieve. First reports about the presence of *Prostephanus truncatus* (Horn) (Col., Bostrichidae) a very destructive maize pest came from this area (Anonymous 1986) and the biological control program of this pest showed its first success in this region (Borgemeister *et al.* 1994).

Soil nutrient-holding capacity is low, and because of the small size of the farms and low productivity, inputs (fertilizer, insecticides etc.) are rarely used. Maize is mostly produced for autoconsumption in this area.

Southern Guinea Savanna (SGS)

The climate in this zone is influenced, like the southern coastal zone, by two rainy seasons one from April to July and the other one from October to November. The annual rainfall varies from 1000 to 1300 mm and the dry season lasts for 4 to 6 months. This is the main maize production zone in Benin, in which two crops of maize are produced during one year.

In this zone high losses occur because of *Mussidia nigrivinella* (Ragonot) (Lepidoptera: Pyralidae) (Setamou *et al.* 1997, in press). Diener *et al.* (1987) found that insects like stemborers and cobborers can cause damage to the grain pericarp which serve as entry points to fungal spores or carry these spores themselves. The tunneling also allows the kernels to dry down to levels more favorable for the growth of *A. flavus* and aflatoxin production than for other fungi. As compared to the north there is a high presence of *P. truncatus*, a post-harvest insect that was accidentally introduced to Africa, which causes losses of up to 16% in Tanzania (Henckes 1992) and up to 10% in Togo (Pantenius 1987). This insect might have an effect on the levels of aflatoxins, because of heavy tunneling and high production of maize meal.

Northern Guinea Savanna (NGS)

The Northern Guinea Savanna (NGS) has one rainy season that lasts from May till October, and the dry season from November till the beginning of May. Annual rainfall is between 900 and 1300 mm. Farmers produce maize only during the rainy season. The NGS has an intensive production system, with a multitude of crops produced. Fertilizer use has become quite widespread. Lele and Stone (1989) remarked, that 22% of the total national consumption of fertilizer in Nigeria is used in this zone. Beninese farmers are rapidly increasing their fertilizer use, especially because of the distribution of fertilizer with the state supported cotton production program and the GLOBAL 2000-Project, which concentrates its activity on the middle and northeastern zone of Benin. This program gives credit to farmers to buy improved maize seed and fertilizer. The use of fertilizers might decrease the risk of fungal infection, because dense populations of plants and reduced fertilization have been reported to result in higher levels of aflatoxins (Jones & Duncan 1981).

Sudan Savanna (SS)

This area is characterized by rainfall of less than 1000 mm per annum and an median relative humidity below 40%, except for the wet season, when humidity rises to 60%. The dry season lasts from October till May. Maize production is low, but will expand into these areas in the years to come. One maize harvest is produced per year. The soil fertility is low and land erosion is a problem in this area. The Sudan Savanna presents ideal climatic environment for mycotoxin infection, dry seasons followed by rain provide optimum conditions for the development of *Aspergillus* fungi and subsequent aflatoxin production. The other factors that increase the risk of contamination are poor soil fertility and periodic drought stress. The possible effect of drought is the elimination of microbial competitors of *A. flavus* (Diener *et al.* 1987), with a subsequent increase in *Aspergillus* spp.. In the north of Benin maize plants are severely attacked by the parasitic weed *Striga hermonthica*. Cobb (1979) linked the presence of weeds to an increased aflatoxin contamination.

Chapter 1 gives an introduction to this work. The objectives for the surveys and the field trials are described. The different agroecological zones are summarized and their distinguishing characteristics are presented and the risk for aflatoxin contamination in the different ecozones is evaluated.

In Chapter 2 the theoretical background to this work is reviewed. The genus *Aspergillus* is described and the developmental path of aflatoxins elucidated. The effect of aflatoxins on human health is presented, with the main effects being their involvement in the development of liver cancer and as co-factor in the malnourishment of children. Then the factors that influence aflatoxin development are given, they can be split up into climatic, agronomic and biotic factors. Climatic factors are the temperature, relative humidity, moisture content of the goods and water activity. The agronomic factors that may influence aflatoxin development are plant stress, irrigation, cropping pattern, variety, planting date, harvest date and storage conditions. Insects may contaminate the stored goods and can help to spread the *Aspergillus* spores in maize. Past surveys for aflatoxins in West-Africa and similar agroecological regions in the world are presented, the work in West-Africa was initiated in the 60's, when peanut exports from West-Africa were taken from the international market, because of too high aflatoxin contamination levels.

In Chapter 3 results from trials that looked at the influence of position in a granary and resultant aflatoxin content are presented. Sampling for aflatoxins in a big lot of grains or cobs is difficult, since not all the cobs show an equal distribution of mycotoxins. Some of the grains can have very high amounts of contamination, whereas others show very low amounts. Two short experiments are described, which were conducted to test if freezing or fumigation had an effect on the fungal flora. These measures were used to arrest insect development in the samples collected during the field surveys. The optimal sample size to determine contamination with *Aspergillus* fungi was determined with the help of literature.

In Chapter 4 maize production in Benin was characterized. The results of questionnaires in 93/94 and 94/95 were presented. The different parts of the maize production process were described, maize variety, usage of fertilizer and pests problems in the field. When maize was harvested, there were differences in the harvesting technique, the field drying period, the sorting of damaged or discolored cobs, the harvesting period and the drying period after

harvest. Farmers in Benin used different storage structures and had multiple storage pest problems. The factors that influenced the farmers choice of a store were agroecological zone, the availability of building material and tribal differences. Pest problems in storage and the farmers solutions to them, were very different from one farmer to another. Farmers consumption habits of maize were evaluated.

Chapter 5 described the methodology used to study the fungal flora and determine the grain moisture content of the collected maize samples. The chemical extraction and the chromatography method used to elucidate aflatoxins was explained. The distribution of *Aspergillus* and related fungi in Benin were presented. Aflatoxin contamination was related to agroecological zone and zonal differences in Benin were pointed out.

The following chapter (Chapter 6) describes the use of regression analysis to determine the influential factors on aflatoxin development in Benin. Factors that typify maize production in the different agroecological zones were regressed against aflatoxin contamination, to determine those factors in maize production, maize harvesting and maize storage that had an increasing or reducing effect on aflatoxins. For each agroecological zone and survey year these factors were different.

In Chapter 7 the traditional storage structures were described and differences between aflatoxin contamination in these stores were presented. The size, form and positioning of the storage structure were determined by ecoregion, the wealth of the farmer and the availability of building material. Many farmers changed their storage structure in the course of a production year. Farmers stored their maize in a field store and later in the season transferred it to a storage structure near the house. Aflatoxin contamination was presented by ecozone and by storage type.

Chapter 8 described the role that post-harvest insects played in the infection of maize with aflatoxins in Benin and which insect species increased the risk of contamination with aflatoxins and which species had an influence on fungal development.

In Chapter 9 field trials are described that related form of storage to aflatoxin contamination. Maize in Benin was stored on the cob with or without the husks, or as grains. On maize stored under Beninese coastal conditions fungal contamination, insect damage and aflatoxin contamination was measured.

The overall discussion follows in Chapter 10. The distribution and importance of aflatoxin contamination in Benin was evaluated. The factors influencing aflatoxins under Beninese

conditions were presented and weighted against each other. Potential solutions to aflatoxin contamination under Beninese conditions were descibed. The conclusions drawn from the evaluation of the different influential factors on the development of aflatoxins under Beninese conditions in the different agroecological zones were presented.

CHAPTER 2   **Literature review**

2.1   The genus *Aspergillus*

The genus *Aspergillus* belongs to the Deuteromycetes (Fungi Imperfecti, Hyphomycetes), their teleomorphs can be found in the Ascomycetes. The fungi of the genus *Aspergillus* are capable of using many commodities as substrates, because of the large numbers of enzymes which they can use for their development. They can form on many plants, on leather, paper, wood, seeds of all kinds and packaging materials (Coker *et al.* 1984).

The genus *Aspergillus* is characterized by the production of asexual spores (conidia) on a specialized structure called an aspergillum. This aspergillum can be either uniseriate or biseriate (Illustration 2.1).

Figure 2.1:   Conidiophore of the *Aspergillus* spp.
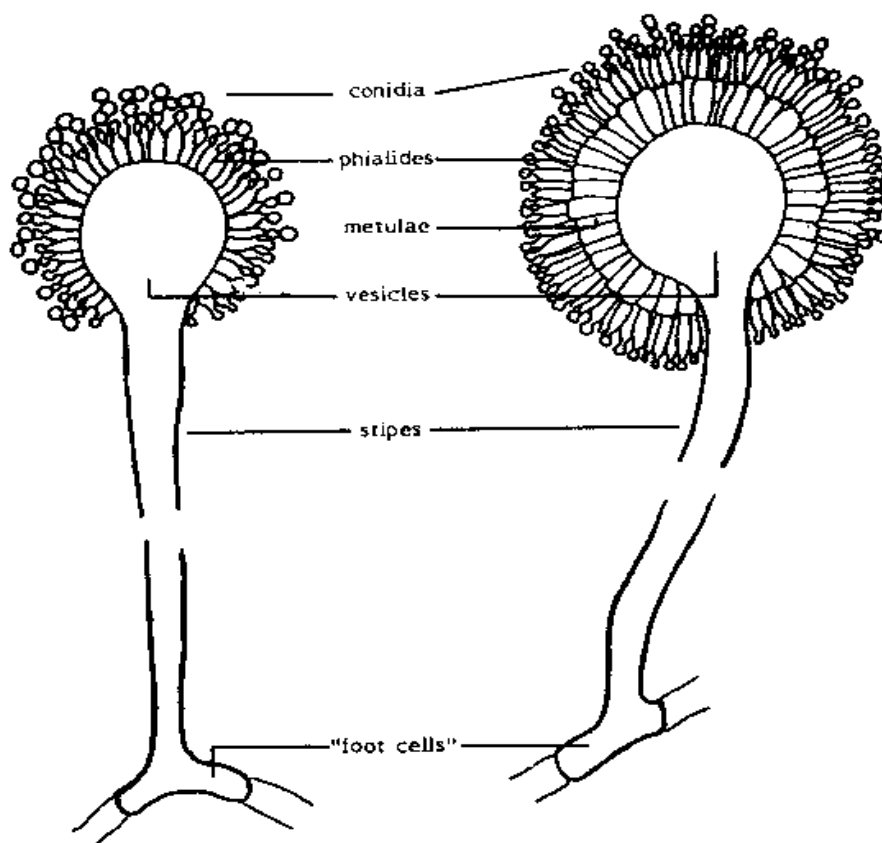
(a) uniseriate species   (b) biseriate species (Klich & Pitt 1988)

The basal portion of the stipe is usually curved and forms a "foot cell". The whole structure including the aspergillum, stipe and foot cell is called a conidiophore (Klich & Pitt 1988).

The contamination of standing corn with *A. flavus* involves three stages. Airborne or insect transmitted conidia contaminates the silk and grows into the developing ear (Figure 2.2).

Figure 2.2:   Development cycle of *A. flavus* in the soil and on the plant (Wicklow & Donahue 1984)
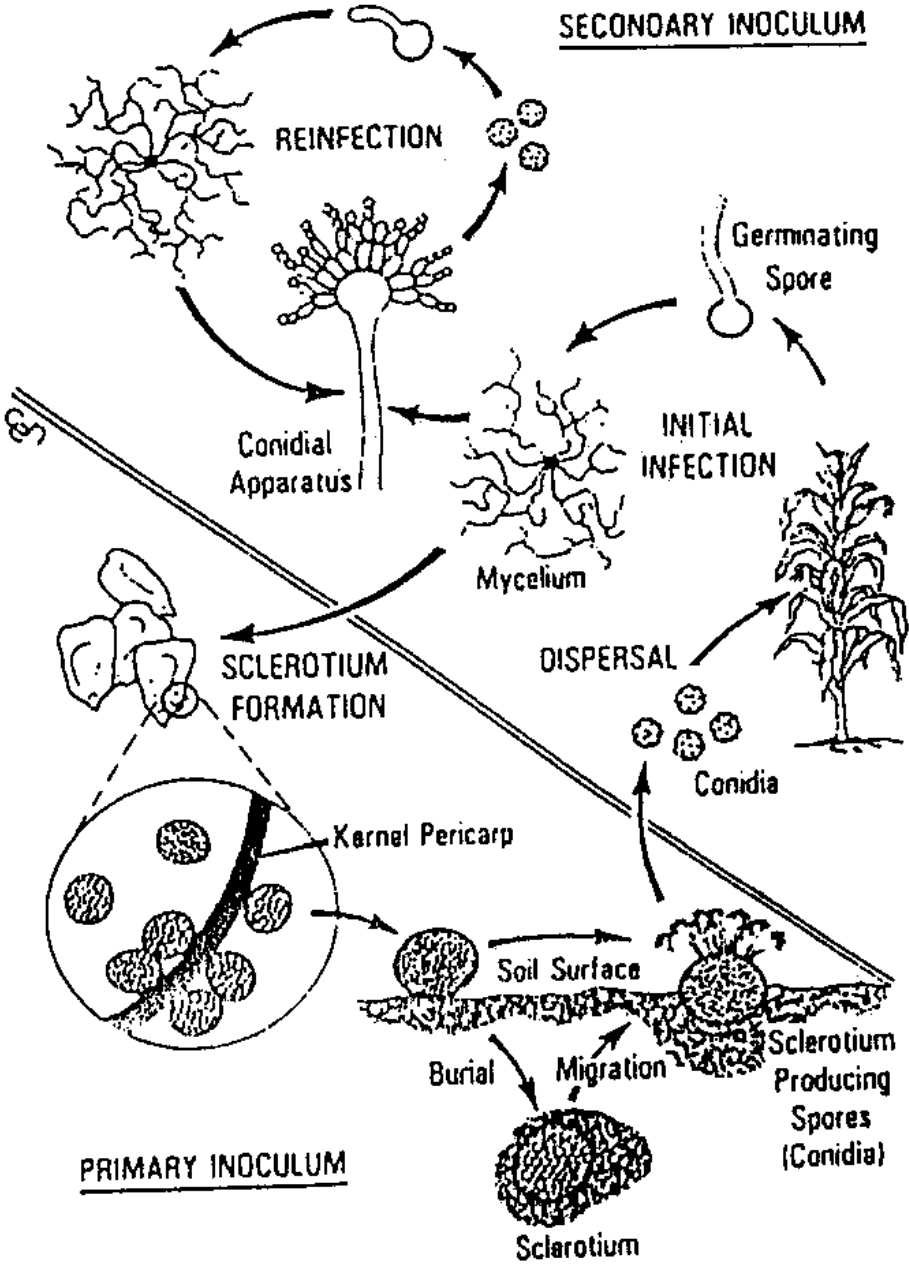
Then kernels in the ear, which were damaged by insects or birds, were infected with *A. flavus* and contaminated with aflatoxins. Climatic and cultural factors which stressed the plant, such as drought and nutrient deficiency increased the susceptibility to fungal contamination (Wicklow 1988). Jones (1979) observed that *A. flavus* entered the developing kernels through the stylar canal. Other authors (Marsh & Payne 1984) suggested that kernels were infected through the scar left by silk detachment. After plating the sporulation of *A. flavus* was recorded for intact kernels. Sporulation was abundant at the kernel (68%) and tip (48%) and least through the endosperm (12%) (Fennel *et al.* 1993). Mycock *et al.* (1992) presented evidence that maize grains internally infected with *A. flavus*, when left to germinate could give rise to plants that were internally infected with the same fungus. The conclusions was that the fungi may be seed-borne, but most of the authors suggested that *A. flavus* was soil-born and that it survived for long periods as sclerotia in the soil (Cotty *et al.* 1994; Saito *et al.* 1989).

For the genus *Aspergillus*, Thom & Church (1926) edited the first complete taxonomic description, which was later expanded by Thom & Raper (1945), and completely revised by Raper & Fennel (1965). Recently some systematic groups have been restructured. Gams & Samson (1986) divided the genus *Aspergillus* into six subgenera each containing one or more sections. More recently taxonomies have been revised by Pitt & Klich (1988) and Kozakiewicz (1989) published a taxonomic key based on identification of the conidial ornamentation with the help of a scanning electron microscope.

Aflatoxins are only produced by two related species: *A. flavus* and *A. parasiticus*, where the latter species also produces aflatoxin of the G type. Because aflatoxins have been shown to be toxic to certain competitor microbes, insects and other animals in the ecosystem a survival benefit to the fungi producing aflatoxins was implied (Bhatnagar & Cleveland 1991).

Scientists have attempted to isolate genes associated with aflatoxin biosynthesis through cloning of genes, in an attempt to understand the enzymes regulating the biosynthesis. Information gained on the regulation of the genes in the pathway could help to develop control strategies through inhibition of these controlling genes (Keller *et al.* 1994). With the use of mutants, Bhatnagar *et al.* (1993) elucidated the biosynthetic pathway of aflatoxins (Figure 2.3) : acetate $\rightarrow$ polyketide $\rightarrow$ norsolorinic acid(NOR) $\rightarrow$ averantin(AVN) $\rightarrow$ averufanin(AVNN) $\rightarrow$ averufin(AVF) $\rightarrow$ 1-hydroxyversicolorone (HVN) $\rightarrow$ versiconal hemiacetal acetate(VHA) $\rightarrow$ versiconal (VAL) $\rightarrow$ versicolorinA(Ver A) $\rightarrow$ demethylsterigmatocystin (DMST) $\rightarrow$ sterigmatocystin (ST) $\rightarrow$ O-methylsterigmatocystin (OMST) $\rightarrow$ aflatoxin B_1 (AFB_1).

Fig. 2.3: Biosynthetic pathway of aflatoxins (Bhatnagar et al. 1993)
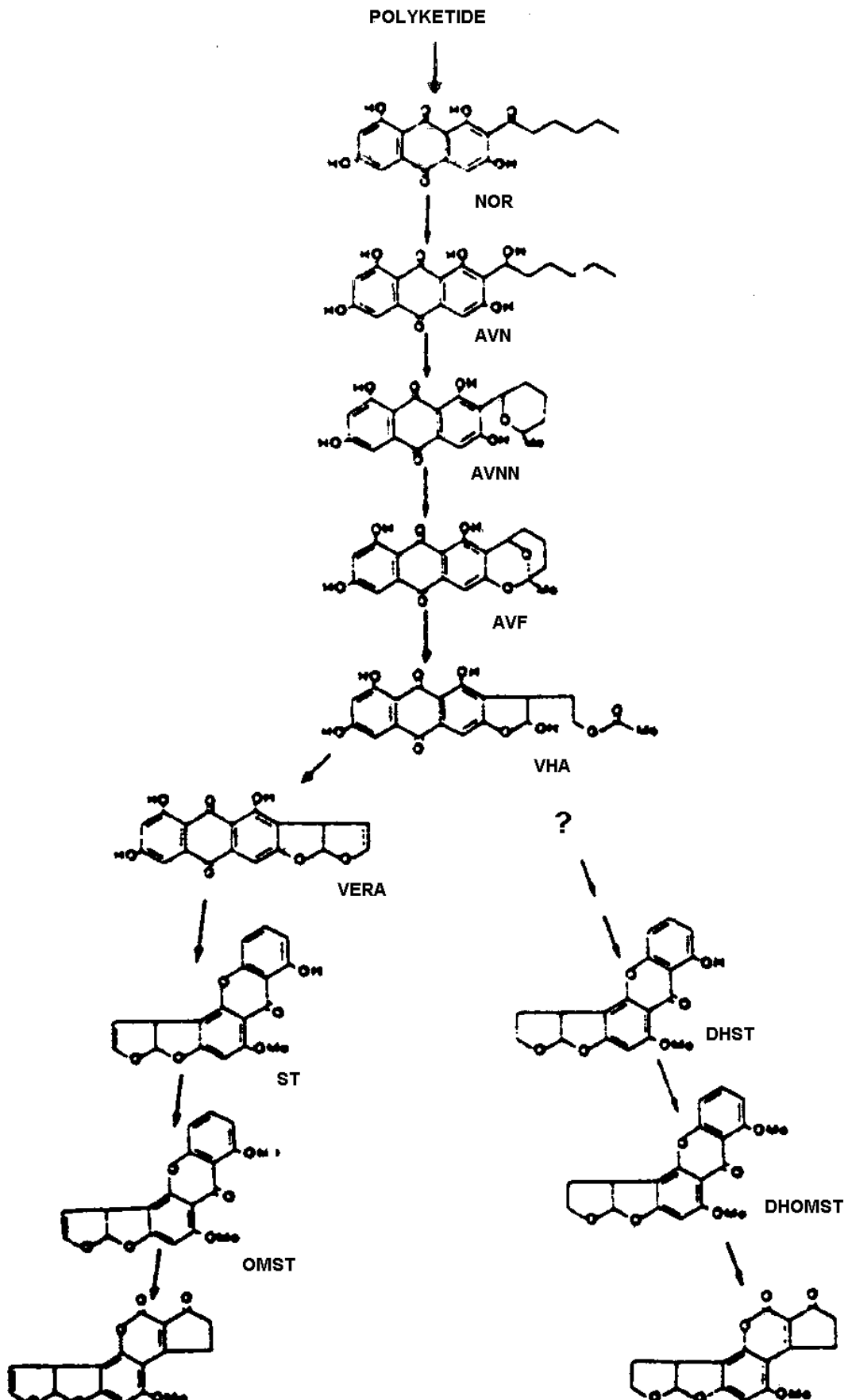
Ingestion of aflatoxins can lead to the development of various sicknesses in man and animal. Aflatoxins affect mainly the liver. They have been found to be carcinogenic and teratogenic in animals, as well as the cause of impairment of protein formation, blood coagulation, weight gain and immunogenesis. A fact that has to be considered is, that aflatoxins taken up by animals are not eliminated out of the food chain, but will eventually reach the consumer and be taken up. The amount of toxins that accumulates in the end consumer and the elimination of these toxic components depends on the individuals' health status and his detoxification capacity.

Staib *et al.* (1983) reported a case of acute aflatoxicosis of a 60 year old male alcoholic suffering of cirrhosis and primary carcinoma of the liver. The autopsy of the body revealed a ball of *A. flavus*-mycelium in the lungs of the victim. Other cases of acute aflatoxicosis were reviewed in the works of Shank *et al.* (1972) in Thailand, by Krishnamachari *et al.* (1975) in India and by Nagindu *et al.* (1982) in Kenya. Aflatoxins have been linked to cancer, Reye's syndrome and Kwashiorkor, sicknesses that appear with a higher incidence in Africa than in other parts of the world (Hendrickse 1985; Wild *et al.* 1991; Bassett *et al.* 1995). Keen & Martin (1971) and Alpert *et al.* (1971) have produced evidence from various parts of Africa that the level of aflatoxin contamination of local foods is directly related to the incidence of liver cancer in the villages. Van Rensburg (1977), showed that there is a highly positive correlation between the level of intake of aflatoxin and liver cancer rate, particularly in Kenya, Thailand, Mozambique and Swaziland. This is corrobated by Peers *et al.* (1976), Shank *et al.* (1972), and Habish & Abdulla (1971). Studies conducted by Alpert *et al* (1971) in Uganda, by Keen & Martin (1971) in Swaziland and by Shank *et al.* (1972) in Thailand revealed a relationship between the frequency of liver cancer in the study areas and the aflatoxin contamination of foods offered for sale in markets and in home stores. Studies carried out by various workers in Thailand, Kenya, Mozambique and Swaziland and reported by Van Rensburg (1974) have related the actual concentrations of aflatoxin in meals consumed, to the incidence of primary liver cancer in the areas from which the meal samples were taken. Bulatao-Jayme *et al.* (1982) concluded that the risk of developing primary liver cancer increases with the aflatoxin load of the ingested food. Similar evidence is reported by Yu *et al.* (1989) who studied significant correlation's between the primary liver cancer mortality rates

and aflatoxin intake in the local foods in five villages in China. Wang *et al.* (1983) analyzed the relationship between climatic data (temperature and humidity), aflatoxin, hepatitis B and primary liver cancer mortality. The authors concluded that climate is an important environmental factor that determines the differences in the distributions of liver cancer. Nwokolo & Okonkwo (1978) reported a high incidence of primary liver cancer in young males under 40 in Nigeria and parts of Africa south of the Sahara. This evidence is supported by Edington & McLean (1965) who described a higher distribution of liver cancer in the Western parts of Nigeria, where 5.9 cases were recorded per 100,000 males as compared to 3.0 in the United Kingdom and 2.7 in the United States (Hutt 1971). Liver cancer constitutes 7.2% of all cancers in the western zones. Edington (1977), described that in the Northern savanna area of Nigeria, liver cancer constitutes 19.2% of all cancers. Bean *et al.* (1989) studied the occurrence of aflatoxin and its metabolites in populations with high liver cancer incidence in 161 Nigerians residing in Lagos. They reported that aflatoxin B_1 occurred in 3.1% of the urine samples and G_1 in 9.9%. The highest mean concentration was found for aflatoxin G_1 at approximately 12ug/100ml urine. They concluded that this confirmed the presence of aflatoxin metabolites in populations with a high incidence of liver cancer. Nwokolo (1977) reported that in the Southern Guinea Savanna, hospital records showed that a district with a population of 150,000, has an annual incidence of 5.0 per 100,000 males of liver cancer. Tsuboi *et al.* (1984) analyzed aflatoxin B_1 content in serum and urine samples from patients with acute or chronic hepatitis and primary hepatocellular carcinoma in Japan, Indonesia and the Philippines. The authors reported that aflatoxin B_1 was detected in 5%, 23%, and 17% of the samples of the acute or chronic hepatitis patients and in 10%, 6% and 9% of the samples of the primary hepatocellular carcinoma patients in Japan, Indonesia and the Philippines respectively. They concluded that aflatoxin B_1 played a role in the expression of acute or chronic hepatitis and primary hepatocellular carcinoma.

In addition to hepatic diseases, other illnesses have been linked to aflatoxin exposure. Hendrickse *et al.* (1982) suggested that children with Kwashiorkor were more affected by aflatoxins, their ability to transform and excrete aflatoxins was impaired by metabolic imbalances associated with Kwashiorkor. Coulter *et al.* (1986) biopsied the liver of 27 Sudanese children suffering from Kwashiorkor and 13 children with miscellaneous liver diseases. They reported that aflatoxin B_1, B_2 and aflatoxicol were detected in the organs from children with Kwashiorkor, but none in those suffering from marasmic Kwashiorkor. They also

reported that aflatoxin G_1 and G_2 were detected in 5 of 12 children with chronic liver disease. Further studies were completed by Hendrickse (1984), in a survey of 469 sera and 468 urine samples from children in Sudan, aflatoxin was detected more often and at higher concentration in sera from children with Kwashiorkor than in other groups with malnourishments. Adhikari *et al.* (1994) reports that the consumption of foods that are contaminated with aflatoxin producing fungi by susceptible Kwashiorkor children leads to a reduced immunity. Hendrickse & Maxwell (1989), remarked that aflatoxins were found most frequently and in a higher concentration in the sera of Kwashiorkor children as compared to other groups. The number of infections in these children increased and the number of days spend in hospital were longer (Adhikari *et al.* 1994).

Wild *et al.* (1990) conducted a survey to determine if there is a correlation between aflatoxin, its metabolites and gastritis in an inland Kenyan village which has a high incidence of symptomatic gastritis in young people. The authors reported that patients with gastritis had a significantly higher level of aflatoxin (5-338ug/ml of urine) than in healthy members of the population. They therefore concluded that there is a strong association between urinary aflatoxin and gastritis.

Another correlation exists between the ingestion of aflatoxin and the expression of the sickness Reye's syndrome, a condition that affects small children. Stora *et al.* (1983) reported that between 120 and 810 ug/g of aflatoxin B_1 was detected in the livers of 5 infants suffering from this disease. They concluded that these children experienced acute intoxication by aflatoxin B_1.

Hadiidane *et al.* (1985), collected food samples from the homes of patients suffering from hepatoma, Reye's syndrome and alimentary toxic aleukia in Tunisia. They reported that aflatoxin B_1 was detected in 50 % of the food samples, aflatoxin B_2 in 55%, aflatoxin G_1 in 20% and aflatoxin G_2 in 5%. The authors concluded that these mycotoxins were probably involved in the development of the patients diseases. Studies in the Gambia revealed that exposure to aflatoxins already starts in pre-natal children. Umbilical cord sera was assessed for the presence of aflatoxin-albumin adduct, with 70% of the cord sera being positive for this adduct (Wild *et al.* 1991).

Inhalation of fungal spores of the *Aspergillus* group can lead to the development of lung cancer, especially in workers that are exposed to airborne fungal spores with a carcinogenic potential (Autrup *et al.* 1991), the same risk exists for the inhabitants of mold and rot infected houses (Holmberg 1984). Mulvey *et al.* (1983) conducted a survey during the 1981 sugarcane

harvest in Louisiana, USA to investigate possible causative mechanisms for the increased risk of lung cancer mortality for sugarcane farm workers. Various fungal isolates among which was *A. flavus*, were identified in the sputum samples of the workers, aflatoxin B_1 was also detected.

2.3　　　　　　　　　Factors influencing *Aspergillus* spp. infection and aflatoxin development in maize

Factors that influence growth of *A. flavus* and the formation of aflatoxins can be classified into three categories climatic factors, agronomic factors and biotic factors (Diener *et al.* 1987).

2.3.1 Climatic factors

Under favorable conditions, spores of the fungi will grow and produce aflatoxin. The mycoflora of stored cereals is influenced by environmental factors, especially temperature, water potential, pH and gas atmosphere (Magan & Lacey 1988). Choudary & Sinha (1993) observed in India that aflatoxin accumulation was highest in maize stored for 52 weeks during the monsoon, a season with high relative humidity. A decline of 33% in the level of aflatoxin B_1 was observed during the winter with lower relative humidity. The weather during the growing season, especially during the grain filling period is closely related to pre-harvest infection and subsequent fungal development (Thompson *et al.* 1980). Especially those varieties that are harvested late in the season will be more susceptible to fungal infection, because of early rains and problems with sufficient drying before storage. Gbodi *et al.* (1985) collected maize samples from farmers stores at three different periods of the year: the dry harmattan period from November to February; the hot and dry period from March to May and lastly the hot, humid and wet period from June to September, from several local government areas of Plateau state located in the Mid-Altitude zone in Nigeria. They reported that the highest level of aflatoxin was found in samples from Langtang local government area (Nigeria) which usually has a hot, humid and wet climate. The aflatoxin B_1 level was 960 ppb; aflatoxin B_2 was 544 ppb.

A. flavus has been reported to grow from 10-12°C to 43°C and aflatoxin is produced over the temperature range of 15°C to 37°C. The temperature requirements for several *Aspergillus* species are given in Table 2.1.

Table 2.1:   Optimum temperatures for the development of different
                              Aspergillus spp. and *Penicillium spp.* (compiled from
                              Lacey & Magan 1991; Wilson & Abramson 1992)

FUNGAL SPECIES	Optimum$^{\circ}$C
Aspergillus restrictus	30-35
Aspergillus glaucus	30-35
Aspergillus candidus	45-50
Aspergillus flavus	30-35
Penicillium	20-25

Cereal grains that are infected by *A. flavus* can start to heat and so favor the fungal growth. By the time that 10% of the kernels have been invaded by *A. flavus* serious spoilage will have occurred (Christensen & Kaufman 1974). Diener & Davis (1970) reported that temperatures as low as 8°C may allow the slow growth of *A. flavus* and aflatoxin production at temperatures of 11°C in sterilized rice and 14°C in unsterile peanuts. Rambo *et al.* (1975) and Sorenson *et al.* (1967) reported that fungal growth or toxin production at minimum temperatures probably occurs only when other factors are nearly optimum i.e. if both temperature and moisture content are sub optimal, the fungus is not likely to grow. They also reported that temperatures of 20°C to 35°C are suitable for aflatoxin production, with 37°C to 43°C probably being the upper limit. Ross *et al.* (1979) reported that if both temperature and moisture are favorable for *A. flavus*, aflatoxin can be produced within 48 hours. Temperature requirements for *A. flavus* infection are quite high. Silk inoculation of corn at 32°C to 38°C had the result that 73% of the kernels were infected, at 21°C to 26°C only 2.5% were infected (Jones *et al.* 1980). A phytotron study confirmed that infection required day and night temperatures above 30°C. Widstrom *et al.* (1990) observed that aflatoxin concentration was significantly correlated with maximum and minimum daily temperatures and net daily evaporation following full silking. Early planting of maize in Georgia is believed to bear the greatest risk of contamination with aflatoxins because seasonal maximum and minimum temperatures are at their highest and net evaporation is at its peak. Similar results were obtained by Thompson *et al.* (1980) who reported that the highest toxin levels were found in kernels inoculated at the latest kernel development stage and grown at the highest post inoculation temperature regime.

Studies in Thailand showed that moisture content of maize samples varied from 16.8% to 30.7% depending on the time of harvest. Maize that was harvested early (113 days) showed the highest levels of aflatoxins (Kawasugi *et al.* 1988). Moisture content of less than 17%

showed no infection with *A. flavus*. As relative humidity increases above 85%, the growth of *A. flavus* increases dramatically. In this range even a small increase in moisture can be very significant in terms of increasing the risk of aflatoxin contamination (Christensen & Mirocha 1976; Sauer & Burroughs 1986). There does not seem to be an upper limit of moisture that restricts growth or aflatoxin production, but under normal conditions, very high moisture levels may be unsuitable for *A. flavus* because of competition from other fungi, yeast's and bacteria (Chang & Markakis 1981). Aflatoxins can be formed at a relative humidity of 88, 90 and 99% (Lillehoj 1983), humidities that are common in the southern parts of West-Africa.

In experimental studies the moisture content of the grain rather than the surrounding relative humidity was measured. Trenk & Hartman (1970) reported that at a moisture content of 16% or less aflatoxin production was not likely, but 17% may be high enough for slow growth and aflatoxin production. Studies have shown that considerably higher moisture contents may be optimal for growth and toxin production (Chang & Markakis 1981). Calderwood & Schroeder (1968) showed that *A. flavus* growth was optimal in freshly harvested maize with a moisture content of 20 to 28%, particularly at high temperatures of 20° to 30°C.

A better measurement for the likelihood of *A. flavus* in colonizing a given substrate, is the water activity (a_W) which indicates the availability of water and is dependent on temperature and increases with relative humidity (Mahmoud *et al.* 1992). Water activity is the ratio of the vapor pressure of water over a substrate (P) to that vapor pressure over pure water at the same temperature and pressure (Po).

$$a_W = \frac{P}{Po}$$

Fernandez-Pinto *et al.* (1991) observed that minimal aflatoxin production occurred at an a_W of 0.895 at above 20°C and the maximum toxin production was recorded at a_W 0.95 at temperatures of 37°C. This report was confirmed by Faraj *et al.* (1991) who reported maximal colony growth of *A. flavus* at 30°C and 0.95 and 0.98 a_W. Table 2.2. shows the water activity that is necessary for the growth of various species:

Table 2.2:   Minimum water activity (a$_W$) for the growth of different
                    Aspergillus spp. and *Penicillium spp.* (compiled from Lacey &
                    Magan 1991)

Fungal Species	Minimum a$_W$
Aspergillus halophilicus	0.68
Aspergillus restrictus	0.70
Aspergillus glaucus	0.73
Aspergillus candidus	0.80
Aspergillus flavus	0.85
Penicillium	0.80-0.90

Cuero *et al.* (1987) reported that aflatoxin production was highest at 0.98 and 0.95 aw at 25°C. Lacey & Magan (1991) concluded that the minimum a$_W$ for germination and growth of *A. flavus* is 0.78 and the optimum 0.95. A water activity of 0.70 which is below the requirements of the *Aspergillus* fungi will successfully protect the grains against fungal attacks. Commodities stored at humidity between 75 and 85% are susceptible to fungal attack within normal storage time.

Growth of different fungi on media with a specific pH showed that *A. parasiticus* was more tolerant of acidic pH than *A. flavus* with optimum growth at a pH from 3 to 8. West-African soils are usually more acidic. *A. flavus* growth was greatest in a range from 5 to 8. All *Aspergillus* species tested grew rapidly at alkaline pH with poor tolerance of very acid pH (Wheeler *et al.* 1991).

2.3.2   Agronomic factors

Agronomic factors that may influence aflatoxin development are plant stress, irrigation, cropping pattern, variety, date of planting, date of harvest and storage conditions (Cotty 1994). The plant characteristics that can affect the development of grain molds before harvest include: susceptibility to insect damage, physical and chemical characteristics of the husk, physical and chemical characteristics of the grain and influence of the climate itself which sometimes is expressed as plant stress (Williams & McDonald 1983).

The susceptibility of different maize varieties towards contamination with *A. flavus* and aflatoxins was studied by Kang *et al.* (1990), with the aim to select for resistant varieties. Several varieties which were less susceptible to contamination with *A. flavus* were identified, but no variety with a full resistance could be selected. Lillehoj *et al.* (1980) tested hybrids at 12

different test sites regarding their aflatoxin producing ability. There were no varieties that showed complete resistance to aflatoxins. The effect of location was very pronounced, mechanical damage of the ears increased the aflatoxin content significantly. Certain varieties were more susceptible to *Aspergillus spp.* infection with increasing relative humidity (Lokesha *et al.* 1987). Zuber *et al.* (1983) observed that open-pollinated varieties showed a higher percentage of aflatoxin infected cobs than hybrids. They speculated that this was because of plant stress during the grain filling period and influenced by higher temperatures during some of the study years. Aflatoxin levels varied from 2 to 605 ppb in hybrid maize varieties. No genotypic response was observed, but there was a significant relationship between irrigation and aflatoxin content (Fortnum & Manwiller 1985). Nwogu & Nwankwo (1979) in a survey to compare white and yellow maize varieties in Port-Harcourt markets in Rivers state in Nigeria, concluded that the yellow maize variety was more susceptible to microbial and most fungal attack, with *A. flavus* being the most predominant fungal species.

Castor *et al.* (1987) observed that Haitian maize market samples from the January harvest consistently revealed more aflatoxin-free samples than the July or October harvest, so that a seasonal effect on aflatoxin contamination could be observed. Bilgrami *et al.* (1992) evaluated the influence of cropping pattern on aflatoxin contamination of maize grown during the monsoon season in India. Toxin levels were the highest at a planting density of 56,000 plants/ha followed by 83,000 plants/ha in inoculated plots. Weeding of the maize fields during the growing season did not influence the contamination with aflatoxins. Lillehoj *et al.* (1983) observed that planting maize so that the silkening stage does not coincide with the conidial spread reduces the development of aflatoxins. In the same study elevated levels of aflatoxins were correlated with heavy insect damage. The planting date plays a role in the development of the toxin, significant reductions in aflatoxin B_1 was associated with early planting (Jones & Duncan 1981) .

Several authors investigated the influence of the date of harvest on the development of aflatoxins (Lee & Chuang 1993; Scott & Zummo 1994; Lynch *et al.* 1991). A confounding factor in the infestation with aflatoxins is the practice of leaving maize unharvested in the field after maturity. Significantly more aflatoxin was found in grains harvested late than those harvested early (Jones & Duncan 1981). Contrary to the above studies, no trend towards an increase in aflatoxin was found in maize in Thailand as field-drying time increased (Nagler *et*

al. 1992). Tanboon-Ek (1989) remarked that field drying, followed by mechanical drying are the most effective means of reducing aflatoxin contamination in maize in the same region.

Growth under stressed conditions, such as dense population of plants or reduced fertilization was reported to have an influence on the incidence of contamination by aflatoxin. Aflatoxin concentration of kernels inoculated with *A. flavus* was negatively correlated with corn yield, silk leaf nitrogen and grain nitrogen. The inoculated kernels that received no nitrogen had an average of 28% more aflatoxin, than those that received fertilizer (Payne *et al.* 1989). There was a positive correlation between high aflatoxin levels in corn and plots receiving low levels of nitrogen (Jones & Duncan 1981). Dense populations of plants and reduced fertilization also resulted in higher aflatoxin levels. Schmitt & Hurburgh (1989) reported that aflatoxin concentration was highest in the southeast and south-central regions of Iowa during the 1983 season coinciding with extreme drought conditions suffered earlier that year. This observation is also corroborated by Wicklow (1988), who reported that drought stress and crowding of corn plants were associated with *A. flavus* contamination and aflatoxin. These results are confirmed in studies by Hill *et al.* (1983) who found an average of 244 ppb in drought stressed sound mature kernels, insect damaged kernels showed even higher levels. Field trials with irrigated and non-irrigated plots of with *A. parasiticus* artificially infested groundnuts showed that irrigation decreased the frequency of all *Aspergillus* species (Brenneman *et al.* 1993). Azaizeh *et al.* (1989) observed that drought stress leads to enhanced colonization of groundnut shells and kernels. Smith & Riley (1992) showed that drought stress and insect damage have a synergistic effect on aflatoxin levels in preharvest corn. In the plots that did not receive irrigation or insecticide application a 48.8% increase in BGYF (Bright greenish yellow fluorescence) observed under black light and an aflatoxin B1 concentration of 191.5 ppb was observed, while the plots that received irrigation and insecticide application had the lowest BGYF (1.1%) and a mean of 0.06 ppb of aflatoxin B1. Water stress during the last growing period contributed to aflatoxin development in sound mature kernels of peanuts, but if these plants were irrigated during the same time no significant aflatoxin was detected (Williams & McDonald 1983). Davis *et al.* (1986) remarks that corn plants exposed to drought stress are more susceptible to infection by *A. flavus* than unstressed plants.

Another factor that contributed to the contamination with *Aspergillus* species was the presence of a weed canopy (Lillehoj 1983). Sander *et al.* (1985) observed that cotton that was standing in weedy patches showed higher amounts of aflatoxins than those that were in plots without

weeds. Maize that is standing in weedy patches faces interspecific competition similar to the stress that is faced by plants through droughts. Jones (1986) remarked that this type of stress would play a role in years that have high rainfall during the early vegetative stages, followed by drought during the reproductive period.

If maize was grown with a crop that was also susceptible to aflatoxin development there is an increased risk of toxin metabolism (Cotty 1994). Cole *et al.* (1982) investigated the effect of a peanut, maize, soybean crop rotation on aflatoxin development. He found more aflatoxin if maize was planted after groundnut. Similar effects were observed by Griffin *et al.* (1981), when maize was cropped in rotation with peanuts, the authors noticed a *A. flavus* population build-up in the soil over the years. Bilgrami *et al.* (1991) observed 23-28% aflatoxin-positive samples in monocropped mustard and 9-13% in mixed cropping. The lack of competitive fungi in monocropped mustard was given as reasons for the high aflatoxin development.

Soil type can also play a role in the contamination with *A. flavus*. Mehan *et al.* (1991) found that groundnuts grown on Vertisols showed much lower concentrations of aflatoxins, than the variety grown on Alfisols. Application of gypsum to groundnuts at 35 days after planting led to a decrease in aflatoxin production and chitin assays showed less fungal biomass (Reding *et al.* 1993). Gypsum used as a fertilizer changes the soil pH to more basidic values and reduced the frequency of *Aspergillus* fungi.

Development of storage fungi in a post-harvest commodity is determined also by length of time in storage (Lillehoj & Zuber 1988). Ahmad (1993) observed that aflatoxin contamination in storage was dependent on the storage system. Levels of aflatoxin were lower in closed metal bins which restricted air exchange and reduced oxygen levels, than in gunny bags that allowed air to flow through stored blackgram seeds. Bhatti *et al.* (1990) reported for Pakistan that seed samples stored for 8 to 12 months at a higher moisture content (12-17%) and those collected from mud plastered stores had a higher incidence of aflatoxin producing *A flavus* strains. Aflatoxin contamination of maize stored in traditional storage structures in Bihar, India was highest in the kothi made out of mud and rice husk, as compared to the mora made from paddy hay ropes wound in a container, gunny bags or iron bins (Prasad *et al.* 1987).

2.3.3   Biotic factors

Several authors observed a relationship between insect damage and aflatoxin formation (Bowen & Mack 1991; Lynch & Wilson 1991; Lynch *et al.* 1991; Gorman & Kang 1991).

Wounding by insects may provide entry points for fungal spores. Barry *et al.* (1992) showed that maize cultivars that had a resistance to ear-infesting insects also produced less aflatoxins in pre-harvest grains. It was reported that under any given environmental conditions, fungal growth may be several times faster in damaged as compared to intact kernels (Tuite *et al.* 1985; Fennel *et al.* 1977). Cracks and breaks in maize are caused mainly by harvesting, although insect feeding may also be responsible for breaks in the pericarp. Zuber *et al.* (1986) reported that insects that feed on maize ears in the field predispose kernels to *A. flavus* infection through the physical damage caused by their feeding. Likewise, insect feeding in stored maize might open the kernels to fungal invasion. Insects may act as vectors by carrying fungal spores on their bodies and by contaminating grain as they move about. McMillian *et al.* (1990) reported that insect damage contributed significantly to enhanced *A. flavus* sporulation and aflatoxin contamination, by transmitting the fungal spores into the kernels. In the same study a significant correlation was found between the yearly means of aflatoxin contamination and *A. flavus* contamination of lepidopterous pests found at the same locations. Nitidulids (sap beetles, picnic beetles etc.), are known to carry toxigenic fungi including *A. flavus* (Dowd 1991). In laboratory studies adult *Carpophilus lugubris* Murray and *C. hemipterus* (L.) preferred damaged to undamaged maize kernels (Dowd 1994). Hardin (1987) reported that damage by earworms rendered the maize cob more attractive to soil-inhabiting nitidulids which feed on crop residues on the soil. The author observed that these beetles carry *A. flavus* spores into earworm damaged cobs. A partial means of control of these beetles, and in consequence of aflatoxin contamination, would be to remove these crop residues from the field after harvest.

Strong correlations were found between the infestation of stored maize with the maize weevil *Sitophilus zeamais* Motsch. and other secondary species and the contamination with *A. flavus* (Wright *et al.* 1992; Sinha & Sinha 1991). The incidence of *A. flavus* fungi and aflatoxin contamination was comparatively higher in insect-damaged maize samples from different localities in India than in insect free samples (Sinha & Sinha 1992). In laboratory trials, rapid insect population growth increased moisture content, dust production, *A. flavus* infection and aflatoxin contamination and decreased germinability. Toxigenic strains of *A. flavus* were isolated from the insects with no difference between species (Sinha & Sinha 1992).

Infestation of maize with insects and *Aspergillus* species is influenced by husk type. Maize varieties that possess relatively tight, complete husk cover had significantly lower mean amounts of aflatoxin, than those with loose, incomplete huskcover following artificial *A. flavus*

infestation. Ears hand-infested with the curculionid *S. zeamais* had higher amount of aflatoxin (329 ppb), than those infested with noctuid *Spodoptera frugiperda* (J.E. Smith) (80 ppb), the pyralid *Ostrinia nubilalis* (Hb.) (71 ppb) or the noctuid *Heliothis zea* (Boddie) (60 ppb) (McMillian *et al.* 1987).

Another factor that influenced the growth of *A. flavus* on its substrate was the competition with other organisms. Zummo & Scott (1992) found that after artificially infecting maize grains with *Fusarium moniliforme* (Sheld.) and *A. flavus* the percentage of grains infected with *A. flavus* was lower than in grains infected with *A. flavus* alone. Ramakrishna *et al.* (1993) remarked that faster growth of one species caused inhibition of slower growing species. In areas of contact of two species discrete colonies were formed, however at 30°C *A. flavus* overgrew the colonies of all other species. In the same trials aflatoxin B_1 production was decreased through the presence of *Penicillium verrucosum* (Dierckx). In kernel simultaneously inoculated with *F. moniliforme* and *A. flavus*, aflatoxin levels were 12 times lower, than kernels that were wound inoculated with *A. flavus* alone (Wicklow *et al.* 1980).

2.4 Aflatoxin survey reports from different agroecological regions

Most of the surveys on aflatoxin were effected in developed countries, data for the developing countries is rather sparse and in West-Africa even more so. Tuite (1984) carried out a survey of 493 fields of dent corn from 67 counties in Indiana, USA and they reported that the average concentration of aflatoxin B_1 was 79.9 ppb while the highest amount of total aflatoxin was 471 ppb. They attributed this high incidence of aflatoxin to the high temperatures and drought that were experienced in those counties. A survey of some maize growing areas of Bihar State, India, found maize infested with mycotoxin producing fungi *A. flavus, A. niger* (van Tieghem), *A. ochraceus* (Wilhelm) and *Fusarium* spp. within different maize samples (Sinha & Ranjan 1990). The authors collected 76 maize samples during flood and post-flood periods in north Bihar, India. They reported that all 76 maize samples were positive for aflatoxin and amounts of aflatoxin B_1 ranged from 66 to 2163 ppb. Their results further confirmed that aflatoxin contamination of corn can occur prior to harvest. Aflatoxin producing fungi had the highest frequency of occurrence in all the cases. It was also reported that out of 248 *A. flavus* isolates screened, 154 produced aflatoxins. Madsen & Rasmussen (1990) collected 581 samples from different countries comprised of 197 samples of maize and maize products and the rest of millet, rice and pulses. They reported that the highest incidence and concentration of aflatoxins

(174 ppb) was in the maize samples, particularly, maize grain. Huang *et al.* (1990) in China collected 28 maize grain samples from local areas. They reported that the grain samples were contamination with *A. flavus* varied from 100% to 14%. They also reported that 17 of the samples produced aflatoxin B_1 and B_2 and other samples produced B_1, B_2, G_1 and G_2.

2.5   Past work on aflatoxins in West-Africa

Studies have been undertaken in different regions of the world, to survey for the presence of aflatoxins, with the result that 42 to 100% of the samples were contaminated (Lillehoj & Zuber 1988). In West-Africa the research work has been mostly concentrated on groundnuts (Jonsyn 1989; McDonald & Harkness 1967; McDonald 1964) and processed food (Nwokolo & Okonkwo 1978, Akano & Atanda 1990, Adebajo *et al.* 1994).

The work on groundnuts in the 60s and 70s in Nigeria was initiated because they were a very important food and cash crop and regulatory limits for aflatoxins were established for their export (Mehan *et al.* 1991). The research to solve the aflatoxin problem on groundnuts focused on artificial drying (McDonald 1976; McDonald & Harkness 1964) and varietal differences (McDonald & Harkness 1967).

Dietary exposure to aflatoxins in Nigeria was studied in 100 samples of 10 commodities by Ibeh *et al.* (1991) 50% of yam flour, 40% of cassava flour, 30% of processed cassava (garri), 20% of beans and 10% of rice showed aflatoxins. These high contamination levels show that aflatoxins are a serious health hazard in Nigeria. Opadokun (1990) in an evaluation of aflatoxin in market samples over the past 28 years, remarked that the most susceptible Nigerian crops to aflatoxin contamination are maize and groundnuts, and to a lesser extent cottonseed and melonseed. Udoh (1995) in a two-year survey of stored maize of 25 farmers each in five agroecological zones observed that in the mid-altitude zone one sample (1994) was contaminated with aflatoxins and in the Northern Guinea Savanna three samples (1995) showed low aflatoxin contamination. In the Humid Forest zone 16% (1994) and 27% (1995) of the samples were contaminated. In the Sudan Savanna 12% (1994) and 25% (1995) and in the Southern Guinea Savanna 24% (1994) of the samples were aflatoxin positive.

A study by Opadokun *et al.* (1979) 39 maize samples were obtained from Kano markets (Nigeria), moisture content from 6.6% to 18.9% were measured. One third of the samples were aflatoxin positive with an average of 106 ppb, 8 samples had more than 30 ppb, the limit for human consumption in Nigeria. In 37 samples that were obtained from farmers stores,

markets and government farms, the moisture content was high at 15 to 26%, levels that allow aflatoxin development (Oyeniran 1973). Aflatoxin content was low in unshelled samples from farmers stores, except those that were preserved heaped on the floor, and very high contamination levels were found in shelled maize from government farms. No reasons were given for the differences in aflatoxin content between the differently stored grain samples. Alozie *et al.* (1980) investigated the presence of aflatoxin in Nigerian beverages. The beverages were "emu aran" and "ogogoro" produced from palm juice, "brukutu" and "pito" made from either sorghum or millet. The authors detected aflatoxin in all four of the beverages and all of them supported the growth of *A. flavus*. Okoye (1986) conducted a survey of traditional breweries in Jos in northern Nigeria and reported that the traditional beers contained a high incidence of aflatoxin B_1.

In the Republic of Benin 32 samples, mostly taken from commercial warehouses, were analyzed by HPLC for aflatoxins and ochratoxins (Bouraima *et al.* 1993). Twenty-one of the samples proved to be positive at levels between 0.54 ppb to 14 ppb of aflatoxin B_1, 0.50 ppb to 6 ppb for B_2, 0.09 ppb to 58 ppb for G_1 and 0.05 ppb to 10 ppb for G_2. Levels higher than 20 ppb were found in 8 samples. Only 5 samples were positive for ochratoxin A at levels between 15 and 45 ppb.

Levels of aflatoxin contamination of local foods and beverages is directly related to the incidence of liver cancer in the various regions of Africa (Linsell 1977; Keen & Martin 1971; Alpert *et al.* 1971). The mycoflora of two fermented foodstuffs of Sierra Leone, "ogi" made from maize and "foofoo" from cassava were determined before, during and after fermentation by Jonsyn (1989). He reported that the maize kernels contained *A. flavus* growth and aflatoxin B_1 and ochratoxin A were extracted from these kernels. The processed "foofoo" also contained aflatoxin, whereas in the "ogi" only traces were detected. Groopman *et al.* (1990) studied different processed food samples in the Gambia and found that groundnut sauce was the primary source of aflatoxin contamination with levels of 2 to 333 ppb. Hendrickse (1984) detected aflatoxins in several commonly eaten foods obtained from local markets in Sudan. In raw food samples from homes 80% were positive for aflatoxins. In surveys in Ghana all the samples from warehouses and silos showed aflatoxins in levels from 10 to 150 ppb (Kpodo 1996). Aflatoxin contamination of foods is polyvalent, but the risk seems to be higher in countries with high humidities and poor maize production systems.

CHAPTER 3 Temporal and spatial distribution of aflatoxins

3.1 Introduction

The distribution of aflatoxin in stored grains is not uniform. A small portion of grains may contain very high quantities of the toxin (Francis *et al.* 1988; Whitaker *et al.* 1979). Dickens (1987) suggested when checking for aflatoxin presence, samples should be biased towards those grains that are likely to contain aflatoxins. Kernels with visible mold growth or those damaged by insects, should be sampled with priority. To estimate the average aflatoxin concentration in a given lot of grains, the sample should be unbiased and collected in a random manner (Dickens 1987). Davis *et al.* (1980) have established protocols for survey sampling and came to the conclusion that a ten pound sample of shelled maize is probably sufficient to determine the average aflatoxin distribution in a lot. Francis *et al.* (1988) compared aflatoxin contamination in different amounts of grains and found that different sample sizes did not give significantly different results, he came to the conclusion that aflatoxin analysis of a 10g sample that was sufficiently ground and blended, would give a statistically similar result than a 50 g sample. To establish a satisfactory sampling procedure for the surveys conducted for this work, experiments were carried out to determine the influence of position in the store and time of sampling after storage on aflatoxin content. Jewers *et al.* (1988) have found that there were no significant differences between samples drawn from different parts of a sack. Another aspect that was elucidated in the pre-trial, was the effect of fumigation and freezing on the resulting mycoflora with the objective of determining if these post-harvest measures could be used to kill the insect fauna right after sampling. In order to study the insect populations, without influencing the fungal flora.

3.2 Materials and Methods

3.2.1 Influence of position in a granary and freezing on aflatoxin content

Experimental granaries were established by the Larger Grain Borer-Project in the Mono-Province in 1992-93, in the Coastal Savanna of Benin. On a platform cylinders out of mesh wire were filled with maize cobs, arranged into 4 different layers from the top to the bottom, which were sampled after 36 weeks of storage. A 20 cob sample replicated three times was taken from each layer, dehusked and degrained and stored at 2°C. A further 100 g subsample replicated three times was frozen (-10°C) for 24 hours to kill the insect fauna. On PDA-plates

50 grains which had been frozen (-10°C) were plated out and the appearing fungi compared to 50 plated refrigerated grains (2°C).

The grains were washed 1 minute in 5% NaClO and then with sterile water. Aflatoxin content was determined through TARGET test kits (TERRATEK Inc.). This aflatoxin field testkit can detect aflatoxin levels of extracted samples in methanol/water (80:20), at 20 ppb and 10 ppb. The column will fluoresce blue under a 365nm lamp if aflatoxins are present. Resulting data was related to the faunistic data and moisture content of the sample data which was collected by the LGB-Working Group, with the aim to see if the presence of insects had any effect on the incidence of *Aspergillus*. Statistical differences between the different samples and treatments were evaluated using the SPSS-Statistical Package (Norusis & SPSS 1993) and means were separated using the Student-Newman-Keuls Test.

3.2.2 Influence of storage treatments on aflatoxin content

Another pre-trial was set up in 1992-93 (Ex-Soprova) to determine if fumigation of samples would have an effect on the mycoflora and resultant mycotoxins. Maize was harvested, dried and fumigated under polyethylene sheets for three days with 0.9 g of Gastoxin® (56.7% aluminium phosphide, Casa Bernardo Limitado, Brasil). Experimental granaries were established in the Mono province by the LGB-Working Group. The four treatments in this trial were fumigated, non-fumigated, visual selection, and fumigation followed by visual selection. Maize cobs were selected on the basis of size, huskcover and presence of visible insect damage and removed before storage. The 20 cob samples replicated three times, were evaluated after 36 weeks of storage for their mycoflora and TARGET test kits determined the aflatoxin levels. Statistical differences were evaluated running the SPSS-Statistical Package (Norusis & SPSS 1993) and means were separated with the Student-Newman-Keuls Test.

3.2.3 Temporal and spatial distribution of *Aspergillus* spp. in an experimental granary

The aim of this study was to determine if a certain position in a granary would have an effect on the resultant fungal contamination and aflatoxins in a sample. Another aspect studied was, if fungal contamination and aflatoxin content changes with storage time. The experiment was set up in the 92-93 season by the LGB-Working Group in Dadohoue (Mono) to determine the effect of cob position on the presence of insects in a granary. To study fungal distribution and aflatoxin content 100 g subsamples were taken from 20 cob samples removed from 3 positions

in the granary: top, bottom and center. The timing was 6 and 9 months after harvest, with 6 replications. The grains were washed 1 minute in 5% NaClO and then with sterile water. Fungi were determined through blotter paper plating (25 kernels per sample) of 3 samples per position, the different fungal species were determined 5 days afterwards. Aflatoxin content was determined through the TARGET-testkit®. Resulting fungal data was related to the faunistic data collected by the LGB-Working Group, with the aim of determining if the presence of insects would have any effect on the incidence of *Aspergillus* spp.. Statistical differences between the different positions in the granary and samples taken at different times after storage were evaluated using the SPSS-Statistical Package (Norusis & SPSS 1993) and means were separated using the Student-Newman-Keuls Test.

3.3 Results

3.3.1 Influence of position in a granary and freezing on aflatoxin content

The analysis of variance showed that there were no differences between the different positions inside a grain granary as related to aflatoxins. There were significant differences between the different positions for the total number of fungi infecting the samples. The top of the granary was significantly more affected by fungi when measured as the total amount of fungi (Table 3.1). If the different fungal species were compared separately there were no differences between the layers.

Table 3.1: Influence of position on the mean fungal contamination of maize stored for 36 weeks (Evaluation of 75 kernels per position)

Position	*Aspergillus*	*Fusarium*	*Penicillium*	Total Fungi
Top	15.6 a	20.0 a	8.9 a	93.3 b
Upper Middle	17.8 a	13.3 a	8.9 a	71.1 ab
Lower Middle	31.7 a	4.3 a	2.0 a	73.3 ab
Bottom	17.8 a	13.3 a	11.1a	53.3 a
F-Value	0.80	1.15	0.69	2.24

Fungal data (%) was arcsine ÷ transformed
Means followed by the same letter are not significantly different from each other(SNK, p = 0.05)

Aflatoxins were only found in the samples of the bottom layer and out of the 3 samples there were 2 that were positive at 10 ppb. No differences could be found between the fungal flora of the refrigerated samples stored at (2^OC) and those that were frozen (-10^OC) for 24 hours. No aflatoxins were found in the samples stored in the freezer, but only one sample per layer was

tested to reduce labor and chemical waste. This indicates that it is possible to freeze grain samples for a short while, to eliminate the insect fauna, without significantly altering the composition of the fungal flora.

3.3.2　　　　　　　　　　　Influence of storage treatments on aflatoxin content

The analysis of variance showed that the samples that were fumigated had significantly (p=0.0109) more *Aspergillus* fungi than the other treatments (Table 3.2).

Table 3.2:　　　　　　　Influence of cob selection and fumigation on *Aspergillus* spp. and mean total fungal contamination of maize stored for 36 weeks (mean of 100 kernels per sample) mean $\pm$ S.E.

Treatment	*Aspergillus*	*Fusarium*	*Penicillium*	Total Fungi	G.M.C.
Fumigation	26.7 b	16.0 a	12.0 a	61.3 b	11.9 a
No Fumigation	16.0 ab	5.3 a	6.7 a	41.3 ab	11.6 a
Selection	10.7 a	6 .7 a	2.7 a	34.7 a	11.8 a
Select+Fumigate	21.3 b	8.0 a	6.7 a	49.3 ab	11.6 a
F-Value	4.07	0.86	0.88	2.74	3.05

G.M.C. = Grain moisture content, Fungal data (%) was arcsine $\div$ transformed
Means followed by the same letter are not significantly different from each other(SNK, p = 0.05)

For the total mean number of fungi, there were significant differences between the treatments (Table 3.2). The fumigated maize had significantly more *Aspergillus* and total fungal development than the maize that was selected for damage. There were no differences for the grain moisture content (G.M.C.), infection with *Fusarium* spp. and total percentage of fungi between the different treatments. Only two out of 12 samples showed a positive result in the TARGET Test®. Repetition 2 of the fumigated treatment was positive at 10 ppb, and Repetition 3 of the select treatment was positive at 20 ppb. But this does not let us draw conclusions about the advantages of fumigation in reducing aflatoxin contamination, or if fumigation results in higher fungal contamination. The aim of the trial was to see if fumigation can eliminate insect contamination while at the same time not interfering with number of fungal organisms encountered on the samples. The selection of grains seemed to have a reducing effect on fungal development with lower total amounts of fungi observed, especially the percentage *Aspergillus* was significantly lower than in the other treatments (Table 3.2).

The number of adult insects in the nonfumigated treatments was much higher than in the fumigated treatments, where the same insect numbers were reached more than three months

later (Figure 3.1). No large differences between the selected and nonselected treatment were observed for the insect numbers (Borgemeister *et al.* 1994).

Figure 3.1:   Density of *Prostephanus truncatus*/kg grain by treatment over 12 sampling occasions (b) density of *Sitophilus zeamais*/kg grain by treatment over12 sampling occasions, and (c) grain losses (%) by treatment over 12 sampling occasions (Borgemeister *et al.* 1994).
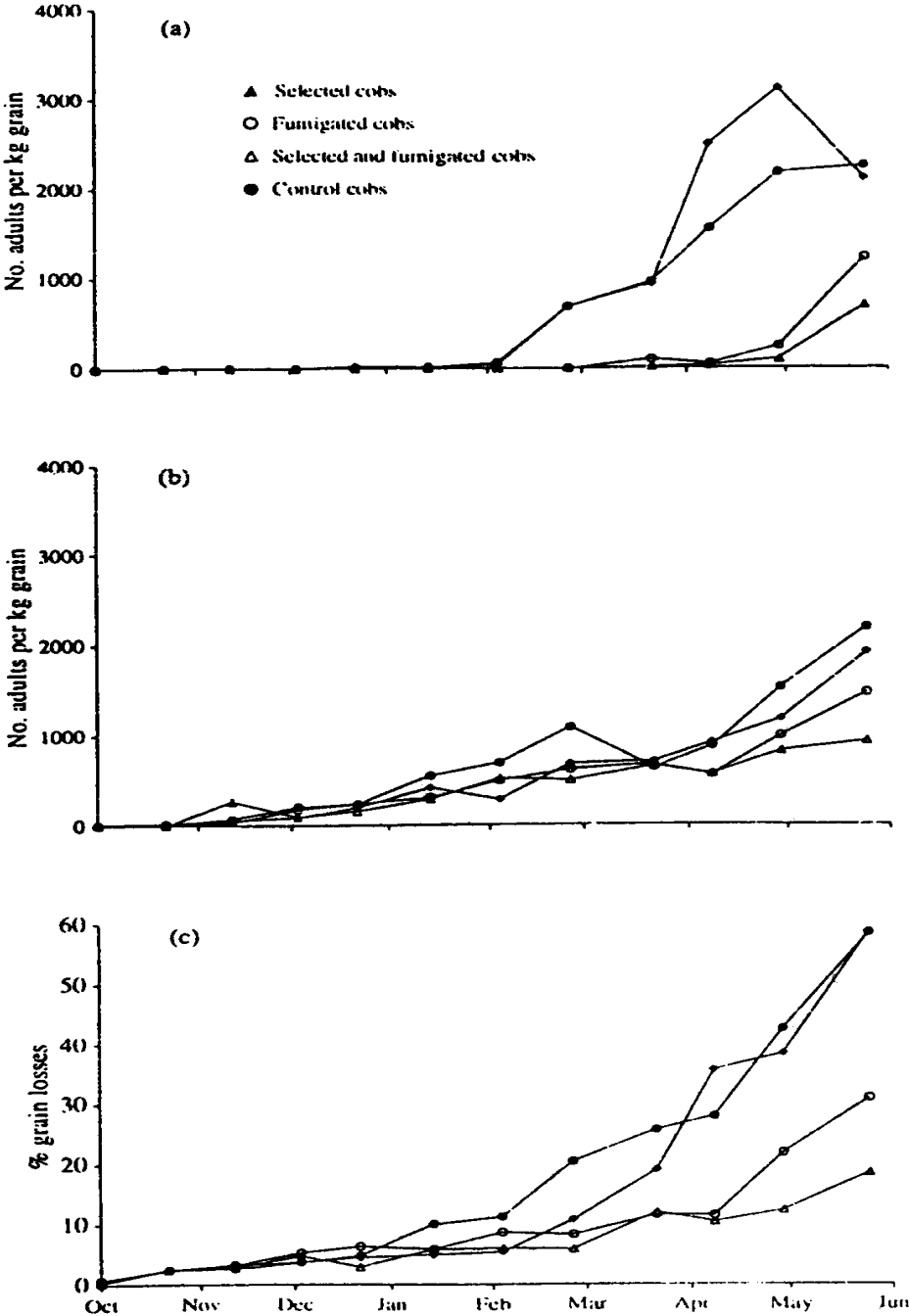
3.3.3 Position and time trial

There were significant differences in the distribution of the fungi between the layers for the sampling after 6 months of storage only for the *Fusarium* spp. (p=0.03) (Table 3.3).

Table 3.3: Fungal distribution at the top, middle and bottom of a maize store after six months of storage (92-93)

Position	*Rhizopus*	*Fusarium*	*Penicillium*	*Aspergillus*	Total Fungi
Top	0.0 a	16.0 b	4.0 a	6.7 a	29.3 a
Middle	2.2 a	13.3 b	6.7 a	7.1 a	32.0 a
Bottom	0.0 a	1.3 a	10.7 a	14.7 a	26.7 a
F-Value	1.03	4.17	0.78	0.34	0.48

Fungal data (%) was arcsine ÷ transformed
Means followed by the same letter are not significantly different from each other(SNK, p = 0.05)

From one sampling to another there was a rapid increase in the fungal population. Whereas at 6 months most of the fungi were found in the bottom layer except for *Fusarium*, at 9 months most of the fungi were found in the top layer (Table 3.4).

Table 3.4: Fungal distribution at the top, middle and bottom of a maize store after nine months of storage (92-93)

Position	*Rhizopus*	*Fusarium*	*Penicillium*	*Aspergillus*	Total Fungi
Top	4.8 a	27.6 b	7.6 a	28.6 a	67.6 b
Middle	6.0 a	16.8 a	9.5 a	23.8 a	57.9 ab
Bottom	6.7 a	17.1 ab	4.8 a	16.2 a	45.7 a
F-Value	0.10	1.95	1.05	1.26	3.09

Fungal data (%) was arcsine ÷ transformed
Means followed by the same letter are not significantly different from each other(SNK, p = 0.05)

Over time the development of fungi in the top layer was more rapid than in the bottom layers. *Aspergillus* spp. contamination increased over time. After 9 months of storage significant differences between the different layers could be observed for the infection with *Fusarium* spp. and the total fungal contamination. Aflatoxin content also increased with time, while none of the samples were aflatoxin positive at 6 months, 33% were at 9 months. The top layer was more heavily affected by fungi over time. This might be specific to this particular storage type, one possible factor for the increased aflatoxin levels could be rain water that may seep into the thatch roof over time.

3.4   Discussion

Freezing had no effect on the contamination with fungi, and there were no differences in the fungal contamination levels between the frozen and the refrigerated samples. The conclusion was that it is possible to freeze samples to kill insects, to aid in determination and counting of these insects. This method was also used in other laboratories to store maize samples before processing, with no adverse effects on the fungal flora and aflatoxin analysis (Cotty, P.J. pers. comm.).

Selection of cobs for damage of insects, size and huskcover and their removal resulted in a decrease of the contamination of the samples with *A. flavus* (Table 3.2). Pelletier and Reizner (1992) described that hand-sorting was more efficient in removing aflatoxin contaminated grains than machine sorting or fluorescent sorting of peanuts. Sorting of visibly infected maize kernels and discolored grains, has been suggested to be a remedy for high aflatoxin contamination (Prete & Cicero 1987). In this experiment the selection of cobs reduced the fungal contamination, but these reductions were not significant.

It would have been expected that fumigation (Table 3.2) would have had a secondary effect on the development of aflatoxin through the reduction of insect damage, as insects facilitate the development of aflatoxins through their boring and predispose the grains to infection with the aflatoxin producing fungi (Bowen & Mack 1991; Gorman & Kang 1991). Wright *et al.* (1992) remarked that, fumigation when weevil populations were high, produced many dead insects on which *A. flavus* propagated, supplying great quantities of inoculum. Also Leitao *et al.* (1987) found no influence of phospine on *A. flavus* and aflatoxin production. This could be one of the reasons why *A. flavus* development was higher in the fumigated stored cobs. No aflatoxin reducing effect when maize was fumigated was noticed in this experiment, rather highest development of *A. flavus* was found in the fumigated treatments. It could have been that the maize that was fumigated under the polyethylene sheets was not dried very well after harvest. The microclimate under the sheets could have induced fungal development.

There was an indication in this study that there was a higher rate of fungal development in the upper parts of the grain stores (Table 3.4 and 3.1). This could be because of the rain penetrating the thatch roof, that will become looser with storage time. Fiagan (1994) remarked that molding can start when rains would infiltrate the stored goods. Another possibility is that the differences in temperature were very pronounced at the top of the stored goods (CEEMAT

1988). Several authors (Payne *et al.* 1988; Widstrom *et al.* 1990) have described that changes in minimum and maximum temperatures will result in higher likelihood of aflatoxin development, than when temperatures do not show such big variations. Grains at the top of the granary will be more likely to absorb heat during the day and cool down during the night. There might be humidity build-up in the top layers because of rain-water seeping in or because of absorption of atmospheric moisture (Ranjan *et al.* 1992).

Christensen & Meronuck (1989) remarked that dry matter losses in stored grains will increase with time of storage, also fungal contamination will increase with time of storage. In this study fungal contamination increased with storage time (Table 3.3 and 3.4). The percentage of total fungal contamination nearly doubled from sampling at six months of storage to nine months of storage (Table 3.3 and 3.4), mostly because of an increase in contamination with *Aspergillus* spp..

Dickens *et al.* (1983) described that there was a high probability that the aflatoxin concentration in a small sample was less than the aflatoxin concentration in the whole lot. When taking samples for mycotoxin analysis, one had to take a big sample out of the bin and then subsample. This methodology was adapted for the presented study.

CHAPTER 4   **Maize production, harvest and storage practices in four agroecological zones in Benin**

4.1   Introduction

The different agroecological zones of Benin (introduced in section 1.3) were surveyed to assess the farmers practices of producing, harvesting and storing maize. The aim of this study was to relate production factors to post-harvest quality of maize. Country-wide surveys have been carried out in Benin, but mostly from an economic point of view (Totongnon 1994) or from the plant protection point of view (SPV 1992). A good overview about maize production and marketing in West-Africa can be found in the proceedings of the FAO-Workshop (1994) and in the thesis from Lutz (1994).

4.2   Materials and Methods

Surveys were conducted in 30 villages in Benin (see Figure 1.1), at the beginning of the storage period 1993, from September till December. Eight villages were chosen per agroecological zone, with six villages in the SS, because of the lower importance of maize production in this area. The choice of the villages was aided through personal contacts with either extension service workers or development aid volunteers which intervened in the visited villages.

The basic questionnaire was adapted from a similar study by Albert (1991) in Togo. The questionnaires were field tested in two villages in northern Nigeria and in two villages in southern Benin and then modified to fit the purposes of this study.

First year questionnaire

The questionnaire was divided into four sections: production factors; harvest factors which included sorting and drying; the storage factors including a section about the storage structure and about the pest problems in storage and the farmers solutions to these problems. The last section was on maize consumption habits of the farmers. A copy of the questionnaire can be found in Annex I. Ten farmers were interviewed per village. In the southern zones of Benin in each village four woman farmers were interviewed. These woman maize farmers were difficult to find in northern Benin, since land ownership and the traditional role of the woman is different in this region, with fewer women taking part in the agricultural activities than in the south.

Second year questionnaire

The same villages as in the first year were visited in the second year. Those farming practices that could change from year to year were reevaluated. A copy of the second year questionnaires and the label used during the second sampling can be found in Annex II. The questionnaire touched on what crops maize was associated with, which type of fertilizer was used, what damaged maize in the field and an evaluation of maize huskcover by the farmer. Questions were asked about the length of drying, the sorting and dehusking of the harvested cobs. The last set of questions asked were on the storage structure, in what form maize was stored, what products were used to protect maize in storage and the storage time. The survey was timed to coincide with the first month of maize storage, so that in the South the villages were visited from September till October 94 and in the north the visits took place in December 94. Five farmers per village were interviewed since the first year questionnaire revealed that farming practices were homogeneous in a village and heterogeneous from one village to another.

For the second sampling at six months after storage in March 95 in the South and in April 95 for northern Benin, only a short label was added to the maize sample (Annex III). This gave information on the date of harvest, the length of drying after harvest, what storage structure was used, the storage period, use of a storage protectant and how the maize was stored (with/without husk or as grains).

4.3   Results

4.3.1   Maize production practices in the four agroecological regions of
                Benin

First Survey Year

The results of the questionnaire showed that in the Forest Mosaic Savanna (FMS) 77.5% and the Southern Guinea Savanna (SGS) 55% of the farmers grew their maize during the two seasons. In the Northern Guinea Savanna (NGS) 85% and 100% in the Sudan Savanna (SS) grew maize during one season, since only one cropping cycle is possible in most northern regions.

41% of the farmers in the FMS and 56.3% in the SGS grew maize after maize. In the northern zones 63.8% (NGS) and 60% (SS) planted maize in a crop rotation on the same field. The crops that were often rotated with maize were in the FMS cassava, cowpea, groundnut, soja.

In the other regions maize was rotated with cotton, yams, sorghum. millet, groundnut, cowpea and soja. There were 20% (NGS) and 26.7% (SS) of the farmers who would plant maize on newly prepared land every year. In all regions the area cultivated with maize, by one farming family varied between one and three hectare. Farmers often intercropped their maize with another crop. In the FMS, 73,5% intercropped maize with another crop, whereas in the other regions it was only between 38% to 46%. In the south the intercrop was usually cassava, whereas in the north it was usually sorghum. 10% of the farmers in the NGS intercropped their maize with groundnut *(Arachis hypogae* L.).

In the FMS of Benin, farmers used mostly "local" varieties (83.8%). Further to the north farmers used "improved" varieties; 43.8% in the SGS, 78.8% in the NGS, and 83.3% in the SS respectively. "Local" varieties are usually propogated by the farmers themselves, the characteristics that are associated with them are smaller, harder grains, small cobs with a good huskcover and lower yields. The improved varieties have been released by the extension services e.g. CARDER (Centre d'action rurale et developpement rurale), IITA, INRAB (Institute National de la Recherche Agronomique du Benin), they are characterized by big grains, longer cobs with less huskcover and higher yields. Maize seeds used for planting were bought at the state organization which distributes agricultural inputs CARDER in the SGS and NGS, in the other zones maize producers mostly use self-propagated seed. Most of the farmers felt that their maize had good husk cover, with a higherer frequency of bad husk cover in the southern zones and a gradual reduction of the trait of bad huskcover towards the north. The amount and tightness of the husk plays a role in protecting cobs against the attack of insects both in the field and in storage.

The seed treatment of maize varied from one region to another. In the FMS zone ash was often used, in the other regions farmers used a product like Thioral® (Thirame + Heptachlor), Benlate® (Benomyl), Basudine® (Diazinon), Gamma-HCH® (Lindane) etc.. Fertilizer was used by 47.5% (FMS), 55% (SGS), 53.8% (NGS) and 83.3%(SS) of the farmers.

The farmers recognized that there were multiple causes for damages to maize in the field. Molds were rarely recognized as a problem. If farmers mentioned larger animals as a problem they cited hares, squirrels, grasscutters, cows, baboons and monkeys. This seems to be a problem in the more forested areas of Benin, where fields often were far away from the villages. The following Table (4.1) shows the different pest problems pointed out by the farmers, multiple responses were possible:

Table 4.1:   Pest problems for % farmers in the different agroecological zones of Benin (93-94)

Pest Problems	FMS	SGS	NGS	SS
Insects	38.8	41.3	51.3	48.8
Rats	67.5	90.0	23.8	6.7
Birds	67.5	80.0	51.3	58.3
Molds	0.0	0.0	2.5	3.3
Larger animals	2.5	7.5	21.3	10.0
Striga	0.0	0.0	15.0	31.7

Second survey year

For the second survey year in the different ecozones, more than 60% of the farmers complained about having problems with insects and birds, while their maize was growing in the field. Only between 10 to 15% of the farmers had problems with fungi in the field. When they complained about fungal contamination, this was often because of delayed harvest and leaving the maize in the field after maturity so that maize started to lodge, water seeped into the cobs and fungi developed.

Fertilizer use was widespread in the northern regions of Benin, 77.5% of the farmers in the SGS used fertilizer, this increased to 80% in the NGS and 93.3% in the SS. The use of NPK and urea was widespread, since these fertilizers were distributed through the cotton production service (SONAPRA) for which inputs are subsidized by the Beninese state. Farmers take a part of the allocation for their cotton crop to fertilize their maize fields. The percentage of farmers that use fertilizer and which type was used in the different regions is listed in Table 4.2.

Table 4.2:   Use of fertilizer for % farmers in the different agroecological zones of Benin (94-95)

Fertilizer Use	FMS	SGS	NGS	SS
Use of fertilizer	33.3	77.5	80.0	93.3
NPK	13.3	35.0	35.0	43.3
Urea	16.7	42.5	40.0	43.3
Phosphate Ammonium	0.0	0.0	2.5	3.3
Double Phosphate	0.0	0.0	2.5	0.0
Manure	3.3	0.0	0.0	3.3

Most of the farmers rotated maize with cotton. Only in the NGS 25% of the farmers planted maize in fields that had yams on them the year before. Around 20% of the farmers in all ecozones were planting maize after maize. In the southern regions 17% (FMS) and 28% (SGS)

rotated maize with groundnut, whereas in the northern regions (NGS and SS) around 7% had this practice.

In all ecoregions maize was intercropped with different types of crops. In the FMS 40% of the farmers planted maize with cassava, in the SGS 10% of the farmers intercropped maize with groundnut, in the NGS 10% of the farmers grew maize with cowpea (*Vigna unguiculata* Walp) and 12.5% intercropped it with sorghum, whereas in the SS maize/sorghum (*Sorghum bicolor* L.) intercrop was practiced by 20%. Regional differences for intercropping of maize in Benin were noticed.

The usage of local and improved varieties of maize in the second survey year followed the same trend as in the first year (Table 4.3).

Table 4.3:   Use of local or improved maize variety for % farmers in the different agroecological zones of Benin (May-April 95)

Variety	FMS	SGS	NGS	SS
Local	85.0	39.0	61.9	51.6
Improved	15.0	61.0	38.1	48.4

In the FMS, most of the farmers used local maize varieties, and in the SGS there were 61% using improved varieties. In the north farmers used mostly local varieties with 61.9% in the NGS and 51.6% in the SS.

Very few farmers complained about poor huskcover (Table 4.4). There were 6.8% of the farmers growing improved varieties that complained about bad huskcover and for those growing local varieties this were 6.5%.

Table 4.4:   Huskcover rating for % farmers in the different agroecological zones of Benin (93-94)

Huskcover Rating	FMS	SGS	NGS	SS
Poor	12.5	10.0	6.3	0.0
Moderately good	3.8	5.0	15.0	8.3
Good	83.7	85.0	78.7	91.7

4.3.2	Maize harvest practices in the four agroecological regions of Benin

First survey year

Maize was harvested by more than 50% of the farmers as green maize for home consumption, then eaten mostly grilled or sometimes boiled. First season maize was mostly harvested green, rarely maize from the second harvest. The part of the total harvest that was collected green, was rarely more than 10% of the total maize harvest, except for 10% of the farmers that sold green maize in the two southern zones of Benin, who sold up to 30% of their maize harvest as green maize.

In the FMS, 71.3% harvested maize when they considered it ripe, this means that they leave maize in the field 2 to 3 weeks after physiological maturity. In the SGS, there were 60%, in the NGS 51.3% and in the SS 68.3% of farmers routinely harvesting "ripe" maize. The reasons for delaying their harvest were: farmers did not have enough manpower to harvest on time, the storage structure had to be prepared first or they had other preoccupying activities like field preparation for other crops. The harvest of cotton was their priority or they were involved in traditional ceremonies. Farmers also gave as reason for a late harvest that maize had to dry on the plant before they stored it.

None of the farmers in the FMS practiced an early harvest, but 42.5% harvested their maize within the recommended period and 57.5% practiced late harvesting. In the SGS 3.8% practiced an early harvest, 42.5% harvested the maize at maturity and 53.8% harvested late. In the NGS, only 3.8% harvested early, 56.3% normal harvest and 38.8% late harvest. In the SS, a high percentage were harvesting late (73.3%), 17.5% harvested at normal time, and 3.3% harvested early.

Farmers took an average of 3 to 9 days to harvest their maize depending on the area planted with maize and the manpower available. Most of the farmers harvested their maize within a short period, except in the SGS where 22.5% harvested in bits, with several days in between the different harvests.

In Benin, farmers had several methods of harvesting, which varied according to region and to ethnic group. In the SS and NGS, methods of harvesting were cutting the plants, putting them in sheaves to dry in the field and collecting the cobs at a later date. Another method was collecting the cobs, assembling them in heaps and dehusking the cobs in the shade beside the

field. Farmers gave as reasons for choosing the way they harvest, that their chosen method was faster or easier, reduced the losses or protected against damages. Several farmers said that they chose their harvesting practice according to their tradition.

One of the main differences between the northern and southern region was the harvesting of maize with or without husk (Table 4.5). Farmers in southern Benin harvested maize with the husk, whereas the majority of farmers in northern Benin would harvest without the husk. During harvesting a nail or another sharp object was used to take the husk off.

Table 4.5:   Harvesting practice for % farmers in the different agroecological zones of Benin (93-94)

Harvesting Practice	FMS	SGS	NGS	SS
With husk	97.5	66.3	38.7	11.7
Without husk	2.5	33.7	61.3	88.3

Second survey year

In the FMS, maize was harvested two times per year, the first harvest was effected in June or August and the second in December to early January (Table 4.6). In the SGS, maize was usually harvested from July to September, with only 12.5% of the farmers growing a second season crop that was harvested from December to January. The harvest in the NGS was delayed because of cotton harvesting, so that farmers harvested from August till December, with the majority of the farmers either harvesting in October or in December. Only in the southern part of the NGS, maize was harvested two times per year. In the SS, only one maize crop per year was harvested from September till November.

Table 4.6:   Harvest period for % farmers in the different agroecological zones of Benin (94-95)

Harvest	FMS	SGS	NGS	SS
June - August	60.0	65.0	16.2	0.0
September - November	0.0	22.5	51.2	100.0
December - January	40.0	12.5	32.6	0.0

4.3.3	Pre-storage practices in the four agroecological regions of Benin

4.3.3.1	Drying practices in the four agroecological regions of Benin

First survey year

Most of the farmers dried their maize in the field for more than 30 days (Table 4.7).

Table 4.7:	Field drying periods for % farmers in the different agroecological zones of Benin (93-94)

Field Drying Period	FMS	SGS	NGS	SS
0 - 20 Days	25.0	2.5	7.5	10.0
20 - 30 Days	26.3	22.5	32.5	33.8
> 30 Days	45.0	68.7	60.0	33.7
No definite period	3.7	6.3	0.0	0.0

Shorter field drying periods were practiced in the FMS, where rain would set in during the harvest, or in the SS where farmers would often dry their maize outside the field. In the northern regions of Benin, farmers usually left maize for long times in the field to dry properly or because they harvested cotton first, but this practice can lead to an increase in the infestation with insects, especially lepidopteran pests.

Farmers dried their maize after harvest to reduce mold and insect attack mostly through solar drying, only one farmer in the FMS zone dried maize over the fire. Wind drying, often in special storage structures called cribs, was practiced by three farmers. Drying of harvested cobs took any time from 1 to 120 days, with most of the farmers drying for 30 days in the SGS (30%) and for 3 to 7 days in the SS (26.7%). In the other regions the length of drying varied. Farmers judged the end of drying by observing the reduced weight of the kernels after drying, another method was to observe if the kernels were making a breaking noise if they were crushed, or they could not be scratched with a fingernail.

Second survey year

During the second survey year more than 85% of the farmers in all the ecozones left maize in the field after maturity to dry on the stalk. Maize was left in the field for three to eight weeks. After harvest 50% in the FMS dried their harvested maize for ten days or less. Post-harvest drying was either done in the field or more often in the house or in the courtyard. In the SGS, 30% dried maize after harvest and in the NGS 22.5% had the same practice. In these ecozones,

farmers preferred to leave maize on the stalk to dry in the field. One third of the farmers in the SS dried maize between 5 to 15 days after harvest.

For the second sampling during the second survey year (Table 4.8), drying of maize was practiced in the SGS, the NGS and the SS, but rarely in the FMS.

Table 4.8: Drying time in the field for % farmers in the different agro-ecological zones of Benin (94-95)

Drying time	FMS	SGS	NGS	SS
Farmers that dry	2.5	17.1	15.0	38.7
1 - 3 days	0.0	4.9	3.7	9.7
4 - 14 days	2.5	4.9	0.0	12.9
15 - 30 days	0.0	4.9	4.3	3.2
> 30 days	0.0	2.4	7.0	12.9

In the NGS, 7% of the farmers dried their maize for more than one month. In the SS, 38.7% of the farmers dried their maize after the harvest, with 12.9% drying it for periods between 4 to 14 days and another 12.9% drying for periods over one month. All the other farmers were not practicing any form of post-harvest drying.

In the South of Benin maize was dried with the husk intact. Whereas in the northern regions, starting in the northern part of the SGS, farmers usually took the husk off when drying maize. In the SS, nearly 50% were drying maize without the husk. In the two southern zones 67.5% (FMS) and 73.8% (SGS) were drying their maize at a later stage in the processing before storage, when maize was already degrained. In the northern zones there were only 42.5% (NGS) and 18.8% (SS) that dried degrained maize.

There were regional differences in the way farmers dried their maize for the second sampling in the second survey year. In the FMS, 89.5% dried maize with the husk, and in the SGS only 16.7% took off the husk before drying the maize (Table 4.9). In the NGS, half of the farmers dried with the husk the other half dehusked before drying. In the SS, only 33.3% left the husk on the cob when drying.

Table 4.9: Drying form in the field for % farmers in the different agro-ecological zones of Benin (94-95)

Drying form	FMS	SGS	NGS	SS
With husk	89.5	80.0	50.0	33.3
Without husk	10.5	16.7	50.0	66.7
Grains	0.0	3.3	0.0	0.0

4.3.3.2   Sorting practices in the four agroecological regions of Benin

First Survey Year

Most farmers in the two southern zones separated cobs that had poor huskcover from the rest at harvest (Table 4.10), whereas in the northern parts of the country no sorting according to this criteria was practiced.

Table 4.10:   Removal of cobs for bad huskcover by % farmers in the different agroecological zones of Benin (93-94)

Removal of cobs	FMS	SGS	NGS	SS
Farmers sort for huskcover	85.0	73.7	28.7	5.0
No sorting for huskcover	15.0	26.3	71.3	95.0

There were usually few or very few cobs taken out because of bad husk cover. Those cobs selected and removed were either thrown away, given as feed to animals, or given to people who had helped with the harvest. Another option was to use these cobs with poor huskcover for immediate consumption in the days after the harvest.

Across ecoregions, except in the SS, one third of the farmers were sorting their maize right at harvest, otherwise they would sort maize before storage. Usually maize cobs that were damaged, were small or had incomplete husks were sorted out. In the first survey year, around 26.7% of the farmers in the FMS sorted at harvest (Table 4.11), 30% and 32.5% of the farmers sorted at harvest in the SGS and NGS. Whereas in the SS, only 10% separated out damaged cobs, they were rather storing most of the crop without any sorting.

Second Survey Year

Farmers recognized that moldy grains were bad and those cobs were removed in all zones with 27.5% in the FMS, 25% in the SGS, 12.5% in the NGS and 56.7% in the SS. Most of the moldy cobs were either thrown away, otherwise damaged cobs were given to animals.

In the second survey year, cobs damaged by insect were often sorted out, also cobs damaged by rats or birds were put aside. The percentage of farmers that sort and the timing of sorting is presented in Table 4.11.

Table 4.11:   Sorting of maize by % farmers in the different agroecological
                       zones of Benin (93-94)

Sorting	FMS	SGS	NGS	SS
Farmers that sort	86.7	80.0	97.5	73.3
at harvest	26.7	30.0	32.5	10.0
before storage	60.0	45.0	60.0	63.3
during storage	0.0	5.0	5.0	0.0

4.3.3.3   Dehusking and degraining practices in the four agroecological
                       regions of Benin

First Survey Year

In the FMS, husks were removed from maize before it was sold or consumed (88.8%). In the
SGS, dehusking was either at harvest (27.5%), before storage (47.5%), or before consumption
or sale (26.3%). Dehusking was effected at harvest (57.5%), or before consumption or sale
(36.3%) in the NGS. These regional variations were because of differences in the storage
structures and storage practices, and availability of labor.

Most farmers in the SGS and NGS would dehusk their maize, when they changed their storage
structure. In the SS, farmers would dehusk and often degrain their maize before storing it in
bags (Table 4.12).

Table 4.12:   Time of dehusking for % farmers in the different agroecological
                       zones of Benin (94-95)

Time of dehusking	FMS	SGS	NGS	SS
during harvest	6.7	22.5	62.5	70.0
before storage	3.3	17.5	5.0	66.6
during storage	0.0	37.5	22.5	1.7
before consumption	90.0	22.5	10.0	1.7

The same variations between the regions were noticed for the degraining of cobs. In the FMS,
maize was degrained just prior to consumption or sale (85%). Farmers degrained either before
consumption or sale (63.8%) or before storage (31.3%) in the SGS. In the NGS and the SS,
farmers degrained their maize either before consumption or sale 76.3% and 61.7%
respectively, or before storage 17.5% and 28.3% respectively. Degraining usually took place in
the house, except in the NGS where 46.3% of the farmers degrained their maize in the field, at
the time when they emptied their drying platforms to store either in bags or clay-structures at
home. Before storage grains were winnowed to clean them of dirt and chaff. After degraining

the cobs, sometimes another sorting was effected, when mostly damaged or colored grains were taken out. This was rarely done in the SS. The grains sorted at this late sorting stage were thrown away or given to animals, but very rarely eaten.

4.3.4   Storage practices in the four agroecological regions of Benin

4.3.4.1   Storage structures in the four agroecological regions of Benin

First Survey Year

Farmers pre-store maize in different locations, mostly because of lack of manpower, other priorities at that time of the year, or the storage structures were not ready (Table 4.13). Pre-storage constituted a mean of drying the maize before storage. The pre-storage of maize in the field was common in the NGS, whereas pre-storage in a room was prevalent in the FMS and the SS. Storage in the field put maize at the risk of bushfires and theft, so that the storage structure was changed shortly before the dry season, usually around February.

Table 4.13:   Pre-storage practices for % farmers in the different agroecological zones of Benin (93-94)

Pre-Storage	FMS	SGS	NGS	SS
Directly stored	68.7	87.5	72.5	61.7
Storage in room	23.8	11.2	11.2	33.3
Storage in field	0.0	0.0	16.3	1.7
Storage in courtyard	7.5	1.3	0.0	3.3

Traditional storage structures in rural Africa were of varied types (Table 4.14). In every ecozone certain storage types were predominant. In the FMS, 71.2% of the farmers stored their maize in the "Ago", with the ebli-va and storage in a room on the floor also often practised. In the SGS, farmers stored maize in the "Ago" (18.8%) conical granary (30%) or over the ceiling (27.3%). In the NGS, maize was often stored in the "secco" (46.3%) or in the conical granary (31.3%). In the SS, 30% used the "secco" and 45% stored in clay granaries.

A more detailed description of the different storage structures is given in Chapter 7 and examples of storage structures are shown in Annex IV. Some of the structures mentioned in Table 4.14 were used as pre-storage structures (see Table 4.13) and then another type of store was used to store maize for the remaining storage period. In the SGS, the conical granary and then the clay granary (22.5%) or bags (7.5%) were used. In the NGS, farmers first used the secco in the field and then the clay granary near the house (8.8%), other farmers used conical

granaries 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%), room on the floor then bags (5.0%) or the conical granary and bags (1.7%).

Table 4.14: Types of storage structures for % farmers in the different agroecological zones of Benin (93-94)

Storage Structure	FMS	SGS	NGS	SS
Crib	2.5	6.3	1.3	1.7
Secco	0.0	0.0	46.3	30.0
Clay granary	0.0	3.8	5.0	45.0
Room on the floor	7.5	5.0	8.8	8.2
Bags	1.3	1.3	3.7	6.7
On the roof	0.0	0.0	0.0	6.7
Conical granary	2.5	30.0	31.3	1.7
Ago	71.2	18.8	0.0	0.0
Over the ceiling	5.0	27.3	0.0	0.0
Ebli-va	8.7	0.0	0.0	0.0
Platform	0.0	7.5	3.7	0.0
Basin	1.3	0.0	0.0	0.0

The Table (4.15) shows the percentage of farmers that stored their maize with husks, without husks or as grains. The fourth category of farmers used two types of storage structure, where first maize was stored with husks and later degrained and stored in another store as grains.

Table 4.15: Storage form for the % farmers in the different agroecological zones of Benin (93-94)

Storage Form	FMS	SGS	NGS	SS
with husk	91.2	38.7	38.7	1.7
without husk	6.3	36.3	45.0	56.6
grains	2.5	1.3	8.8	30.0
with husk then grains	0.0	23.7	7.5	11.7

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

The time of storage varied between the ecozones (Table 4.16). Storage for 5 to 12 months was common in the FMS and SGS. In the FMS, 13.7% stored maize for more than 12 months. In this area the size of maize stores is used by the population to evaluate the wealth and social

prestige of their owners. Maize was usually stored between 3 to 8 months in the NGS. In the SS, a longer storage period of 7 to 12 months was practiced, because there was only one maize harvest per year.

Table 4.16:   Storage period for % farmers in the different agroecological zones of Benin (93-94)

Storage Period	FMS	SGS	NGS	SS
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

Second Survey Year

In the FMS, farmers predominately stored their maize in the "Ago" (Table 4.17). Around 16.6% stored their maize on a platform (Ebli-va). In the SGS, farmers used a wide variety of different stores, they either used the "Ago" often made out of bamboo, stored their maize under the roof, used the modern improved crib (FAO 1994), stored maize with the husk on top of a platform or put it in conical stores. In the NGS, 32.5% of the farmers used conical stores, and 45% used the "secco" to store their maize. In the SS, 33.3% of the farmers stored maize in the "secco", 40% stored their maize in earthenware containers made out of clay. One third of the farmers stored their maize in the room either in bags or just on the floor.

Table 4.17:   Storage structures for % farmers in the different agroecological zones of Benin (Sept.-Dec. 94)

Storage Structure	FMS	SGS	NGS	SS
Crib	6.7	10.0	5.0	6.7
Secco	0.0	0.0	45.0	33.3
Clay granary	0.0	0.0	0.0	40.0
Room on the floor	0.0	5.0	2.5	30.0
Bags	0.0	0.0	7.5	0.0
Conical granary	0.0	25.0	32.5	3.3
Ago	69.0	27.5	0.0	0.0
Over the ceiling	6.7	20.0	0.0	0.0
Ebli-Va	16.6	0.0	0.0	0.0
Platform	0.0	10.0	7.5	0.0
Baskets	0.0	2.5	0.0	0.0

Many farmers in the northern zones changed their storage structure after an initial field storage (Table 4.18), often this was after 3 to 6 months and it coincided with a period of low labor demand for agricultural field operations.

Table 4.18: Types of secondary storage structures for % farmers in the different agroecological zones of Benin (Sept.-Dec. 94)

Secondary Storage	FMS	SGS	NGS	SS
Bags	3.3	30.0	65.0	33.3
Ago bamboo	0.0	5.0	0.0	0.0
Clay granary	0.0	17.5	10.0	3.3

This was rarely done in the FMS. In the SGS, 30% stored their maize in bags after a certain time, and in the NGS zone, 65% moved their maize to bag storage. In the SGS, 17.5% would leave degrained maize in clay stores the "Kozoun". Often farmers would also change the storage form during the storage period, farmers that would change to bags or clay granaries would store maize as grains.

As in the first survey, most of the farmers in the FMS stored their maize in the "Ago", "Ebli-va" or over the ceiling and 10% had transferred their maize into bags (Table 4.19).

Table 4.19: Types of storage structures for farmers in the different agroecological zones of Benin (March-April 95)

Storage Structure	FMS	SGS	NGS	SS
Crib	7.5	10.0	4.7	2.7
Secco	0.0	0.0	25.5	10.0
Clay granary	0.0	2.5	2.3	30.0
Room on the floor	5.0	7.5	7.0	20.0
Bags	10.0	22.5	23.5	23.3
On top of the roof	0.0	0.0	0.0	6.6
Conical granary	0.0	12.5	25.5	0.0
Ago	50.0	12.5	0.0	0.0
Over the ceiling	10.0	15.0	2.3	3.3
Ebli-va	15.0	0.0	0.0	0.0
Platform	2.5	7.5	4.6	3.3
Baskets	0.0	10.0	4.6	0.0

In the SGS, 22.5% had transferred their maize into bags. Otherwise as at the beginning of storage, farmers stored their maize after six months of storage in the "Ago" made out of bamboo, under the roof, in the improved crib or in a conical store. In the NGS, where farmers at the beginning of storage in the were mainly storing in the secco or in the conical store, 23.5% stored their maize in bags after six months of storage. The predominant storage

structures in the SS, were storage on the floor in a room, clay stores and storage in the "secco". The percentage of farmers that stored maize in bags in this zone, increased to 23.3% after six months of storage.

At the beginning of storage in the second survey year, in the southern zones, maize was stored with the husk, and further to the north, maize was stored without the husk, and in the SS increasingly as grains (Table 4.20).

Table 4.20:   Storage form for % farmers in the different agroecological zones of Benin (Sept.-Dec. 94)

Maize Storage Form	FMS	SGS	NGS	SS
with husk	86.7	62.5	30.0	10.0
without husk	10.0	32.5	62.5	73.3
grains	3.3	5.0	7.5	16.7

After 6 months of storage during the second survey year more than 90% of the farmers in the FMS were storing their maize with the husk. In the SGS, 46.3% were storing maize as grains. This trend was similar in the NGS, where 58.1% of the farmers stored degrained maize. In the SS, 38.7% of the farmers degrained maize before storage, and 48.4% were storing without husk (Table 4.21).

Table 4.21:   Storage form for % farmers in the different agroecological zones of Benin (March-April 95)

Storage form	FMS	SGS	NGS	SS
with husk	92.5	22.0	16.3	12.9
without husk	5.0	31.7	25.6	48.4
grains	2.5	46.3	58.1	38.7

In the FMS, farmers stored for shorter periods (Table 4.22).

Table 4.22:   Storage period of maize for % farmers in the different agroecological zones of Benin (Sept.-Dec. 94)

Storage period	FMS	SGS	NGS	SS
3 - 5 months	6.7	20.0	7.5	14.3
6 - 7 months	16.7	25.0	12.5	7.2
8 - 10 months	40.0	12.5	45.0	17.8
11 - 12 months	36.6	42.5	35.0	60.7

This was because of two maize growing seasons. Maize was stored for six to eight months by 16.7% and 36.6% of the farmers stored for twelve months. In the SGS, half of the farmers

stored for four to seven months and 42.5% stored it till the next harvest would come in. In the NGS, farmers usually stored for eight to twelve months. In the SS, farmers, were obliged to store for even longer periods, between nine and twelve months.

For the second sampling the same trend as during the first sampling was shown (Table 4.23).

Table 4.23: Storage period of maize for % farmers in the different agroecological zones of Benin (March-April 95)

Storage period	FMS	SGS	NGS	SS
3 - 5 months	15.0	2.5	2.5	0.0
6 - 7 months	20.0	7.5	2.5	0.0
8 - 10 months	27.5	25.0	45.0	46.7
11 - 12 months	37.5	55.0	50.0	50.0
> 12 months	0.0	10.0	0.0	3.3

Farmers in the south stored their maize for shorter periods and farmers in the north usually stored for longer periods. In the FMS, 37.5% stored for a period of 12 months, in the SGS 50% stored maize for an equally long period. In the NGS and SS, a twelve months storage was practiced by 50%.

4.3.4.2 Storage Problems in the four agroecological regions of Benin

First Year Survey

The majority of the farmers in the FMS (82.5%), SGS (82.5%) NGS (93.8%) and SS (86.7%) complained about storage problems. Farmers noticed primarily insects and rats (see Table 4.24).

Table 4.24: Storage problems for % farmers in the different agroecological zones of Benin (93-94)

Storage problems	FMS	SGS	NGS	SS
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

Storage problems were discovered by the farmers in the FMS and SGS zone at the beginning of the storage period, or within the first 2 months. Farmers in the NGS and SS zone, noticed these problems usually after 1 to 3 months. Farmers reacted to these problems differently.

Approximately 50% of the questioned farmers in the different ecozones, did not do anything against storage problems (Table 4.25). Those that did treat storage problems used commercial insecticides, either especially formulated for stored grains like, Sofagrain® (pyrimiphos-methyl and permethrin), Actellic® (pyrimiphos-methyl) or Percal M® (permethrin and malathion) or insecticides commonly used for cotton production like Actellic® (pyrimiphos-methyl), Decis® (deltamethrin), Dursban® (chlorpyriphos) and Nuvacron® (monocrotophos). Few farmers used rodenticides. Another method of avoiding storage problems was to sell the stored maize as soon as possible. Farmers also used certain traditional means of plant protection e.g. neem leaves, pepper, ash mixed with sand, ash alone, petrol, smoke or manure to protect their stored maize. The farmers satisfaction with the storage protectant used, differed in the regions. 51.3% of the farmers in the FMS zone had success with their method of grain conservation. In the SGS, 48.8% of the farmers were satisfied, while in the NGS only 27.5% and in the SS 30% felt that their preservation method gave satisfaction.

Farmers used hygienic measures to protect their maize. They cleaned the storage structure before loading it with new maize and all the old grains were removed. Farmers noticed that under certain conditions, e.g. heavy rains or bad roofing, their maize germinated while in store. To avoid germination farmers often redid the floor of their storage structure or changed the branches, which were used to cover the stores.

Table 4.25: Storage treatment for % farmers in the different agroecological zones of Benin (93-94) (Multiple answers were possible)

Storage treatment	FMS	SGS	NGS	SS
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	10.0	10.0	3.8	10.0
Sale	5.0	0.0	21.3	16.7
Sorting	2.5	0.0	5.3	6.7
Ash + Sand	1.3	8.8	7.5	5.0
Smoke	3.75	0.0	11.3	3.3
Mechanical means	0.8	2.5	3.8	1.7
Traps against rats	0.0	3.8	2.5	3.3
Redo storage structure	0.0	1.3	1.3	0.0

Farmers reacted to storage problems with the listed measures (Table 4.25), usually attack by storage pests was treated, the other pests were not treated. There were 10 to 18.7% that used

the recommended storage insecticides, only in the SGS a higher percentage of farmers were following the suggestion. 17.5% in the FMS used cotton pesticides, insecticides that are not recommended for use in storage. In the NGS (21.3%) and the SS (16.7%) of the farmers resorted to the sale of their maize if they detected a pest problem. Mechanical means to protect maize against storage pests, were rat guards on the stores, traps or sorting of maize grains. In the FMS, SGS and SS 10% of the farmers were using traditional plants. In the SGS (8.8%) and the NGS (7.5%) used a mixture of sand and ash. In the NGS 11.3% used smoke.

Second Year Survey

In the second survey year the storage problems encountered by the farmers were as follows (Table 4.26), multiple answers were possible.

Table 4.26:   Storage problems for % farmers in the different agroecological zones of Benin (September-December 94)

Storage problems	FMS	SGS	NGS	SS
Insects	60.0	62.5	77.5	56.7
Rats	43.3	20.0	2.5	0.0
Birds	53.3	55.0	47.5	43.3
Badly formed	20.0	25.0	27.5	13.3
Spoilage	33.3	40.0	55.0	46.7

Over 60% of the farmers in the FMS and the SGS complained about insects and birds that attacked their stored maize. Rats were only a problem in the southern regions (FMS and SGS). The percentage of farmers that noticed storage problems in the second survey year, was much higher than in the first year. About 20% of the farmers across all ecoregions said, that their maize was badly formed, which meant that the huskcover was incomplete, the cobs were not completely formed and not all the kernels were filled in. In the FMS, 20% raised this problem and in the other regions there were 25% (SGS), 27.5% (NGS) and 13.3% (SS) respectively. Another problem mentioned was that maize cobs would spoil, this meant that insects had attacked the cobs or that moisture had entered the cobs.

During the second survey most farmers found insects destroying their maize, these were 86.7% (FMS), 95% (SGS), 90% (NGS) and 73.3% (SS) respectively (Table 4.27), multiple answers were given. In the NGS 20% and in the SS 13.3% complained about lepidopterous pests in their stored maize. Also 16.7% of the farmers in the SS had problems with fungi, this was surprising since the SS is the driest area of Benin.

Table 4.27:   Storage Pests for % farmers in the different agroecological zones
                     of Benin (March-April 95)

Storage Pests	FMS	SGS	NGS	SS
Insects	86.7	95.0	90.0	73.3
Lepidoptera	0.0	0.0	20.0	13.3
Rats	40.0	22.5	47.5	33.3
Molds	6.7	2.5	0.0	16.7

The recommended storage treatment was used by only 13.3% during the second year (FMS),
by 45% in the SGS, by 40% in the NGS and by 16.7% in the SS. In the FMS, the use of
insecticides destined for the treatment of cotton in maize storage was especially widespread
(30%). In the SGS, 17.5% and in the SS 10% used these highly toxic insecticides to protect
stored maize. In the NGS none of the farmers used these products, even though this is the main
cotton producing area. Neem leaves (*Azadirachta indica*) were used by 16.7% in the FMS and
10% used ash. In the SGS 10% used ash. In the SS, 10% of the farmers stored their maize
over fire and used smoke to ward off insects.

Table 4.28:   Storage treatment for % farmers in the different agroecological
                     zones of Benin (March-April 95)

Storage Treatment	FMS	SGS	NGS	SS
No treatment	40.0	45.0	82.5	73.6
Storage insecticide	15.0	35.0	15.0	6.6
Cotton insecticide	20.0	0.0	0.0	3.3
Neem + ash	5.0	2.5	0.0	6.6
Cotton insectide + traditional means	2.5	7.5	0.0	3.3
Cowpea insecticide	5.0	0.0	0.0	0.0
Petrol	7.5	0.0	0.0	0.0
Smoke	5.0	0.0	0.0	3.3
Traditional plants	0.0	0.0	2.5	3.3

After 6 months of storage in the second survey year (Table 4.28), very few farmers treated
their maize. When they did treat, they either used the recommended storage product or cotton
insecticides. The use of traditional products in the stores was rare, farmers usually complained
that these methods were not very successful as compared to commercial insecticides.

4.4.3.5   Consumption practices in the four agroecological regions of
                               Benin

First Year Survey

In order to evaluate the daily intake and the health risk of aflatoxin exposure, farmers were
asked how many times per day they ate a maize-based meal (Table 4.29). Around 50% of the
farmers ate maize three times a day, except for the SS. About 20 to 30% of the farmers across
ecozones said that maize consumption varied with season. This was because different crops
were ready for harvest and consumption at different times of the year. Also certain ethnic
groups had a preference for certain crops.

Table 4.29:   Number of maize meals eaten per day by % farmers in the
                               different agroecological zones of Benin (93-94)

Daily intake of maize meals	FMS	SGS	NGS	SS
1 time a day	1.3	5.0	1.3	11.7
2 times a day	18.7	26.3	18.8	31.7
3 times a day	46.2	43.7	48.7	26.7
4 times a day	1.3	5.0	12.5	1.7
Frequency depends on season	32.5	20.0	18.8	28.2

4.3.6   Discussion

Differences could be noticed between the first and the second questionnaire year. Farmers
would have gotten used to the way the survey team was asking the questions and at the same
time the survey team was becoming used to the farmers answers. The answers to the questions
in the second year were very accurate, with more details given regarding the production and
the storage practices. Also the questions of the second year questionaire were structured so
that farmers practices that were unclear from the first survey year, could be further elucidated.

Maize Production

Farmers in Benin have developed a rather intricate system of producing, harvesting and storing
maize. In the northern regions maize production has only been recent. Because of the
decreasing land availability for agricultural production in southern Benin, more than 75% of
the farmers in the FMS and 50% of the farmers in the SGS grow maize in the same field from
year to year. Cole *et al.* (1982) studied the effect of crop rotation on aflatoxin load in different
commodities, when maize was planted after maize aflatoxin load was higher than maize that
was cropped in a rotation. The intercropping of maize with peanuts or the inclusion of peanuts

in a maize crop rotation had the potential of increasing the risk of aflatoxin contamination through an increased spore potential in the soil (Mehan *et al.* 1991). In Benin 10% of the farmers associated maize with groundnuts, both in the first and second survey year. Another crop that seemed to have the same effect of enhancing aflatoxin development was cotton (Diener *et al.* 1987, Cotty *et al.* 1994). Cotty and Lee (1989) found that cottonseeds were contaminated with aflatoxin levels of 8900 to 16800 ppb. Cotton is one of the major cash crops with 144,114 tons exported from Benin in 1996 (ONASA 1997). The cotton crop may serve to build up an *A. flavus* spore potential in the soil, since Beninese farmers often left cotton debris in the field before burning it the next year when they prepared the field for the next crop. Also *A. flavus* spores from infected cotton bolls could be dispersed during harvesting (Wicklow 1988), which coincides with the silkening stage of late planted second season. The accepted model of *A. flavus* infection is that insect-transmitted or airborne spores contaminate the silk and germinate, forming a mycelium that infects damaged maize kernels (Wicklow & Donahue 1984).

It has been demonstrated that maize varieties show differences in their ability to resist infection with *A. flavus* and aflatoxin expression (King & Scott 1982; Zuber *et al.* 1983). In Benin there existed regional contrasts for the usage of maize varieties, in the south farmers preferred local varieties and in the north farmers rather planted improved varieties, both of which were open-pollinated. Reasons for these regional differences can be found in the better storability of local varieties because of better huskcover and higher yields for improved varieties (Kossou *et al.* 1993). The insect pressure in the south of Benin was higher than in the north (Borgemeister *et al.* 1997). The north is a region where farmers produced maize on a commercial basis, with more inputs, 83.3% of the farmers in the SS used fertilizer. Whereas farmers in the south produced maize for home consumption (FAO 1994).

Pest problems noticed by the farmers were multiple. It was interesting that most of the farmers would perceive in their fields rather large pests, such as rats and birds. Molds that were frequently encountered on the collected samples through visual observation, were rarely cited by them. It seems to be that there is a lack of information about the multiple causes that can damage their maize. Animals that are seen around their field are attributed to damages found on the maize, even though they are not necessarily the primary causal agent.

Maize harvest

According to the recommendations of the FAO (CEEMAT 1988) early maize harvest was supposed to avoid insects, rats and bird damage of maize. Very few farmers in Benin followed this recommendation, which is also an extension message of the CARDER, the Beninese rural extension service. The reasons for harvesting late were lack of manpower, other priorities like the cotton harvest or farmers felt that maize had to dry in the field. Scott & Zummo (1994) reported that maize harvested late was more contaminated with aflatoxins than maize harvested early. The FAO-Report (1994) proposed that maize under humid West-African conditions should be dehusked before storage. This occurred only in the northern regions, where over 50% of the farmers stored their maize dehusked. Studies by Meikle *et al.* (1997 in press) revealed that five of eight varieties stored without husk in simulated maize cribs in southern Benin experienced significantly higher percent grain losses because of insects than maize that was stored with the husk. The authors postulated that varietal differences occured because of variability in huskcover, and not because of inherent grain characteristics, like hardness, size and chemical characteristics. The farmers preference for storage with husk in the south of Benin revealed in this survey, could be validated through the described experiments.

Sorting of maize can reduce the contamination with aflatoxins, even though not all grains that are contaminated with aflatoxins will also show visible fungal infection or discoloration (Prete & Cicero 1987). Another farmers practice that could reduce aflatoxin content of stored grain, was the habit of sorting cobs according to size, huskcover and husk extension. It is well known that huskcover plays a role in the protection of cobs against insects pests (Kossou *et al.* 1993; Vowotor *et al.* 1994), but it also impedes rapid drying of cobs (Vowotor *et al.* 1994). Most of the farmers dried their maize for periods of more than 30 days. This drying was mostly effected as field drying on the stalk. During the second sampling of the second year it was observed that once maize was harvested, farmers only in the SGS (17.1%) and the SS (38.7%) dry their maize for another period of time. Pre-storage structures such as the improved crib, the conical granary and the platform are storage structures that aid in drying the harvested goods (FAO 1992), and so the actual drying period and the percentage of farmers that dry is actually higher than the figures here presented.

Maize Storage

The storage of maize in an intermediary structure may lead to the contamination of maize through pests and pathogens, often farmers left their maize on the floor in a corner of the room or in the courtyard (Fiagan 1994), with the floor being in immediate contact with the maize cobs, and increasing the risk of infection with *Aspergillus* fungi (Smith 1991).

The majority of farmers in Benin complained about storage insects that affect their maize, with rats also being a constant problem (SPV 1992). This could be confirmed by this study, where in all zones except the SGS more than 40% of the farmers cited insects as major pests in storage, and around 20% cited rats and insects. In the second year the percentage of farmers that complained about insect pests was even higher. The pressure of insects on stored maize under West-African conditions is very high, with grain losses of up to 30% recorded in Togo for a storage season from 6 to 9 months (Pantenius 1987; Pantenius 1988). About 50% of all the farmers in this study used a multitude of treatments to control pests in storage. The percentage of farmers that used the recommended storage insecticides in the different regions varied around 15%, except for the NGS where in the first year 26.3% and in the second year 34.2% used these components. Farmers complained about the limited efficacy of storage insecticides (Schulten 1990). About 10% of the farmers used traditional plants to protect their stored maize. When farmers 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 for economic reasons.

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

Maize consumption

To assess the risk of aflatoxin ingestion and the health risk due to these toxins in Benin the frequency of consumption of maize-based meals was observed. This varied between 1 to 5

meals a day, and varied with season, ethnic group and region. In a study of 295 persons in Benin 61% ate maize every day of the week and 23% consumed maize five to six times a week (Lutz 1994). The northern ethnic group only consume about 10 kg/person of maize per annum whereas in the south this can reach up to 136 kg/person per year (FAO 1994). Considering the high frequency of consumption of maize in Benin, the contamination of maize meals with aflatoxins can represent a danger to human health, especially since this toxin is a co-factor in many diseases as described in section 2.2.. Allen *et al.* (1992) observed seasonal differences in Gambian children, in the levels of aflatoxin albumin adduct. These adducts of aflatoxin bound to periphal blood albumin provides a measure of aflatoxin exposure in the 2 to 3 months before blood sampling (Wild *et al.* 1990). Seasonal differences could also exist in Benin. Foods consumed at the end of the storage period might have higher aflatoxin levels than goods eaten right after harvest.

CHAPTER 5   **Distribution of *Aspergillus* spp. and aflatoxin contamination in four agroecological zones in Benin**

5.1   Introduction

In 1993 and 1994 thirty villages were visited in the Republic of Benin. Maize farmers in the villages were asked questions concerning their maize production, harvest and storage practices and storage problems (Chapter 4). At the same time that the questionnaire was administered, samples were taken from the farmers stores to assess the microbial flora and contamination with aflatoxins. The different *Aspergillus* spp. were studied and identified, because only a few species can develop aflatoxins (see Chapter 2). Also the accompanying flora was examined, other fungi may have an inhibitory or an additive effect on *A. flavus* and aflatoxin development (Ramakrishna *et al.* 1993).

There exist several methods to assess aflatoxin levels in commodities. Minicolumn methods were used for rapid screening and can only give a qualitative result. There are several chromatography methods that are used to quantify toxins, they can be distinguished as:

- open column chromatography

- thin layer chromatography

- high performance liquid chromatography

- gas liquid chromatography (Shotwell 1983).

For the extraction of samples solvents, like chloroform were used. The extraction of the samples with liquid solvents lead to a recovery of 70 to 110 percent of the mycotoxins present in the sample (Soares 1992). The clean-up procedures have been based on precipitation with with lead acetate or iron (III) hydroxide (Francis *et al.* 1988). Multitoxin detection was also possible with TLC-Methods, these have been reviewed by Soares (1992).

Surveys of aflatoxins have been completed in many West-African countries Nigeria (Ibeh *et al.* 1991; Opadokun 1990; Udoh 1995), Ghana (Kpodu 1996) and Benin (Bouraima *et al.* 1993, Setamou 1996). But the influence of agronomic practices on aflatoxin contamination in post-harvest maize under West-African conditions have not been analyzed. The objective of the study described in this chapter was to assess fungal contamination and aflatoxins in the four agroecological zones of Benin.

5.2   Materials and Methods

5.2.1   Sampling procedure

The sample size for the pre-trials, surveys and field trials was determined with the help of literature, it was concluded that 20 cobs per store taken from a larger sample would be adequate to give a representative evaluation of the fungi and amount of aflatoxins present in the samples and the grain stores from which these samples were taken. Instead of taking a large amount of cobs we opted to sample many farmers in the respective agroecological region so that a good representation of the respective agroecoregion was given. Also the size of the farmers grain stores was very variable with some farmers storing maize in baskets that rarely had more than 60 to 80 cobs and other farmers were storing 1 to 2 tons in a store. The amount of cobs also had to be small since this represented parts of the farmers food for the whole year, taking a very large sample would have taken too much away from their food source for the year.

The granaries of 300 farmers were sampled in Benin (see 4.2) at the beginning of storage (August-December 93). Access to the maize in the farmers maize stores was sometimes difficult because of the size, height and form of the storage structure. Where possible cobs were taken from different positions in the granary, otherwise most of the cobs came from the top of the grain store. Subsamples of 20 cobs were taken from a larger amount of cobs and taken to the laboratory. If maize was stored as grains an equivalent amount was taken, the weight of the composite sample was between 0.8 to 1.5 kg. All the cobs were degrained, the grains were mixed and then a subsample was taken for the fungal analysis. The rest was ground in a separating mill (Romer) and used for aflatoxin extraction..

Sampling was repeated 6 months after harvest from February 94 till April 1994, an average time of storage for the farmers and the time they usually contemplate selling or opening the granary for self consumption. The number of farmers stores sampled was reduced to 150, since it was noticed that farmers practices were very homogenous within a village but differences could be recognized between villages. In the 94-95 season, the same farmers as in the survey after 6 months of storage in 93-94 were sampled. Where these farmers could not be found, samples were taken from the initial 10 farmers in a village. Sampling was again done shortly after the harvest from September till December 94 and at 6 months of storage from March till April 95.

5.2.2 Microbiological evaluation

The blotter method developed by Doyer in 1938 and later included in the International Seed Testing Association Rules (I.S.O. 1979) was used to determine the mycoflora on the stored grains. Twenty-five (25) maize grains were subsampled from each of the samples, they were surface sterilized in 10% Sodium Hypochlorite (Merck Chemicals) solution for 30 seconds and rinsed in distilled water. Five (5) grains were then placed in each of 5 Petri dishes on moistened filter paper (WHATMAN FILTER No.1) and incubated at 27^OC with a 12 hours light and 12 hours darkness rhythm for 5 days.

After 5 days, the grains were observed under the stereo-microscope for any fungal growth. The appearing fungi were transferred onto Czepak Yeast Extract Agar (CYA), Malt Extract Agar (MEA) and Czepak Yeast Extract with 20% sucrose (CY20S). The *Aspergillus* spp. were identified with the key by Klich & Pitt (1992). Other occurring fungi were determined according to the books of Domsch *et al.* (1980), Samson *et al.* (1995), Barnett & Hunter (1972) and Ellis (1971).

5.2.3 1 Grain moisture content (G.M.*C.*)

The moisture of the samples was determined according to the International Standards Organisation routine method (I.S.O. 1979). A subsample from the original sample was ground (Tekmar IKA-A10, Analytical Mill), transferred to a metal container and the weight determined. Then the sample was dried for two hours at 130^oC, and reweighted. The corn moisture was determined through the following formula:

$$MC = 100 \cdot \frac{Wi - Wd}{Wi}$$

MC = Moisture content

Wi = initial weight

Wd = weight after drying

5.2.4　　　　　　　　Aflatoxin analysis

5.2.4.1　　　　　　　Extraction of the samples

Extraction was done by the method of Thomas *et al.* (1975) and modified by Singh *et al.* (1991). The samples were ground (WARING Blender, later ROMER Mill), and to 50 g of the ground powder 250 ml of methanol/water (60:40, v/v) were added. This was shaken for 30 minutes on a mechanical shaker (Lab-Line Multi-Wrist Shaker). The solution was left to sediment and filtered through a WHATMAN Filter No.1. Of the resulting filtrate, 125 ml were poured into a separating funnel and 30 ml of sodium chloride and 50 ml of hexane were added. This solution was manually shaken for 2 minutes and left to separate.

The lower methanol water layer was collected in another separating funnel, 50 ml of chloroform were added, and shaken vigorously giving frequent vents to allow the developing gases to emerge. The lower chloroform layer was let out into a beaker containing 5 g of cupric carbonate, shaken and left to settle.

The solution was filtered through a WHATMAN Filter Paper No. 42, which had a bed of anhydreous sodium sulfate. The cupric carbonate was washed again with 25 ml of chloroform and filtered, these two extracts were combined, evaporated under the hood airflow and preserved in a plastic container in the refrigerator (5°C), to be used in thin-layer chromatography.

5.2.4.2　　　　　　　Thin-layer chromatography

Silica-gel pre-coated 20 cm x 20 cm plates were used (SIGMA Chemical), they were activated in a drying oven and placed in a desiccator cabinet till used. To the dried refrigerated aflatoxin extract 1 ml of chloroform was added and 5, 10, 15 µl of the extract spotted with a 10 µl HAMILTON syringe on the base line of the plate. These spots were dried with the help of a hair dryer, so that their size did not exceed 0.5 cm. For comparison 1 µl, 5 µl, 10 µl of the mixed B_1, B_2, G_1, G_2 Aflatoxin standard (SIGMA Chemical) were spotted on the same baseline, with 1 cm in between. Opposite sides of the TLC-plate carried a baseline, with this method it was possible to accommodate 10 samples on the same plate. The plate was developed unidimensionally in a TLC-tank in a chloroform/acetone (96:4, v/v) solvent system till the spot migrated to a level of 10 cm, then the plates were dried. The intensity of the fluorescence of the sample spot was observed in the Chromato-Vue cabinet under longwave

UV-Light (365 nm) and related to that of the standard aflatoxin spots. The process was repeated until the sample spots fluorescence matched the fluorescence of the aflatoxin standard. When the intensity of the fluorescence of the lowest concentration of the sample was too intense to match the standard, the sample extracts were diluted and re-chromatographed.

The aflatoxin concentrations were determined mathematically according to the following formula:

$$\text{Aflatoxin concentration} = \frac{S \times Y \times V}{W \times Z}$$

S =   volume of the aflatoxin standard
Y =   concentration of aflatoxin standard in µg/ml
V =   volume of solvent in µl required to dilute final extract
Z =   volume of the sample extract in µl, required to give
       fluorescence intensity comparable to that of Sµl of the standard
W =   weight of the original sample in g

The confirmatory test also followed the guidelines of Singh *et al.* (1991). Concentrated ethyl alcohol (95%) and hydrochloride were mixed 90:10 (v/v) and sprayed on the dried TLC-plates. The plates were observed under 365 nm light and those spots that gave off a yellowish-green fluorescence showed that aflatoxins were present.

5.2.5   Statistical analysis

The SPSS-Statistical Package (Norusis & SPSS 1993) was used to determine if agroecological differences in fungal infection, aflatoxins levels and grain moisture content existed. The statistical procedures used was means comparison using the Student Newmans Keuls test (SNK). The percentage of stores that were aflatoxin positive for each zone was calculated, and the percentage of aflatoxin-positive stores that showed contamination levels higher than 20 ppb was determined. Aflatoxin (ppb) amount was $\log(x+1)$-transformed before analysis to normalize data, since a lot of the samples had no aflatoxin in them and some of the aflatoxin results were very high. Fungal data, measured as percentage of grains contaminated with a certain species, was arcsine squareroot transformed (Zar 1974). The aflatoxin, fungal and insect data was presented untransformed in the tables.

5.3   Results

Comparisons across zones

Fungi from three main fungal genera *Aspergillus*, *Fusarium* and *Penicillium* were found in the infected stored maize samples. Other fungi such as *Rhizopus* spp., *Diplodia* spp., and unidentified species were encountered and grouped as "other".

First Survey Year

In the SGS significantly higher ppb aflatoxin than in the other ecoregions were detected (Table 5.1) and the percentage of *Aspergillus* fungi in the plated samples was also the highest in this zone at the beginning of storage. The FMS had less fungal contamination than the rest of the zones. The percentage of *Fusarium* spp. that developed on the plated grains was overall high, with the highest contamination in the SGS. The percentage of *Penicillium* spp. that developed in the plated grains was significantly lower in the two northern zones than in the FMS, with the SGS showing the highest percentage of *Penicillium* spp.. The percentage of other fungi, that did not belong to the main groups of storage fungi (*Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp.) was low. It was the highest in the SGS and the lowest in the SS. When the total percentage of fungi that grew out of the plated kernels were evaluated, the two northern zones SS and NGS revealed the lowest quantity of total fungi.

Aflatoxin concentration, in samples collected during the second sampling of the 1993-1994 season, gradually increased from the south to the north. But there were no significant differences between the results for the three southern zones. The highest levels of aflatoxin was detected in the SS after 6 months of storage (Table 5.2).

The percentage of *Aspergillus* spp. that developed on the plated kernels was very high with all zones having a mean of over 50% of the kernels infected by *Aspergillus* spp.. The SGS, as in the first sampling, had the highest *Aspergillus* spp. infection and the percentage of kernels infected had tripled within three months. The percentage of *Fusarium* fungi that developed on the kernels, in contrast was lower, than at the first sampling occasion. The two southern zones, FMS and SGS, had lower amounts of *Fusarium* infection, than the two northern zones, NGS and SS. The NGS had an equivalent percentage of kernels infected as six months before. The percentage of *Penicillium* spp. was higher in the SGS and the FMS, but there were no

significant differences between the zones. Only the SS showed significantly lower percentages of infection with other fungi at the beginning of storage. For the total fungal infection of the kernels, significant differences were observed between the middle zones, SGS and NGS, and the two other zones. Overall, more than 80% of the kernels had fungal growth. The grain moisture content decreased from the south to the north. Sampling at the beginning of the storage period, showed that grain in the two southern zones had in the mean two percent more grain moisture than the other zones (Table 5.1).

Table 5.1:   Aflatoxin levels (mean ppb) and accompanying fungi (% contamination, N= 25 grains) in 300 grain stores in the four agroecological zones of Benin during August-December 1993

Zone	Aflatoxin	*Aspergillus*	*Fusarium*	*Penicillium*	Other Fungi	Total Fungi	G.M.*C.*
FMS	12.7 a	10.8 a	48.7 b	14.6 b	9.6 a	71.3 b	14.54 ab
SGS	20.3 b	21.8 b	56.5 c	20.1 c	16.3 b	90.8 c	15.34 b
NGS	12.2 a	20.1 b	41.2 a	7.3 a	10.3 a	64.9 ab	12.26 a
SS	4.6 a	14.6 ab	38.2 a	7.2 a	8.5 a	60.0 a	11.61 a
F-VALUE	4.77 (p=.003)	3.96 (p=.009)	6.99 (p≤.001)	19.13 (p≤.001)	2.86 (p=.037)	17.86 (p≤.001)	40.75 (p≤.001)

Aflatoxin (ppb)was log(x+1)-transformed before analysis, fungal data (%) was arcsine ÷transformed, G.M.*C.* = Grain moisture content

Means followed by the same letter are not significantly different from each other(SNK, p = 0.05)

means are presented untransformed, Means were calculated for each zone with FMS = 80 stores, SGS = 80 stores, NGS = 80 stores, SS = 60 stores

Table 5.2:   Aflatoxin levels (mean ppb) and accompanying fungi (% contamination, N= 25 grains) in 150 grain stores in the four agroecological zones of Benin during April-May 1994

Zone	Aflatoxin	*Aspergillus*	*Fusarium*	*Penicillium*	Other Fungi	Total Fungi	G.M.*C.*
FMS	30.0 a	62.8 a	28.8 a	12.3 a	14.4 b	86.0 a	13.1 d
SGS	28.4 a	78.5 b	29.4 a	12.4 a	21.0 b	95.9 b	12.2 c
NGS	44.0 a	67.7 ab	50.6 b	7.2 a	17.8 b	94.7 b	11.2 b
SS	125.5 b	54.4 a	38.1 a	8.1 a	5.7 a	80.3 a	10.0 a
F-VALUE	4.60 (p=.004)	4.58 (p=.004)	11.2 (p≤.001)	2.23 (p=.087)	6.25 (p≤.001)	8.76 (p≤.001)	94.92 (p≤.001)

Aflatoxin (ppb)was log(x+1)-transformed before analysis, fungal data (%) was arcsine ÷transformed, G.M.*C.* = Grain moisture content

Means followed by the same letter are not significantly different from each other(SNK, p = 0.05)

means are presented untransformed, Means were calculated for each zone with FMS = 40 stores, SGS = 40 stores, NGS = 40 stores, SS = 30 stores

Table 5.3: Aflatoxin levels (mean ppb) and accompanying fungi (% contamination, N= 25 grains) in 150 grain stores in the four agroecological zones of Benin during September-December 1994 (survey 3)

Zone	Aflatoxin	*Aspergillus*	*Fusarium*	*Penicillium*	Other Fungi	Total Fungi	G.M.*C*.
FMS	4.6 a	34.7 ab	44.8 a	23.4 ab	5.2 a	80.5 ab	15.2 b
SGS	23.1 a	47.0 b	43.8 a	32.1 b	1.6 a	88.1 b	15.1 b
NGS	19.0 a	27.1 a	57.9 b	20.9 a	1.2 a	81.0 ab	12.9 a
SS	12.9 a	33.3 ab	40.0 a	15.7 a	1.9 a	72.3 a	12.6 a
F-VALUE	1.21 (p=.309)	3.85 (p=.011)	4.03 (p=.009)	4.87 (p=.003)	1.92 (p=.129)	3.60 (p=.015)	45.11 (p≤.001)

Aflatoxin (ppb)was log(x+1)-transformed before analysis, fungal data (%) was arcsine ÷transformed, G.M.*C*. = Grain moisture content

Means followed by the same letter are not significantly different from each other(SNK, p = 0.05)

means are presented untransformed, Means were calculated for each zone with FMS = 40 stores, SGS = 40 stores, NGS = 40 stores, SS = 30 stores

Table 5.4: Aflatoxin levels (mean ppb) and accompanying fungi (% contamination, N= 25 grains) in 150 grain stores in the four agroecological zones of Benin during April-May 1995 (survey 4)

Zone	Aflatoxin	*Aspergillus*	*Fusarium*	*Penicillium*	Other Fungi	Total Fungi	G.M.*C*.
FMS	2.2 a	8.3 a	47.1 b	11.3 a	0.3 a	61.6 a	11.0 a
SGS	18.7 b	23.6 b	34.2 a	27.9 b	1.7 ab	66.0 a	11.4 ab
NGS	9.1 ab	24.4 b	41.3 a	4.0 a	3.6 b	63.1 a	11.7 b
SS	18.5 ab	25.5 b	29.5 a	3.7 a	2.1 ab	55.5 a	11.8 b
F-VALUE	3.99 (p=.009)	4.11 (p=.008)	3.70 (p=.013)	7.45 (p≤.001)	3.49 (p=.017)	1.16 (p=.326)	5.56 (p≤.001)

Aflatoxin (ppb)was log(x+1)-transformed before analysis, fungal data (%) was arcsine ÷transformed, G.M.*C*. = Grain moisture content

Means followed by the same letter are not significantly different from each other(SNK, p = 0.05)

means are presented untransformed, Means were calculated for each zone with FMS = 40 stores, SGS = 40 stores, NGS = 40 stores, SS = 30 stores

Second Survey Year

The amounts of aflatoxins found in the 94-95 samples at the beginning of the storage period, showed no significant differences among agroecological zones. The mean ppb aflatoxin in samples fom the SGS was higher than in the other zones and the sample that had the highest amount of aflatoxin found (2500 ppb) came from this zone (Table 5.3). The percentage of *Aspergillus* fungi in the SGS was significantly higher than in the NGS zone. The two northern zones showed lower *Aspergillus* spp. infection rates than the southern zones though the SS was not significantly different from either FMS or SGS. The percentage of *Fusarium* spp. that developed on the plated kernels was significantly higher in the NGS than in the other zones, overall there were more than 40% of the kernels in each zone that developed *Fusarium* fungi during the first sampling in the 1994-95 season. The two southern zones showed higher quantities of *Penicillium* infection than the North, with the SGS being significantly different from the SS. Only the FMS showing a mean of 5.2% of other fungi and there were no significant differences between the zones. Evaluating the total fungal contamination in the different ecozones significant differences were observed between the SGS (88.1%) and the SS (72.3%).

Evaluating the aflatoxin content of the samples collected 6 months after harvest in the 1994-95 season (Table 5.4), significant differences were detected between the ecozones. Mean aflatoxin content of the samples from the FMS were significantly lower than in the other zones and those were much lower than in the survey after harvest of the previous year. The percentage of kernels that developed *Aspergillus* fungi was in general low, with the FMS having significantly lower infection rates than the other zones. Contamination with *Fusarium* spp. revealed significant differences between the agroecological zones, but overall the percentage of *Fusarium* fungi that developed on the kernels was high. Percent kernel infection with *Penicillium* spp. was higher in the SGS, as compared to the other zones. The percentage of kernels that showed other fungal contaminants was low, with the FMS zone having the least. There were no significant differences between the agroecological zones for the total fungal contamination; in all the zones there were more than 60% of the kernels that showed fungal development. The grain moisture content in the two southern zones was again significantly different than the other two zones, six months later the differences between the zones were not so pronounced, but still they existed.

Comparison across years

The percentage of stores that were aflatoxin positive (Table 5.5) was between 5 and 25% for the first sampling after harvest in 1993-94, but for the same sampling a high percentage of samples 50 to 85%, depending on the ecoregion, were aflatoxin positive at rates of more than 20 ppb. When maize was evaluated after 6 months of storage in 93-94 there were more stores contaminated (25 to 57%), but the percentage of stores that showed levels of more than 20 ppb remained about the same.

Table 5.5: Percent stores that were aflatoxin positive and percentage of samples >20ppb in the different ecoregions in 93-94 and 94-95

Survey	Beginning 93-94		6 months 93-94		Beginning 94-95		6 months 94-95	
	%Stores positive	positive >20ppb	%Stores positive	positive >20ppb	%Stores positive	positive >20ppb	%Stores positive	positive >20ppb
FMS	10.0	78.8	24.4	60.0	36.7	18.2	22.5	11.1
SGS	25.0	85.0	35.0	78.6	55.0	54.6	58.5	45.5
NGS	10.0	50.0	35.0	57.1	72.5	31.0	60.5	15.4
SS	5.0	66.7	56.7	76.5	56.7	35.3	56.7	23.5

In the second survey year (94-95) the percentage of stores showing aflatoxin was higher than in the first year (Table 5.5), but the number of samples with more than 20 ppb was lower, only in the SGS more than 50% of the positive samples showed high levels greater than 20 ppb. Six months later in the SGS, NGS, and SS there were 60% of the samples that showed aflatoxins with only the SGS showing a high percentage of samples that had more than 20 ppb of aflatoxins.

5.4 Discussion

Thin layer chromatography was chosen for the determination of aflatoxin levels in stored goods, because of its simplicity, speed, ability to handle many samples simultaneously and the costs of analysis were lower than more sophisticated methods. This is a method that was employed in many surveys and in mycotoxin testing programs especially in developing countries (Zuber *et al.* 1987). The visual observation of the sample fluorescence and comparing it to the aflatoxin standard is a semi-quantitative method that gives a good result for the aflatoxin levels. This method was used by mycotoxin analysis laboratories all over the world to determine aflatoxin levels (Shotwell 1983). The detection limit with the method by Thomas *et al.* (1975) used in this study was given as 2 ppb (Soares 1992). When

TLC-techniques with simple visual evaluation of the fluorescence were applied, the detection limit was equivalent to the lowest amount of mycotoxin visible on a TLC plate, which corresponded to a level at which 50% of the cases would be observed (FAO 1990). Confirmation of the presence of aflatoxins was necessary since there could be some compounds that fluoresce in a similar manner as aflatoxin

The results for the 94-95 season differed from the 93-94 results. There was no increase in the contamination with aflatoxins from the first to the second sampling period. Rather mean aflatoxin contamination decreased in the FMS, the SGS and NGS from one sampling to another. Like in the first sampling year mean aflatoxin in the SS was higher after 6 months of storage than at the beginning of storage. These findings suggested that environmental conditions may have had a great impact on the process of aflatoxin development, which was also observed by Faraj *et al.* (1991), Gorman & Kang (1991) and Payne (1992). The effect of temperature on toxin production was well documented (Trenk & Hartmann 1970; Magan & Lacey 1988; Trucksess *et al.* 1988). The SS is the hotter zone of Benin with an average temperature of 28°C throughout the year, and high temperature from 34° to 38°C in the hot months from December to April. This could be compared with the results of Gonzales *et al.* (1988) who reported that the growth rate of *A. niger*, *A. flavus* and *A. parasiticus* rose with increasing temperature.

For the second survey the highest amounts of aflatoxins could be found in the SS, this is the driest agroecological zone of Benin. Cotty (1994) remarked that aflatoxins occur in dry environments rather than in zones that have high rainfall and high temperatures. There was also more aflatoxin development in regions that have high daily temperatures than those growing at lower temperatures (Thompson *et al.* 1980). Fungal contamination varied for the agroecological zones and survey years. In the first year *Aspergillus* spp. incidence consistently rose from the sampling period at the beginning of storage to the second sampling six months later in storage. It might be that the fungus continually invaded the maize stock as the storage season progressed. The percentage of kernels that were contaminated with *Fusarium* spp. was still high after six months of storage. At the beginning of storage more than 70% of the kernels across the ecozones were contaminated with fungi, but six months later the percentage of kernels that showed fungal growth decreased to around 20% in all the ecozones. *Aspergillus* contamination of stored grain was highly correlated with the relative humidity (Christensen & Mirocha 1976; Sauer &

Burroughs 1986). Also the harvest in Benin was effected in the middle of the rainy season, making drying difficult. With the lower fungal contamination after 6 months of storage explained by the dry season setting in after the harmattan around the second sampling period. The regional differences observed for the *Aspergillus* spp. distribution did not hold for the other fungi observed. A decrease for the contamination with *Fusarium* spp. was observed from one sampling period to another. *Fusarium* fungi need high humidity for their development. They are considered more of a field pathogen that infects maize in the field and have the potential to develop mycotoxins (Lacey & Magan 1991). There was no differences observed between the two sampling period for the infection with *Penicillium* spp..

The presented results confirm the findings of Setamou *et al.* (1997 in press) who studied aflatoxin contamination in maize before harvest in Benin. In their study *A. flavus* was found in over 60% of the fields in 1994 and 1995, with the SGS and the NGS having higher infection levels than the other zones. One can conclude from these results, that maize was already predisposed to aflatoxin development in the field. In the same study, field contamination with aflatoxins in maize cobs was evaluated, relatively low levels were recorded in the FMS and in the SS. In the SGS and NGS high levels were recorded (Setamou 1996). The conclusion from these studies and the here described results were that the same zones that showed high infestation in the field also had high levels of aflatoxin in storage. There were indications in the presented study that the Guinea Savannas had higher levels of aflatoxins than the FMS, the high mean aflatoxin contamination after 6 months of storage in the SS in 1993/94 has to be investigated further. A third year of data might have given a clearer picture of the agroecological zones, that are especially at risk for aflatoxin contamination in Benin.

In a similar study by Udoh (1995) in Nigeria in the SGS, NGS, SS and the Humid Forest Zone, the wet and humid zone of eastern Nigeria, more than 25% of the stored grain samples showed *A. flavus* growth. The only zone that showed low *A. flavus* infection (11.2%), was the Mid-Altitude Zone (MAZ), which is one of the coolest parts of Nigeria. Contrary to the here described study (Chapter 5) no aflatoxin was found in maize from the NGS in 1994 in Nigeria, in the other zones the percentage of maize samples that were aflatoxin positive was below 20%, except for the SGS where 24% of the samples showed aflatoxins. Mean aflatoxin levels in Nigeria were higher than in Benin. These result are

contrary to the study by Aja-Nwachukwa & Emejuaiwe (1994) who found more than 80% of the maize samples from southeastern Nigeria showing fluorescence because of aflatoxin B_1. For the 1995 season in Nigeria mean aflatoxin contamination levels were lower, with 18-28% of the samples being aflatoxin positive, except in the MAZ where no aflatoxins were analyzed (Udoh 1995). In Nigeria a similar trend as in Benin was observed, with high aflatoxin contamination in the dry and hot areas, as opposed to the hot and humid areas.

CHAPTER 6   **Influence of agronomic practices on aflatoxin contamination**

6.1   Introduction

The factors that were thought to influence growth of *A. flavus* and the formation of aflatoxin could be divided into three categories climatic factors, agronomic factors and biotic factors (Diener *et al.* 1987). The agronomic factors that may have influenced aflatoxin formation were plant stress, irrigation, cropping pattern, variety, date of planting, date of harvest and storage conditions (Cotty 1994). The biotic factors that influenced *A. flavus* infection and aflatoxin formation were damage because of insects, rodents, birds and other animals (McMillian *et al.* 1990). Abramson (1991) suggested that multivariate techniques should be used to evaluate how physical factors (temperature, rapidity of drying, moisture content etc.) affected the production of mycotoxins at harvest time. The plant characteristics that affected the development of grain molds before harvest included: susceptibility to insect damage, physical and chemical characteristics of the husk, physical and chemical characteristics of the grain and influence of the climate itself which could be expressed as plant stress (Williams & McDonald 1983). Smith & Riley (1992) showed that drought stress and insect damage had a synergistic effect on aflatoxin levels in pre-harvest corn. Development of storage fungi in a post-harvest commodity may be affected by the duration of storage (Lillehoj & Zuber 1988). Ahmad (1993) observed 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" with paddy hay ropes wound into a container, gunny bags and iron bins (Prasad *et al.* 1987). The mean aflatoxin B_1 concentration of maize stored in the "kothi was 1840 ppb, the "mora" had a mean of 1280 ppb, in samples from the iron bin 430 ppb were measured and in maize stored in jute bags 520 ppb were measured.

In this chapter, the agronomic factors (presented in Chapter 4) that influence the contamination of maize with aflatoxins (presented in Chapter 5) were determined using regression analysis.

6.2 Materials and Methods

Questionnaires (see annex I, II) were administered in the 30 survey villages in Benin (see figure 1.1), to evaluate the influence of production, harvest, drying, and storage practices on aflatoxin development. Analysis was run seperately for the production, harvest and storage factors. The sampling of stored maize differed for the various agroecological zones, to coincide with specific storage periods. Sampling at the beginning of the storage period was carried out in the south from August to September 93, and in the north from November till December 93. The results of the aflatoxin analyses (see chapter 5) were used as the dependent variable, and the survey questionnaire data (see Chapter 4) were the independent variables. For the sampling of grain after 6 months of storage, in the south samples were taken in February till March 1994 and in the north in April 1994. Aflatoxin results were log(x+1)-transformed to normalize data before analysis. The survey were repeated in 1994-95 at the beginning of storage and at six months of storage.

Most of the answers to the survey questions were entered as binomial values. Each answer in the survey was recorded in a separate column, a "yes" was recorded as 1 or "no" as 0. For answers that described a hierarchical scale, like good, moderately good, bad answers were entered as a scale e.g. 1 to 3. Answers to questions that were personal opinions, were left out of the statistical analysis. The statistical package used was the SPSS-Statistical Package (Norusis & SPSS 1993). Stepwise linear regression was used to select variables that describe variability in aflatoxin levels in the farmers maize samples.

The model is expressed as:

$$Y' = B_0 + B_1 * X_1 + ... + B_{n-1} * X_{n-1} + B_n * X_n$$

With: Y = dependent variable, X = indepedent variables

B_0 = intercept, B_1 = slope

Before accepting the results from a multiple regression analysis one has to know enough about the biological phenomenon under study, biological sense has to be used to evaluate if the results describe a chance pattern or it describes a valid model of the relationship between the variables (Sokal & Rohlf 1995).

6.3   Results

6.3.1   Production factors

1993/94 Survey

Regression analysis of the samples from the beginning of storage showed, that there was a higher risk of aflatoxin development in maize from stores in the SGS (Table 6.1).

Table 6.1: Regression analysis of production factors that influence aflatoxin
           content of maize sampled at the beginning of storage (season 93-94)

Regression analysis		b_0	b_i	t
Across all agroecological zones		0.65		
X_1	Store located in SGS		0.63	3.59***
X_2	Maize intercropped with cowpeas		2.15	4.75***
X_3	Farmers aware of poor huskcover		-0.31	2.43*
$R^2 = 0.13$, N = 300				
FMS	no variable significantly affects aflatoxin levels			
SGS		0.78		
X_1	Maize intercropped with cowpeas		2.28	2.23*
$R^2 = 0.10$, N = 80				
NGS		1.20		
X_1	Farmers planted maize in other field from year to year		-0.62	2.28*
X_2	Maize intercropped with cowpeas		2.45	3.85***
X_3	Farmers aware of poor huskcover		-0.38	2.24*
$R^2 = 0.27$, N = 80				
SS		0.76		
X_1	Improved maize variety		-0.76	2.49*
X_2	Maize was intercropped with cowpeas		2.20	4.89***
$R^2 = 0.33$, N = 60				

* significant at < 0.05, *** significant at < 0.001

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, ($P \leq 0.05$ to add, $P \geq 0.10$ to remove)
b_0 = regression koefficient, b_i = standard error for each x, t = significance level

Across agroecological zones, the farmers awareness of bad huskcover of their maize was related to lower aflatoxin contamination. The relationship between increased aflatoxin content and maize that was intercropped with cowpea was noticed across ecozones and in the three northern zones (SGS, NGS, SS). In the FMS, no production variable was found that significantly related to aflatoxin levels (Table 6.1). In the NGS, rotating maize to different fields from one year to another and when farmers sorted out damaged cobs before

storing was associated with low aflatoxin measurements in the analyzed samples. In the SS, use of improved maize varieties led to lower aflatoxin quantities.

Across agroecological zones, aflatoxin levels six months after harvest were positively associated with the complaint of farmers that larger animals, like cane rats, cows or baboons were damaging their fields. Also high aflatoxin levels were associated with samples from the SS (Table 6.2). For the within zone analysis, no variable was found that could explain high or low aflatoxin contamination in maize samples from the SGS and SS. Planting maize in the same field from year to year was associated with high aflatoxin levels in the FMS. In the NGS, damage of maize plants through larger animals was related to high aflatoxin content.

Table 6.2:   Regression analysis of production factors that influence aflatoxin
             content of maize sampled after 6 months of storage (season 93-94)

Regression analysis			b_0	b_i	t
Across all agroecological zones			0.47		
	X1	Large animals damaged maize in the field		0.55	2.14*
	X2	Store located in SS		0.68	3.61***
R 2 = 0.11, N = 150					
FMS			4.87 E-17		
	X1	Farmers planted maize in the same field from year to year		0.60	2.19*
R 2 = 0.11, N = 40					
SGS		no variable significantly affects aflatoxin levels			
NGS			0.38		
	X1	Large animals damaged maize in the field		0.89	2.76**
R 2 = 0.17, N = 40					
SS		no variable significantly affects aflatoxin levels			
* significant at < 0.05, *** significant at < 0.001					

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, (P < 0.05 to add, P > 0.10 to remove)
b0 = regression koefficient, bi = standard error for each x, t = significance level

1994/95 Survey

Across ecozones at the beginning of storage a positive relationship between aflatoxins and farmers who complained about problems with fungi in the field (Table 6.3) was found. Maize/groundnut intercrop was associated with high aflatoxin content. When farmers applied fertilizer, specifically Double-Ammonium-Phosphate (DAP), the risk of aflatoxin contamination was reduced. In the FMS, maize/cassava intercrop or the rotation of maize with cowpea was associated with high aflatoxin. In the NGS, high aflatoxin contamination

was related to big animals damaging maize and intercropping maize with cassava, but low aflatoxin levels were found when maize was planted in a mixed cropping system e.g. maize with tomato, pepper, okra and cowpeas.

Table 6.3: Regression analysis of production factors that influence aflatoxin content of maize sampled at the beginning of storage (94-95)

Regression analysis		b_0	b_i	t
Across all agroecological zones		0.53		
X1	Farmers noticed fungal damage of maize in the field		0.44	1.18*
X2	Maize intercropped with groundnut		0.85	2.55*
X3	DAP-fertilizer was applied in the field		-0.14	3.43**
$R^2 = 0.16$, N = 150				
FMS		6.59 E-17		
X1	Maize intercropped with cassava		1.30	4.66***
X2	Maize rotated with cowpea		0.44	2.18*
$R^2 = 0.65$, N = 40				
SGS		0.60		
X1	Maize rotated with groundnut		1.03	2.66*
$R^2 = 0.16$, N = 40				
NGS		0.47		
X1	Large animals damaged maize in the field		0.94	4.21***
X2	Maize planted in mixed cropping		-1.41	2.48*
X3	Maize was intercropped with cassava		1.98	3.65**
$R^2 = 0.46$, N = 40				
SS	no variable significantly affects aflatoxin levels			
* significant at < 0.05, ** significant at <0.01, *** significant at < 0.001				

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, (P < 0.05 to add, P > 0.10 to remove)
b0 = regression koefficient, bi = standard error for each x, t = significance level

Maize samples from the FMS after 6 months of storage in 94-95, like at the beginning of the storage period, had lower aflatoxin contamination than samples from the other zones (Table 6.4). In the FMS, aflatoxin content of the samples was lower when farmers used local maize varieties. In contrast, in the NGS, aflatoxin contamination of the sampled grains was reduced in improved maize varieties. In the SGS and SS, no production variables were found that significantly affected aflatoxin levels.

Table 6.4: Regression analysis of production factors that influence aflatoxin
content of maize sampled after 6 months of storage (season 94-95)

Regression analysis			b_0	b_i	t
Across all agroecological zones			0.53		
	X1	Store located in FMS		-0.35	3.01**
R 2 = 0.06, N = 150					
FMS			1.11		
	X1	Local maize variety		-0.98	3.87***
R 2 = 0.28, N = 40					
SGS		no variable significantly affects aflatoxin levels			
NGS			1.35		
	X1	Improved maize variety		-0.96	3.78***
R 2 = 0.26, N = 40					
SS		no variable significantly affects aflatoxin levels			
** significant at<0.01, *** significant at < 0.001					

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, (P < 0.05 to add, P > 0.10 to remove)
b0 = regression koefficient, bi = standard error for each x, t = significance level

6.3.2   Harvest factors

1993/94 Survey

Table 6.5: Regression of harvest factors that influence aflatoxin content of maize
sampled at the beginning of storage (season 93-94)

Regression analysis			b_0	b_i	t
Across all agroecological zones			0.13		
	X1	Store located in SGS		0.70	3.84***
	X2	Maize harvest took 1 to 5 days		0.42	2.60**
R 2 = 0.06 , N = 300					
FMS		no variable significantly affects aflatoxin levels			
SGS		no variable significantly affects aflatoxin levels			
NGS			0.15		
	X1	Sorting later in processing before storage, not right after harvest		0.58	2.09*
R 2 = 0.05, N = 80					
SS		no variable significantly affects aflatoxin levels			
* significant at < 0.05, ** significant at<0.01, *** significant at < 0.001					

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, (P < 0.05 to add, P > 0.10 to remove)
b0 = regression koefficient, bi = standard error for each x, t = significance level

When the maize harvest practices were regressed against aflatoxin content of maize sampled
at the beginning of storage, there was a positive relationship between the presence of
aflatoxins in the samples and the SGS zone. Also, when harvest takes between 1 to 5 days,
the risk of aflatoxin contamination increased (Table 6.5). In the NGS, sorting of maize later

in storage processing not right after harvest, increased the risk of aflatoxin contamination. Late sorting (see Table 4.11) was due to the farmers use of temporary stores (see Table 4.13) or the change from a field store to storage in or near the house. Some farmers sorted maize at a later processing stage, when they dehusked or degrained cobs. In the south of Benin this preceded the sale of the crop (see section 4.3.3.3).

Regression analysis of data from sampling 6 months after storage (Table 6.6) showed that in the SS, there was an increased risk of aflatoxin contamination. Also maize harvested in August showed a tendency to be associated with high aflatoxin levels. In the FMS zone, when the whole maize-plant was cut at harvest, laid on the ground and the cobs were collected later, there was a high risk of aflatoxin contamination. In the NGS, drying of maize in the field for more than 30 days after maize maturity, was associated with high levels of aflatoxin.

Table 6.6: Regression of harvest factors that influence aflatoxin content of maize sampled after 6 months of storage (season 93-94)

Regression analysis		b_0	b_i	t
Across all agroecological zones		0.37		
X1	Store located in SS		0.83	4.21***
X2	Harvest in August		0.42	2.38*
R 2 = 0.11, N = 150				
FMS		0.37		
X1	Maize was cut at harvest		2.03	2.59*
R 2 = 0.15, N = 40				
SGS	no variable significantly affects aflatoxin levels			
NGS		0.05		
X1	Drying in the field >30 days		0.66	2.62*
R 2 = 0.34, N = 40				
SS	no variable significantly affects aflatoxin levels			
* significant at < 0.05, *** significant at < 0.001				

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, (P < 0.05 to add, P > 0.10 to remove)
b0 = regression koefficient, bi = standard error for each x, t = significance level

1994/95 Survey

Country-wide factors that reduced the contamination of the samples with aflatoxins, were drying maize without the husk and sorting of maize before storage (Table 6.7). Samples that originated from stores in the FMS were associated with low aflatoxin levels. Also in the same ecozone, aflatoxin was negatively associated with the practice of farmers to sort out

spoiled cobs, usually those cobs that showed fungal growth, insect damage and rat damage before storing the cobs (see section 4.3.3.2).

Table 6.7:   Regression of harvest factors that influence aflatoxin content of maize sampled content of maize sampled at the beginning of storage (94-95)

Regression analysis		b_0	b_i	t
Across agroecological zones		1.04		
X1	Maize was sorted after harvest		-0.33	1.96ns
X2	Maize was dried without husk		-0.31	2.20*
X3	Store located in the FMS		-0.41	2.74**
R 2 = 0.11, N = 150				
FMS		0.51		
X1	Sorting out of spoiled cobs		-0.47	2.99**
X2	Sorting out of rat damaged cobs		-0.37	0.35*
X3	Field drying for 5 to 10 days		0.87	3.65**
X4	Drying of harvested cobs for 60 to 90 days		0.52	2.11*
R 2 = 0.54, N = 40				
SGS		1.02		
X1	Field drying for > 7 days		0.76	2.06*
X2	Maize was winnowed bevore storage		0.79	2.42*
X3	Sorting of damaged, discolored or not well formed cobs before storage		-0.49	2.25*
X4	Maize was harvested with husk		-0.66	2.85**
R 2 = 0.49, N = 40				
NGS		0.62		
X1	Maize was harvested with husk		-0.62	2.35*
R 2 = 0.23, N = 40				
SS		1.04		
X1	Sorting out of insect damaged cobs		-0.64	2.96**
X2	Maize dried without husk		-0.68	2.71*
X3	Maize dried for more than 30 days in the field		1.22	2.86**
R 2 = 0.44, N = 30				

ns =not significant, * significant at < 0.05, ** significant at<0.01

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, (P < 0.05 to add, P > 0.10 to remove)
b0 = regression koefficient, bi = standard error for each x, t = significance level

Aflatoxin contamination of the samples increased with drying in the field for 5 to 10 days and drying of the harvested maize cobs for long periods of 60 to 90 days. In the SGS, when maize was dried in the field for periods of more than 7 days aflatoxin content increased, the same effect was noticed when maize was winnowed before storage. There was a negative relationship between aflatoxin content in the samples, harvesting with the husk and sorting

of maize cobs that were damaged, discolored or not well formed before storage. In the NGS, aflatoxin contamination was reduced when maize was harvested with the husk. In the SS, aflatoxin contamination was reduced through sorting out of cobs that showed insect damage and drying without the husk. Aflatoxin contamination in this zone increased when maize was left to dry in the field for more than one month.

Comparing the results of the country-wide regression at 6 months after harvest in 1994-95, maize samples from the FMS and harvesting maize in December was related to lower aflatoxin levels (Table 6.8). No harvest variables affected aflatoxin levels in the FMS and the SS. In the SGS, maize harvested in August had lower aflatoxin levels, whereas in the NGS this was true for maize harvested in December.

Table 6.8: Regression of harvest factors that influence aflatoxin content of maize sampled after 6 months of storage (season 94-95)

Regression analysis		b_0	b_i	t
Across all agroecological zones		0.58		
X1	Store located in FMS		-0.30	2.58*
X2	Maize was harvested in December		-0.29	2.27*
R 2 = 0.09, N = 150				
FMS	no variable significantly affects aflatoxin levels			
SGS		0.90		
X1	Maize was harvested in August		-0.54	2.34*
R 2 = 0.13, N = 40				
NGS		0.66		
X1	Maize was harvested in December		-0.51	2.75*
R 2 = 0.16, N = 40				
SS	no variable significantly affects aflatoxin levels			
* significant at < 0.05				

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, (P < 0.05 to add, P > 0.10 to remove)
b0 = regression koefficient, bi = standard error for each x, t = significance level

6.3.3 Storage factors

1993/94 Survey

Across all ecozones, aflatoxin content was negatively related with the use of insecticides in store. Aflatoxin levels were higher when maize was stored for short periods of three to five months in the SGS (Table 6.9). In the FMS, lower aflatoxin levels were found when stored maize was treated with insecticides. In the SGS, aflatoxin content increased when farmers stored their maize for short periods of three to five months. Maize that was stored in the

NGS in the "Ago" (FAO 1994), and in the "Crib" in the SS, had a higher risk of aflatoxin contamination (see section 4.3.1 and Chapter 7 for description of the storage structures).

Table 6.9: Regression of storage factors that influence aflatoxin content of maize sampled at the beginning of storage (season 93-94)

Regression analysis			b_0	b_i	t
Across all agroecological zones			0.63		
	X1	Store located in SGS		0.61	3.37**
	X2	Maize was stored for 3 - 5 months		0.46	2.15*
	X3	Insecticides were used as storage protectants		-0.59	3.57***
$R 2 = 0.10, N = 300$					
FMS			0.78		
	X1	Insecticides were used as storage protectants		-0.78	2.23*
$R 2 = 0.07, N = 80$					
SGS			0.78		
	X1	Maize was stored for 3 - 5 months		1.89	3.31**
$R 2 = 0.12, N = 80$					
NGS			0.26		
	X1	Maize was stored in the "Baskets"		1.73	3.53***
$R 2 = 0.14, N = 80$					
SS			0.16		
	X1	Maize was stored in the "Crib"		2.24	2.55*
$R 2 = 0.10, N = 60$					
* significant at < 0.05, ** significant at < 0.01, *** significant at < 0.001					

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, (P < 0.05 to add, P > 0.10 to remove)
b0 = regression koefficient, bi = standard error for each x, t = significance level

The country-wide regression analysis of data from sampling 6 months after harvest showed (Table 6.10), that there was a positive relationship between the SS and aflatoxin content. Those farmers that were aware that they had storage problems were less likely to have aflatoxin contamination in their maize. In the SGS, maize that was stored in the same store as sorghum and maize stored as grains was positively related to high aflatoxin content. The factor that reduced aflatoxin contents in this zone was cleaning of the store before the storage of the new harvest. In the SS, aflatoxin content was reduced by storing maize as grains, and farmers that noticed that their grains were germinating in the store had lower aflatoxin quantities. When maize was attacked by insects during storage, there was a higher risk for aflatoxin contamination.

Table 6.10: Regression of storage factors that influence aflatoxin content of maize sampled after 6 months of storage (season 93-94)

Regression analysis			b_0	b_i	t
Across all agroecological zones			0.96		
	X1	Store located in SS		0.69	3.66***
	X2	Farmers aware of storage problems		-0.35	2.04*
R 2 = 0.11, N = 150					
FMS		no variable significantly affects aflatoxin levels			
SGS			1.59		
	X1	Storage structure cleaned before storing the next harvest		-1.54	3.09**
	X2	Maize stored with sorghum		2.17	3.15**
	X3	Maize stored as grains		0.66	2.99*
R 2 = 0.45, N = 40					
NGS		no variable significantly affects aflatoxin levels			
SS			1.22		
	X1	Farmers noticed that their maize germinates in storage		-1.25	2.55*
	X2	Maize damaged by insects in storage		0.92	2.79**
	X3	Maize stored as grains		-0.86	2.44*
R 2 = 0.45, N = 30					
* significant at < 0.05, ** significant at<0.01, *** significant at < 0.001					

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, (P < 0.05 to add, P > 0.10 to remove)
b0 = regression koefficient, bi = standard error for each x, t = significance level

1994/95 Survey

Across the country the level of aflatoxin in stored maize was negatively related to the usage of cotton insecticides, storing maize as grains, storing maize over smoke and storing it in the "Ago" made from bamboo or under the roof (Table 6.11). Aflatoxin content was positively related to short storage of three to five months. In the SGS, there was an increased risk of aflatoxin development if maize was stored under the roof. If maize in the NGS was stored in the secco or the conical store high aflatoxin levels were found in the grain. Storing maize in bags after an initial storage in another container reduced aflatoxin contamination. If the leaves and bark of the *Khaya senegalensis* tree were used to protect maize against insects, there was an increased risk of aflatoxin development. In the SS, farmers that noticed a build-up of insects in stored maize had lower aflatoxin levels, while those that stored maize on top of the roof had more.

Table 6.11:   Regression of storage factors that influence aflatoxin content of maize sampled at the beginning of storage (94-95)

Regression analysis		b_0	b_i	t
Across all agroecological zones		0.71		
X1	Maize stored for 3-5 months		0.39	2.21*
X2	Smoke used to protect stored maize		-0.74	2.12*
X3	Farmers used cotton insecticide as storage protectants		-0.54	3.22**
X4	Maize stored as grains		-0.64	2.70**
X5	Maize stored in "Ago" of bamboo		-0.46	2.12*
X6	Maize stored under the roof		0.50	2.40*
$R^2 = 0.21$, N = 150				
FMS	no variable significantly affects aflatoxin levels			
SGS		0.60		
X1	Maize stored under the roof		0.75	2.24*
$R^2 = 0.12$, N = 40				
NGS		0.32		
X1	Farmers used *Khaya senegalensis* - bark to protect their stored maize		2.13	3.73***
X2	Farmers used bags as secondary storage		-0.55	2.74*
X3	Maize stored in "Secco"		0.74	3.18**
X4	Maize stored in "Conical stores"		1.07	4.16***
$R^2 = 0.46$, N = 40				
SS		1.44		
X1	Farmers aware of insects in storage		-0.65	2.51**
X2	Maize stored on the roof		1.28	2.85***
$R^2 = 0.27$, N = 30				

significant at < 0.05, ** significant at <0.01, *** significant at < 0.001

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, ($P < 0.05$ to add, $P > 0.10$ to remove)
b0 = regression koefficient, bi = standard error for each x, t = significance level

Across agroecological zones, the aflatoxin content of samples for the last survey in March-April 95 (Table 6.12) was positively related to maize stored as grains, the use of traditional plants to protect grain stores and the use of storage containers that were older than 5 years. The aflatoxin levels in samples from the SGS was higher than in the other zones. In the FMS, the use of traditional measures to control storage pests, as did the usage of storage containers that were more than 5 years old increased the risk of aflatoxin contamination. Aflatoxin levels were lower when farmers noticed that grains germinated in storage. In the SGS, high aflatoxin measurements were positively related to maize being stored with cowpea. A reduction of aflatoxin content was achieved through the use of mechanical means, such as smoke or the use of rat guards to combat storage pests. In the NGS, storage of maize for 3 to 5 months reduced aflatoxin contamination, whereas the use of traditional

plants to protect the store against pests and storage of maize for 6 to 7 months increased aflatoxins. In the SS, aflatoxin content was positively related to maize being protected with traditional leaves, maize being stored on top of the roof and storage in containers that were more than 5 years old, and negatively related to storing maize in the improved crib.

Table 6.12: Regression of storage factors that influence aflatoxin content of maize
            sampled after 6 months of storage (season 94-95)

Regression analysis			b_0	b_i	t
Across all agroecological zones			0.22		
	X1	Farmers used traditional plants to protect their maize from insects		1.64	3.88***
	X2	Maize stored as grains		0.26	2.61*
	X3	Storage containers were more than 5 years old		0.74	2.78**
	X4	Store located in SGS		0.26	2.40*
$R2 = 0.22, N = 150$					
FMS			0.14		
	X1	Storage containers>5 years old		1.11	3.33**
	X2	Farmers used traditional plants to protect their stored maize		0.62	3.47**
	X3	Grains germinated while in storage		-0.18	-2.08*
$R2 = 0.41, N = 40$					
SGS			0.62		
	X1	Maize stored with cowpea		0.61	2.38*
	X2	Farmers used mechanical means to protect their stored maize		-0.80	2.74**
$R2 = 0.23, N = 40$					
NGS			0.66		
	X1	Maize stored for 3 - 5 months		-0.50	3.02**
	X2	Farmers used traditional plants to protect their stored maize		1.25	2.43*
	X3	Maize stored for 6 -7 months		1.22	2.37*
$R2 = 0.41, N = 40$					
SS			0.16		
	X1	Farmers used traditional plants to protect their stored maize		2.18	7.36***
	X2	Storage containers were more than 5 years old		2.01	9.39***
	X3	Maize stored in the "Crib"		-2.16	6.09***
	X4	Maize stored on top of the roof		0.70	2.66**
$R2 = 0.85, N = 30$					

* significant at < 0.05, ** significant at<0.01, *** significant at<0.001

Y = log (aflatoxin (ppb) +1), stepwise forward selection procedure, (P < 0.05 to add, P > 0.10 to remove)
b0 = regression koefficient, bi = standard error for each x, t = significance level

6.4   Discussion

Some farming practices were found to increase the likelihood of aflatoxin contamination in stored maize, while others had a reducing effect. However in some of the regressions the explained variance (r2) was very low. This could be because many other factors like climate, also influence aflatoxin levels in maize, which were not incorporated in the regression analysis. Farming practices and environmental conditions under which maize is grown in sub-Saharan Africa were extremely variable (Cardwell *et al.* 1997 in press). Reasons for not measuring intensively the environmental factors that influence aflatoxins, lay in the nature of this survey work, that was intended to gain a broad insight into aflatoxin presence or absence in maize and the influence of farming practices. The variables selected in the regression analysis describe trends and these have to be verified in on-station trials. Also interactions between different variables were not taken into account, so that some of the relationships described could be much more complex.

6.4.1   Production factors

There was a pronounced zonal effect on aflatoxin development in Benin, maize samples that originated in the SGS or SS were related to higher aflatoxin levels, whereas samples from the FMS showed lower levels. The different ecozones have different weather conditions and it was not surprising to find different aflatoxin concentrations in the different zones. Lillehoj (1983) found variations in aflatoxin content of maize from diverse locations, aflatoxin incidence and levels were highest in maize from southern U.S. locations. The authors drew the conclusions that temperature and relative humidity may be critical factors influencing the infection process and toxin production. The effect of environment on aflatoxin contamination in maize has been well established (Fortnum 1986; Thompson *et al.* 1980; Jones *et al.* 1980, Lillehoj 1983).

Another factor that has been known to influence toxin production was plant stress, because of factors like poor soil fertility and drought (Lillehoj 1983). Land use intensity in the SGS was high, especially in areas that have been agriculturally exploited for a long time (Meikle 1992). These high land use intensities leads gradually to a negative negative nutrient balance in the agricultural ecosystem and deteriorated soil resources (Stahr *et al.* 1993) this might have been one of the reasons for high aflatoxin levels in this zone.

Maize samples that originated in the SS were linked to high aflatoxin contamination. This could be an indication that aflatoxin development in Benin, was influenced more by variations in temperature and drying stress, than by high relative humidity. Temperatures in the SS could rise up to 45ºC and the dry season starts in October and rains only resume in May (Adam & Boko 1983). Wicklow (1988) had extensively described the relationship between stress and aflatoxin. Smart *et al.* (1990) suggested that stress cracks, due to poor kernel filling under water stress might provide the cracks for *A.flavus* to infect the kernels. Under hot and dry conditions, like those that exist in the SS of Benin, *A. flavus* will outcompete many saprophytic fungi (Cotty 1994).

Our results showed that intercropping of maize with crops such as cowpea, groundnut and cassava (Manihot esculenta Crantz) in the field lead to an increase in the aflatoxin levels in stored maize. Aspergillus spp. were isolated from cowpea in Nigeria (Gill *et al.* 1983; Umechuruba 1985). Intercropping of cowpea and maize, which both supported the growth of *A. flavus* (Sinha & Ranjan 1990) might have increased the *A. flavus* spore potential in the soil (Diener *et al.* 1987) and subsequently infection with this fungi and aflatoxin development. In this study the presence of groundnut in a crop rotation with maize was positively correlated with aflatoxin content in stored maize. The occurrence of aflatoxins on groundnuts was well documented (Mehan *et al.* 1991). Cole *et al.* (1982) described an additive effect on aflatoxins if maize was planted after groundnut. Similar effects were found by Griffin *et al.* (1981), when maize was cropped in rotation with peanuts, the authors noticed a *A. flavus* population build-up in the soil over the years. The observed high aflatoxin levels in this study, when farmers rotated groundnut with maize, might be due to the described additive effects.

When maize in Benin was planted in a mixed cropping system that included vegetables, aflatoxin content was reduced. In India on monocropped mustard seeds a higher development of *A. flavus* was observed, than on seeds grown in a mixed cropping (Bilgrami *et al.* 1991). There were 23-28% aflatoxin-positive samples in monocropped mustard and 9-13% in mixed cropping. The lack of competitive fungi in monocropped mustard was given as a reason for the high aflatoxin development. This can be compounded with the results of Zummo & Scott (1992), who observed lower aflatoxin load on maize kernels inoculated with both Fusarium moniliforme Sheld. and *A. flavus* as compared to kernels infected with *A. flavus* alone. But the role of intercropping or rotation in the development of aflatoxins in

maize in Benin has to be clarified in on-farm tests, since one of the recommendations of the National Extension Service advises farmers to intercrop maize with cowpea to increase soil fertility (SPV 1992). The results from this study indicated an augmentative effect on aflatoxin content when maize was rotated or intercropped with another crop.

The role that maize variety played in aflatoxin development differed with agroecological zone. In the FMS, the use of local varieties was negatively correlated with aflatoxin development. The local varieties usually had smaller and harder grains and a tighter huskcover, and thereby, a better protection against rain and the attack by insects. Barry *et al.* (1986) showed that there were significant differences in aflatoxin content between those maize varieties that were rated as having a tight huskcover and those with a loose huskcover. In studies in India a negative relationship was found between maize that had good huskcover and aflatoxin (Bilgrami *et al.* 1978). The same conclusions were drawn by McMillian *et al.* (1987) who found in 2 out of 3 years lower aflatoxin contamination in maize that was judged to have tight huskcover. The relationship between lower aflatoxin levels in local maize varieties in the FMS might be because of a good huskcover that protects the cob against insects and fungal contamination. Meikle *et al.* (1997, in press) observed that Beninese local varieties have a better huskcover and a longer husk extension.

In the NGS, aflatoxin was negatively correlated to improved maize varieties. This may be because of the farmers practice that improved varieties were usually grown on productive soils and often received fertilizer, which was rarely done for local varieties. Lack of fertilizer may actually increase the risk of aflatoxin development. Several authors have provided evidence that higher levels of aflatoxin were associated with lower levels of soil applied nitrogen (Jones & Duncan 1981, Cotty 1994). Aflatoxin concentration in maize, wound inoculated with *A. flavus*, was negatively correlated with maize yield, silk leaf nitrogen and grain nitrogen in a two-year study in North Carolina (Payne *et al.* 1989). Likewise during the survey, aflatoxin was negatively related with application of DAP (Double Ammonium Phosphate) in the maize fields, which seems to indicate *A. flavus* suppression after a nitrogen application. There seems to be a trend that Aspergillus strains from Benin produced more toxins when nitrate was their nitrogen source than ammonium (Cardwell, K.F. & Cotty, P.J. pers. comm.). Many farmers in the south did not use any fertilizer (see Table 4.2), the majority of farmers that applied fertilizer would use part of their cotton-

fertilizer (N-P2O5-K2O: 14-23-14) and urea to fertilize their maize. The role of nitrogen source in toxin production of Beninese strains has to be clarified.

In the literature damage to maize plants in the field has been described as one of the most important factors associated with aflatoxin development (Barry *et al.* 1992; Gorman & Kang 1991; McMillian *et al.* 1987). In this study damage by birds, larger animals and fungal attack in the field, was positively related to high aflatoxin measures in the samples. The influence of insect damage on aflatoxin development was well documented (Widstrom *et al.* 1995; Smith & Riley 1992). Surprisingly in Benin, farmers that noticed insect damage in the field, had no higher aflatoxin content in their stored maize. However there seemed to be a bias of farmers towards noticing damage of rats, birds and larger animals in their fields. There were only 40 to 50% of the farmers that noticed insect damage, versus 70 to 90% noticing rats or birds in their field (Table 4.1).

6.4.2   Harvest factors

Aflatoxin content in the analyzed samples increased, when farmers cut their maize plant at harvest, laid down the maize plants on the soil and collected the cobs later. This farmers practice in Benin could aid in establishing the contact between the cobs and the soil. The source of A flavus inoculum was often soil (Diener *et al.* 1987). The practice of heaping maize to dry the cobs and collecting the cobs later had resulted in high aflatoxin levels. Farmers would leave heaped maize in the field for extended periods and this could increase the time of possible exposure to Aspergillus spores. When the levels of *A. flavus* spores in the air were evaluated in India, seasonal differences were observed with the highest relative density of spores present during the monsoon followed by the summer and lowest levels were observed during winter (Bilgrami & Choudhary 1990). Maize was planted in Benin in May at the beginning of the rainy season and would mature during the summer months to be harvested from August till October depending on the planting date. If similar seasonal patterns for the levels of *A. flavus* spores as in India existed in Benin, cobs that are harvested in the rainy season in August, would have a higher likelihood of becoming contaminated with *A. flavus* spores.

The summary from studies in Thailand were, that fungal infection occurred mostly between harvest and the final drying stage. It was concluded that handling methods at this time should be improved to reduce fungal contamination (Tsuruta *et al.* 1985), these findings

were similar to Lee & Chuang (1993) who reported that harvesting maize late was positively related to high aflatoxin content. Aflatoxin contamination in maize increased in Benin when harvesting took one to five days. Tanboon-ek (1989), proposed that field drying on the stalk before harvest, followed by mechanical drying after shelling, were the most effective ways of reducing aflatoxin contamination of maize. Field-drying for 2 to 4 weeks may have a significant role in controlling levels of aflatoxin in Thai maize (Nagler *et al.* 1992). But in Benin the opposite effect was noticed. Drying in the field that took more than 5 to 90 days increased aflatoxin contamination. Maize in Benin that was left in the field for a longer time had a higher risk of insect infection, with most of the grain losses in storage attributed to Prostephanus truncatus (Borgemeister *et al.* 1997 submitted). Moreover Setamou (1996) observed high aflatoxin levels in maize that was harvested late insect damage, especially due to Mussidia nigrivinella increased. The conclusion from the presented study are, that high aflatoxin levels in maize that was harvested late or where drying was delayed, might be because of insect infestation from the field on.

The timing of harvest may have played a role in the development of aflatoxins in this study. This was also observed in India where immature maize kernels harvested during the rainy season did become contaminated with aflatoxin B1 and mechanical damage increased the level of contamination. Maize harvested in the dry season showed no aflatoxin (Asanuma & Vayuparn 1985). This is supported by studies from Bilgrami & Choudhary (1993) who observed the highest amount of *A. flavus* spores in soil samples collected during the rainy season. Analysis of data from this study revealed a positive relationship between harvest in August and aflatoxins in the 93-94 season, whereas in the following season maize harvest in August resulted in the SGS in a reduction of aflatoxins. The rainy season in Benin starts in May and finishes in July, so that maize which was planted in April/May will grow and mature all through the rainy period. Farmers in the south of Benin harvest their maize during the short dry season in August. It could be that some of the maize was harvested with a high moisture content, and because of the frequent rains in August drying of maize was incomplete. Leaving a moisture content of more than 17%, which facilitates the growth of *A. flavus* (Kawasugi *et al.* 1988., Siriacha *et al.* 1990). The December harvesting period was related to lower aflatoxin levels in stored maize, but this could be a factor that was due to interactions with other factors, since there are no reasons for maize that was harvested in the dry season to have especially high aflatoxin loads.

Furthermore it was revealed in this study that harvesting of maize with the husk was a practice that reduced aflatoxin contamination and the drying of maize without the husk would have the same effect. The importance of huskcover to protect against insect attack was described by (Meikle *et al.* in press) and good huskcover was related to lower aflatoxin content in studies as described in 6.4.1.. The effect of huskcover on aflatoxin content in stored maize was further evaluated in on-farm trials described in Chapter 9.

Farmers in Benin sorted their maize at different stages after harvest. This processing reduces fungal and insect contamination. Pelletier & Reizner (1992) remarked that hand sorting of peanuts was the most effective means of removal of aflatoxin contaminated groundnuts. Maize cobs that were damaged, discolored or had insufficient huskcover were usually sorted out manually in Benin. Also maize cobs that had an inferior size would be put to the side, to be consumed first or stored for only short periods. Farmers sorted maize at different stages after harvest, sorting of maize late in processing had an increasing effect on aflatoxins, whereas when maize was sorted immediately after the harvest this reduced aflatoxin levels. It seems to be, that sorting was a cheap and easy way of reducing aflatoxin load in stored maize.

The winnowing of maize before storage might actually aided in spore dispersal from one grain to another. During winnowing spores from grains which were infected with *A. flavus*, may be dispersed with the wind to uncontaminated grains and may lead to aflatoxin development. Wind dispersal of spores was the principal means of distribution of spores from the soil to maize cobs (Wicklow & Donahue 1984).

6.4.3 Storage factors

The influence of storage time on aflatoxin content was only noticed for a storage period from 3 to 5 months, which generally increased the aflatoxin levles in the maize samples. It seems to be, that farmers who stored maize for such a short period, did not take as many precautions and care as those that were storing maize for a long period. For the later, farmers tended to sort out damaged cobs. These were consumed first and only the well developed and undamaged cobs were stored. In southern Benin, maize was mostly stored with the husk, farmers in this area selected cobs for good huskcover before storing them.

Storage of maize under the roof of the house had a positive relationship with aflatoxins content. Maize was collected from the field and then either stored with husk or without

husk under metal roofs, rarely forming a layer bigger than 30 cm. Often maize would come into storage with humidities that were above the "safe" level for fungal growth. *A. flavus* did not exhibit extended growth below the aw (see section 2.3.1) of 0.85 (Sauer & Burroughs 1980). High temperatures that could build up under a metal sheeting roof, would accelerate the growth of fungi. Gonzales *et al.* (1988) observed that growth of *A. flavus*, A. niger and A. parasiticus increased with increasing temperature. The advantages and disadvantages of storing under the roof are further discussed in Chapter 7.2..

Another type of storage, associated with higher aflatoxin content, was storing on top of the roof and the "Crib" in the SS (storage types are discussed in Chapter 7). In the NGS, the "Secco" and conical stores seemed to be prone to develop higher aflatoxin levels, than the other types of stores. It seems to be that these relationships were because of other factors than the storage structure itself, since not all the factors that could have influenced fungal development in storage were measured and interactions between factors were not taken into account. FAO (1992) reported that the "Crib" and the "Secco" were mostly used as a drying structure and they allowed sufficient air circulation. Like described in section 6.4.2, insufficient drying seems to be one of the main causal factors of aflatoxin development. So that well aerated stores should rather be associated with lower aflatoxin levels.

Storage structures that had a negative relationship with aflatoxins were, the Ago made out of bamboo and secondary storage in bags. An FAO-Project introduced an improved bamboo storage container that had the same form as the "Ago", which gave good results in drying of stored maize with humidity reductions from 20% at harvest to 14% after 3 months of storage (FAO 1992). In this study the use of bags as a secondary storage structure, later in the maize storage season were related to lower aflatoxin content. The change in storage structure usually was accompanied by maize processing either dehusking or degraining, also most farmers sorted maize at this point in time (see 4.3.3.3). In section 6.4.2 the function of sorting in reducing aflatoxin load in stored maize in Benin is explained. Another reason for the lower aflatoxin rates, observed in this study when maize was stored in bags, might be because of the bags themselves. Siriacha *et al.* (1990) evaluated the use of different types of packaging for grain maize with respect to the development of *A. flavus* and aflatoxins. Thick polyethylene (PE) bags (125 µm thick) and high density PE bags (40 µm thick) inhibited the growth of *A. flavus* for more than 20 days and no aflatoxin was detected. Wet

maize kernels with various maturity levels (97-125 days after planting) and moisture content of 21.3-33.4% were protected from *A. flavus* infection in these thick PE bags.

In this study, there was a negative relationship between farmers that complained about storage problems and aflatoxins. It seems that farmers that noticed that their maize was damaged by pests or fungi used either traditonal, mechanical or chemical means to reduce these problems (the different methods are described in section 4.3., 4.2). The same held true for those farmers that noticed that their grain germinated in store, which was negatively related to aflatoxin content. This relationship can not be well explained by the data collected during this survey, since germination of grains was because of high moisture content and high moisture content was related to high aflatoxin load in stored grains (Kawasugi *et al.* 1994, Sinha & Sinha 1992, Trucksess *et al.* 1988).

Since the early 60's, researchers have related insect damage to high levels of aflatoxin (Sinha & Sinha 1991). The same authors (1992) showed that *A. flavus* infection in insect-damaged grain was 87% and in insect-free samples 25%. It is well documented that insects propagate Aspergillus spores in the stores (McMillian 1987; McMillian *et al.* 1990; Lynch & Wilson 1991; Lynch *et al.* 1991; Gorman & Kang 1991). Wright (1992) remarked that *A. flavus* contamination was strongly correlated with high densities of weevils. In this study insect damage was related positively to aflatoxin. This can be compounded by the study of Mutiro *et al.* (1992) who 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. There was an aflatoxin reducing effect of insecticides (Table 6.11, 6.13).

One of the negative effects of pesticide use in Benin was the widespread use of cotton insecticides for stored grains (SPV 1992). Cotton pesticides in Benin are distributed on credit basis through the state extension service and are more readily available than the recommended storage insecticides (Actellic Super®, Sofagrain®) (SPV 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, with a lot of adverse health effects and even the possibility of fatal poisonings (SPV 1992). In this study the use of cotton insecticides was related to lower aflatoxin content in stored

maize, but because of the health hazards of these potent insecticides, this is a practice that shouldn't be encouraged.

In this study, application of traditional plants to protect against storage insects was positively related to aflatoxin concentrations 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 (Dube *et al.* 1990; Bhatnagar & McCormick 1988; Cardwell & Dongo 1994). It seems that the in-vitro effect could not be achieved by applying plants directly to the stored cobs. It was reported that Aspergillus fungi would grow on medicinal plants, and could lead to aflatoxins on them (Roy & Kumari 1991; Narita *et al.* 1988). Thus the mixing of plant substances with stored cobs may actually increase the risk of aflatoxin development instead of controlling it. Also plants may increase relative humidity inside the grain store through their biomasse, and consequently increase fungal growth.

With increasing age of the storage structure, the risk of aflatoxin contamination increased in Benin. Most of the storage structures had a life span of 1 to 3 years, before major parts of the storage structure had to be reconstructed. Traditional stores that have life spans of more than 5 years were made out of clay or wood. In surveys of traditional storage structures in India, the amount of aflatoxin contaminating the stored cereal samples was between 430-2830 p.p.b., and the greatest amount was detected in cereals stored in the "kothi" made out of clay (Prasad *et al.* 1987). In clay stores humidity build-up might be possible, this could allow Aspergillus spores to persist for a longer time in these types of stores. But this hypothesis needs to be further studied. In rare cases farmers would light a fire under the storage structure to reduce the humidity and control insects inside the store. This smoking of stored goods had a negative effect on aflatoxin contamination. Daramola (1988) observed in Nigeria that smoking was a very effective mean of protecting maize against storage insects and compared well with chemical insecticides like Actellic® (Pirimiphos-Methyl). Udoh *et al.* (1994) reported that between 3.6 and 12% of the farmers in the different agroecological zones of Nigeria used smoke to protect their maize and aflatoxin contamination in such grains was lower.

The regression analysis revealed that aflatoxin was reduced when farmers cleaned their grain stores. Grains that were left in the store after the storage season were often infested by insects and fungi. The cleaning of grain stores of remaining grains and debris from the

previous harvest, was one of the basic hygiene measures to combat storage problems (Smith 1991).

The influence of storage form on aflatoxin development in this study was not clear. There was a negative relationship between aflatoxin development and storage as grains in the SS after 6 months of storage in 93-94 and at the beginning of storage 94. On the contrary storage as grains would increase aflatoxin risk in the SGS and across zones after 6 months of storage in 94-95. There are some reports of higher development rates of insects on maize stored as loose grains (Kossou *et al.* 1992; Vowotor *et al.* 1995), this might have an influence on increasing aflatoxins in maize stored as grains. Further studies need to clarify the impact of storage form on aflatoxin development, it seems to be that this relationship is highly dependent on agroecological region. On-farm storage trials that looked at the influence of storage form on aflatoxin contamination are presented in Chapter 9.

A positive effect between aflatoxin contamination and the storage of maize with cowpea was observed in this study. The relationship between high aflatoxin levels and intercropping of maize with cowpea in the field was already presented in section 6.4.1. Cowpea may become infected with *A. flavus* as described by Gill *et al.* (1983) and Umechuruba (1985). But in the literature aflatoxin contamination of cowpeas has not been described. The same trend of increased aflatoxin contamination was noticed for the storage of maize with sorghum. It has been reported that sorghum can be infected with *A. flavus* (Usha *et al.* 1994) and infection of pre-harvest grains with this fungi may result in the development of aflatoxins (McMillian *et al.* 1983). No correlation between the storage of groundnuts and maize in one store and an increase in aflatoxin content was observed.

CHAPTER 7   **Storage structure, storage form and aflatoxin**

7.1   General introduction

In rural Africa, 60-80% of all grain produced was stored at farm or village level (Compton *et al.* 1993). Maize is being stored for periods of up to two years or longer, to ensure self-sufficiency and nutrition over periods of drought. Most stores in West-Africa were family stores. Communal stores as promoted by development projects have been difficult to manage and farmers discontinued the use after a short while (FAO 1992). Losses in stored grains because of insects and fungi can reach 10 to 15% for second season maize and can attain levels of 25 to 30% for first season maize (Fiagan 1994).

Traditional storage structures in rural parts of Africa were of varied types. Their shape was determined by a multitude of factors. In villages with divers ethnic groups, different storage structures could be found. Cost of the storage structure and climatic conditions were also determining factors for a farmer to choose a certain type. Another was the availability of building material. In southern coastal zones of West-Africa, granaries were made out of locally available materials, like palm, neem and coconut branches, ribs of palm trees and wooden poles. In less populated zones where wood was easily available, most of the storage structures were made from wood. Building materials were selected for their accessibility, resistance to insects and pliability when woven. Imported materials such as cement, metal and wire mesh were only used by wealthy farmers or those that received support from a development project. The different storage types could be characterized by their aeration, facility and rapidity of drying and the type of building material used. The commodities could be either stored directly from the field into the storage container, or they would be deposited in a room to be stored later. The reasons for using a particular storage structure were: that it provided protection against animals and theft, was less expensive, provided good drying and allowed a good and long storage (FAO 1992).

Description of the different storage structures

The different storage types could be classified according to their aeration, ease of drying and the type of building material used. Stores that could be typified as being well aerated were the crib, the conical granaries, and the platform. These types of stores were also used

as drying structures. When storing over the ceiling, in clay granaries or in the secco, the stored commodities were not as well aerated as in other storage types. Illustrations of some of the indigenous stores used in Benin were shown in the annex III.

The disadvantages of traditional storage structures mentioned by the farmers were: the store was difficult to construct, it could break, it may burn or was not durable. Another inconvience was that it had poor ventilation and pests were a problem. The period a particular storage structure could be used varied, depending on the materials used for construction. Durable structures, usually made of clay, could last up to 30 to 40 years. More than 50% of the farmers used their storage structures for 1 to 4 years, before rebuilding them.

Stores from plant materials

Ago

The "Ago" was the most common structure in the southern parts of Benin. It has the form of a giant basket made out of locally available material, the base could be made from bamboo, wood or the midribs of palm trees. The platform was raised about 20 to 40 cm off the ground, higher in areas where rats were a problem. The walls of the basket were clad out with palm branches, bamboo or with pliable branches from trees like *Azadirachta indica* A. Juss [Meliaceae], *Mallotus oppositifolius* (Gers.) Müll. arg. [Euphorbiaceae], *Cassia siamea* Lam. [Cesalpiniaceae]. Farmers believe that neem leaves also deter insects. Sometimes these stores were constructed in a square form, more like a big room. Air flow was possible through the walls, but could be reduced because of the tightly packed cobs. The top of the basket was covered with a thatched roof made of *Imperata cylindrica* in most cases or *Panicum* spp.. The roof could be taken off or raised so that farmers would sun their stored goods to ensure good drying. These roofs were not very durable and heavy rains during the rainy season could lead to seeping in of water and subsequent development of fungi. Maize was stored in the "Ago" mostly with the husk. The stores had a life-span of one to three years. Parts of it, like the palm front walls, were rebuilt every year.

Ebli-va

This type of storage structure was common to the southern parts of Benin, Togo, Ghana and Cote d'Ivoire. It consists of a round platform that was raised slightly off the ground and

maize with the husk was stored on top of it. The biggest cobs with the end towards the outside were used to make up the wall and in between smaller cobs were interspaced giving the wall stability and the rest of the maize was poured into the middle. These platforms could have a diameter of 1 to 4 meters. The size of the storage structure was a measure of the wealth of the farmer. Farmers often kept their maize for a period of 2 to 3 years. The top of the store was covered by a thatch roof.

Conical stores

Conical stores consisted of logs placed in a round circle towards a low center. The whole structure formed a cone, these structures were found in Benin, Togo and Ghana. This type of store was often built in the field as a drying structure, where maize was stored just after the harvest until farmers found time to dehusk or the store was taken down because of the menace of bush fires. The bottom pillar, was lifted slightly off the ground to give good aeration and to prevent humidity and pests from attaining the stored product. Studies in Togo have shown that this type of storage structure gave a very good aeration as compared to the more common circular types (Smith 1991). The store could have a diameter of 1 to 4 meters. The top was either left open during the dry season or covered with a thatched hood made out of grass. Especially during the rainy season farmers sometimes cover their maize with plastic sheets. In the conical stores maize was stored with the husk in southern Benin and in the middle region husks were taken off to achieve better drying. If the stored maize was not sufficiently dried, this could lead to a rapid development of storage fungi because of humidity build-up under the plastic sheets.

Platform

Farmers in West-Africa often stored their grain and legume crops on platforms, that were sometimes build over the outside kitchen fire. The fire dried the maize and the smoke deterred insects. The platform was often covered with a thatched roof, in the form of a hat. These stores were also used for field storage of grain, when maize was stored for short periods of 2 to 4 months. Thereafter, with the onset of the dry season, maize was taken to the farmhouse, degrained and stored in bags in the house or stored in smaller clay storage containers called "AKA" or "KOZOUN".

The platform store had wooden posts that were usually 1 to 1.5m high, rat guards were rarely found on these stores. The stores usually last for 6 to 7 years, but parts of it such as the roof were redone every year. This type of store could be found in all the ecozones of Benin.

Secco

The "SECCO", also called "OBA" in Nigeria, was made out of grass such as *Androgogon* spp. and *Pennisetum* spp., or sorghum stalks that were woven to form a basket, which enclosed the stored maize or sorghum. The basket was deposited on a platform, that was raised 30 cm to 1 m above the ground. The diameter of the store varied between 2 to 4 m. This was a typical storage structure in the NGS. This store was mostly used as a drying structure, to store maize for up to 3 to 4 months and thereafter maize was stored permanently in bags or in clay stores. Maize was stored without the husk. The store was often only used during the dry season, so it would be left open to the sun and possibly rain, and was rarely covered by plastic sheeting or a thatch roof out of *Hypparhinia diplandra*. The biggest problems in stored maize in this region were losses because of birds, either already in the field or later on in store.

Improved Storage Types

Crib

This storage type was introduced to West-Africa by the FAO (1992). The storage space was built like an elongated box, with even spaced openings. It was usually made from locally available materials like wood or bamboo. The expensive models were covered with mesh wiring. The store had legs that were about 1m off the ground. Very often rat guards made from metal were placed on the legs. The roof was made from metal sheeting or straw. This structure gave good aeration to dehusked maize. It could also be used to store sorghum, cowpea, groundnuts and other commodities. The FAO-recommendation were that farmers harvested their maize early, and used the crib as a drying structure, before storing maize as grains in bags. The "grenier amelioree" was a local adaptation of this store, made with bamboo and other locally available materials.

The program to adopt the crib as a pre-storage and storage structure failed in Benin. Between 1967 to 1987 more than 149 cribs and 160 "grenier ameliorée" (improved stores)

were constructed, it was reported that less than 5% of the installed stores were used in 1992 (FAO 1992). As reasons for the low adoption rate were given: high cost of this store, maize had to be dehusked before storing and pesticides had to be applied 2 to 3 times during the storage period because of the higher risk of insect attack on dehusked cobs adding to the overall cost of this type of store. Farmers also did not appreciate that the stored goods were easily visible by other people, which may provoke jealousy and tempt people to steal.

Bags

The storage of maize in bags was becoming more and more popular in West-Africa. Bags were made of polyethylene or jute and were often used at a later stage in storage. Farmers harvested maize with the husk, stored it in the house on the floor or in their field stores, either on the platform or in conical stores, and after 4 to 10 weeks they dehusked, shelled and then bagged maize to store for a further 3 to 6 months.

Farmers often treated their maize with a solitary or binary insecticide before bagging it. Bags were usually stored on cement or clay floor in the house. Fungal growth and mycotoxin build-up could occur when bags were placed on the floor. This could be easily stopped by placing a wooden platform under the bags. Also the lack of cleanliness in rooms where bags were stored, could lead to rat damage.

Store room, floor

The store room was either especially constructed to store farm products, or a part of the house was transformed into a store. Farmers kept the store-room more or less clean. Rats could be a problem in this type of storage. Maize could be stored either with or without the husk or as grains. The stored product could be put into sacks or poured onto the floor, which was either made from clay or cement.

Store rooms could also be made of locally available materials like wood and palm tree branches and be located in the field, where they had all the disadvantages of the more traditional stores, such as easy access for insects and rats.

Earthen Storage Types

Clay Stores

These storage containers looked like a big earthenware pot. They were called "rhumbu" in Nigeria, and "bourarou", "grenier atacora" or "banco" in Benin. The stores could have a different shape, size and compartmentation. The base of the store was very important in protecting stored goods against pests, mounting humidity and consequent fungal development. It could have differently shaped feet, either with three or four divisions. Stones, earth or wood were used to build the base of the store. Sometimes the base of the store was hollow and served as a poultry-house. Chickens would eat termites and impede them from entering the stored goods. The opening of the store was covered with a wooden cover or a piece of metal sheeting. Most of the stores would also have a straw roof that could be taken off. In the store there was often a compartmentation that served as a stepping ladder when entering the store, and also divided the different stored goods like maize, groundnut, cowpea, pidgeonpea (*Cajanus cajan* (L.) Huth), sorghum, fonio (*Digitaria exilis* (Kipp.) Stapf), and beans like bambara beans (*Voandzeia subterranea* (L.) Thou ex D*C.*) and soyabeans (*Glycine max* (L.) Merr).

The walls were made from clay that was often mixed with straw or cow dung and could be clad out with cement. Farmers often used termite hill clay to build their stores, because of the stability of this material. The outside walls that were 5-6 cm thick and could be decorated, painted or otherwise embellished. Clay stores in the NGS and the SS of Benin were up to 3 m high and had a diameter of 2-3 meters.

Clay stores found in the SGS, were much smaller and were called "Kozoun". The "Kozoun" was usually less than 1 m high and had a capacity of 0.3 to 0.5 t, and lasted only 3 to 4 years. The clayey soils that were used to build the "Kozoun" were rarely mixed with straw and were thus less durable, so that the walls had to be 10 to 15 cm thick. The walls were not very smooth, so that they sheltered insects and gave access to rats and termites. Many farmers complained that these stores collapsed easily, because rain which degraded the outer walls, was much more abundant in this region.

Small quantity storage containers

Under the roof, Plafond

Farmers often stored maize on top of their ceiling, under a corrugated iron roof, especially in the region around Bohicon, Abomey and Cove (see Figure 1.1). Some ethnic groups like the Peulh or Fulani, stored under the roof of their traditional hut. The maize cobs were usually stored without the husk. During the survey, fungal deterioration of cobs stored under the roof was often noticed. This might have been because ventilation under the roof was poor and stored maize could not dry properly. Fungal deterioration could be accelerated through solar heating of the roof.

On top of the roof, Roof

In very dry areas like the SS maize farmers stored maize on top of their houses on mud roofs. The maize husks were usually left on the cobs and sometimes the stalks were conserved in the same way to serve as animal fodder.

Barrels

Barrels were often used to store cowpea, beans or seed maize, when farmers did not store large quantities. This is a very good way of storing goods, if barrels were only opened when goods were to be used. The barrels found during the survey, were either old metal barrels or made out of plastic or even cardboard. In some cases farmers treated maize with insecticides in powder form, or with neem oil or other types of vegetable oils that had an insecticidal effect. The high cost and low availability of barrels in rural areas and their small size have however reduced their usage.

Bottles

Small quantities of grains which were usually preserved as seed material for the next season, may be stored in bottles. They were often closed with a corn spindle.

Bowls

Women farmers often used either metal, plastic or calabash bowls to store small quantities of grains, for 2 to 4 months. Maize was stored as grains or without the husk.

Baskets

Farmers stored their grains in the house in baskets. The baskets size could vary from 50 cm to 2 m. Storing maize in baskets was only a temporary solution, and the storage time rarely exceeded 3 months. Maize was often stored with the husk and rats could be a problem when storing in baskets.

Tied to a tree or roof

Maize could be tied to a tree or from the roof of the house. Often the husk was used to attach one cob to another, but some of the husks were still left on to protect the cobs against bird damage. This way of storing the cobs allowed good aeration and drying. Farmers often stored seeds for next years planting in this way.

7.2   Materials and Methods

Over two years (93/94) and (94/95) samples were collected from traditional storage structures and their aflatoxin concentration analyzed. These surveys were already extensively described in section 5.2. Thirty villages in Benin were visited. The types of storage structures were recorded and the storage time and storage form was noted. Aflatoxins were extracted and quantified with the method described in section 5.2.4. Simple descriptive statistics were used to present differences between the distribution, range and mean aflatoxin content of storage structures in different ecozones.

7.3.   Results

Aflatoxin contamination in storage structures in agroecological zones

In each ecoregion there were storage structures that were predominant. In the FMS, maize was stored by 64% of the farmers in the "Ago", 10% in the "Ebli-va" otherwise they used small containers like bottles, bowls or calabashes. In the SGS, farmers commonly used the "Ago" (18.9%), under the roof (19.4%), bags (12.4%), clay structures (9.0%) and conical granaries (14.9%). Like described in section 4.3.4 farmers changed their storage structure during the storage period. At the beginning of the season a more traditional store like the "Ago", the conical store or under the roof were used. Towards the end of the season, maize would be transferred to clay containers or into bags. In the NGS, maize was stored in bags (25%), in conical stores (24%) or in the secco (33%). The most frequent storage structure

in the SS, was the clay store (37.5%) followed by the secco (23%) and storing "on the floor" (17.1%).

In the FMS (Table 7.1), there were 20% of the samples that were aflatoxin positive. The analysis of variance for the mean aflatoxin contamination between the different storage types, showed no significant differences. Storing maize in bags resulted in the highest amount of aflatoxin in maize, but only few farmer use this type of storage. When farmers used the "Ago" or "Ebli-va", 32 % of the stores showed aflatoxins, with high means of 71.1 ppb and 32 ppb respectively.

Table 7.1: Utilisation of different storage structures and their aflatoxin content in the FMS of Benin

Storage Type	Number. of stores	Number stores aflatoxin positiv	Mean aflatoxin (ppb)	Range (ppb)	Standard Deviation
Ago	120	27	71.09	1-375	101.3
Ebli-Va	25	6	32.01	10-50	18.2
Crib	9	2	8.33	8.33	4.5
Plafond	8	1	12.50	12.5	2.9
Bags	6	1	250.00	250.00	0.0
Baskets	8	1	1.67	1.67	0.0
Floor	9	0	0.00	0.00	0.0
Conical	4	0	0.00	0.00	0.0
Platform	2	0	0.00	0.00	0.0
Total	191	38 (19.89%)	12.92	1-250	49.43

In the SGS, aflatoxin contamination was generally high (Table 7.2), but this seemed to be a zonal effect, rather than an effect of the storage structure itself. Storage over the roof (plafond) and storage in bags resulted inhigher aflatoxin content than storage in baskets, conical stores or in the "Ago". There was a high number of aflatoxin positive stores in the SGS (Table 7.2), where 40% of the stores were contaminated. High aflatoxin contamination was found in clay structures, but with a low percentage of stores being aflatoxin positive. The crib showed the highest mean of 394.7 ppb, which could be explained by one sample having 2500 ppb. Storing in baskets, on the floor and on platforms showed low mean aflatoxin levels. Storage in bags showed significantly more aflatoxins than storage in baskets, conical stores and in the "Ago".

Table 7.2:   Utilisation of different storage structures and their aflatoxin content in the SGS of Benin

Storage Type	No. of stores	Number stores aflatoxin positiv	Mean aflatoxin (ppb)	Range (ppb)	Standard Deviation
Ago	38	9	50.23	6.67-165	49.1
Crib	12	7	394.66	1-2500	929.8
Plafond	39	23	73.39	1-375	103.5
Bags	25	17	69.15	1-250	67.3
Baskets	16	2	17.17	1-33.33	22.9
Clay	18	3	112.50	62.5-150	45.1
Floor	10	4	37.33	1-85	35.3
Conical	30	10	24.47	1-75	28.4
Platform	13	5	22.40	1-66.67	28.1
Total	201	80 (39.8%)	34.61	1-2500	182.5

The highest mean aflatoxin content in the NGS (Table 7.3) could be found in baskets (133.9 ppb), on platform (75.2 ppb) or in bags (67.7 ppb). The highest mean aflatoxin content was found in "Baskets", but this was a storage structure that was rarely used in the northern parts of Benin. The crib, conical store, platform, secco and storage in bags all had more than 40% of the stores contaminated with aflatoxins. Maize in the NGS was stored frequently in conical stores, in the secco or in bags in the NGS. Mean contamination levels were lower than in the SGS, but there were 52.1% of the samples that were aflatoxin positive.

Table 7.3:   Utilisation of different storage structures and their aflatoxin content in the NGS of Benin

Storage Type	Number of stores	Number stores aflatoxin positiv	Mean aflatoxin (ppb)	Range (ppb)	Standard Deviation
Crib	5	2	9.4	2-16.7	10.4
Plafond	7	0	0.0	0	0.0
Bags	48	20	67.7	1-250	100.0
Baskets	6	2	133.9	17.7-250	164.3
Clay	10	2	18.4	16.7-20	2.3
Floor	9	1	1.0	1	0.0
Conical	46	23	20.2	1-250	53.5
Platform	7	3	75.2	1-250	156.8
Secco	64	24	47.1	1-563	124.4
Total	201	77 (38.3%)	19.3	1-563	64.7

In the SS, the conical store showed the highest percentage of stores contaminated, but had low levels of 11 ppb (Table 7.4). The highest contamination levels were found in the clay stores (116.4 ppb), the secco (98.8 ppb) and in bags (125.5 ppb). Overall in the SS, more than 35.5% of the stores were contaminated with aflatoxins. In the SS, when maize was stored in bags, in the secco, on top of the roof and in clay containers high aflatoxin contamination was recorded.

Table 7.4:   Utilisation of different storage structures and their aflatoxin content in the SS of Benin

Storage Type	Number of stores	Number stores aflatoxin positiv	Mean aflatoxin (ppb)	Range (ppb)	Standard Deviation
Crib	3	2	9.3	2 - 16.7	10.4
Bags	19	8	125.5	1 - 250	124.9
Baskets	1	0	0.0	0	0.0
Clay	57	18	116.4	1 - 500	39.0
Floor	26	12	50.1	1 - 166.7	59.9
Conical	1	1	11.0	11	0.0
Platform	1	0	0.0	0	0.0
Secco	35	10	98.8	1 - 500	52.3
Roof	12	3	100.7	1 - 250	131.7
Total	152	54 (35.53%)	32.95	1-500	91.3

7.4   Discussion

Grain stores in Benin were usually adapted to the climatic conditions of the region they evolved in. Grain storage is not only the storage structure, which was its central unit, but it is an assembly of different handling steps that start at harvest, incorporate the different processing steps before storage and the protection of the stored product through phytosanitary measures (Fiagan 1994). Programs by the American Peace Corps to improve the storage system in Benin with the introduction of the "Dichter-Silos", big cylindrical cement stores and "Brooks-Dryers", dryers that were fired with wood to dry maize and other commodities rapidly, were started in the 70's in Benin, but these structures were rarely adopted by the rural population (Fiagan 1994) since the lack of aeration in the silos would lead to rapid fungal deterioration of maize. The dryers were using too much wood and were too expensive to run. The introduction of community stores through aide programs to ensure food security was rarely successful, since the management of these stores was a problem (FAO 1992).

In section 7.3 the results of the aflatoxin contamination of the different storage structures were presented for the agroecozones. Bouraima *et al.* (1993) recorded especially in the southern regions of Benin fast fungal development, so that stored products were not consumable after storage (SPV 1992). Aflatoxin and ochratoxin development was analysed in samples that came from cribs, bag storage and stored on the floor. Aflatoxin was detected in 44% of the samples.

The results presented here showed high aflatoxin development in stores that were located in the two northern zones (Table 7.3 and 7.4). In each zone different stores showed high contamination with no storage type showing high aflatoxin in all the zones. Any attempt to reduce aflatoxin through the improvement of the storage structure has to be specific to the zone. In the FMS, the traditional stores "Ago" and "Ebli-va" could be improved to make them less susceptible to influx of rain water through the roof. Also the long periods of storage of up to 2 to 3 year could have an effect on aflatoxin contamination through accumulated insect damage. But long storage periods in these regions were a form of earning social prestige (Smith *pers. comm.*).

In the SGS, maize stored on the platform, on the floor and under the roof (plafond), was contaminated in 60% of the cases and with high mean contamination (Table 7.2). Farmers who stored in these containers should dry their goods well before storage. Storing maize under the roof in the SGS resulted in aflatoxins, it seemed to be that the tin roof heats up the stored commodity during the day and there was rapid cooling down during the night favorising the growth of *Aspergillus* spp.. The crib had high aflatoxin levels, but this should be further examined, since cribs was supposed to be drying structure, that were used to bring down the moisture content of freshly harvested maize to lower more storable levels (FAO 1992).

In the NGS, high contamination levels were measured when maize was stored in bags, usually a method that was used by modern progressive farmers that had better farming and management practices. Maize that was stored in bags would be degrained later in the season, when maize had dried down to levels that did not allow growth of *Aspergillus* fungi and aflatoxin development. It seemed, that conditions in intermediary storage, with little care taken to protect maize against the influx of humidity, insect and pest attack, would predispose maize for aflatoxin development. In a similar study in Nigeria opposite trends

were found, storage in bags in the SGS, NGS and SS rather decreased the likelihod of maize to have high aflatoxin content (Udoh 1995).

As a general trend, it was remarked that storage types that weren't indigenous to a agroecological region, like the "Ago" in the northern zones of Benin showed high aflatoxin contamination. In the NGS and SS, traditional stores like the conical store, the secco and clay stores showed a high percentage of stores with aflatoxins, but their mean aflatoxin levels were not very high. Prasad *et al.* (1987) observed that maize was highly contaminated with aflatoxins when stored in clay structures. High contamination levels in these stores could be explained by the ability of the fungi to survive on organic matter (Coker *et al.* 1984) that was mixed with the soil used for building the stores. Under favourable conditions these spores would germinate into the stored grains of the new harvest. Also mean aflatoxin levels in the clay structures were high in the SGS, probably for similar reasons. This could be remedied through hygienic measures, such as cleaning of the stores after the storage season and then lighting a fire inside to kill any residual fungal spores or insects. In the SS, high aflatoxin contamination levels were measured in bags, clay stores, the secco and on top of the roof. This seemed to be an ecozone effect since this zone showed consistently high aflatoxin contamination in the two survey years.

CHAPTER 8   **Insect infestation of stored maize in four agroecological regions in Benin and contamination with aflatoxins**

8.1   Introduction

The relationship between insect damage and aflatoxin formation had been reported by many authors (Bowen & Mack 1991; Lynch & Wilson 1991; Lynch *et al.* 1991; Gorman & Kang 1991). Insect feeding made grains more vulnerable to invasion by storage fungi including *A. flavus.* Insects could provide entry holes for fungal spores and mycelium (Tuite *et al.* 1985; Fennel *et al.* 1977). The later surveyed fields in the southern U.S. and found that many of the insects collected were infected with *A. flavus* both internally and externally. Lynch & Wilson (1991) remarked that insects could act as vectors, by carrying fungal spores on their bodies and contaminated grain as they moved about. *Heliothis zea* (Lepidoptera: Noctuidae) and *Ostrinia nubilis* (Hubner) (Lepidoptera: Pyralidae) were implicated in the transmission of *A. flavus* inoculum to corn ears in studies from the southern United States (McMillian *et al.* 1990). In the same trials 12 to 87% of the *H. zea* moths captured in light traps were contaminated with *A. flavus* spores. The Nitidulids (e.g. *Carpophilus lugubris* Murrey, *C. freemani* Dobson) appeared to be important vectors of *A. flavus* on maize (Lussenhop & Wicklow 1991). This insect species were shown to consume *A. flavus* spores without any negative effect of the fungi on the insect (Wicklow 1988).

Barry *et al.* (1992) showed that maize cultivars that were resistant to ear-infesting insects also had less aflatoxins in grains sampled before harvest. Strong correlations were found between the infestation of stored maize with the maize weevil *Sitophilus zeamais* Motschulsky (Curculionidae) and other secondary species and the contamination with *A. flavus* (Wright 1992; Sinha & Sinha 1991). In India the incidence of fungi of the *A. flavus* group and aflatoxin contamination was comparatively higher in insect-damaged maize samples from different localities than in insect free samples (Sinha & Sinha 1992). In laboratory studies *S. zeamais* were treated topically with *A. flavus* spores and raised on maize. When the aflatoxin B_1 content was analyzed in the corn, significantly higher levels were observed in the *S. zeamais* treatment, than in mechanically damaged corn that had been inoculated with *A. flavus* spores or than undamaged maize that had been inoculated. Corn moisture content increased to 19-20% after 30 days (Beti *et al.* 1995) levels that allowed *A. flavus* to grow abundantly (Sauer & Burroughs 1980). In this chapter insect

infestation of the survey samples (Chapter 5) will be presented and the insects role in the development of aflatoxins will be evaluated.

8.2   Materials and Methods

The samples from the nation-wide survey at the beginning of storage in 1993/94 were used to evaluate insect damage (see Chapter 4, 5, 6). Sampling procedures as described in section 5.2.1 were used. The number of maize cobs that were damaged by coleopteran pests, lepidoptera or visibly infected with fungi was recorded. The following coleopteran species were determined: *Sitophilus zeamais* Motschulsky (Curculionidae), *Prostephanus truncatus* Horn (Bostrychidae)*, Cathartus quadricollis* Guerin (Cucujidae), *Carpophilus dimidiatus* Fabricius (Nitidulidae). The presence/absence of a certain insect species and any discoloration on the cobs was noted. Ten percent of the samples were collected as grains. For these samples the number of grains visibly damaged by insects and by fungi, and the number undamaged grains from a 1000 grain sample were recorded and used in the analysis. This evaluation was not possible for the samples collected six months after harvest, since most of the samples were stored as grains and most of the insect species were no more present in the samples.

In 1994/95, cobs were dehusked in the lab and the insect species determined, their number and the percentage of cobs infested by a certain species recorded (e.g. % cobs *C. l.*). In addition to the coleoptera previously mentioned, the lepidoptera: *Cryptophlebia leucotreta* (Lep.: Tortricidae), *Eldana saccharina* Walker (Lep.: Pyralidae), *Mussidia nigrivinella* (Lep.: Pyralidae), *Busseola fusca* (Lep.: Noctuidae), *Sesamia calamistis* Hampson (Lep.: Noctuidae) were determined. The amount of damage atttributable to these insects was assessed visually, as a mean percentage of the cob area damaged, the individual cob results were pooled for one sample (% Ins). At the same time fungal species visually present were noted, and the cob area they covered assessed. For those samples that were taken as grain samples, these counts were done on the basis of a 1000 grain sample, to get the percentage of grain attacked by fungi, insects and the undamaged grains.

Statistical analysis

Percent cobs damaged by insects were grouped into damage classes and related to aflatoxin contamination for the 1993/94 data. Aflatoxin load on samples damaged by a certain insect

species were compared to undamaged samples. Differences between damaged and undamaged samples were evaluated using the Student Newmans Keuls test (SNK) (p=0.05).

Means comparisons evaluated differences in insect infestation between zones in 1994/95and means were separated using the Student Newmans Keuls test (SNK) (p=0.05). The statistical programme made adjustments for unequal sample size (Norusis & SPSS 1993, p. 278). Correlations were used to determine the factors affecting aflatoxin contamination and *Aspergillus* fungi development and to establish relationships between variables. Data from each sampling date was analysed separately. Stepwise regression analysis was used to determine which factor contributed to aflatoxin development. Total aflatoxins (B_1, B_2) as x = ppb of sample were transformed to log (x + 1), and all percentages for insect and fungal contamination were arcsine-transformed before analysis (Zar 1974). The data is presented untransformed in the tables. Analyses were performed with the SPSS for Windows program (Norusis & SPSS 1993).

8.3   Results

1993/94 Season

Only 6% of the samples collected at the beginning of storage in 93/94 were free of insects. The lepidoptera were found in 36% of the samples. Of the coleopteran species collected on the maize cobs, *S. zeamais* and *C. quadricollis* were the most abundant, found in 50% and 41% of the samples, whereas the nitidulid beetle *Carpophilus* spp. was found in 20% of the samples. Overall 13.4% of the samples from the first survey were contaminated with aflatoxins, with a mean of 96.8 ppb, for the aflatoxin positive samples.

The classification of the samples according to the percentage of cobs damaged by insects (Table 8.1) revealed, that no aflatoxin was detected in maize free from insect damage. Both the percentage of aflatoxin positive samples and the mean concentration of aflatoxin gradually increased with increasing percentage of cobs damaged by insects. Samples with more than 40% damage, had the highest percentage of aflatoxin positive samples (21%) and the highest mean of 21.9 ppb and a range from 0 to 563 ppb. In maize samples in which all the cobs were damaged by insect the mean grain moisture content was 15.74% ($\pm$ 2.88) (Table 8.1).

Table 8.1:   Grain moisture content (%) and aflatoxin contamination (ppb) for insect cob damage classes on maize beginning of storage (1993/94)

% cobs damaged	N/damage class	Grain moisture content (±Sd)	Mean aflatoxin (±Sd)	Range aflatoxin (ppb)	% positive samples
0	19	10.90 (1.40)	0.0	0-0	0.0
0-20	42	13.41 (2.95)	0.32 (2.05)	0-13	2.4
21-40	61	13.58 (2.21)	8.36 (35.31)	0-250	9.8
>40	138	13.97 (2.18)	21.92 (72.61)	0-563	21.0

N= no. of samples

Comparison of lepidopterean free samples (N=125) and those damaged by lepidopterean species (N=175) showed that the latter contained significantly more toxins than the previous ones. The mean for the damaged samples was 20.4 ppb and for the undamaged samples 8.4 ppb (F=11.14**, P=0.001). Infestation of coleopteran species without considered individual species did not significantly affect aflatoxin contamination, but *Carpophilus* sp. infestation increased aflatoxin contamination significantly in the samples. The mean aflatoxin content of samples free from *Carpophilus* spp. (N=266) was 9.5 ppb, which was significantly lower than the 26.6 ppb detected in *Carpophilus* spp. infested samples (N=23) (F=5.05*, P=0.02). The two most abundant species *S. zeamais* and *C. quadricollis* spp. had no effect on aflatoxin contamination.

94/95 Season (Beginning of storage)

In 1994/95, only 7.7% of the samples were insect free. *B. fusca* only occurred in low numbers in the SGS and the FMS, and *E. saccharina* only in the NGS, the mean numbers were so low that they were not presented in the table. There were no differences for the mean aflatoxin contamination between the different agroecozones. The numbers of *C. leucotreta* were significantly lower in the SS (Table 8.2). There were no significant differences between the ecoregions for the percentage of cobs damaged by *C. leucotreta*. The number of *M. nigrivinella* was significantly higher in the NGS. The percentage of cobs damaged by this pest (% Mus.) was high in the SGS with a mean of over 40% of the cobs damaged by *M. nigrivinella* and 60% in the NGS. The number of *S. calamistis* was very low in the SS, with a mean of 0.09. Damage attributable to insects was significantly higher in the NGS and SS than in the other zones.

Correlations of data at the beginning of storage showed that aflatoxin was highly correlated to the visible infection with *Aspergillus* spp. fungi (r=.477*, N=140). Correlations were found between the percentage area visibly infected with *A. flavus* and the mean number of *M. nigrivinella* found on the cobs (r=.239*, N=140).

Table 8.2   Mean aflatoxin(ppb), percentage of aflatoxin positive samples per ecozoen (% Pos.),
mean numbers of insects species (e.g. *C. l.*), mean cobs/sample infected with insect species (e.g. % C. l.)
in the four agroecological zones at the beginning of storage in the year 94/95 (untransformed data)

Zone	N	Aflatoxin	% Pos.	*C. l.*	*M. n.*	*S. c.*	Coleop	% C.l.	% M.n.	% S. c.	Dam Ins.
FMS	30	4.6 a	36.7	.883 b	3.37 b	.876 b	0.189 a	18.50 a	26.98 a	5.52a	0.72a
SGS	40	23.1 a	55.0	.796 b	3.53 b	.622 b	0.225 a	22.29 a	42.50 b	4.23a	3.25a
NGS	40	19.0 a	72.5	.562 b	5.44 c	.334 b	1.596 a	20.41 a	59.03 c	4.03a	16.46b
SS	30	12.9 a	56.7	.435 a	1.53 a	.088 a	1.571 a	16.87 a	25.70 a	1.51a	13.27b
F-VALUE		2.30		3.76	7.43	3.20	2.40	0.90	14.62	3.31	6.35

N = number of samples per zone, % Pos. = percentage of samples aflatoxin positive per zone,
C. l. = mean number of *Cryptophlebia leucotreta* , *M.n.* = mean number of *Mussidia nigrivinella, S. C.* = mean number of *Sesamia calamistis,*
Coleop.= mean number of Coleoptera, %Cry = Mean % cobs damaged by *Cryptophlebia leucotreta*, % Muss. = Mean % cobs damaged by *Mussidia nigrivinella*,
% Ses = Mean % cobs damaged by *Sesamia calamistis*, Dam. Ins. = Mean cob area damaged by Insects
Aflatoxin (ppb)was log(x+1)-transformed before analysis
Means followed by the same letter are not significantly different from each other(SNK, p = 0.05)

Table 8.3   Mean aflatoxin(ppb), percentage of aflatoxin positive samples per ecozoen (% Pos.),
mean numbers of insects species (e.g. *Cry.l.*), mean cobs/sample infected with insect species (e.g. % Cry)
in the four agroecological zones at six months of storage in the year 94/95 (untransformed data)

Zone	N	Afla.	%Pos.	*C. q.*	*Carp.*spp.	*M. n.*	*S. z.*	% *C. q.*	% Carp.	%*M. n.*	% *S. z.*	Dam. Ins.
FMS	40	2.2 a	22.50	7.55 b	.034 a	1.70 ab	10.26 b	47.82 b	1.56 a	28.16 ab	48.83 c	14.54 b
SGS	41	18.7 b	58.54	4.18 ab	.108 b	3.38 c	7.84 b	39.91 b	5.78 b	50.85 b	52.40 c	9.98 b
NGS	43	9.1 ab	60.47	1.76 ab	.007 a	1.64 b	2.74 a	37.78 b	0.67 a	46.72 b	32.08 b	5.37 ab
SS	31	18.5 ab	45.16	0.32 a	000 a	0.14 a	0.16 a	11.84 a	0.00 a	8.15 a	11.62 a	2.37 a
F-VALUE		3.99		5.39	3.32	8.96	9.36	7.15	4.25	13.73	11.12	8.04

C. q. = mean number of *Carthartus quadricollis, Carp.*spp.= mean number of Carpophilus spp., *M.n.* = mean number of *Mussidia nigrivinella,*
S. z. = mean number of *Sitophilus zeamais,* % Carth = Mean % cobs damaged by *Carthartus quadricollis*, % Carp= Mean % cobs damaged by Carpophilus spp.
% Muss. = Mean % cobs damaged by *Mussidia nigrivinella*, % *S.* = Mean % cobs damaged by *Sitophilus zeamais*, Dam. Ins. = Mean cob area damaged by Insects

1994/95 Season (6 months of storage)

Six months after harvest 6.5% of the samples were without insect damage. *E. saccharina* damage was infrequent and only found on maize that was stored with the husk in the FMS, SGS and NGS. The mean infestation and the percentage of cobs infested with *C. leucotreta* showed no differences between the agroecoregions. The highest number of *M. nigrivinella* larvae was found in the SGS. Mean percentage of cobs from one sample infested with *M. nigrivinella* tended to be higher in the SGS and NGS, than in the other regions. The highest number of *Carpophilus spp.* was observed in the SGS (Table 8.3), but the percentage of cobs infested was no different between the regions. The number of *C. quadricollis* was significantly higher in the FMS, whereas the percentage of cobs with this species was significantly lower in the SS. *P. truncatus* was rarely recorded in the maize samples. There was a mean of 5.29% and 8.41% percentage of cobs damaged by *P. truncatus* in the FMS and in the SS respectively. Significantly more *S. zeamais* were found in the FMS and SGS than in the other zones. For the percentage of cobs with damage by this species a similar trend was recorded with more cobs damaged in the FMS and SGS, than in the NGS and SS. The damage levels because of insects were significantly higher in the FMS and the lowest in the SGS.

At six months after harvest a strong relationship existed between aflatoxin and the percentage area visibly infected by *Aspergillus* (r=.219*, N=150) and the area visibly infected by *Penicillium* (r=.330*, N=150). Correlations existed between aflatoxin and the area damaged by *S. calamistis* (r=.245*, N=150), *C. leucotreta* (r=.260*, N=150) and *S. zeamais* (r=.222*, N=150). The same relationship existed between occurrence of the toxin and damage attributable to insects (r=.201*, N=150).

8.4   Discussion

At the beginning of the storage season the most abundant specie was *M. nigrivinella*, this corrobates the findings by Moyal (1995) in the Côté d'Ivoire who found *M. nigrivinella* to be the most damaging pest of standing maize. There was a significant relationship between the visual observation of *Aspergillus* spores on the cobs and the number of cobs destroyed by *M. nigrivinella*. This confirms the results by Setamou (1996) who reported that *M.*

nigrivenella damage significantly increased the risk of aflatoxin development in maize before harvest in Benin. In this study the highest number of earborers were found in the SGS, this may be because of the abundance of wild host plants in this region as observed by Schulthess & Setamou (1997).

Positive correlations were found between aflatoxin and the number of *S. calamistis* and *C. leucotreta*. Lepidopteran pests have been known to carry fungal spores (McMillian *et al.* 1990) and might aid in infecting maize plants with fungal spores. In Kenya a survey was carried out to investigate the micro-organisms present on stem- and cobborers, there 7% of the larvae were infected with *Aspergillus* fungi (Odindo *et al.* 1989). *C. leucotreta* could be implicated in vectoring *Aspergillus* spores into maize cobs, since this species is known to lay their eggs on the husk of maize cobs (Moyal 1995). When the first generation larvae hatch they might carry the spores into the cobs. Damage caused by this maize pest occurs mostly on the tip of the cob, this is also the site where *Aspergillus* spores are known to enter the maize cobs (Wicklow & Donahue 1984). The observed correlations between lepidopterous pests and aflatoxins might be accredited to insects acting as vectors of fungal spores. The same mechanism could explain the significant relationship that existed between insect damage and aflatoxins, which was observed in the FMS and the SGS.

Another factor that was correlated in this study with aflatoxin, was the number of *S. zeamais* in the maize samples. This relationship has previously been reported, maize infected with *S. zeamais* and *A. flavus* spores had significantly higher Aflatoxin B_1 levels than maize that was only inoculated with *A. flavus* (Beti *et al.* 1995, Mc Millian 1987). Also the relationship between *Carpophilus* spp. and aflatoxin has been observed earlier. This storage insect was found in stored grains with high moisture content that was heavily contaminated with fungi (Vega *et al.* 1995). Likewise, in laboratory studies adults of *C. lugubris* and *C. hemipterus* preferred damaged to undamaged maize kernels (Dowd 1994). Their presence in the survey samples from Benin might be an indicator of grains with a high moisture content that were about to degrade. These beetles were known to consume the spores and mycelium of *A. flavus*, and it had been observed that spores could germinate after passage through the nitidulids gut (Wicklow 1988).

CHAPTER 9   **Storage trials**

9.1   Introduction

To verify some of the trends revealed in the surveys, on station storage trials were established, to test which form of storage was most adapted to the climatic conditions in southern Benin. When the survey data was evaluated (Chapter 6), the role of storage form in aflatoxin contamination of stored grains was not clear. In 1993-94 storage as grains in the SGS and SS led to higher aflatoxin contamination of maize measured after 6 months of storage, whereas in 1994-95 storage as grains was associated across ecozones with lower aflatoxin content of maize sampled at the beginning of storage. Storage form was reported to have an effect on the infestation with insects (Vowotor 1992; Kossou *et al.* 1992). The relationship between insect infestation and aflatoxin has been summarized in section 2.2.3 and reviewed in articles by Wicklow (1988) and McMillian (1987). When maize was stored dehusked in traditional cribs in southern Benin there were significantly more weevils present than in the undehusked treatment. Comparison of the number of weevils on dehusked and shelled grains showed, that there were no significant differences between the treatments (Kossou *et al.* 1992). In laboratory trials by Vowotor *et al.* (1995) the mean development time of maize weevils was 25.2 days on unshelled maize and 22.8 days on shelled grains, the authors drew the conclusion that storing maize unshelled with the husk intact would reduce the possibility of a weevil population build-up.

9.2   Materials and Methods

A trial was established at the Biocontrol Station, Abomey-Calavi in December 1994, to determine the effect of different storage forms on resulting aflatoxins. Second season maize was used for the experiment. Farmers in southern Benin often used first season maize as "green maize" to be consumed fresh or it was used till the next harvest came in. Second season maize was stored for longer periods from December till the next years maize was planted in May. The sampling dates chosen, were 3 months after storage (3/4/1995) and 6 months (28/6/1995) after storage. The microbiological and aflatoxin analysis were effected as described in section 5.2.

The following treatments were replicated four times:

T1 = stored as grains, dehusk and degrain out of the field

T2 = stored without the husk, dehusk in the field

T3 = stored without the husk, dehusk out of the field

T4 = stored with the husk

The insect damage levels and species composition was evaluated like described in section 8.2. The variables observed were visible damage because of insects, mean numbers of insects of a certain species and percentage of cobs from one sample that were attacked by an insect species. On samples that were taken as grains, the number of insect species found on the sample was recorded by separating them with a No. 5 sieve (4 mm) and counting the different insect species. The damage because of insect species were observed on 1000 kernels and recorded as a percentage,

The storage trial was repeated in December 1995, with a reduction of the treatments, sampling was done after 3 (15/4/1996) or 6 months (18/6/1996). The following treatments were replicated five times.

T1 = stored as grains

T2 = stored without the husk

T3 = stored with the husk

For both trials, maize cobs were stored in baskets of 60-70 cm circumference, which were placed on elevated platforms, protected with ratguards and covered with a roof made out of thatch. For each treatment 100 cobs were placed in the basket and at each sampling 20 were taken out and evaluated like described above (Chapter 5.2).

Total aflatoxins (B_1, B_2, G_1, G_2) as x = ppb of sample were transformed to log (x + 1), and all percentages from the mycological observations and insect data were arcsine-transformed before analysis. Analyses were performed with SPSS-Statistical Package (Norusis & SPSS 1993). Data were subjected to treatment mean comparisons using the Student Newman Keuls test. Correlations were calculated to establish relationships between variables. Data from each year were first analyzed separately and then were pooled together. The aflatoxin, fungal and insect data are presented untransformed in the tables.

9.3   Results

1994/95 Season

When comparing the aflatoxin contamination of maize stored in different storage forms (Table 9.1), after 3 months of storage significantly higher aflatoxin content was observed in maize stored as grains (70.1 ppb), than when storing with the husk (11.8 ppb) or dehusking in the field (20.1 ppb). No significant differences could be observed between dehusking outside the field (40.4 ppb) and storage as grains.

Table 9.1:   Means comparison for aflatoxin (ppb), fungal contamination (%), grain moisture content (%) for treatments (94/95 season) after 3 months of storage

Treatment	Aflatoxin	*Aspergillus*	*Fusarium*	*Penicillium*	Other Fungi	Total Fungi	G.M.C
Dehusk(Outside)	40.4 ab	69.3 a	29.6 a	9.1 a	0.3 a	86.1 a	13.3 a
Dehusk(Field)	20.1 a	68.0 a	20.0 a	10.7 a	1.3 a	80.0 a	13.6 a
With Husk	11.8 a	65.3 a	36.0 a	13.3 a	0.0 a	90.7 a	13.4 a
Grains	70.1 b	93.3 a	12.0 a	5.3 a	0.0 a	100.0 a	13.0 a
p-Value	.0263	.3089	.0963	.5116	.4833	.1314	.3854

Aflatoxin (ppb)was log(x+1)-transformed before analysis, fungal data (%) was arcsine ÷ transformed
Means followed by the same letter are not significantly different from each other (SNK, p = 0.05)

After 6 months of storage no significant differences existed for the aflatoxin content measured in the different storage treatments (Table 9.2). Very high infection levels with *Aspergillus* fungi were observed after 3 months, the range was between 65.33% and 93.33% with no significant differences between the treatments. After 6 months of storage significantly more *Aspergillus* fungi were found when maize was stored as grains. Overall levels slightly decreased when compared to the sampling at 3 months of storage, this might be due environmental conditions that were unfavorable for the growth of the studied fungi, but what contradicts this hypothesis was the rise in grain moisture content. The *Fusarium* infection levels rose slightly from sampling after 3 months of storage to sampling after 6 months of storage. Fusarium infection was significantly higher when maize was dehusked in the field or when storing with the husk (Table 9.2). No significant differences were recorded between the treatments for *Penicillium* spp. infection levels and grain moisture content. Maize stored as grains after 6 months of storage showed a significantly higher fungal infection rate and a higher percentage of "other" fungi.

Table 9.2: Means comparison for aflatoxin (ppb), fungal contamination (%), grain moisture content (%) for treatments (94/95 season) after 6 months of storage

Treatment	Aflatoxin	*Aspergillus*	*Fusarium*	*Penicillium*	Other Fungi	Total Fungi	G.M.C
Dehusk(Outside)	24.8 a	40.0 a	25.3 a	22.7 a	1.3 a	45.3 a	11.4 a
Dehusk(Field)	71.8 a	63.3 a	45.3 b	26.7 a	0.0 a	69.3 b	11.8 a
With Husk	22.2 a	64.0 a	48.0 b	38.7 a	0.0 a	76.7 b	11.5 a
Grains	93.5 a	92.7 b	32.0 a	14.7 a	22.7 b	96.0 c	10.9 a
p-Value	.2298	.0076	.0104	.1913	.0396	.0203	.4619

Aflatoxin (ppb)was log(x+1)-transformed before analysis, fungal data (%) was arcsine ÷ transformed
Means followed by the same letter are not significantly different from each other (SNK, p = 0.05)

No significant differences between the different storage forms were observed for many insect variables, so that only variables with significant differences are presented in the tables. Significant differences could be found between the different storage treatments (Table 9.3) for the visible insect damage, the mean number of *C. quadricollis, M. nigrivinella* or *S. zeamais*.

Table 9.3: Means comparison for insect variables for treatments (94/95 season) at 3 months after storage

Treatment	Damage Insects	Mean No. *C. quadricollis*	Mean No. *M. nigrivinella*	Mean No. *S. zeamais.*
Dehusk(Home)	13.38 a	16.20 a	0.20 a	16.53 a
Dehusk(Field)	19.65 a	11.45 a	0.22 a	24.78 ab
With Husk	56.85 c	47.98 c	0.53 b	53.38 c
Grains	29.96 b	25.21 b	0.32 ab	31.57 b
p-Value	.0000	.0000	.0417	.0002

Means followed by the same letter are not significantly different from each other (SNK, p = 0.05)

For the presented variables, the number of insects were the highest when maize was stored with the husk, lowest on dehusked maize. There were no differences between maize that was dehusked in the field and dehusking at home, except for the number of *C. quadricollis* and *S. zeamais* but these differences were not significant.

After 6 months of storage insect damage rose to around 90% in all the treatments (Table 9.4), with significantly lower damage levels observed inmaize that was dehusked at home than dehusking in the field. This increase was mostly because of an increase in the numbers of *P. truncatus*, at 3 months of storage mean numbers of this storage pest across treatments were 0.77 and at 6 months 5.92. But no significant differences between the treatments were

observed for the infestation levels with *P. truncatus*. Mean numbers *of M. nigrivinella* were significanlty higher when maize was stored with the husk, whereas means of *S. zeamais* were significantly lower in maize dehusked in the field.

Table 9.4:   Means comparison for insect variables for treatments (94/95 season) at 6 months after storage

Treatment	Damage Insects	Mean No. *C. quadricollis*	Mean No. *M. nigrivinella*	Mean No. *S. zeamais.*
Dehusk(Home)	83.6 a	12.4 a	0.63 a	30.2 b
Dehusk(Field)	94.9 b	4.7 a	0.67 a	15.2 a
With Husk	93.1 ab	16.1 a	3.28 b	41.6 b
Grains	90.5 ab	11.1 a	1.53 a	28.9 b
p-Value	.0476	.1694	.0011	.0029

Means followed by the same letter are not significantly different from each other (SNK, p = 0.05)

1995/96 Season

As in the 94/95 season, maize stored as grains showed the highest contamination with aflatoxins (Table 9.5).

Table 9.5:   Means comparison for aflatoxin (ppb), fungal contamination (%), grain moisture content (%) for treatments (95/96 season) at 3 months after storage

Treatment	Aflatoxin	*Aspergillus*	*Fusarium*	*Penicillium*	Other Fungi	Total Fungi	G.M.C
With Husk	3.3 a	54.4 a	4.0 a	0.0 a	8.8 a	64.8 a	13.5 a
Dehusked	2.0 a	64.0 a	8.0 a	0.8 a	11.2 a	83.2 a	13.9 b
Grain	15.5 b	74.4 a	1.6 a	0.0 a	0.8 a	77.6 a	13.7 ab
p-Value	.0444	.4672	.1725	.3966	.4556	.4018	.0218

Aflatoxin (ppb)was log(x+1)-transformed before analysis, fungal data (%) was arcsine ÷ transformed
Means followed by the same letter are not significantly different from each other (SNK, p = 0.05)

After 3 months of storage maize stored as grains had significantly higher aflatoxin levels than the other treatments (p=.044, N=15), 6 months later high aflatoxin content was found in maize stored as grains and in dehusked maize, the lowest aflatoxin levels were measured in maize that was stored with the husk (p=.0003, N=15) (Table 9.6).

Maize stored with the husk had an aflatoxin content of 3.3 ppb and 6 months later this rose to 4.1 ppb. In maize stored dehusked, after 3 months only 2 ppb were detected and after 6 months this rose to 14.1 ppb. The highest amount of aflatoxins could be found in maize stored as grains, for the first sampling 15.5 ppb were found and later 37.1 ppb. In the 95/96

season, there were no significant differences between the different treatments for the percentage of *Aspergillus* found in the samples either at 3 months (p=.467, N=15) or at 6 months (p=.116, N=15). After 6 months of storage all the maize kernel that were stored either dehusked or as grains developed *Aspergillus* fungi. The percentage of kernels that were infected with *Fusarium* spp. was low with no significant differenced between the treatments. After 6 months of storage, maize that was stored dehusked showed a higher infection rate with *Penicillium* spp. and "other" fungi. (Table 9.6). Grain moisture did not differ between the treatments.

Table 9.6: Means comparison for aflatoxin (ppb), fungal contamination (%), grain moisture content (%) for treatments (95/96 season) at 6 months after storage

Treatment	Aflatoxin	*Aspergillus*	*Fusarium*	*Penicillium*	Other Fungi	Total Fungi	G.M.C
With Husk	4.1 a	93.6 a	0.8 a	0.8 a	48.0 b	96.8 a	11.7 a
Dehusked	14.1 b	100.0 a	0.0 a	7.2 b	60.8 b	100.0 a	11.4 a
Grain	37.1 b	100.0 a	0.0 a	0.0 a	6.4 a	100.0 a	11.7 a
p-Value	..0038	.1162	.3966	.0136	.0008	.3966	.3520

Aflatoxin (ppb)was log(x+1)-transformed before analysis, fungal data (%) was arcsine ÷ transformed
Means followed by the same letter are not significantly different from each other (SNK, p = 0.05)

In 1994-95 much higher numbers and damages due to insects were observed, only the insect variables that were significantly different from each other are listed in Table 9.7 and 9.8. After 3 months of storage (Table 9.7), damage because of insects was lower in maize stored with the husk than stored dehusked, the same was observed for the mean numbers of *P. truncatus* and *S. zeamais*, but some of these relationships were not significant. The number of *C. quadricollis* on the contrary was slightly higher when maize was stored with the husk, but this was not a significant relationship.

Table 9.7: Means comparison for insect variables for treatments (95/96 season) after 3 months of storage

Treatment	Damage Insects	Mean No. *Carpophilus spp.*	Mean No. *C. quadricollis*	Mean No. *P. truncatus*	Mean No. *S. zeamais*
With Husk	81.09 a	5.28 a	24.31 a	29.04 a	14.16 a
Dehusked	98.95 b	4.83 a	21.41 a	45.54 a	37.56 b
Grain	90.02 ab	5.06 a	22.86 a	37.29 a	25.86 ab
p-Value	.0325	.9148	.8319	.1237	.0040

Means followed by the same letter are not significantly different from each other(SNK, p = 0.05)

After 6 months of storage, damage because of insects was high, with no significant differences recorded between the treatments (Table 9.8). Mean numbers of *Carpophilus spp* and *C. quadricollis* was the highest in maize stored with the husk, lower in maize stored as grains and the lowest in maize stored dehusked. The mean number of *P. truncatus* and *S. zeamais* was significantly higher when storing with the husk than dehusked, but insect abundance on maize stored as grains was not significantly different from the other storage forms.

Table 9.8:   Means comparison for insect variables for treatments (95/96 season) after 6 months of storage

Treatment	Damage Insects	Mean No. *Carpophilus spp.*	Mean No. *C. quadricollis*	Mean No. *P. truncatus*	Mean No. *S. zeamais*
With Husk	99.9 a	26.60 c	80.17 c	42.74 b	45.72 b
Dehusked	100.0 a	7.03 a	31.86 a	12.02 a	28.86 a
Grain	99.9 a	16.82 b	56.02 b	27.38 ab	37.29 ab
p-Value	.4633	.0018	.0000	.0283	.0074

Means followed by the same letter are not significantly different from each other(SNK, p = 0.05)

9.4   Discussion

The highest aflatoxin levels in the 94/95 trial were found in maize that was stored as grains (Table 9.1, 9.2), the same trend was observed in 95/96, with significantly more aflatoxin content measured in maize stored as grains than dehusked maize and storage with the husk showed the lowest concentration (Table 9.5, 9.6). The same observation was made by Lacey & Mora (1992) who studied aflatoxin contamination of maize in Costa Rica, samples from maize that were stored on the cob had almost no aflatoxin contamination but shelled maize was frequently highly contaminated. When the authors studied the percentage of kernels that were damaged by *A. flavus*, they found a mean of 7% on shelled cobs compared to 0.26% when stored on the cob.

Aflatoxin content in maize that was dehusked in the field, was higher than maize that was dehusked at home after 6 months of storage. This was an expected result since maize dehusked in the field could come into contact with the soil that bears an important spore potential (Cotty *et al.* 1994). The evaluation of the development of the measured factors with time revealed, that the aflatoxin and fungal contamination increased with time. Several authors have reported that fungal infection increased with storage time (Payne *et al.* 1988; Christensen & Meronuck 1989). The later authors also found a correlation between fungal

contamination, moisture content and storage time. The relationship between increase of moisture content over storage time could not be observed in this study, rather the moisture content decreased with time. Temperature could have a big influence on aflatoxin development (Lacey & Magan 1991; Wilson & Abramson 1992), the average temperature in southern Benin during the day for the trial period was mostly above 30°C, temperatures that were optimal for the growth of *A. flavus* (Jones *et al.* 1980). In the same year (Table 9.2) high development rates of "other" fungi were observed after 6 months, when maize was stored as grains, these were mostly "moisture-loving" fungi such as *Rhizopus* spp. and *Curvularia* spp.. The relative humidity is above 80% all year round in southern coastal Benin, except for the drier "Hamattan" months of December to January (Adam & Boko 1993). So that the contamination with these fungi might be induced by high relative humidities. On the other hand grain moisture content decreased with time, which contradicts this theory. The decrease in grain moisture can be explained with further drying in the storage structures while air passes through them. This is supported by Smith (1991) who observed that grain moisture content in the rather open structures in southern Togo decreased with time of storage. This might have the effect of reducing *A. flavus* growth like observed in the 94/95 trial, but this trend could not be observed in the 95/96 trial so that other mechanisms should be at the base of the differences in grain moisture content.

Insect development followed a different trend than the development of fungi and aflatoxin, the highest number of insects in this study were found on maize stored with the husk, then maize stored as grains and dehusked maize showed the least insects, except for sampling after 3 months of storage in the 94/95 season. This was contrary to the studies of Kossou *et al.* (1993) who found that dehusked ears were more susceptible to weevils than cobs stored with the husk, and grains had the highest number of insects. When maize was stored as grains there was a higher development rate of insects (Kossou *et al.* 1992).

Vowotor *et al.* (1995) suggested that "by storing unshelled maize the developmental period of the maize weevil was prolonged, reducing the chance of build up of destructive populations of the insect." On the contrary, in the presented study higher *S. zeamais* numbers were reached on maize that was stored as cobs with the husk. The husk could serve as a refuge to the weevils (Kossou *et al.* 1992) and *S. zeamais* would find better conditions for their development. This theory was supported by higher *S. zeamais* number

which were observed in this study in maize stored with the husk after 6 months of storage in 95/96 (Table 9.8).

P. truncatus normally attacks maize late in the season, their highest flight activity in Benin was in the months of December to January and another peak was from May to June (Borgemeister *et al.* 1997), this trial was established in December and from very early on these insects could be observed in the stored maize. Tunneling by this insect was very intensive with a lot of maize flour produced (Pantenius 1988), this might be an ideal substrate for *Aspergillus* fungi, especially when insect activity leads to the heating of maize (Dix & All 1987). Further studies have to show if *P. truncatus* has the ability to increase toxin levels in stored grains.

C. quadricollis was mostly considered a secondary storage beetle, on stored commodities this beetle was often found together with primary storage insects such as *Sitophilus* spp. (Allotey 1991). This seemed to be one of the reasons, why this insect was only collelated with high aflatoxin content later in the storage period in the presented study. But their numbers on maize could be very high especially together with *P. truncatus* (Pantenius 1987). This can be confirmed by this tiral, where after 6 months of storage a mean of 80.17 *C. quadricollis* were found in maize stored with the husk.

CHAPTER 10   **Conclusion and potential solutions to the aflatoxin problem in Benin**

Fungal infection was quantified and aflatoxin contamination was assessed by thin layer chromatography in maize samples from 300 Beninese farmers stores in four agroecological zones in 1993/94 and 1994/95. Information about farmers maize production practices were collected. A plan for sampling maize from storage was based on pretrials that looked at spatial and temporal distribution of aflatoxin in stores. Then farmers stores were sampled twice during the storage season, shortly after harvest and 6 months later.

Aflatoxin contamination in stored maize in Benin was shown to be a fairly common problem. Out of 742 samples collected during the two survey years, there were 186 samples (25%) that were aflatoxin positive and from these positive samples 60 percent were contaminated with levels of more than 20 ppb, the limit set by the WHO (Van Egmond 1991). Maize that was contaminated with levels of more than 20 ppb are unsuitable for human consumption.

Farmers' perception of aflatoxin problems

Maize farming practices varied with agroecological zones and were quite heterogeneous among villages. Differences in agronomic practices were also due to ethnic group, gender, wealth and level of education, but they rarely varied greatly within a village.

Most farmers knew that the consumption of discoloured grains was detrimental to their health and they sorted out these grains (Table 4.11). The link between fungal contamination and these discolorations was rarely made. Very few farmers mentioned molds as a problem in stored maize. Farmers questioned about their field and storage pest problems, cited mostly birds, rodents and insects that were easily recognized by them. Damage found on maize plants was connected to these pests, even when they rarely caused the observed problem. Between 45 to 50% of the Beninese farmers did nothing against storage problems. If farmers treated they used commercial insecticides, either those destined for plant protection in cotton, or storage insecticides. Traditional means of plant protection (Table 4.25 & 4.28) were used in rural areas, where tradition was still respected. Farmers judged these measures as inefficient and often asked for pesticides.

Distribution and importance of fungi and aflatoxin in stored maize in the different agroecological zones

High aflatoxin contamination during both survey years were found in the SGS at the beginning of storage. In 1993/94, at 6 months after harvest, high aflatoxin levels were noticed in the NGS and SS. In 1994/95, for both sampling periods all zones had mean levels below 20 ppb, the regulatory limit determined by the WHO, except in the SGS where 45.5% of the aflatoxin positive samples had aflatoxin levels of more than 20ppb. Setamou (1996), analysed aflatoxin content in standing maize in the same agroecological regions in Benin, the same zones that had high *A. flavus* infection in the field also had high levels of aflatoxin in storage as observed in this study. High aflatoxin contamination was found in the SS after 6 months of storage in 1993/94. It has to be further investigated if this was related to the high temperatures that prevail in this region or if in this region certain stress factors decrease the plants potential to resist *A. flavus* infection and aflatoxin development.

Factors influencing aflatoxin production

a) Abiotic factors influencing aflatoxins
In the presented study, in both years, grain moisture at the beginning of the storage period was significantly higher in the two southern zones. 6 months later grain moisture content was reduced. One of the reasons might be that inside the granary drying occurs, either because of the openness of the grain store or because of the high median temperatures. In 1994/95 at 6 months after storage, the grain moisture content in all the zones was around 11% with slightly higher moisture content in the Guinea Savannas. No indications of fungal competition was found in this study. The maize samples that were strongly contaminated with fungi other than *Aspergillus* did not necessarily show low *Aspergillus* growth and aflatoxin.

b) Biotic factors influencing aflatoxins
Of all the samples collected during the storage surveys only 6-10% were insect free (Chapter 8). The evaluation of insect infestation at the beginning of storage in 1993/94, showed that lepidopterous pests had a significant effect on aflatoxin contamination in stored maize in Benin. In a similar study of maize before harvest, Setamou (1996) found a positive correlation between insects per cob and aflatoxin, the field samples of maize that showed aflatoxin contamination were also damaged by *M. nigrivinella*.

The most damaging coleopteran species in the stored grain samples were *S. zeamais* and *C. quadricollis*. In the presented study *Carpophilus spp.* increased significantly the amount of aflatoxin in maize. Dowd (1994, 1991) found that *A. flavus* was vectored by *Carpophilus dimidatus*. This insect species seemed to be associated with fungal growth, and preferred cobs which had a higher grain moisture content.

c) Agronomic practices
Regression analysis revealed several agronomic practices that were associated with an increased risk of aflatoxin contamination and other farmers practices that were linked to lower aflatoxin content in stored maize in Benin (Table 10.1). Continuous maize cropping was associated with higher aflatoxin content. In the present study associations of maize with cowpea, groundnut or cassava had the same effect. Harvest factors that were linked to high aflatoxin content in stored maize were long drying periods in the field, and a delay in drying, sorting and storage of maize. Storage structures that seemed to encourage fungal infection and aflatoxin development, were storage over the ceiling, on top of the roof, "Ago" (Chapter 7) in the SS and NGS, and in storage containers that were more than 5 years old. One possible explanation for the increased risk of aflatoxin development in older grain stores was that a spore potential remained in the stores from year to year and this helped to infect the new grains. In addition to this, stores might become less air-tight with time.

The results of the presented study gave indications that if farmers were informed about the aflatoxin problem and ways to impede fungi from entering and developing in standing or stored maize, the risk of aflatoxin development could be considerably reduced. The farming practices that were associated with lower aflatoxin levels in stored maize are presented in Table 10.1. The use of fertilizer, in this study use of double ammonium phospate (DAP) was related to lower aflatoxin content in stored maize. Drying seemed to play a vital role in reducing aflatoxin contamination in stored maize. In grain from Thailand that was not dried after mechanical shelling, aflatoxin contamination prevailed if the moisture content was higher than 20% it was suppressed if moisture content was lower than 17% (Siriacha *et al.* 1990). Farmers practices that might help to reduce the risk of aflatoxin contamination, were storage in either the secco, conical stores, the crib in the SS, Ago made from bamboo and bags as secondary storage. Use of insecticides or smoking was associated with lower aflatoxin contamination. Probably this was because of the effect on insect pests and notdue to a direct effect on *A. flavus*.

Table 10.1 Farming practices that were associated with higher and lower
aflatoxin levels in stored maize in Benin (93/94 and 94/95)

	Higher aflatoxin levels	**Lower aflatoxin levels**
Production Practices		
	Maize monocropping	Crop rotation
	Improved variety in South	Local variety in South
	Local variety in North	Improved variety in North
	Maize/cowpea, groundnut or cassava intercrop	Maize in mixed cropping
	No fertilizer	DAP fertilizer
	Maize is damaged in the field	Farmers aware of bad huskcover
Harvest Practices		
	Delayed harvest	Harvest at crop maturity
	Harvest maize in heaps, cobs collected later	Harvest of maize with the husk
	"Field" drying on the plant	Sun drying on platform
	Delayed drying	Drying of maize without the husk
	No sorting at harvest	Immediate removal of damaged cobs
Storage Practices		
	No preparation of the storage structure	Cleaning of the storage structure
	Maize stored for 3-5 months	
	No insect control	Smoke or insecticide use
	Maize stored in Ago, Crib, conical store, on roof	Maize stored in Ago (Bamboo), bags

Influence of storage form on aflatoxin

Another aspect of the presented work, looked at the influence of storage form on aflatoxin contamination. Maize stored with the husk showed low mean levels of aflatoxin and grains had high levels of the toxin (Chapter 9). High insect infestation was found when maize was stored with the husk in 1994/95 at the beginning of storage, and six months later all treatments were heavily infested with insects. Good huskcover could reduce the damage of insects to stored maize cobs, but this was highly dependent on ecoregion and the insect species that was predominant on stored grains. The results from the storage form trials seemed to give an indication that aflatoxin contamination was not a function of the storage

form, but rather was influenced by the treatment of maize before storage e.g. drying, sorting. All the measures to decrease aflatoxin contamination or a combination of the measures presented in section 10.2.3 might be more valuable in reducing aflatoxin contamination than only the storage form.

What should be done next?

Some of the potential solutions to the aflatoxin problem in Benin, are the technology packages presented in section 10.2.3 and Table 10.1. These packages have to be field tested with the farmers to evaluate their feasibility and acceptability. Simultaneously the impact of aflatoxins on the health status of the concerned populations has to be assessed. Also the populations in West-Africa have to be informed about the detrimental effects of eating grains and other foodstuff that are contaminated with mycotoxins.

The risk of multitoxin exposure in Africa is also very high with some of the goods contaminated with several fungi and their respective toxins. This effect of multiple toxin exposure has to be studied further. Fumonisin analysis on 80 samples from the sampling at the beginning of storage in 1993/94, showed that in all the samples fumonisins were detected at levels from 20 to 8770 ppb. There were strong correlations between aflatoxin and fumonisin development on stored grains, indicating that as grain degrades the risk of maize becoming contaminated with various toxins increased (Hell *et al.* 1996). Another aspect that should be studied in depth is the passage of *Aspergillus* spores from the soil to the plant. The factors that regulate spore propogation have to be clarified and what possible role insects play in this process.

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Annex I

Original questionnaire was administered in french, here the english translation is shown.

Questionnaire at the beginning of storage in 93-94

GENERAL INFORMATION

Agroecological Zone
Farmers' Name   Interviewers' Name
Ethnic Group
Location   Village

SAMPLE NO.

    Date of collection

    When harvested

What do you aspire to achieve, what is the most important thing in life?
    good health
    money
    good farming
    spiritual atonement

What contributes to this?

How do you plan to achieve it?
    Invest in farming
    Save money
    Sell goods

GENERAL CROPPING INFORMATION

1) What are the most important crops (list in order of importance)?
    1
    2
    3
    4
    5

2) Why do you grow each of these crops (tick the one which is more important)?

        1 food   sell

        2 food   sell

        3 food   sell

        4 food   sell

        5 food   sell

MAIZE PRODUCTION

1) Do you grow maize each season?

        main growing season

        short growing season (If yes, get a sample)

        Time of harvest

               Sample No.

        Intercropping/Crop Rotation

2) Give an estimation of your harvest?

3) Do you intercrop maize?

        No   Yes   Crop

4) Do you ever grow maize after maize in the same field?

        No   Yes

5) What crops do you grow after maize?

6) What crops do you grow before maize?

        1 year before

        2 years before

7) Do you still have maize from last year harvest in storage?

        No   Yes

        Time of harvest

        Sample No.

        Intercropping/Crop Rotation

GET A SAMPLE OF MAIZE, ALL THE FOLLOWING QUESTIONS REFER TO THIS SAMPLE

8) Did you intercrop the maize (sample), describe the crop rotation?

        No   Yes   Crop

9) What seed did you use?

        own seed

        bought   source

        gift

10) What variety did you use?
> local
> improved

12) Did you treat the seed at planting with pesticides?

No	Yes	Brand

13) Did you use fertiliser?

No	Yes	Type

14) Did you use pesticides when the maize was still growing in the field?

No	Yes	Brand

15) Do pests attack the maize while still standing in the field?
> insects
> mice/rats
> birds
> moulds
> others

HARVEST PRACTICES

1) When is the maize ready for harvesting?
> - silk falls out
> - cobs and husk are completely dry
> - cobs fall down
> - grain can't be scratched with the fingernail
> - other

2) Are you able to harvest as soon as the crop is mature?

No	Yes
Why not?	
	No labor available
	Other activities in this period
	Other

3) How do you harvest?
> - cutting the whole stalk
> - collecting the ears
> - bending the stalk before harvest to let it dry then harvest
> - other

Why do you harvest this way?
> - prevent insect attack
> - dry the cob completely
> - other

How long did the harvest take?

All at once or in bits?

4) Do you harvest green maize?
        No   Yes

        What part of you crop of maize is harvested green?

        For sale   For household consumption

5) Do you leave the cobs on the plant to dry before harvesting?
        No   Yes

        How long?

   Why do you do this?
                no labor force
                still has to dry
                other

6) When do you remove the husk?

Where?   in the field
                in the house
                other

7) Do you separate cobs that are not well covered by the husk?
        No   Yes

Where do you do this?
                field
                house
                other
Were there many?
                No   Yes, how many?
What do you do with these cobs?
                - throw them away
                - feed to animals   which animals
                - eat themselves
                - sell
                - other

TRY TO GET SAMPLES OF THE DISCARDED COBS

8) Do you sort out other kinds of damaged cobs?
        No   Yes

What is your sorting criteria?
            colour
            cob size
            grain size
            damage
            other

What types of damage would you sort out?
            insect
            rodent
            birds
            moulds(discoloration)
            other

What do you do with the damaged cobs?
            - throw them away
            - feed them to animals   which animals?
            - eat themselves
            - sell
            - other

Were there many last harvest?
 Many   About half   Few   None

TRY TO GET SAMPLES OF THE DAMAGED COBS

9) When do you remove the grain from the cob?

        Where is this done?
                field
                house
                other
        Do you clean the grain at this stage?

        winnowing

        sorting   what criteria   - colour
                                                            - cob size
                                                            - grain size
                                                            - damage
                                                            - other

                    what do you do with the sorted grains
                    throw them away
                    feed to animal   which?
                    eat themselves
                    sell
TRY TO GET SAMPLES OF THE SORTED OUT GRAINS

10) How do you dry your maize?

                solar drying
                professional dryer
                drying over the fireplace
                other

       describe the structure?
                drying arena
                on the roof

       where is the drying done?
                field
                house
                other

11) How long does drying take?

12) How do you know if the grain is really dry?

                can't be scratched by fingernail
                gives a breaking sound when chewed
                other

13) Is it important that the grain is really dry?

       Why?   prevent insect damage
                                                no problems with moisture
                                                other

14) Drying is

                                                without husk
                                              with husk

STORAGE PRACTICES

1) When is the harvested maize put into storage?

       Is it put directly into storage or do you put it elsewhere for a few days?

       Why?

2) How long do you usually store?

3) What method of storage do you usually use?
       on a raised platform on the farm
       on a raised platform near the house
       in a room in the house
       in a crib
       over the fire place
       on the ceiling

4) Where is your storage structure located?

        in the courtyard
        behind the house
        in the field
        inside the house
        others

5) Construction material for storage structure?

        wood
        clay
        metal

why did you use this material?

6) How many seasons have you used this type of store?

7) Do you store maize in it every season?

        No,why?   Yes

8) Do you use it to store other foodstuffs?

        No   Yes, which ones?

STORAGE PROBLEMS

1) Do you have problems with storage?

        No   Yes

2) Which is the most important problem?

        moisture
        insects
        rodents
        mould
        theft
        others

3) When did you observe this problem?

4) What did you do to solve this problem?

5) Does the grain germinate in storage?

        No   Yes

6) Do you clean the storehouse before storage?

        No   Yes

Do you remove old grains?

        No   Yes

What else did you do to the storehouse before storage?

        apply insecticides on the floor

        reinforece the floor with cement

        others

7) If you treated the storehouse before storage, what methods do you use?

        ash

        sand

        smoke

        manure

        neem

        insecticides

        others (specify)

8) Were the methods used successful?

        No   Yes

9) How did you store your maize?

        grain

        in the husk

        dehusked

        other

10) In which form is the maize stored the most?

        grain

        in the husk

        dehusked

        other

11) Do you use pesticides during storage?

        If yes, give name:

12) Do you take any other precautions?

        which ones?

CONSUMPTION PRACTICES

1) Who eats maize?

            adults

            children

            babies

2) How is it prepared?

      do you sort before preparing?

      what do you do with the bad grain?
            give to animals (name them)
            sell
            discard
            eat
            others (specify)

3) How often do you eat prepared maize?

4) Do you use maize for animal feed, which animals?

5) Do you buy maize in the market?
      how often
      what time of the year

Annex II
Original questionnaire was administered in french, here the english translation is shown.

Questionnaire at the beginning of storage in 94-95

Agroecological zone: Date:
Village:
Farmer: Sex:

Crop association   Cassava
                Groundnut
                Cowpea
                Sorghum
                Sesame
                Yam

Fertilizer   NPK
                Urea
                Ammonium Phosphate
                Double Ammonium Phosphate
                Manure

Agents of field damage
                Insects
                Rodents
                Birds
                Fungi
                Big animals
                Striga
Huskcover
What variety did you use?
                local
                improved
Do the husk cover the cob well?   Yes
                No

If NO, how many of the cobs were not well covered?   Many
                        About half
                        Few
Drying
After Maturity
For how long did you leave the maize to dry in the field before harvesting it?
After harvest
For how long did you leave the maize to dry outside the field before storing it?
Number of Days:
Storage form?   with husk
                        without husk
                        grains

Sorting

Do you sort?   Yes
                                                  No

      When do you sort?
            at harvest
            before storage
            during storage
            before consumption

Give the reasons for sorting?

When do you dehusk?
            at harvest
            before storage
            during storage
            before consumption

Storage

What storage structure(s) do you use?

Name of storage structure

Storage form   with husk
                      without husk
                      grains

During the storage period do you change your storage structure?   Yes
                                                                          No

Name of storage structure

Storage form   with husk
                      without husk
                      grains

What storage problems do you have?
      Insects
      Rodents
      Birds
      Fungi

What storage protection do you use?
            Commercial
            Traditional

For how many months do you store maize before the stock is exhausted?

Number of months:

<u>INFORMATIONS ABOUT THE SAMPLE</u>
Storage structure:
Size of the store:
Date of harvest:

Storage   with husk
                                                                                without husk
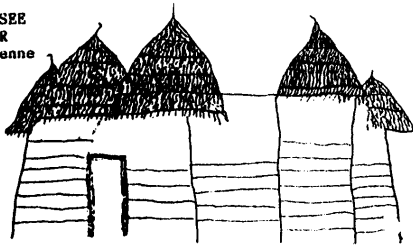   as grains

Annex III

Original questionnaire was administered in french, here the english translation is shown.

Questionaire after 6 months of storage in 94-95

Agroecological zone:
Village:
Farmer:

1) What variety did you use?
          local
          improved

2) For how long do you leave maize in the field to dry before harvesting it?
          Number of days:

3) Do you sort your maize?   Yes
                                               No

4) What storage structure(s) do you use?
          Storage structure(s):

          Size of the storage structure(s):

          Date of harvest:

5) Storage   with husk
                        without husk
                        grains

6) Do you use insecticides or traditional methods to protect your stored maize?

7) For how many months do you store your maize?

          Number of months:

Illustrations of storage structures in Benin (FAO 1992)

PARTIE UTILISEE
COMME GRENIER
Capacité moyenne
0,8 tonne

Maison tata Somba dont la partie supérieure est aménagée sous forme de grenier en terre en vue du stockage de grains de céréales et de cossettes de manioc/igname
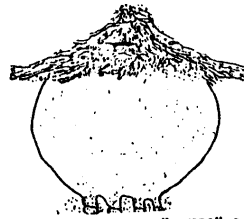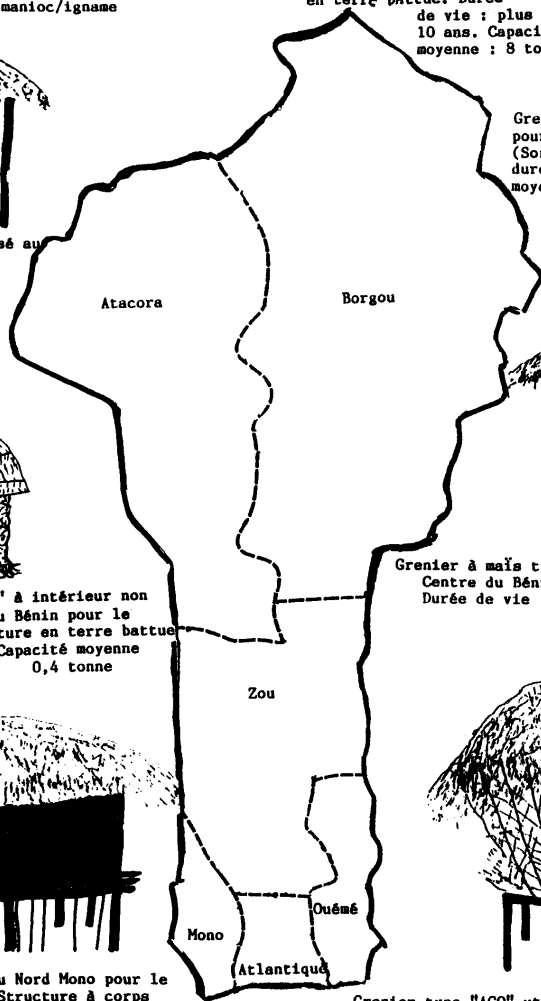
Grenier en terre type "BANCO" à intérieur cloisonné, utilisé pour le stockage des grains de céréales et de cossettes de manioc /igname. Structure en terre battue. Durée de vie : plus de 10 ans. Capacité moyenne : 8 tonne
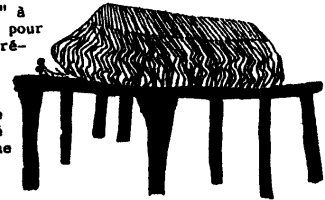
Grenier "PLATE-FORME" utilisé au champ pour stockage provisoire des Céréales (Sorgho/Mil et Maïs) en épis, le contenant dure une campagne agricole, le support en moyenne 3-5 campagnes agricoles - Capacité 1 tonne
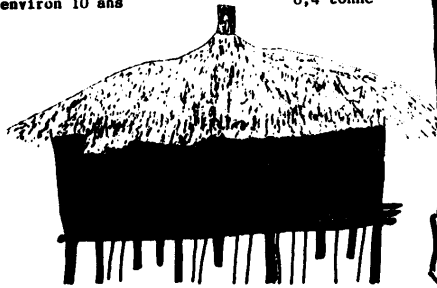
Grenier type "SECCO" utilisé au Nord du Bénin pour le stockage du Sorgho, Mil et le Maïs en épis. Structure en matériaux végétaux. Durée de vie : 1-2 ans Capacité moyenne : 1 tonne

Atacora

Borgou

Grenier en terre type "KOZOUN" à intérieur non cloisonné utilisé au Centre du Bénin pour le stockage du maïs grain. Structure en terre battue. Durée de vie : environ 10 ans   Capacité moyenne 0,4 tonne
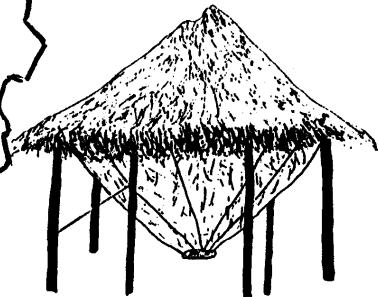
Grenier à maïs type "ZINGO à fond cônique utilisé au Centre du Bénin. Structure en matériaux végétaux Durée de vie : 1-2 ans - capacité moyenne : 1 tonne

Zou

Mono

Ouémé

Atlantique

Grenier type "AVA" utilisé au Nord Mono pour le stockage du maïs en spathe. Structure à corps constitué par les spathes. Durée de vie du support : 2-3 ans. Capacité moyenne : 2 tonnes
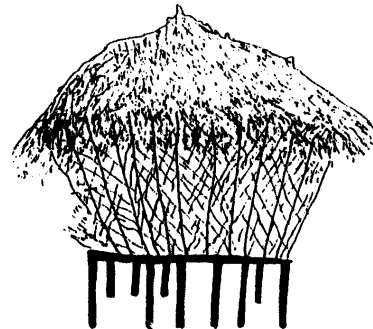
Grenier type "AGO" utilisé au Sud du Bénin pour le stockage du maïs en spathe. Structure en matériaux végétaux à durée de vie courte (moins de 2 ans). Capacité moyenne comprise entre 0,8 et 2 tonnes