

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

**Von dem Fachbereich Gartenbau
der Universität Hannover
zur Erlangung
des akademischen Grades eines**

Doktors der Gartenbauwissenschaften

**-Dr. rer. hort.-
genehmigte Dissertation
von**

Dipl. Ing agr. Kerstin Hell

geboren am 28.7.1962 in Bevensen

1997

Referent: **Prof. Dr. H.-M. Poehling**

Korreferent: **Prof. Dr. S. Vidal**

Tag der Promotion: **19. Dezember 1997**

Table of contents	Page
CHAPTER 1 Introduction	1
1.1 Background of the research topic	1
1.2 Objectives of this study	2
1.3 Agroecological zones of Benin	3
1.4 Outline of this study	7
CHAPTER 2 Literature Review	10
2.1 The genus <i>Aspergillus</i>	10
2.2 Aflatoxins and their effects on human health	13
2.3 Factors influencing <i>Aspergillus</i> spp. infection and aflatoxin development in maize	17
2.3.1 Climatic factors	17
2.3.2 Agronomic factors	20
2.3.3 Biotic factors	24
2.4 Aflatoxin survey reports in agroecological regions	26
2.5 Past work on aflatoxins in West-Africa	26
CHAPTER 3 Temporal and spatial distribution of aflatoxins	29
3.1 Introduction	29
3.2 Materials and methods	29
3.2.1 Influence of position in a granary and freezing on aflatoxin content	29
3.2.2 Influence of storage treatments on aflatoxin content	30
3.2.3 Temporal and spatial distribution of <i>Aspergillus</i> spp. in an experimental granary	31
3.3 Result	31
3.3.1 Influence of position in a granary and freezing on aflatoxin content	31
3.3.2 Influence of storage treatments on aflatoxin content	32
3.3.3 Position and time trial	35
3.4 Discussion	36
CHAPTER 4 Maize production, harvest and storage practices in four agroecological zones in Benin	38
4.1 Introduction	38
4.2 Materials and methods	38
4.3 Results	40
4.3.1 Maize production practices in the four agroecological regions of Benin	40
4.3.2 Maize harvest practices in the four agroecological regions of Benin	43
4.3.3 Pre-storage practices in the four agroecological regions of Benin	45

4.3.3.1	Drying practices in the four agroecological regions of Benin	45
4.3.3.2	Sorting practices in the four agroecological regions of Benin	47
4.3.3.3	Dehusking and degraining practices in the four agroecological regions of Benin	48
4.3.4	Storage practices in the four agroecological regions of Benin	49
4.3.4.1	Storage structures in the four agroecological regions of Benin	49
4.3.4.2	Storage problems in the four agroecological regions of Benin	55
4.4.4.3	Consumption practices in the four agroecological regions of Benin	59
4.4	Discussion	59

CHAPTER 5

Distribution of <i>Aspergillus</i> spp. and aflatoxin	64
contamination in four agroecological zones in Benin	

5.1	Introduction	64
5.2	Materials and Methods	65
5.2.1	Sampling procedure	65
5.2.2	Microbiological evaluation	66
5.2.3	Grain moisture content (G.M.C.)	66
5.2.4	Aflatoxin analysis	67
5.2.4.1	Extraction of the samples	67
5.2.4.2	Thin-layer chromatography	67
5.2.5	Statistical analysis	68
5.3	Result	69
5.4	Discussion	74

CHAPTER 6 Influence of Agronomic practices on aflatoxin contamination 78

6.1	Introduction	78
6.2	Materials and Methods	79
6.3	Results	80
6.3.1	Production Factors	80
6.3.2	Harvest Factors	83
6.3.3	Storage Factors	87
6.4	Discussion	92
6.4.1	Production Factors	92
6.4.2	Harvest Factors	95
6.4.3	Storage Factors	97

CHAPTER 7 Storage structure, storage form and aflatoxin 102

7.1	General introduction	102
7.2	Materials and Methods	109
7.3.	Results	109
7.4	Discussion	112

CHAPTER 8 Insect infestation of stored maize in four agroecological regions in Benin and contamination with aflatoxins	115
8.1 Introduction	115
8.2 Materials and Methods	116
8.3 Results	117
8.4 Discussion	121
CHAPTER 9 Storage trials	123
9.1 Introduction	123
9.2 Materials and Methods	123
9.3 Results	125
9.4 Discussion	129
CHAPTER 10 Conclusion and potential solutions to the aflatoxin problem in Benin	132
References	137
Acknowledgements	
Curriculum vitae	
Annex	

List of Figures	Page
CHAPTER 1 Introduction	
Figure 1.1: The four agroecological zones of Benin with survey sites	4
CHAPTER 2 Literature Review	
Figure 2.1: Conidiophore of the <i>Aspergillus</i> spp.	10
(a) uniseriate species (b) biseriate species (Klich & Pitt 1988)	
Figure 2.2: Development cycle of <i>A. flavus</i> in the soil and on the plant (Wicklow & Donahue 1984)	11
Figure 2.3: Biosynthetic pathway of aflatoxins (Bhatnagar <i>et al.</i> 1993)	13
CHAPTER 3 Temporal and spatial distribution of aflatoxins	
Figure 3.1: Density of <i>Prostephanus truncatus</i> /kg grain by treatment over 12 sampling occasions (b) density of <i>Sitophilus zeamais</i> /kg grain by treatment over 12 sampling occasions, and (c) grain losses (%) by treatment over 12 sampling occasions (Borgemeister <i>et al.</i> 1994).	34

List of Tables	Page
CHAPTER 2 Literature Review	
Table 2.1: Optimum temperatures for the development of different <i>Aspergillus</i> spp. and <i>Penicillium</i> spp. (compiled Lacey & Magan 1991; Wilson & Abramson 1992)	18
Table 2.2: Minimum water activity (aw) for the growth of different <i>Aspergillus</i> spp. and <i>Penicillium</i> spp. (compiled Lacey & Magan 1991)	20
CHAPTER 3 Temporal and spatial distribution of aflatoxins	
Table 3.1: Influence of position on the mean fungal contamination of maize stored for 36 weeks (Evaluation of 75 kernels per position)	32
Table 3.2: Influence of cob selection and fumigation on <i>Aspergillus</i> spp. and mean total fungal contamination of maize stored for 36 weeks (mean of 100 kernels per sample) mean + S.E.	32
Table 3.3: Fungal distribution at the top, middle and bottom of the maize store after six months of storage (92-93)	35
Table 3.4: Fungal distribution at the top, middle and bottom of the maize store after nine months of storage (92-93)	35
CHAPTER 4 Maize production, harvest and storage practices in four agroecological zones in Benin	
Table 4.1: Pest problems for % farmers in the different agroecological zones of Benin (93-94)	41
Table 4.2: Use of fertilizer for % farmers in the different agroecological zones of Benin (94-95)	42
Table 4.3: Use of local or improved maize variety for % farmers in the different agroecological zones of Benin (May-April 95)	42
Table 4.4: Huskcover rating for % farmers in the different agroecological zones of Benin (93-94)	43
Table 4.5: Harvesting practice for % farmers in the different agroecological zones of Benin (93-94)	44
Table 4.6: Harvest period for % farmers in the different agroecological zones of Benin (94-95)	45
Table 4.7: Field drying periods for % farmers in the different agro-ecological zones of Benin (93-94)	45
Table 4.8: Drying time in the field for % farmers in the different agro-ecological zones of Benin (94-95)	46
Table 4.9: Drying form in the field for % farmers in the different agro-ecological zones of Benin (94-95)	47
Table 4.10: Removal of cobs for bad huskcover by % farmers in the different agroecological zones of Benin (93-94)	47
Table 4.11: Sorting of maize by % farmers in the different agroecological zones of Benin (93-94)	48
Table 4.12: Time of dehusking for % farmers in the different agroecological zones of Benin (94-95)	49

Table 4.13:	Pre-storage practices for % farmers in the different agro-ecological zones of Benin (93-94)	50
Table 4.14:	Types of storage structures for % farmers in the different agro-ecological zones of Benin (93-94)	50
Table 4.15:	Storage form for the % farmers in the different agroecological zones of Benin (93-94)	51
Table 4.16:	Storage period for % farmers in the different agroecological zones of Benin (93-94)	51
Table 4.17:	Storage structures for % farmers in the different agroecological zones of Benin (September-December 94)	52
Table 4.18:	Types of secondary storage structures for % farmers in the different agroecological zones of Benin (September-December 94)	53
Table 4.19:	Types of storage structures for farmers in the different agroecological zones of Benin (March-April 95)	53
Table 4.20:	Storage form for % farmers in the different agroecological zones of Benin (September-December 94)	54
Table 4.21:	Storage form for % farmers in the different agroecological zones of Benin (March-April 95)	54
Table 4.22:	Storage period of maize for % farmers in the different agro-ecological zones of Benin (September-December 94)	54
Table 4.23:	Storage period of maize for % farmers in the different agro-ecological zones of Benin (March-April 95)	55
Table 4.24:	Storage problems for % farmers in the different agroecological zones of Benin (93-94)	55
Table 4.25:	Storage treatment for % farmers in the different agroecological zones of Benin (93-94) (Multiple answers were possible)	56
Table 4.26:	Storage problems for % farmers in the different agroecological zones of Benin (September-December 94)	57
Table 4.27:	Storage Pests for % farmers in the different agroecological zones of Benin (March-April 95)	58
Table 4.28:	Storage treatment for % farmers in the different agroecological zones of Benin (March-April 95)	58
Table 4.29:	Number of maize meals eaten per day by % farmers in the different agroecological zones of Benin (93-94)	59

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

Table 5.1:	Distribution of aflatoxins (mean ppb) and accompanying fungi (% of 25 grains) in 300 grain stores in the four agroecological zones of Benin during August-December 1993 (survey 1)	71
Table 5.2:	Distribution of aflatoxins (mean ppb) and accompanying fungi (% of 25 grains) in 150 grain stores in the four agroecological zones of Benin during April-May 1994 (survey 2)	71
Table 5.3:	Distribution of aflatoxins (mean ppb) and accompanying fungi (% of 25 grains) in 150 grain stores in the four agroecological zones of Benin during September-December 1994 (survey 3)	72

Table 5.4:	Distribution of aflatoxins (mean ppb) and accompanying fungi (% of 25 grains) in 150 grain stores in the four agroecological zones of Benin during April-May 1995 (survey 4)	72
Table 5.5:	Percent stores that were aflatoxin positive and percentage of samples >20ppb in the different ecoregions in 93-94 and 94-95	74

CHAPTER 6 Influence of Agronomic practices on aflatoxin contamination

Table 6.1:	Regression analysis of maize production factors that influence aflatoxin at the beginning of storage (93-94)	80
Table 6.2:	Regression analysis of maize production factors that influence aflatoxin after six months of storage (93-94)	81
Table 6.3:	Regression analysis of maize production factors that influence aflatoxin at the beginning of storage (94-95)	82
Table 6.4:	Regression analysis of maize production factors that influence aflatoxin after six months of storage (94-95)	83
Table 6.5:	Regression of harvest factors that influence aflatoxin at the beginning of storage (93-94)	84
Table 6.6:	Regression of harvest factors that influence aflatoxin after six months of storage (93-94)	84
Table 6.7:	Regression of harvest factors that influence aflatoxin at the beginning of storage (94-95)	86
Table 6.8:	Regression of harvest factors that influence aflatoxin after six months of storage (94-95)	87
Table 6.9:	Regression of storage factors that influence aflatoxin at the beginning of storage (93-94)	88
Table 6.10:	Regression of storage factors that influence aflatoxin after six months of storage (93-94)	89
Table 6.11:	Regression of storage factors that influence aflatoxin at the beginning of storage (94-95)	90
Table 6.12:	Regression of storage factors that influence aflatoxin after six months of storage (94-95)	91

CHAPTER 7 Storage structure, storage form and aflatoxin

Table 7.1:	Utilisation of different storage structures and their aflatoxin content in the FMS of Benin	110
Table 7.2:	Utilisation of different storage structures and their aflatoxin content in the SGS of Benin	111
Table 7.3:	Utilisation of different storage structures and their aflatoxin content in the NGS of Benin	111
Table 7.4:	Utilisation of different storage structures and their aflatoxin content in the SS of Benin	112

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

Table 8.1:	Grain moisture content and aflatoxin contamination for insect cob damage classes on maize beginning of storage (1993/94)	118
------------	--	-----

Table 8.2	Mean aflatoxin, mean numbers of insects, mean cobs/sample infected with insect species in the agroecological zones at the beginning of storage in the year 94/95 (untransformed data)	120
Table 8.3	Mean aflatoxin, mean numbers of insects, mean cobs/sample infected with insect species in the agroecological zones at six months of storage in the year 94/95	120

CHAPTER 9 Storage trials

Table 9.1:	Means comparison for aflatoxin (ppb), fungal contamination (%), grain moisture content (%) for treatments (94/95 season) after three months after storage	125
Table 9.2:	Means comparison for aflatoxin (ppb), fungal contamination (%), grain moisture content (%) for treatments (94/95 season) after six months after storage	126
Table 9.3:	Means comparison for insect variables for treatments (94/95 season) at three months after storage	126
Table 9.4:	Means comparison for insect variables for treatments (94/95 season) at six months after storage	127
Table 9.5:	Means comparison for aflatoxin (ppb), fungal contamination (%), grain moisture content (%) for treatments (95/96 season) at three months after storage	127
Table 9.6:	Means comparison for aflatoxin (ppb), fungal contamination (%), grain moisture content (%) for treatments (95/96 season) at six months after storage	128
Table 9.7:	Means comparison for insect variables for treatments (95/96 season) after three months of storage	129
Table 9.8:	Means comparison for insect variables for treatments (95/96 season) after six months of storage	129

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

Table 10.1	Farming practices that were associated with higher and lower aflatoxin levels in stored maize in Benin (93/94 and 94/95)	135
------------	--	-----

Annex

- I. Questionnaire 1993-94
- II. Questionnaire 1994-95 (August - December 1994)
- III. Questionnaire 1994-95 (March -April 1995)
- VI. Illustrations of storage types

List of Abbreviations:

aw	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/Ministère 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	Société National sur la Promotion Agricole
SS	Sudan Savanna

Abstract (Deutsch)

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

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% wiederum 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 Fruchfolge 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 assoziiert: 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 assoziiert 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.