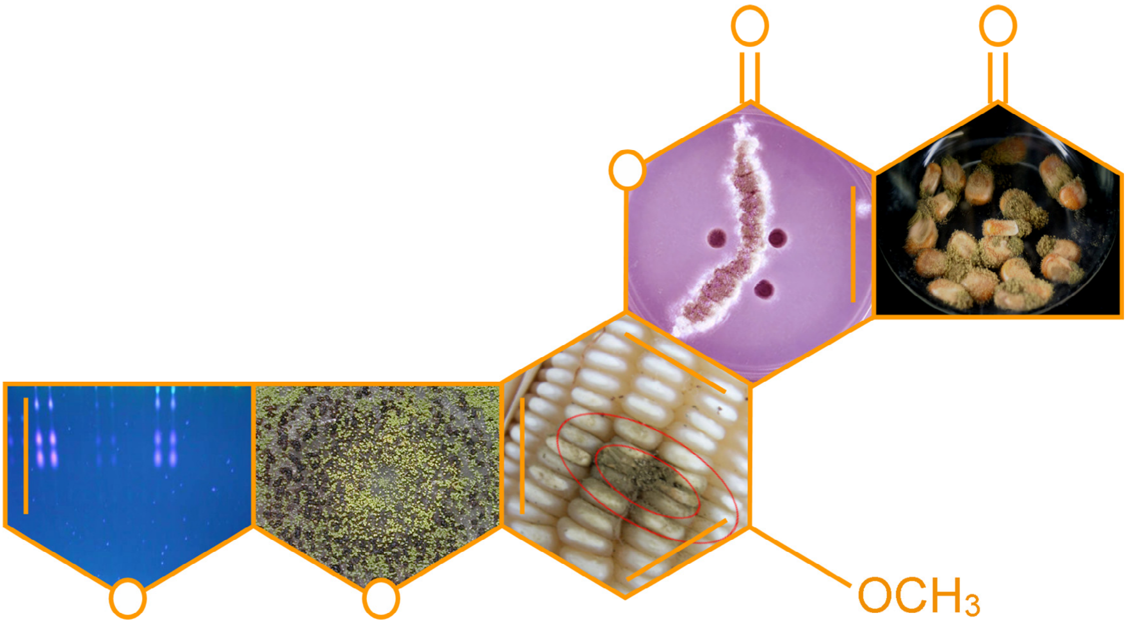
Annotated Bibliography of IITA Publications on Mycotoxins



Compiled by
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Contents

Foreword

Contamination of food by toxin-producing fungi and the mycotoxins they produce are of common occurrence in Africa. Chronic and lifelong exposure of a large proportion of African population to foodborne mycotoxins, probably beginning *in utero*, is a reality and a serious problem. There is an increasing mass of literature that documents the wide ranging health impact of mycotoxins in humans and animals that includes cancer, impairment of growth and development of children, immune suppression, reduced nutrient uptake and utilization, birth defects, and death. Due to their severe effects on health, several countries including a few in Africa, have adopted legislations barring trade in agricultural products exceeding permissible levels of mycotoxins. However, most countries in Africa lack capacity to enforce food safety regulations, and consequently face frequent rejection of exportable products leading to economic loss

In early 1990s the International Institute of Tropical Agriculture (IITA) began active research on mycotoxins. A multidisciplinary research team, often referred as 'IITA Mycotoxin Group' in partnership with advanced research institutes in the US and Europe as well as many national programs in Africa has been devoted in developing viable options to reduce mycotoxin contamination in staple crops and its impact on health and economy. Prominent among the IITA partners are the United States Department of Agriculture (USDA)–Agriculture Research Service in New Orleans and at the University of Arizona, University of Leeds (UK), Kansas State University (USA), University of Georgia (USA), University of Bonn (Germany), Institute of Sciences of Food Production (ISPA) – National Research Council (Italy), Università Cattolica del Sacro Cuore (Italy), University of Natural Resources and Applied Life Sciences (Austria), University of Aarhuus (Denmark) and national institutions in Benin, Burkina Faso, Cameroon, Ghana, Mozambique, Nigeria, Senegal, Tanzania, Togo, and Zambia. Students and visiting scientists from several universities and national programs in Africa have enriched mycotoxin research at IITA.

Research-for-Development areas of this group encompassing pathologists, post-harvest biologist, breeders, molecular biologist, socio-economists, medical epidemiologist and communication specialists included assessment of the extent of mycotoxin contamination in different agricultural commodities from a systems perspective (biotic, abiotic and socioeconomic aspects), ecology and distribution of the causal agents, insect-fungus-toxin interactions, effects on health especially children, impact on trade, and management of contamination through host resistance, biocontrol, cultural, post-harvest, food processing, and raising awareness and sensitizing policy makers.

Efforts of this research group has contributed to valuable knowledge and products, which is captured in this annotated bibliography, It is a compilation of citations and abstracts of publications authored or co-authored by IITA staff since 1995 with the aim of sharing the breadth of IITA research-for-development activities on mycotoxins. The bibliography is partitioned into different sections: health, host plant resistance, biocontrol/ecology, survey, management, insect-fungus-toxin interactions and awareness for easy access to different publications. Citations within each section are arranged chronologically.

I take this opportunity to acknowledge the IITA core donors, BMZ (Germany), Austrian Development Agency (Austria), Sorghum and Millets Collaborative Research Support Program of USAID (INTSORMIL, USA), USDA–Foreign Agriculture Service, DANIDA (Denmark), Rotary International (USA), the African Agriculture Technology Foundation, and Standards and Trade Development Facility of the World Trade Organization, as well as private sector investors such as Nestle for funding mycotoxin research at IITA. IITA will continue its endeavor to tackle this recalcitrant problem affecting millions of poor farmers and agriculture economies of countries in sub-Saharan Africa.

Finally, I would like to congratulate Drs Ranajit Bandyopadhyay, Kerstin Hell and the entire Mycotoxin Group for their effort in preparing this document. I hope that this compilation would be useful to those interested in conducting research and ameliorate mycotoxin problem worldwide.

Paula Bramel
Deputy Director General
Research-for- Development, IITA
16 March 2009

1. Health

Ewuola, E.O., Gbore, F.A., Ogunlade, J.T., Bandyopadhyay, R., Niezen, J., and Egbunike, G.N. 2008. **Physiological response of rabbit bucks to dietary fumonisin: performance, haematology and serum biochemistry**. *Mycopathologia* 165: 99-104.

Maize grains contaminated with fumonisin, a metabolite of *Fusarium verticillioides* was incorporated into matured male rabbits' diet to evaluate its effects on performance, haematology and serum biochemistry in rabbits. Thirty individually caged crossbred adult rabbit bucks averaging 1.36 ± 0.01 kg (about 22–24-week-old) were randomly allotted to three treatment diets comprising a control diet (containing 0.35 ± 0.02 mg fumonisin/kg) and two test diets containing 12.30 ± 0.16 and 24.56 ± 0.14 mg fumonisin/kg, constituting treatments 1 (low infection), 2 (medium infection) and 3 (high infection), respectively, in a five-week feeding trial. Results showed that the dry matter intake (DMI) (g/rabbit) at the end of the feeding trial μwas significantly ($P < 0.05$) influenced. The DMI declined with increasing dietary fumonisin by a significant 80% and 95% ($P < 0.05$) for high and medium levels of dietary fumonisin, respectively, relative to the mean weekly DMI of 609.93 ± 45.08 g by rabbits fed diet with low level of fumonisin. The weekly weight gain tended to decrease with increased dietary fumonisin levels, while the haematological and serum biochemical components examined, were not statistically influenced among the diets when fed to male rabbits for a period of 5 weeks.

Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R.V., Breiman, R., Brune, M-N., DeCock, K., Dilley, A., Groopman, J., Hell, K., Henry, S.H., Jeffers, D., Jolly, C., Jolly, P., Kibata, G.N., Lewis, L., Liu, X., Luber, G., McCoy, L., Mensah, P., Miraglia, M., Misore, A., Njapau, H., Ong, C-N., Onsongo, M.T.K., Page, S.W., Park, D., Patel, M., Phillips, T., Pineiro, M., Pronczuk, J., Rogers, H.S., Rubin, C., Sabino, M., Schaafsma, A., Shephard, G., Stroka, J., Wild, C., Williams, J.T., and Wilson, D., 2006. **Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries.** *Environmental Health Perspectives* 114: 1898-1903.

Consecutive outbreaks of acute aflatoxicosis in Kenya in 2004 and 2005 caused > 150 deaths. In response, the Centers for Disease Control and Prevention and the World Health Organization convened a workgroup of international experts and health officials in Geneva, Switzerland, in July 2005. After discussions concerning what is known about aflatoxins, the workgroup identified gaps in current knowledge about acute and chronic human health effects of aflatoxins, surveillance and food monitoring, analytic methods, and the efficacy of intervention strategies. The workgroup also identified public health strategies that could be integrated with current agricultural approaches to resolve gaps in current knowledge and ultimately reduce morbidity and mortality associated with the consumption of aflatoxin-contaminated food in the developing world. Four issues that warrant immediate attention were identified: *a*) quantify the human health impacts and the burden of disease due to aflatoxin exposure; *b*) compile an inventory, evaluate the efficacy, and disseminate results of ongoing intervention strategies; *c*) develop and augment the disease surveillance, food monitoring, laboratory, and public health response capacity of affected regions; and *d*) develop a response protocol that can be used in the event of an outbreak of acute aflatoxicosis. This report expands on the workgroup's discussions concerning aflatoxin in developing countries and summarizes the findings.

Cardwell, K.F., and Henry, S.H. 2005. **Risk of exposure to mitigation of effects of aflatoxin on human health: a West African example.** Pages 213-235 *in* Abbas, H.K. (Ed.), Aflatoxin and Food Safety. Taylor & Francis Group, Bota Racon, Florida.

This book chapter examines the relative risk of exposure of different human populations to foodborne aflatoxins, the types of health impact that may be incurred by dietary exposure to aflatoxins, and the possible strategies likely to mitigate risks to human health. Risk of exposure is examined in a global context comparing the risk of toxin exposure by levels of national socioeconomic development. The risk of exposure is then reexamined in the context of agroecology, distribution of toxigenicity of *Aspergillus flavus*, and social factors that influence food management practices. The effects of aflatoxins on human health are explored in three sections: human disease and nutritional status, carcinogenicity, and child growth and development. The section concerning mitigation of the effects of aflatoxins on human health contrasts efficacy of regulation, food basket modification, and production-side agriculture interventions. It is concluded that the risk of hepatocellular carcinoma in developing countries, such as West Africa, may be addressed by vaccination for hepatitis B virus and other public health options. Young children in West Africa who are chronically exposed to aflatoxins in foods and who consume nutritionally deficient diets have been shown to be stunted and underweight, as measured by World Health Organization z-scores.

Egal, S., Hounsa, A., Gong, Y.Y.,Turner**,** P.C., Wild**,** C.P., Hall, A.J., Hell, K., Cardwell, K.F. 2005. **Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa.** *International Journal of Food Microbiology* 104:215-224.

Aflatoxins are a family of fungal toxins that are carcinogenic to man and cause immunosuppression, cancer and growth reduction in animals. We conducted a cross-sectional study among 480 children (age 9 months to 5 years) across four agro-ecological zones in Benin and Togo to identify the effect of aflatoxin exposure on child growth and assess the pattern of exposure. Prior reports on this study [Gong et al., 2002. BMJ 325, 20–21; Gong et al., 2003. Int. J Epid. 32, 556–562] showed that aflatoxin exposure among these children is widespread (99%) and that growth faltering is associated with high blood aflatoxin-albumin adducts (AF-alb adducts), a measure of recent past exposure. The present report demonstrates that consumption of maize is an important source of aflatoxin exposure for the survey population. Higher AF-alb adducts were correlated with higher *A. flavus* (CFU) infestation of maize (p = 0.006), higher aflatoxin contamination (ppb) of maize (p < 0.0001) and higher consumption frequencies of maize (p = 0.053). The likelihood of aflatoxin exposure from maize was particularly high in agro-ecological zones where the frequency of maize consumption (Southern Guinea Savanna and Coastal Savanna), the presence of aflatoxin in maize (Southern Guinea Savanna) or the presence of *A. flavus* on maize (Northern Guinea Savanna and Southern Guinea Savanna) was relatively high. Socio-economic background did not affect the presence of *A. flavus* and aflatoxin in maize, but better maternal education was associated with lower frequencies of maize consumption among children from the northernmost agro-ecological zone (Sudan Savanna) (p = 0.001). The impact of groundnut consumption on aflatoxin exposure was limited in this population. High AF-alb adduct levels were correlated with high prevalence of *A. flavus* and aflatoxin in groundnut, but significance was weak after adjustment for weaning status, agro-ecological zone and maternal socio-economic status (p = 0.091 and p = 0.083), respectively. Ingestion of *A. flavus* and aflatoxin was high in certain agro-ecological zones (Sudan Savanna and Southern Guinea Savanna) and among the higher socio-economic strata due to higher frequencies of groundnut consumption. Contamination of groundnuts was similar across socio-economic and agro-ecological boundaries. In conclusion, dietary exposure to aflatoxin from groundnut was less than from maize in young children from Benin and Togo. Intervention strategies that aim to reduce dietary exposure in this population need to focus on maize consumption in particular, but they should not ignore consumption of groundnuts.

Gong, Y.Y., Hounsa, A., Egal, S., Turner, P.C., Sutcliffe, A.E., Hall, A.J., Cardwell, K.F., and Wild, C.P. 2004. **Postweaning exposure to aflatoxin results in impaired child growth: A longitudinal study in Benin, West Africa**. *Environmental Health Perspectives* 112: 1334-1338.

Aflatoxins are dietary contaminants that are hepatocarcinogenic and immunotoxic and cause growth retardation in animals, but there is little evidence concerning the latter two parameters in exposed human populations. Aflatoxin exposure of West African children is known to be high, so we conducted a longitudinal study over an 8-month period in Benin to assess the effects of exposure on growth. Two hundred children 16–37 months of age were recruited from four villages, two with high and two with low aflatoxin exposure (50 children per village). Serum aflatoxin–albumin (AF-alb) adducts, anthropometric parameters, information on food consumption, and various demographic data were measured at recruitment (February) and at two subsequent time points (June and October). Plasma levels of vitamin A and zinc were also measured. AF-alb adducts increased markedly between February and October in three of the four villages, with the largest increases in the villages with higher exposures. Children who were fully weaned at recruitment had higher AF-alb than did those still partially breast-fed ($p < 0.0001$); the major weaning food was a maize-based porridge. There was no association between AF-alb and micronutrient levels, suggesting that aflatoxin exposure was not accompanied by a general nutritional deficiency. There was, however, a strong negative correlation ($p < 0.0001$) between AF-alb and height increase over the 8-month follow-up after adjustment for age, sex, height at recruitment, socioeconomic status, village, and weaning status; the highest quartile of AF-alb was associated with a mean 1.7 cm reduction in growth over 8 months compared with the lowest quartile. This study emphasizes the association between aflatoxin and stunting, although the underlying mechanisms remain unclear. Aflatoxin exposure during the weaning period may be critical in terms of adverse health effects in West African children, and intervention measures to reduce exposure merit investigation.

Gong, Y.Y., Egal, S., Hounsa, A., Turner, P.C., Hall, A.J., Cardwell, K.F., and Wild, C.P. 2003. **Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning**. *International Journal of Epidemiology* 32: 556-562.

Background: Dietary exposure to high levels of the fungal toxin, aflatoxin, occurs in West Africa, where long-term crop storage facilitates fungal growth.

Methods: We conducted a cross-sectional study in Benin and Togo to investigate aflatoxin exposure in children around the time of weaning and correlated these data with food consumption, socioeconomic status, agro-ecological zone of residence, and anthropometric measures. Blood samples from 479 children (age 9 months to 5 years) from 16 villages in four agro-ecological zones were assayed for aflatoxin-albumin adducts (AF-alb) as a measure of recent past (2–3 months) exposure.

Results: Aflatoxin-albumin adducts were detected in 475/479 (99%) children (geometric mean 32.8 pg/mg, 95% CI: 25.3–42.5). Adduct levels varied markedly across agro-ecological zones with mean levels being approximately four times higher in the central than in the northern region. The AF-alb level increased with age up to 3 years, and within the 1–3 year age group was significantly (P = 0.0001) related to weaning status; weaned children had approximately twofold higher mean AF-alb adduct levels (38 pg AF-lysine equivalents per mg of albumin [pg/mg]) than those receiving a mixture of breast milk and solid foods after adjustment for age, sex, agro-ecological zone, and socioeconomic status. A higher frequency of maize consumption, but not groundnut consumption, by the child in the preceding week was correlated with higher AF-alb adduct level. We previously reported that the prevalence of stunted growth (height for age Z-score HAZ) and being underweight (weight for age Z-score WAZ) were 33% and 29%, respectively by World Health Organization criteria. Children in these two categories had 30–40% higher mean AF-alb levels than the remainder of the children and strong dose–response relationships were observed between AF-alb levels and the extent of stunting and being underweight.

Conclusions: Exposure to this common toxic contaminant of West African food increases markedly following weaning and exposure early in life is associated with reduced growth. These observations reinforce the need for aflatoxin exposure intervention strategies within high-risk countries, possibly targeted specifically at foods used in the post-weaning period.

Gong, Y.Y., Cardwell, K.F., Hounsa, A., Egal, S., Turner, P.C., Hall, A.J., and Wild, C.P. 2002. **Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study.** *British Medical Journal* 325: 20-21.

Fetal and early childhood environment, including the nutritional status of the pregnant mother and the infant, are considered critical for growth and risk of disease in later life. Many people in developing countries are not only malnourished but also chronically exposed to high levels of toxic fungal metabolites such as carcinogenic and immunotoxic aflatoxins that cause growth retardation in animals. High exposure to aflatoxins occurs throughout childhood in the West African region, suggesting that growth and development could be critically affected. We assessed exposure to aflatoxins in relation to anthropometric measures in children in Benin and Togo. We studied 480 children (aged 9 months to 5 years) from 16 villages in four geographic zones (four in each zone): Sudan Savanna, Northern Guinea Savanna, Southern Guinea Savanna, and Coastal Savannah. We detected aflatoxin-albumin adducts in 475/479 (99%) samples, with a geometric mean concentration of 32.8 (range 5 to 1064) pg/mg albumin. Children with stunting or who were underweight had 30 to 40% higher mean aflatoxin-albumin concentrations. In a categorical analysis, the association with aflatoxin-albumin concentration was significant, with clear dose-response relations with height for age and weight for age z scores.

This study revealed a striking association between exposure to aflatoxin in children and both stunting (a reflection of chronic malnutrition) and being underweight (an indicator of acute malnutrition). In West Africa, people are chronically exposed to high levels of aflatoxins starting *in utero* and continuing throughout life. In this study, children still partially breast fed had lower exposure, almost certainly reflecting lower toxin levels in milk than in weaning and family foods. Thus growth faltering occurs at a time of change to solid foods, when there is co-exposure to aflatoxin and a plethora of infectious hazards (for example, malaria, diarrhea, respiratory infections). Whether the association between aflatoxin exposure and impaired growth is a direct result of aflatoxin toxicity or reflects consumption of fungus affected food of poor nutritional quality cannot be confirmed from the cross sectional design. However, these observations emphasize the need to investigate this question and to develop strategies to reduce exposure to aflatoxin, possibly involving interventions targeted at the post-weaning period in African children.

Cardwell, K.F. 2001. **Mycotoxin contamination of foods in Africa: Anti-nutritional factors**. *Food and Nutrition Bulletin* 21: 488-492.

Mycotoxins come under regulatory limits in foods and feeds because they are carcinogens. Nevertheless, in addition to tumorigenic properties, many mycotoxins are anti-nutritional factors causing unthrifty growth and development of young animals. In the developed world, human exposure, and particularly exposure of children, to dietary mycotoxins is virtually non-existent because of regulatory standards. In developing countries, monitoring and enforcement of standards is rare. People are being exposed to unsafe levels of various mycotoxins, often in mixtures, and the consequences in terms of public health burden have been ignored. This paper presents information on the health effects that have been attributed to mycotoxin exposure from medical research literature; data on existing mycotoxin levels in west and central Africa; and nutritional indicators on Béninoise children with co-incidental appearance of symptoms of mycotoxin exposure with the weaning of children onto solid foods. The International Institute of Tropical Agriculture, in its maize IPM project, has recognized mycotoxins as one of the most important constraints to the goal of fostering human health and well being through agriculture. An overview of various research and development activities is given.

2. Host Plant Resistance

Brown, R.L., Chen, Z-Y., Cleveland, T.E., Menkir, A. 2008. **Identification of maize breeding markers through investigations of proteins associated with aflatoxin-resistance**. Paper presented at the American Chemical Society Symposium. April 8-10, 2008, New Orleans, Louisiana, USA. (Abstract)

The goal of a collaborative research project between International Institute of Tropical Agriculture (IITA) in Nigeria and ARS-Southern Regional Research Center (SRRC) in New Orleans is to develop maize inbred lines with resistance against aflatoxin contamination by *Aspergillus flavus*. A second goal is to identify gene markers in these lines to facilitate their use in U.S. breeding and African national programs. To accomplish this, comparative proteomics of near-isogenic lines varying in aflatoxin accumulation is being employed to identify kernel proteins associated with resistance (RAPs). A number of RAPs have been identified and several further characterized through physiological and biochemical investigations conducted to determine a potential role in resistance and, therefore, fitness as breeding markers. Three RAPs, a trypsin inhibitor, pathogenesis-related protein and glyoxalase I are being investigated as well, using RNAi gene silencing and plant transformation. Results of proteome and characterization studies are discussed.

Menkir, A., Brown, R.L., Bandyopadhyay, R. and Cleveland, T.E. 2008. **Registration of six tropical maize germplasm lines with resistance to aflatoxin contamination**. *Journal of Plant Registrations* 2: 246-250.

Six tropical maize (*Zea mays* L.) germplasm lines, TZAR101 (Reg. No. GP-568, PI 654048), TZAR102 (Reg. No. GP-569, PI 654049), TZAR103 (Reg. No. GP-570, PI 654050), TZAR104 (Reg. No. GP-571, PI 654051), TZAR105 (Reg. No. GP-572, PI 654052), and TZAR106 (Reg. No. GP-573, PI 654053), with resistance to aflatoxin contamination were developed by the International Institute of Tropical Agriculture through a collaborative breeding project with Southern Regional Research Center of the USDA-ARS. The lines were derived from biparental crosses and backcross populations involving aflatoxin-resistant tropical elite and temperate inbred lines as parents. These lines had aflatoxin levels similar to or lower than a resistant U.S. inbred check, MI82, in both preliminary and confirmation tests conducted in the laboratory using a kernel-based screening assay. Further field tests of the six lines under artificial inoculation with an African strain of *Aspergillus flavus* in Nigeria revealed that these lines had lower levels of aflatoxin compared with elite tropical commercial inbred lines used as checks. These lines also had good agronomic traits and resistance to important diseases in the lowlands, including southern corn leaf blight [caused by *Bipolaris maydis* (Nisikado & Miyake) Shoemaker], southern corn rust (caused by *Puccinia polysora* Underw.), and ear rot.

Menkir, A., Brown, R.L., Bandyopadhyay, R., Chen, Z., and Cleveland, T.E. 2008. **Breeding maize for resistance to mycotoxins at IITA**. Pages 277–286 *in* "MYCOTOXINS: Detection Methods, Management, Public Health and Agricultural Trade." J. F. Leslie, R. Bandyopadhyay and A. Visconti, eds. CABI Publishing, Wallingford, UK.

Ear-rot-causing fungi, including *Aspergillus* and *Fusarium* spp., are common in maize in West and Central Africa. These fungi contaminate maize with mycotoxins that pose serious potential health hazards to humans in these areas. A collaborative germplasm screening project was initiated between the International Institute of Tropical Agriculture (IITA) and the Southern Regional Research Center (SRRC) of the USDA's Agricultural Research Service in 1998 to develop maize germplasm with resistance to aflatoxin contamination. In a laboratory screen, some IITA inbred lines had potential levels of resistance to aflatoxin production as high as or higher than the best lines from the United States. These results prompted the initiation of a breeding project to combine resistance factors from the IITA lines with resistance factors from the US inbred lines. Several crosses and backcross populations were made from selected resistant or potentially resistant inbred lines from the US and IITA. Sixty-five S5 lines were developed from the backcross populations and 144 S5 lines were derived from the F1 crosses. Kernels from these lines were screened in a laboratory assay. Significant differences in aflatoxin accumulation were detected amongst the lines within each group. Several S5 lines in which aflatoxin contamination was significantly less than in either parent were selected for resistance-confirmation tests. We found pairs of S5 lines with 88-97% common genetic backgrounds that differed significantly in aflatoxin accumulation. These pairs of lines are being used for proteomic analyses to identify the proteins and the corresponding genes that limit aflatoxin accumulation. We also found significant differences in fumonisin accumulation amongst 58 elite maize inbred lines in which variation in aflatoxin accumulation was found. Both the new inbreds and the elite lines can be exploited as new genetic sources in breeding programs in which the objective is to develop maize cultivars/hybrids that accumulate lesser amounts of mycotoxins.

Afolabi, C.G., Ojiambo, P.S., Ekpo, E.J.A., Menkir, A., and Bandyopadhyay, R. 2007. **Evaluation of maize inbred lines for resistance to Fusarium ear rot and fumonisin accumulation in grain**. *Plant Disease* 91: 279-286.

Fusarium ear rot and fumonisin contamination is a major problem facing maize growers worldwide, and host resistance is the most effective strategy to control the disease, but resistant genotypes have not been identified. In 2003, a total of 103 maize inbred lines were evaluated for Fusarium ear rot caused by *Fusarium verticillioides* in field trials in Ikenne and Ibadan, Nigeria. Disease was initiated from natural infection in the Ikenne trial and from artificial inoculation in the Ibadan trial. Ear rot severity ranged from 1.0 to 6.0 in both locations in 2003. Fifty-two inbred lines with disease severity ≤3 (i.e., ≤10% visible symptoms on ears) were selected and reevaluated in 2004 for ear rot resistance, incidence of discolored kernels, and fumonisin contamination in grain. At both locations, ear rot severity on the selected lines was significantly ($P < 0.0020$) higher in 2004 than in 2003. The effects of selected inbred lines on disease severity were highly significant at Ikenne ($P = 0.0072$) and Ibadan ($P < 0.0001$) in 2004. Inbred lines did not affect incidence of discolored kernels at both locations and across years except at Ikenne ($P = 0.0002$) in 2004. Similarly, significant effects of inbred lines on fumonisin concentration were observed only at Ikenne ($P = 0.0201$) in 2004. However, inbred lines 02C14585, 02C14593, 02C14603, 02C14606, 02C14624, and 02C14683 had consistently low disease severity across years and locations. Fumonisin concentration was significantly correlated with ear rot only at Ikenne ($R = 0.42$, $P < 0.0001$). Correlation between fumonisin concentration and incidence of discolored kernels was also significant at Ikenne ($R = 0.39$, $P < 0.0001$) and Ibadan ($R = 0.35$, $P = 0.0007$). At both locations, no significant inbred × year interaction was observed for fumonisin concentration. Five inbred lines, namely 02C14585, 02C14603, 02C14606, 02C14624, and 02C14683, consistently had the lowest fumonisin concentration in both trials. Two of these inbred lines, 02C14624 and 02C14585, had fumonisin levels <5.0 µg/g across years in trials where disease was initiated from both natural infection and artificial inoculation. These lines that had consistently low disease severity are useful for breeding programs to develop fumonisin resistant lines.

Brown, R.L., Menkir, A., Bandyopadhyay, R., Cleveland, T.E. and Chen, Z. 2007. **Comparative proteomics of near-isogenic maize inbred lines to identify potential aflatoxin-resistance markers**. *Phytopathology* 97: S14. (Abstract)

Several maize near-isogenic inbred lines were subjected to comparative proteomics to identify kernel proteins associated with resistance. These lines were developed in Nigeria through a joint project between ARS-SRRC and the International Institute of Tropical Agriculture. The goal of this project is to develop aflatoxin-resistant inbreds for use in Central and West African national programs and U.S. breeding programs to combat aflatoxin contamination of maize. Parental lines that produced these inbreds were U.S. aflatoxin-resistant lines crossed with Central and West African lines selected for moderate to high ear rot resistance. Lines were selfed and selected for foliar and ear rot resistances and for good agronomic characteristics until the S4 generation where selection began for aflatoxin accumulation using a laboratory-based kernel screening assay (KSA) and field trials. Recently, inbred lines developed through the above-protocol were re-examined with the KSA and near-isogenic lines varying significantly in aflatoxin accumulation were identified. These lines were subjected to comparative proteomics and differences in protein expression between these near-isogenics were identified and results are discussed.

Brown, R.L., Menkir, A., Bandyopadhyay, R., Cleveland, T.E., and Chen, Z-Y. 2007. **Developing resistant maize inbreds: A progress review with future projections**. Pages 83-84 *in* Proceedings of the 2007 Annual Multicrop Aflatoxin/Fumonisin Elimination & Fungal Genomics Workshop, Atlanta, Ga, October 22-24, 2007. (Abstract)

One goal of the research collaboration between Southern Regional Research Center (SRRC) and the International Institute of Tropical Agriculture (IITA) is to develop maize inbreds, through breeding and selection that have good resistance against aflatoxin contamination by *Aspergillus flavus* in useful agronomic backgrounds. U.S. parents for the initial crosses contributed by SRRC were CI2, MI82, T115, GT-MAS:gk, OH516, SD18, Mp420, B73xTex6 and MO17xTex6; 4001, 1368, Babangoyo, KU1414, 9071, and 9450 comprised the parents contributed by IITA. The former group had demonstrated resistance to aflatoxin contamination in numerous field trials while the latter group, selected in Central and West Africa for moderate to high resistance to fungal ear rots (by several fungi including *A. flavus* and *Fusarium verticillioides*), showed potential for aflatoxin-resistance in the Kernel Screening Assay (KSA) and potential for contributing new traits to the pool of resistance genes. Two populations were made from crosses: a temperate population consisting of 75% U.S. germplasm and a tropical population with a 50% contribution from each source. Presently, 8 inbred lines in S8 to S10 stages of inbreeding are being registered and released as sources of resistance. The selection of these lines for release was based not only on aflatoxin-resistance, but on good agronomic characteristics as well. These lines can be used in U.S. breeding programs for development of commercial cultivars with aflatoxin-resistance and in African national programs. They may also be useful in combating foliar diseases. More aflatoxin-resistant inbred lines are expected to be released over the next several years through the IITA-SRRC collaborative project.

The second goal of the research collaboration is to identify markers in the resistant inbred lines coming out of the breeding program. Towards this goal, comparative proteomics is being employed to identify proteins associated with resistance (RAPs) in near-isogenic lines (varying in aflatoxin accumulation by the KSA), discovered among program breeding materials. Most RAPs identified are constitutively produced and fall into one of four protein categories: 1) antifungal; 2) storage; 3) stress-related; or 4) other (including putative regulatory proteins). Previous investigations have demonstrated that while inducible proteins are needed for kernels to resist aflatoxin accumulation, production of a high level of constitutive proteins in resistant kernels is a major factor differentiating them from susceptible kernels.

Brown, R.L., Chen, Z., Menkir, A., and Cleveland, T.E. 2006. **Proteomics to identify resistance factors in corn - a review**. *Mycotoxin Research* 22: 22-26.

The host resistance strategy for eliminating aflatoxins from corn has been advanced by the discovery of natural resistance traits such as proteins. This progress was aided by the development of a rapid laboratory-based kernel screening assay (KSA) used to separate resistant from susceptible seed, and for investigating kernel resistance. *A. flavus* GUS transformants have also been used, in conjunction with the KSA, to assess the amount of fungal growth in kernels and compare it with aflatoxin accumulation. Several proteins associated with resistance (RAPs) have been identified using 1 D PAGE. However, proteomics is now being used to further the discovery of RAPs. This methodology has led to the identification of stress-related RAPs as well as other antifungals. Characterization studies being conducted, including RNAi gene silencing experiments, may confirm roles for RAPs in host resistance.

Menkir, A., Brown, R.L., Bandyopadhyay, R., Chen, Z-Y, and Cleveland, T.E. 2006. **A USA–Africa collaborative strategy for identifying, characterizing, and developing maize germplasm with resistance to aflatoxin contamination**. *Mycopathologia* 162: 225-232.

Aflatoxin contamination of maize by *Aspergillus flavus* poses serious potential economic losses in the US and health hazards to humans, particularly in West Africa. The Southern Regional Research Center of the United States Department of Agriculture, Agricultural Research Service (USDA-ARS-SRRC) and the International Institute of Tropical Agriculture (IITA) initiated a collaborative breeding project to develop maize germplasm with resistance to aflatoxin accumulation. Resistant genotypes from the US and selected inbred lines from IITA were used to generate backcrosses with 75% US germplasm and F_1 crosses with 50% IITA and 50% US germplasm. A total of 65 S4 lines were developed from the backcross populations and 144 S4 lines were derived from the F_1 crosses. These lines were separated into groups and screened in SRRC laboratory using a kernel-screening assay. Significant differences in aflatoxin production were detected among the lines within each group. Several promising S4 lines with aflatoxin values significantly lower than their respective US resistant recurrent parent or their elite tropical inbred parent were selected for resistance-confirmation tests. We found pairs of S4 lines with 75-94% common genetic backgrounds differing significantly in aflatoxin accumulation. These pairs of lines are currently being used for proteome analysis to identify resistance-associated proteins and the corresponding genes underlying resistance to aflatoxin accumulation. Following confirmation tests in the laboratory, lines with consistently low aflatoxin levels will be inoculated with *A. flavus* in the field in Nigeria to identify lines resistant to strains specific to both US and West Africa. Maize inbred lines with desirable agronomic traits and low levels of aflatoxin in the field would be released as sources of genes for resistance to aflatoxin production.

Chen, Z-Y., Brown, R.L., Menkir, A., Damann, K., and Cleveland, T.E. 2005. **Proteome analysis of near isogenic maize lines differing in the level of resistance against *Aspergillus flavus* infection/aflatoxin production**. *Phytopathology* 95: S19. (Abstract)

Maize kernels of 63 S4 lines with resistance to foliar diseases were extracted from populations developed from various crosses between aflatoxin resistant U.S. lines and African lines demonstrated potential resistance in the Kernel Screening Assay (KSA). KSA analysis identified 5 pairs of lines with contrasting levels of aflatoxin between members of each pair, although the genetic background of each pair member was very similar. Proteome analysis was performed on these lines. Embryo and endosperm proteins were extracted separately from each of these lines, resolved using large format 2-D gels, and compared for qualitative and quantitative differences within a pair and across 5 pairs using Progenesis Discovery gel analysis software. Over 30 proteins that are unique or significantly differentially expressed in more than one pair were then sequenced using ESI-MS/MS. Some of these identified proteins are storage proteins, some are stress-related proteins, and some are antifungal proteins or proteins involved in signal transduction processes. One such protein is currently being cloned and further characterized to determine whether it plays a direct role in maize resistance to A. flavus infection and aflatoxin production.

Brown, R.L., Chen, Z-Y., Menkir, A., and Cleveland, T.E. 2004. **Identification of Aflatoxin-Resistance and Potential Markers in Maize Breeding Materials Developed in West Africa.** Page 43 in Proceedings of the 4th Fungal Genomics, 5th Fumonisin Elimination and 17th Aflatoxin Elimination Workshops, October 25-28, 2004, Sacramento, CA. (Abstract)

The breeding program at the International Institute of Tropical Agriculture (IITA) had originally developed maize populations from crosses of US aflatoxin-resistant inbreds with IITA resistant inbreds. Populations formed had 75%, 50%, or 25% US germplasm. A total of 65 S_5 (~98% towards inbred development) lines extracted from 75% US × 25% IITA germplasm and 63 S_5 lines derived from 50% US × 50% IITA germplasm with resistance to lowland leaf rust, leaf blight and *Curvularia* leaf spot, were advanced to S_6. Seed samples of the first 65 lines were sent to the USDA-ARS laboratory in New Orleans (SRRC) and were screened for resistance to aflatoxin contamination using the laboratory-based kernel screening assay (KSA). Based on the results of KSA screening, nine inbred lines with significantly lower levels of aflatoxin production compared to their respective US resistant parents and with good agronomic features were selected. Following confirmation of the levels of resistance of these lines, they will be candidates for release to the public as sources of resistance genes to aflatoxin production. These lines are also being tested in the field in Nigeria under artificial inoculation with *Aspergillus flavus* to assess the consistency of their resistance across the different strains of *A. flavus*. Several closely-related pairs of lines, differing significantly in aflatoxin accumulation, were identified at SRRC among IITA temperate (75% US) breeding materials. When kernel embryo proteins of these pairs were subjected to differential proteomics, several resistance-associated proteins (RAPs), categorized as stress-related, were found to be more abundantly produced in resistant lines. Also, a putative regulatory protein was identified. When endosperm proteins of closely related pairs were compared, stress proteins, antifungals and putative regulatory proteins were identified. Among the antifungals were a 14 kDa trypsin inhibitor and zeamatin, both previously highlighted in our lab as important to kernel resistance. The discovery of abundant stress-related proteins, constitutively expressed in resistant lines, supports the findings of an earlier investigation we conducted. This may be an important find, since drought and high nighttime temperatures are known to enhance aflatoxin accumulation in maize. Possession of unique or higher levels of the identified stress-proteins, may put resistant lines in an advantageous position over susceptible ones in the ability to defend against pathogens while under stress. Future studies include cloning corresponding genes, performing characterization experiments and studies, such as RNAi gene silencing experiments, to investigate the role of RAPs in resistance. These investigations will aid in selecting the best molecular markers for use in breeding strategies and transformation protocols involving IITA-bred germplasm.

Brown, R.L., Chen, Z., Menkir, A., White, D.G., and Cleveland, T.E. **Identification of natural resistance to aflatoxin elaboration in maize.** 2004. Proceedings of the 15th International Plant Protection Congress, Beijing, China, May 11-16, 2004, pp. 367. (Abstract)

In the past 15 years, several maize genotypes, resistant to *Aspergillus flavus* infection/aflatoxin production, have been identified, and their discovery has enhanced the potential of controlling aflatoxin contamination through the deployment of a host resistance strategy. Several of these maize lines have also been investigated further to gain understanding of the resistance mechanisms operating therein. These investigations were performed using a rapid, laboratory-based, kernel screening assay (KSA) that can separate resistant from susceptible maize lines (comparing favorably with field evaluations), and facilitate host-pathogen interaction studies for illuminating kernel resistance mechanisms. The KSA is also being used to identify aflatoxin-resistant lines among maize germplasm produced in a West African breeding program that combined U.S. aflatoxin-resistant lines with ones selected in Central and West Africa for ear rot resistance. In the above-studies, protein involvement in kernel resistance was implicated. Using 1-dimensional PAGE, certain antifungal proteins have been associated with resistance. Recent use of proteomics has also identified an association between stress-related protein expression and resistance. This last discovery may be an important one, since drought and high night-time temperatures have been observed to greatly increase aflatoxin accumulation. Gene discovery through proteomics, especially using near-isogenic lines produced in West Africa, may lead to the development of markers for maize resistance-breeding programs, and to the identification of candidate genes for plant transformation protocols involving maize as well as other species. Current efforts to confirm the *in vivo* function of putative resistance genes include QTL analysis, and tobacco transformation.

Brown, R.L., Chen, Z-Y., Menkir, A., and Cleveland, T.E. 2003. **Using biotechnology to enhance host resistance to aflatoxin contamination of corn**. *African Journal of Biotechnology* 2: 557-562.

Host resistance is the most widely explored strategy for eliminating aflatoxin contamination by *Aspergillus flavus*. Breeding strategies for developing resistant corn germplasm have been enhanced by the development of new screening tools for field inoculation and for laboratory screening. RFLP analysis of corn populations has highlighted the possibility that different resistance traits can be successfully pyramided into agronomically useful germplasm, while proteomics has impacted the identification of proteins associated with resistance (RAPs). The identification of RAPs has also been enhanced by the discovery of near-isogenic corn lines in progeny generated in a West African breeding program. The characterization of genes of the aflatoxin biosynthetic pathway has provided a foundation for a genomics investigation aimed at understanding the biochemical function and genetic regulation of aflatoxin biosynthesis. Successful inhibition of aflatoxin elaboration may require not only the action of antifungal compounds, but of compounds that block biosynthesis of toxins as well.

Brown, R.L., Chen Z.-Y., Cleveland, T.E., Gonzalez, P., Jackson. T., Menkir, A., Damann, K.E., Rajasekaran, K. 2003. **Progress in the identification and characterization of maize resistance traits against aflatoxigenic Fungi.** Page 58 in Proceedings of the 3rd Fungal Genomics, 4th Fumonisin, and 16th Aflatoxin Elimination Workshops, October 13-15, 2003, Savannah, Georgia. (Abstract)

The main thrust of our research has been to identify resistance markers in maize germplasm demonstrating resistance to aflatoxin contamination by *Aspergillus flavus*. Our aim is to use this information to enhance host resistance in maize lines with superior agronomic characteristics. Our primary strategy has been to identify, using comparative proteomics (resistant vs. susceptible germplasm), resistance-associated proteins (RAPs) and their corresponding genes, further characterize the proteins/genes, and perform confirmation studies to determine their actual role in resistance. Using this approach, we have identified a number of RAPs (stress-related and antifungal) in U.S. lines, and characterized trypsin inhibitor, glyoxalase and pathogenesis-related (PR-10) proteins. To evaluate their role in resistance, we are currently designing an RNAi gene silencing vector and vector constructs involving genes encoding the above three proteins, for maize transformation. Aflatoxin-resistance breeding program at IITA-Nigeria has advanced lines by selfing and selecting for agronomic characteristics, ear rot resistance, and aflatoxin-resistance, to the S5 generation (98% homogeneity). One group of lines originate from crosses involving 75% U.S. and 25% African genetic backgrounds (temperate), and a second group are from crosses involving 50% of each (tropical). Recently, S4 lines of the temperate materials were screened at SRRC for aflatoxin accumulation using the kernel screening assay (KSA). Five different closely-related pairs of lines, with individuals within each pair differing significantly in aflatoxin levels, were identified during screening, and were subsequently subjected to proteome analysis. Results show both qualitative and quantitative kernel embryo protein differences between resistant and susceptible members of the closely-related pairs. Rapid progress made in breeding at IITA has facilitated the advancement of proteomics investigations at SRRC, by providing closely-related germplasm differing in aflatoxin levels. Evaluating these for resistance-associated differences should prove more fruitful than evaluating lines from very diverse genetic backgrounds, which, heretofore, was standard protocol.

Brown, R.L., Menkir, A., Bandyopadhyay, R., Chen, Z.Y., and Cleveland, T.E. 2003. **The development of aflatoxin-resistant maize germplasm and the identification of potential markers.** Page 64 in Proceedings of the 2006 Annual Multicrop Aflatoxin/Fumonisin Elimination & Fungal Genomics Workshop, Ft. Worth, Tx, October 16-18, 2006. (Abstract)

For the last 6 years, the Southern Regional Research Center (SRRC) and the International Institute of Tropical Agriculture (IITA) – Ibadan, Nigeria, have been collaborating on the development of aflatoxin resistant maize inbred lines and on the identification of breeding markers in those lines. Two breeding populations were originally developed from crosses between U.S. aflatoxin-resistant lines and ear rot resistant lines selected in Central and West Africa that accumulated low aflatoxin in the kernel screening assay (KSA). One population contained 50% U.S/ 50% African germplasm, while the other was a backcross population containing 75% U.S. germplasm. In the S4 generation, lines were selected on ear rot and foliar resistance and agronomic qualities. S4 lines and after have been tested by the KSA for aflatoxin accumulation and the best performers, field tested in Nigeria for resistance and agronomic performance. Presently, over 50 S7 lines are being evaluated in confirmation field trials. Also, a number of hybrids generated from inbreds that supported low toxin have produced excellent yields and demonstrated good combining ability. Comparative proteomics is being used to identify potential markers in IITA-SRRC lines. This approach is based on the belief that tracking protein expression under relevant conditions may provide a quicker way of identifying genes involved in resistance. Also, it is believed that a subtractive approach, based on side-by-side comparisons of aflatoxin-resistant with -susceptible lines, is an efficient way of isolating proteins potentially involved in resistance. The recent availability of near-isogenic lines has enhanced the search for resistance genes, rendering unnecessary the development of composite profiles for homogenizing nonresistance-related differences. Investigation on proteins associated with resistance (RAPs) has, thus far, focused on those that are constitutively expressed. The appearance of several stress-related proteins among RAPs is interesting given the enhancing effect of drought on aflatoxin accumulation in maize. Several RAP genes have been cloned and characterized; PR 10, glyoxalase (GLXI), and trypsin inhibitor (TI) have been inserted in RNAi gene silencing vectors which were used to transform maize plants to determine gene function. A 2 year field study involving 3 resistant and 3 susceptible maize genotypes and *Aspergillus flavus* infection was performed; nine different time points were used for sampling. The expression of TI, GLXI, PR 10, peroxiredoxin (PER1) and a protein kinase was studied using RT-PCR. These discoveries have enhanced our understanding of the complexities involved in maize kernel response to fungal infection and aflatoxin formation. Further investigation is needed to understand the actions of plant stress proteins in the host plant environment following fungal infection. Comparative proteomics will be used next to identify kernel proteins induced as a result of fungal infection.

Brown, R.L., Zhi-Yuan, C., Menkir, A., and Cleveland, T.E., Cardwell, K.F., Kling, J.G., and White, D.G. 2001. **Resistance to aflatoxin accumulation in kernels of maize inbreds selected for ear rot resistance in West and Central Africa**. *Journal of Food Protection* 64: 396-400.

Thirty-six inbred lines selected in West and Central Africa for moderate to high resistance to maize ear rot under conditions of severe natural infection were screened for resistance to aflatoxin contamination using the previously established kernel screening assay. Results showed that more than half the inbreds accumulated aflatoxins at levels as low as or lower than the resistant U.S. lines GT-MAS:gk or MI82. In 10 selected aflatoxin-resistant or aflatoxin-susceptible inbreds, *Aspergillus flavus* growth, which was quantified using an *A. flavus* transformant containing a GUS-β-tubulin reporter gene construct, was, in general, positively related to aflatoxin accumulation. However, one aflatoxin-resistant inbred supported a relatively high level of fungal infection, whereas two susceptibles supported relatively low fungal infection. When kernels of the 10 tested lines were profiled for proteins using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, significant variations from protein profiles of U.S. lines were observed. Confirmation of resistance in promising African lines in field trials may significantly broaden the resistant germplasm base available for managing aflatoxin contamination through breeding approaches. Biochemical resistance markers different from those being identified and characterized in U.S. genotypes, such as ones inhibitory to aflatoxin biosynthesis rather than to fungal infection, may also be identified in African lines. These discoveries could significantly enhance the host resistance strategy of pyramiding different traits into agronomically useful maize germplasm to control aflatoxin contamination.

3. Biocontrol / Ecology

Donner, M., Sikora, R.A., Bandyopadhyay, R., and Cotty, P.J. **Molecular characterization of atoxigenic strains of *Aspergillus flavus* for biological control of aflatoxins in Nigeria.** *International Journal of Food Microbiology* (submitted)

Aflatoxins are highly toxic, carcinogens produced by several species in *Aspergillus* section Flavi. These fungi are distributed throughout West Africa where they frequently infect maize and cause aflatoxin contamination both during crop development and in storage. Strains of *A. flavus* that do not produce aflatoxins, called atoxigenic strains, have been used commercially in North America as tools for limiting aflatoxin contamination. A similar aflatoxin management strategy is being pursued in Nigeria. Initial efforts in Nigeria have focused on 18 atoxigenic *A. flavus* Vegetative Compatibility Groups (VCGs) collected from naturally infected maize. In the current study loci across the 68 kb aflatoxin biosynthesis gene cluster were compared among the 18 atoxigenic VCGs from Nigeria, an atoxigenic strain used commercially in North America, and several aflatoxin producers. Five of the VCGs from Nigeria had large deletions (37 kb to 65 kb) extending from the teleomeric side of the aflatoxin biosynthesis cluster. In one case (isolate AV0222) the deletion extended through the cluster to the adjacent sugar cluster. The remaining twelve atoxigenic VCGs, including the VCG used for aflatoxin management in North America, contained all of the aflatoxin pathway genes. However, none of the African VCGs had the SNP known to cause atoxigenicity in the North American VCG. Comparison of pathway genes revealed more changes in atoxigenic than in aflatoxin-producing VCGs and several non-synonymous changes that are unique to atoxigenics, observations that support existence of atoxigenicity for long periods. However, for some atoxigenic VCGs, additional sequencing and experimentation will be required to determine precise causes of atoxigenicity. Diversity of atoxigenic VCGs was assessed with phylogenetic analyses. Although some atoxigenics have common recent ancestry, several are more closely related to aflatoxin producers than to other atoxigenics. Our data support atoxigenicity having evolved multiple times and reflects diversity among atoxigenics that might be exploited during selection of VCGs for biocontrol of aflatoxin contamination. The sequence information developed in the current work provides both insight into the relative stability of the atoxigenicity and a basis for rapid molecular monitoring of the atoxigenic VCGs in the environment. The current study demonstrates VCGs of *A. flavus* in West Africa with diverse mechanisms of atoxigenicity and with potential value in aflatoxin management.

Donner, M., Atehnkeng, J., Bandyopadhyay, R., Sikora, R.A., and Cotty, P.J. 2009. **Distribution of *Aspergillus* section Flavi in soils of various maize fields among three agroecological zones of Nigeria.** *Soil Biology and Biochemistry* 41: 37-44.

Fungal communities in soils of Nigerian maize fields were examined to determine distributions of aflatoxin-producing fungi and to identify endemic atoxigenic strains of potential value as biological control agents for limiting aflatoxin contamination in West African crops. Over 1000 isolates belonging to *Aspergillus* section Flavi were collected from soil of 55 Nigerian maize fields located in three agroecological zones by dilution plating onto modified Rose Bengal agar. The most common member of *Aspergillus* section Flavi (85% of isolates) was the *A. flavus* L-strain followed by the unnamed taxon known as strain S_{BG} (8%), *A. tamarii* (6%) and *A. parasiticus* (1%). Highest incidence of S_{BG} was in Zaria district, and lowest was in Ogbomosho and Ado-Ekiti districts. Only 44% of 492 A. flavus isolates produced aflatoxins in liquid fermentation (limit of detection 5 ng g^{-1}). Thirty-two percent of the *A. flavus* isolates produced >1 mg g^{-1} total aflatoxins but no *A. flavus* isolate produced G aflatoxins. When the agroecological zones were compared, significantly ($P < 0.05$) greater proportions of aflatoxigenic *A. flavus* isolates were found in the Northern Guinea Savannah (61%) than in Southern Guinea Savannah (31%). The Derived Savannah was intermediate between the other two agroecological zones. Each of the regions had atoxigenic strains of potential value as biological control agents. All S_{BG} and *A. parasiticus* isolates produced both B and G aflatoxins and greater than 300 mg g^{-1} total aflatoxins. S_{BG} and *A. parasiticus* isolates were the greatest contributors to the aflatoxin-producing potential of fungal communities in regions where these isolates occurred.

Atehnkeng, J., Ojiambo, P.S., Donner, M., Ikotun, T., Sikora, R.A., Cotty, P.J., and Bandyopadhyay, R. 2008. **Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agroecological zones in Nigeria**. *International Journal of Food Microbiology* 122: 74-84.

Maize samples were collected during a survey in three agro-ecological zones in Nigeria to determine the distribution and aflatoxin-producing potential of members of *Aspergillus* section Flavi. The three agro-ecological zones were, Derived Savannah (DS) and Southern Guinea Savannah (SGS) in the humid south and North Guinea Savannah (NGS) in the drier north. Across agro-ecological zones, *Aspergillus* was the most predominant fungal genera identified followed by *Fusarium* with mean incidences of 70 and 24%, respectively. Among *Aspergillus*, *A. flavus* was the most predominant and L-strains constituted >90% of the species identified, while the frequency of the unnamed taxon S_{BG} was <3%. The incidence of atoxigenic strains of *A. flavus* was higher in all the districts surveyed except in the Ogbomosho and Mokwa districts in DS and SGS zones, respectively, where frequency of toxigenic strains were significantly (P<0.05) higher than that of atoxigenic strains. The highest and lowest incidence of aflatoxin positive samples was recorded in the SGS (72%) and NGS (20%), respectively. Aflatoxin contamination in grain also followed a similar trend and the highest mean levels of B-aflatoxins were detected in maize samples obtained from Bida (612 ng g^{-1}) and Mokwa (169 ng g^{-1}) districts, respectively, in the SGS. Similarly, the highest concentrations of G-aflatoxins were detected in samples from Akwanga district in the SGS with a mean of 193 and 60 ng g^{-1}, respectively. When agro-ecological zones were compared, B-aflatoxins were significantly (P<0.05) higher in SGS than in NGS, and intermediate in maize samples from the DS agro-ecological zone.

Atehnkeng, J., Ojiambo, P.S., Ikotun, T., Sikora, R.A., Cotty, P.J., and Bandyopadhyay, R. 2008. **Evaluation of atoxigenic strains of *Aspergillus flavus* as potential biocontrol agents for aflatoxin in maize**. *Food Additives and Contaminants Part A* 25: 1254-1271.

Aflatoxin contamination resulting from maize infection by *Aspergillus flavus* is both an economic and a public health concern. Therefore, strategies for controlling aflatoxin contamination in maize are being investigated. The abilities of eleven naturally occurring atoxigenic isolates in Nigeria to reduce aflatoxin contamination in maize were evaluated in grain competition experiments and in field studies during the 2005 and 2006 growing seasons. Treatments consisted of inoculation of either grains in vials or ears at mid-silking stage in field plots, with the toxigenic isolate (La3228) or atoxigenic isolate alone and co-inoculation of each atoxigenic isolate and La3328. Aflatoxin B_1 + B_2 concentrations were significantly (p<0.05) lower in the co-inoculation treatments compared with the treatment in which the aflatoxin-producing isolate La3228 was inoculated alone. Relative levels of aflatoxin B_1 + B_2 reduction ranged from 70.1% to 99.9%. Among the atoxigenics, two isolates from Lafia, La3279 and La3303, were most effective at reducing aflatoxin B_1 + B_2 concentrations in both laboratory and field trials. These two isolates have potential value as agents for the biocontrol of aflatoxin contamination in maize. Because these isolates are endemic to West Africa, they are both more likely than introduced isolates to be well adapted to West African environments and to meet regulatory concerns over their use throughout that region.

Atehnkeng, J., Ojiambo, P.S., Donner, M., Ikotun, T., Cotty, P.J., and Bandyopadhyay, R. 2008. **Occurrence of Aspergillus section Flavi in stored maize and prospects from a biocontrol strategy in Nigeria**. Page 420 in Programme and Abstract Book of the 21st International ICFMH Symposium FOOD MICRO 2008, 1-4 September 2008, Aberdeen, Scotland. (Abstract)

Maize samples were collected during a survey in three agroecological zones in Nigeria to determine the distribution and aflatoxin-producing potential of members of *Aspergillus* section Flavi and identify atoxigenic strains for biological control of aflatoxin contamination. Strategies for controlling aflatoxin contamination in maize were investigated with 11 naturally occurring atoxigenic isolates obtained in Nigeria. These isolates were evaluated in grain competition experiments and in field studies during the 2005 and 2006 growing seasons. Across agroecological zones, *Aspergillus* was the most predominant fungal genera identified followed by *Fusarium*. Among *Aspergillus*, *A. flavus* was the most predominant and L strains constituted > 96% of the species identified. The incidence of atoxigenic strains of *A. flavus* was higher in all survey districts in except in the Ogbomosho and Mokwa districts in DS and SGS zones, respectively, where toxigenic strains were significantly (P < 0.05) higher than atoxigenic strains. Among the atoxigenics, two isolates from Lafia, La3279 and La3303, were most effective at reducing aflatoxin B_1+ B_2 concentrations in both laboratory and field trials. These two isolates have potential value as agents for the biocontrol of aflatoxin contamination in maize because they are well adapted and endemic to West Africa, and to meet regulatory concerns over their use throughout that region.

Moore, G., Horn, B.W., Elliott, J., Hell, K., Chulze, S., Wright, G., Naik, M., Carbone, I. 2008. **Recombination, balancing selection and geographic subdivision among worldwide populations of Aspergillus flavus.**
http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=226986 (Abstract)

Aspergillus flavus is a global agent of aflatoxin contamination of economically important crops such as corn, peanuts, and cottonseed. Extensive studies have elucidated the biochemical and regulatory mechanisms of aflatoxin production, but basic knowledge of the evolutionary processes that maintain toxicity in *A. flavus* is lacking. We sequenced 21 intergenic regions in the aflatoxin gene cluster of 166 isolates of *A. flavus* representing geographically isolated populations from five countries (Argentina, Australia, Benin, India and United States). Linkage disequilibrium analyses of molecular variation revealed a different block-like organization for each population suggesting that populations are reproductively isolated. Detailed compatibility analyses for a single population from Georgia showed that all blocks, with the exception of *aflM/aflN* (*hypE*), shared the same evolutionary history. Further analyses on the entire sample revealed a signature of balancing selection within *hypE* and *aflW/aflX*, which acts to stabilize the nonaflatoxigenic phenotype. Although we detected significant geographic differentiation in noncluster regions, balancing selection in *hypE* and *aflW/aflX* decreased genetic differentiation among Australia, India and Georgia. These results may offer insight into the stability of potential biocontrol strains as well as those currently in use.

Tedihou, E., Hell, K., Hau, B., Olatinwo, R., Hoogenboom, G. 2007. **Effect of *Aspergillus flavus* inoculum size, maize variety, and cowpea intercropping on aflatoxin production during maize storage.** Page 123 *in* Proceedings of the 2007 Annual Multicrop Aflatoxin/Fumonisin Elimination & Fungal Genomics Workshop, Atlanta, Ga, October 22-24, 2007. (Abstract)

Aflatoxin contamination of maize (*Zea mays*) by *Aspergillus flavus* is a serious problem with significant health implications in many parts of West Africa. Post harvest aflatoxin contamination is usually influenced by prevailing conditions during maize growth in the field. The main goal was to identify factors that directly or indirectly enhance production of aflatoxin during maize storage. The objective of this study was to determine the effects of initial inoculum level, type of variety and impact of intercropping on the rate of aflatoxin production in maize during storage. The two maize varieties used in this study were *Gbogbe*, a local maize variety with a maturity period of 90 days, and *TZSR-W* (Tropical *Zea mays* Streak Resistant White), an improved variety with a maturity period of 120 days. A local variety of cowpea (*Vigna unguiculata* (L) Walp) was used as intercrop with the maize varieties. Experimental plots were inoculated with *Aspergillus* (*T-strain*). The field experiment was conducted between June and September 2006. The improved variety (TZSR-W) had significantly higher level of Colony Forming Unit (*CFU)* (P = 0.0212), aflatoxin B1 (P = 0.0038), and aflatoxin B2 (P = 0.0014) contamination compared to the local variety (*Gbogbe*). The *Aspergillus* (*T-strain*) inoculation level and variety selection had major impact on the production of aflatoxin in stored maize. Intercropping of maize with cowpea during the growing season did not show any significant impact on *CFU* level. The initial inoculum level and the water content of maize after harvest may have played significant role in *A. flavus* infection initiation and development during storage. These factors could serve as useful inputs in developing a model for predicting the rate of aflatoxin production and level of contamination during maize storage. Avoidance of environmental conditions that favors infection development in the field, utilization of management practices that minimizes primary inoculum or sources of inoculum, and harvesting when the water content is optimal may help prevent or reduce aflatoxin contamination in maize.

Sobowale, A.A., Cardwell, K.F., Odebode, A.C., Bandyopadhyay, R., Jonathan, S.G. 2007. **Persistence of *Trichoderma* species within maize stem against *Fusarium verticillioides***. *Archives of Phytopathology and Plant Protection* 40: 215-231**.**

The ability of four *Trichoderma* isolates to colonize maize stem and persist therein in presence of *Fusarium verticillioides* were tested in the field. These were three strains of *Trichoderma pseudokoningii* and one strain of *T. harzianum*. There were three pairing methods based on the commonly used toothpick inoculation method. This ensures direct introduction of pathogen and antagonist using toothpicks dressed separately with pathogen and antagonists. Maize stems were inoculated with antagonist alone, pathogen alone and sterile toothpicks served as controls. The proportion of antagonist recovered was plotted against distance moved within maize stem. All *Trichoderma* isolates had endophytic growth within the maize stem in all pairing methods, giving a significant quadratic response with distance from the inoculation point. Recoveries of antagonists were slightly more in lower than upper internodes. Simultaneous inoculation of pathogen and antagonist gave the best endophytic growth of antagonists. *T. harzianum* strain 2 and *T. pseudokoningii* strain 4 had the best endophytic growth in all pairing methods. The pathogen had no effect on endophytic growth of *T. pseudokoningii* strain 4. *T. pseudokoningii* strain 4 and *T. harzianum* strain 2 improved in endophytic growth in the presence of pathogen. Competitive exclusion of *F. verticillioides* from maize plant using endophytic *Trichoderma* spp. could be a possibility.

Donner, M., Atehnkeng, J., Bandyopadhyay, R., Kiewnick, S., Sikora, R., Cotty, P. 2006. **Characterisation of aflatoxin-producing and non-producing strains of *Aspergillus* Section *Flavi* in Nigeria**. Poster presented at the Deutscher Tropentag, "Prosperity and Poverty in a Globalised World—Challenges for Agricultural Research", October 11-13, 2006, Bonn. (http://www.tropentag.de/2006/proceedings/node163.html#3785). (Abstract)

Aflatoxins are toxic metabolites produced by fungi of the genus *Aspergillus*. *Aspergillus flavus* is the most common toxin producing species, but different strains produce different amounts of aflatoxin and some produce none. In West Africa these fungi cause aflatoxin contamination in maize both during crop development and in storage. In a joint project with the International Institute for Tropical Agriculture, the potential of atoxigenic *A. flavus* strains as biological control agents was assessed for the reduction of aflatoxin contamination of maize in Nigeria. Over 1000 fungal isolates belonging to *Aspergillus* Section Flavi were collected by dilution plating on modified Rose Bengal agar. The isolates originated from 56 soil samples that were collected from Nigerian maize fields located in five agroecological zones. These isolates were screened for their aflatoxin producing ability in liquid fermentation. Of over 600 isolates screened, 48% produced detectable quantities of aflatoxin. The aflatoxin B1 production of *A. flavus* isolates varied from 33 ppb up to 19,000 ppb. From each soil sample the producing habit of sclerotia and spores was investigated for high aflatoxin producers and atoxigenic isolates. Isolates that produced high levels of toxin in liquid fermentation produced proportionally less spores and had a higher sclerotia mass on Czapeks agar (31C, 25 days), while isolates with high spore mass and less sclerotia mass produced less aflatoxin. The current research involves molecular genetic characterization of the isolates. Portions of the aflatoxin biosynthetic pathway genes, *aflR*, as well as genes from the *A. flavus* genome (taka amylase and *pecA*) were amplified by PCR and sequenced. Phylogenetic trees were constructed from the sequence data to assess relationships among the toxigenic and atoxigenic isolates. The study demonstrates the differences between aflatoxin producing and atoxigenic *Aspergillus* Section Flavi isolates.

Bandyopadhyay, R., Kiewnick, S., Atehnkeng, J., Donner, M., Cotty, P.J., and Hell, K. 2005. **Biological control of aflatoxin contamination in maize in Africa.** Proceedings of the Conference on International Research for Development, October 11-13, 2005, Hohenheim, Germany.
http://www.tropentag.de/2005/abstracts/full/398.pdf.

Aflatoxin contamination of maize, the major cereal in African diets, is a major risk for health and well being of African people, primarily children. Aflatoxin-producing fungi *Aspergillus flavus* and *A. parasiticus* can infect grains from pre-harvest stages in the field through to post-harvest stages in the stores. Based on past work by IITA and our collaborators, several pre- and post-harvest strategies are being tested to reduce risk of aflatoxin contamination. One of the management strategies being developed is biological control using the competitive exclusion mechanism, which has been successfully implemented on cottonseed in Arizona. Natural population of *A. flavus* consists of toxigenic strains that produce copious amount of aflatoxin and atoxigenic strains that lack the capacity to produce aflatoxin. In the competitive exclusion mechanism, introduced atoxigenic strains out compete and exclude toxigenic strains from colonizing grains thereby reducing aflatoxin production in contaminated grains. We have collected more than 4200 isolates of *A. flavus* from different agroecozones in Nigeria to identify atoxigenic strains. Until now, we have identified about 50 candidate atoxigenic strains out of 1500 strains screened so far. Twenty-four of these atoxigenic isolates have been tested under field conditions in Ibadan, Nigeria to identify a few effective strains that can exclude toxigenic strains. These atoxigenic strains are being evaluated for a set of selection criteria to further narrow down the numbers to a few for further use in biocontrol field experiments. One of the important selection criteria will ensure that the candidate atoxigenic strains belong to unique vegetative compatibility groups (for which testers have been developed) that are unable to produce toxigenic progenies in the natural environment. Propensity to multiply, colonize and survive are other selection criteria to make sure that few reapplications will be required once the atoxigenic strains are introduced in the environment. Environmental safety of most promising atoxigenics would be also evaluated. Research to develop atoxigenic strains is resource intensive and will further require downstream development activities. Nevertheless, biological control holds promise of offering a long-term solution for reducing aflatoxin contamination in maize.

Donner, M., Atehnkeng, J., Cotty, P., Bandyopadhyay, R., Sikora, R. A., and Kiewnick, S. 2005. **Aflatoxin producers from soil of maize producing regions in Nigeria**. Poster presented at the Deutscher Tropentag, "The Global Food & Product Chain—Dynamics, Innovations, Conflicts, Strategies", October 11-13, 2005, Hohenheim. http://www.tropentag.de/2005/proceedings/node427.html. (Abstract)

Aflatoxins are toxic fungal metabolites produced by several members of *Aspergillus* section Flavi. Aflatoxin producing fungi disperse from the soil to infest maize crops on which they frequently cause aflatoxin contamination both in the field and in storage. Several aflatoxin producing species are known to occur in West Africa, but the frequencies and distributions of these strains in the major maize producing regions of Nigeria were previously unknown. Over 1,000 fungal isolates of *Aspergillus* section Flavi were collected from the soil by dilution plating of 51 soil samples from Nigerian maize fields onto a modified Rose Bengal agar. Section Flavi averaged 1159 colony forming units per gram (CFU/g) soil and ranged from 2 to 16,660 CFU/g. The L morphotype of *A. flavus* occurred in all samples, whereas the S morphotype occurred in only 19 samples, but was present in every district, ranging from 0 to 45% of the fungi isolated. The highest S strain incidence was found in the Lafia district, and the lowest incidence was in the districts of Ogbomosho and Ado-Ekiti. Soil pH of the sampled soils ranged from 5.1 to 8.7 pH. Isolates varied widely in ability to produce both aflatoxins and sclerotia. Both the shape and size of sclerotia varied with some *A. flavus* strains tending to produce sclerotia embedded in the substrate. The importance of sclerotia embedded in substrates to the potential toxicity of *A. flavus* strains on crops should be considered. The distribution of aflatoxin-producing species in soils will be contrasted with the frequencies on crops grown in the sampled fields.

Sobowale, A.A., Cardwell, K.F., Odebode, A.C., Bandyopadhyay, R., and Jonathan, S.G. 2005. **Growth inhibition of *Fusarium verticillioides* (Sacc.) Nirenberg by isolates of *Trichoderma pseudokoningii* strains from maize plant parts and its rhizosphere**. *Journal of Plant Protection Research* 45: 249-265.

Ability of five strains of *Trichoderma pseudokoningii* (antagonists) to suppress radial growth of *Fusarium verticillioides* (Sacc.) Nirenberg (=*Fusarium moniliforme* Sheldon) was examined *in vitro*. These were *T. pseudokoningii* strain1 (IMI 380933), strain2 (IMI 380937), strain3 (IMI 3809 39), strain4 (IMI 380940) and strain5 (IMI 380941). Each strain was paired with pathogen by inoculating at opposite ends of 9 cm petri plates using three pairing methods. Gradings were assigned to varied growth inhibition of pathogen by antagonists and analysed using GLM procedure (SAS). Growth suppression of *F. verticillioides* by all strains of *T. pseudokoningii* was significantly different (R^2 = 0.98, p = 0.05) from control in all pairing methods. It differed significantly (p>0.0003) among the strains in all pairing methods. Growth suppression also differed significantly among (p>0.0001) and within (p>0.018) pairing methods. Growth suppression was best when antagonists were inoculated before pathogen. Suppression mechanisms include mycoparasitism and competition for space and nutrients. *T. pseudokoningii* strains 3 and 4 had the best (p = 0.05) growth suppression of *F. verticillioides* and could be used as biocontrol agents for endophytic *F. verticillioides* in maize plant. This experiment was conducted in the search for resident microorganisms that might be capable of checking *F. verticillioides* within maize plant by competitive exclusion in subsequent experiments.

Bandyopadhyay, R., and Cardwell, K.F. 2003. **Species of *Trichoderma* and *Aspergillus* as biological control agents against plant diseases in Africa.** Pages 193–206 *in* Biological Control in Integrated Pest Management Systems in Africa (Neuenschwander, P., Borgemeister, C., Langewald, J., eds.). Wallingford, U.K.: CABI Publishing.

The development of biological control agents as a key component of integrated disease management has tremendous potential for application in the African context for the reduction of losses from plant diseases. Several biological control agents can suppress diseases as effectively as fungicides, an input that is often prohibitively expensive to be of value to resource-poor farmers. In Africa, fungal biological control agents, such as species of *Trichoderma*, *Fusarium* and *Aspergillus*, are efficacious in reducing damage caused by pathogens on maize and cowpea in research station trials. *Trichoderma koningii* and *T. harzianum* were effective in controlling damping-off of cowpea caused by *Macrophomina phaseolina*, and effective dosage and application methods have been standardized in greenhouse trials to control the disease. *Fusarium verticillioides* is an endophytic fungus that enhances growth of maize, but becomes pathogenic to cause root and stalk rot, damping-off and ear rot when the plants undergo stress. Two strains of *T*. *harzianum* and *T*. *pseudokoningii* have been shown to reduce the stalk rot phase caused by the pathogen. These two *Trichoderma* species can penetrate the plant, move systemically within the stalk to occupy the same niche as *F. verticillioides*, and competitively exclude the pathogen. In greenhouse trials, the two species reduced stalk rot either when introduced into the stalk through injured sites or after seed treatment. Aflatoxin contamination of maize and groundnut is a serious problem in West Africa. Aflatoxins are produced by aflatoxigenic strains of *Aspergillus flavus*. Non-aflatoxigenic strains of *A. flavus* can colonize the grains, but cannot produce the mycotoxin. Reduction in soil-borne inoculum of aflatoxigenic strains of *A. flavus* has been demonstrated after soil application with more competitive non-aflatoxigenic strains. As a result, fields receiving aflatoxigenic strain of *A. flavus* had reduced aflatoxin level in grains. These are a few examples that reveal biological control as an effective adjunct in integrated disease management. However, much more work needs to be done to demonstrate field efficacy of biological control agents, their persistence, safety and commercial feasibility, before practical application of biological control agents for plant disease control in Africa becomes a reality.

Cardwell, K.F., and Cotty, P.J., 2002. **Distribution of *Aspergillus flavus* section *Flavi* among soils from the four agroecological zones of the Republic of Bénin, West Africa**. *Plant Disease* 86: 434–439.

Certain members of *Aspergillus* section Flavi produce carcinogenic and immunotoxic metabolites called aflatoxins. These fungi perennate in soils and infect maize grain in the field and in storage. The distribution of *Aspergillus* section Flavi across the four different agroecologies of Bénin Republic was determined. The four agroecological zones range from humid equatorial tropics in the south to the dry savanna near the Sahara desert in the north. Soil samples collected in 1994 to 1996 from 44 different maize fields in Bénin were assayed over 3 years (88 samples total) for fungi in *Aspergillus* section Flavi. All soils tested contained *A. flavus.* Isolates (1,454 total) were collected by dilution plate from the soils and existed in populations ranging from <10 to >200 CFU/g of soil. CFU counts did not differ from year to year or change significantly with cropping systems within a zone, but differed significantly among zones. Incidence of *A. flavus* strain isolations varied from south to north, with greater number of CFU of L strain isolates in southern latitudes and higher numbers of CFU of S strain isolates found in the north. The L strain isolates occurred in 81 of 88 samples, whereas S strain isolates were in only 41 of 88 soil samples. Of 96 L strain isolates tested, 44% produced aflatoxins. Only B toxins were produced, and toxigenic isolates averaged over 100 µg of aflatoxin B_1 per 70 ml of fermentation medium (~1.4 ppm). All S strain isolates produced both B and G aflatoxins, averaging over 557 µg of aflatoxin B_1 per 70 ml (8 ppm) and 197 µg of aflatoxin G(1) per 70 ml of fermentation medium (2.8 ppm). *A. parasiticus* and *A. tamarii* were present in less than 10% of the fields and were not associated with any particular agroecological zone.

Cotty, P. J., and Cardwell, K. F. 1999. **Divergence of West African and North American Communities of *Aspergillus* section *Flavi***. *Applied and Environmental Microbiology* 65: 2264-2266.

West African *Aspergillus flavus* S isolates differed from North American isolates. Both produced aflatoxin B_1. However, 40 and 100% of West African isolates also produced aflatoxin G_1 in NH_4 medium and urea medium, respectively. No North American S strain isolate produced aflatoxin G_1. This geographical and physiological divergence may influence aflatoxin management.

4. Survey

Hell, K., Gnonlonfin, B.G.J., Kodjogbe, G. Lamboni, Y., and Abdourhamane, I.K. **Mycoflora and occurrence of aflatoxin in dried vegetables in Benin, Mali and Togo, West Africa.** *International Journal of Food Microbiology* (submitted)

Fungal infection and aflatoxin and fumonisin contamination was evaluated on 180 samples of dried vegetables such as okra, hot chilli, tomato, melon seeds, onion and baobab leaves from Benin, Togo and Mali collected from September to October 2006. All these products are processed to increase their availability during lean periods and decrease their perishability. After plating on selective media a total of 561 fungal isolates, ranging from 18 in tomato and 218 in baobab leaves were identified. Baobab leaves, followed by hot chilli and okra showed high levels of fungal count compared to the other dried vegetables, while shelled melon seeds, onion leaves and tomato had lower levels of fungal contamination. Species of *Aspergillus* were found to be dominant on all marketed dried vegetables from any country. Mycotoxin assessment by Reversed-Phase High Performance Liquid Chromatography showed that only okra and hot chilli sampled were naturally contaminated with aflatoxin B_1 and aflatoxin B_2 at a total concentration of 6.0 µg/kg on okra and 3.2 µg/kg on hot pepper. This is the first time that mycotoxigenic fungi and resultant toxins were found on dried vegetable products from African markets. Previous reports have mostly highlighted the risk of exposure in Africa from staple crops, with other crops and products being affected there is a high risk for consumers to be exposed to aflatoxins and fumonisins through multiple sources necessitating preventive measures.

Essono, G., Ayodele, M., Akoa, A., Foko, J., Filtenborg, O., and Olembo, S. 2009. **Aflatoxin-producing *Aspergillus* spp. and aflatoxin levels in stored cassava chips as affected by processing practices.** *Food Control* 20: 648-654.

Cassava chips (cassava balls, and cassava pellets) are derived cassava products traditionally produced by farmers in sub-Saharan Africa following fermentation, and drying of fresh roots of cassava, and are widely consumed in Cameroon. Once produced, this food commodity can be stored for more than two months and contaminated by a wide array of harmful microbes. In order to assess persistence of toxigenic fungi in cassava chips, aflatoxin-producing fungi (*Aspergillus flavus, Aspergillus nomius,* and *Aspergillus parasiticus*) and aflatoxins were contrasted at regular intervals in home-stored cassava chips collected in two locations of southern Cameroon throughout a two-month monitoring period. Three hundred and forty-six isolates of aflatoxin-producing fungi were found to be associated with all samples. *A. flavus* contaminated more samples in both types of chips (267 isolates in 53 samples), followed by *A. nomius* (58 isolates in 15 samples), whereas *A. parasiticus* was rarest. A direct competitive Enzyme-linked immunosorbent assay (ELISA)-based method was implemented to quantify the content in aflatoxins. Eighteen of the samples contained some aflatoxins at detectable levels whereas 54 did not. The levels of aflatoxin ranged between 5.2 and 14.5 ppb. The distribution of aflatoxin in positive samples depended on 8 parameters including pH, moisture content, storage duration, types of chips, level of contamination by aflatoxin-producing fungi, processing practices and storage facilities. From analysis of variance results, only pH ($p < 0.01$), duration of storage ($p < 0.01$), population of aflatoxin-producing species (0.0001) and the chip type ($p < 0.05$) were significantly related to aflatoxin in positive samples. A stepwise regression analysis (forward selection procedure) indicated that aflatoxin levels were significantly ($p < 0.01$) correlated with processing practices, storage facilities, and storage duration of the chips.

Kankolongo, M.A., Hell, K., and Nawa, I.N. 2009. **Assessment of fungal, mycotoxin and insect spoilage in maize stored for human consumption in Zambia.** *Journal of the Science of Food and Agriculture* (In press)

Maize constitutes the main staple food and most important crop grown in Zambia. However, maize incurs considerable losses both in field and storage due to pathogens and insects. Some of the pathogens and resultant mycotoxins reduce the nutritional quality of the product. Mycotoxins are toxigenic fungal compounds that can cause cancer and suppress growth. In spite of this health hazard, there has been very little research to document their occurrence. Maize grains stored for human consumption were sampled from different agro-ecosystems (forest, valley and plateau areas) of three agroecological zones (high, mid and low altitude). Several fungal genera were recovered among which *Aspergillus flavus*, *A. niger*, *Fusarium verticillioides*, *F. solani*, *Rhizopus stolonifer* and *Penicillium* spp. were prevalent. The weevil *Sitophilus zeamais* and the larger grain borer *Prostephanus truncatus* were the most damaging. Enzyme-linked immunosorbend assay (ELISA) tests yielded fumonisins and aflatoxins ranging between 0.02-21.44 ppm and 0.7-108.39 ppb in 96.4% and 21.4% of samples, respectively. Fumonisin was more pronounced in villages in forest areas whereas aflatoxin was highest in valley and forest areas in Zone II. Strategic interventions to curtail fungal, mycotoxin and insect contamination should be directed towards improved agronomic and postharvest practices of maize from fields to consumers.

Manjula, K., Hell, K., Fandohan, P., Abass, A., and Bandyopadhyay, R. 2009. **Aflatoxin and fumonisin contamination of cassava products and maize grain from markets in Tanzania and Republic of the Congo**. *Journal of Toxicology, Toxin Reviews* (In press)

Food safety and compliance with international standards is a major challenge for achieving food security in sub-Saharan Africa. The present study evaluated the occurrence of *Aspergillus flavus*, *Fusarium* spp., and related fungi, and resultant aflatoxins and fumonisins in dried cassava and maize samples from various markets and villages in Tanzania and Congo. The relationship between mycotoxins and length of storage period was also elucidated. The levels of aflatoxin B_1 varied from 0.3 to 4.4 ppb in cassava chips and flour, and from 0.1 to 13.0 ppb in stored cassava samples, with relatively high levels of contamination found in cassava stored for 4 months. Maize kernels showed high aflatoxin concentrations, with means ranging from 0.04 to 120 ppb. On maize, the dominant mycoflora were *Aspergillus* spp. (3.3–39.5%) and *Fusarium* spp. (42–70.5%), potentially causing serious health risks to consumers of these products. Low levels of fumonisin ranging from 0 to 0.07 ppm were found in cassava chips and flour with mean values ranging from 0.001 to 0.006 ppm. Maize recorded relatively higher fumonisin levels ranging from 0.02 to 9.4 ppm, indicating that maize is potentially a more serious risk to consumer health than cassava. This needs to be taken into account when developing strategies to reduce toxin contamination and improve health of populations. Aflatoxin in maize is a chronic problem in the two countries surveyed, limiting marketability and income. Nevertheless the collected cassava samples are also prone to aflatoxin contamination, but not fumonisin contamination.

Essono, G., Ayodele, M., Akoa, A., Foko, J., Gockowski, J., and Olembo, S. 2008. **Cassava production and processing characteristics in southern Cameroon: An analysis of factors causing variations in practices between farmers using Principal Component Analysis (PCA)**. *African Journal of Agricultural Research* 3: 49-59.

A questionnaire-based survey study was carried out during a 3 month period, from January to April 1998, in 45 villages belonging to three locations (Yaoundé, Mbalmayo, and Ebolowa) of southern Cameroon. The survey was aimed at collecting constraints and processing practices related information from farmers growing cassava and transforming it into chips. Information in the questionnaire contained some characteristics associated with cassava chips production (processing methods, forms of chips produced, their end uses, drying and storage facilities used, the major problems associated with their production) and cassava cultivation (cassava varieties, harvesting periods of these varieties, and their preferred attributes). A total of 225 farmers were interviewed and the results obtained showed that farmers in Yaoundé and Mbalmayo processed and stored chips in similar ways. Similarly, harvesting periods after planting also differed between Ebolowa and both Yaoundé and Mbalmayo. Storage methods and storage facilities were mainly related to the different forms of chips produced. Chips' discoloration frequently reported by the majority of farmers (87%) was as a result of their insufficient drying. Principal component analysis was implemented to determine those factors accounting for differences observed in farmers' practices. Eleven principal components were derived from the variables used in analysis. Five principal components accounting for 72.7% of the total variations were associated with the data set collected in Yaoundé and Mbalmayo. An equal number expressing 78.2% of the overall variance was likewise obtained at Ebolowa. For a number of reasons such as traditional patterns of nutrition, market purposes, the relative proximity with the nearest city, these components suggested that storage methods, end uses, and production constraints were differently perceived by the respondents. They also showed that the different forms of chips produced were more market oriented, and that a number of constraints experienced by farmers were closely related to the way they managed their fields, or market outlets.

Essono, G., Ayodele, M., Foko, J., Akoa, A., Gockowski, J., Ambang, Z., Bell, J.M., and Bekolo, N. 2008. **Farmers' perceptions of practices and constraints in cassava (*Manihot esculenta* Crantz) chips production in rural Cameroon.** *African Journal of Biotechnology* 7: 4172-4180

A survey aimed at collecting information on practices and constraints in the production of cassava (*Manihot esculenta* Crantz) chips, a transformed cassava product obtained through fermentation and drying of its fresh roots was carried out in 45 villages located in three geographical regions (Yaoundé, Mbalmayo, and Ebolowa) of the humid forest zone of Cameroon. A structured questionnaire to interview farmers was employed. Out of 225 farmers sampled, 212 (94%) relying on chips as food and source of income were women. Overall, 51% of all farmers marketed chips locally. Three distinct forms of chips such as broken pulp (62%), balls (25%), and pellets (13%) were cited as being locally produced by farmers. These were obtained either through air fermentation (cassava pellets), or submerged fermentation (broken pulps and balls), using starters or fermenting agents (31% of responses) or without using them (69%). Chips were mainly home-stored in jute and or/plastic bags (43% of responses), open or closed containers (36%), or on devices hanging over the fireplace (21% of responses) for as long as 180 days. Dark spots or discolouration occurring as a result of chips damage were reported by the majority of respondents (82%) as frequent on cassava chips. These were related to insufficient drying (42%), the use of infected cassava roots by plant pathogenic microbes from the fields (12%), or too long drying of chips under sun light (11% of responses). To avoid dark spots and/or discolouration, 112 farmers out of a total of 185 who were aware about chips damage, practiced sundrying, and 21% of this total dried their chips over the fireplace to control chips damage. Pests and diseases problems (47% of responses), mainly related to the incidence of *Stictococcus vayssierei* (Homoptera: Stictococcidae) and lack of market (26%) were cited by farmers as the most important constraints in cassava chips production. From the results obtained, this study outlined that the potential utilization of cassava and its derived products for industrial purposes is not yet exploited in the locations investigated. Additionally, the study also raised concerns about the safety and hygiene associated with traditionally processed and stored cassava chips in the investigated areas.

Gnonlonfin, G.J.B., Hell, K., Fandohan, P., and Siame, A. B.. 2008. **Mycoflora and natural occurrence of aflatoxins and fumonisin B_1 in cassava and yam chips from Benin, West Africa**. *International Journal of Food Microbiology* 122: 140-147.

The presence of fungi, aflatoxins and fumonisin B_1 in cassava and yam chips (during 28 processing and storage) were evaluated during two consecutive seasons in two agroecological zones of Benin (Northern Guinea Savannah, NGS and Sudan Savannah, SS). The Benin samples were assessed for moisture content, fungal infestation and total aflatoxin and fumonisin B_1 contamination. During the two seasons, samples collected from the NGS, had moisture contents ranging from 10.0 to 14.7% in cassava chips and from 11.4 to 15.3% in yam chips. In samples from the SS, moisture content ranged from 10.1 to 14.5% and 11.1 to 14.5% in cassava and yam chips, respectively. *A. flavus* was the predominant fungal species. The maximum cfu/g in cassava and yam chips was 8950 and 6030, respectively. Other fungal species isolated included *P. chrysogenum*, *Mucor piriformis*, *Phoma sorghina*, *F. verticillioides*, *Rhizopus oryzae* and *Nigrospora oryzae*. High performance liquid chromatography analysis of both cassava and yam chips showed no contamination by either aflatoxins or fumonisin B_1.

Ngoko, Z., Daoudou, I.H., Kamga, P.T., Mendi, S., Mwangi, M., Bandyopadhyay, R., and Marasas, W.F.O. 2008. **Fungi and mycotoxins associated with food commodities in Cameroon**. *Journal of Applied Biosciences* 6: 164 - 168.

Spoiled maize grains and numerous types of snacks that are consumed in the Western Highlands of Cameroon are infected by several mycotoxin producing fungi. The extent of contamination of these food commodities by secondary metabolites of fungal origin has not been well studied. This study aimed to identify the microorganisms that infect maize grains and snacks sold at road side markets, and to sensitize the population on the health risks that are associated with consumption of contaminated commodities. Maize and snack samples were collected from various locations in Cameroon. Contaminating microorganisms were isolated and identified using conventional techniques. *Staphylococcus* and *Salmonella* species were the most frequently isolated bacteria while *Fusarium* and *Aspergillus* species were isolated in highest frequency ranging from 20 to 100 % presence in the samples analyzed. Chemical analyses revealed the presence of fumonisins (50-26000 ng g^{-1}), Deoxynivalenol (100-1300 ng g-1) and zearalenone (50-180 ng g^{-1}) in the sampled maize. Contamination of agricultural products by microbial toxins is an important but often underestimated risk to public health and can have long-term health implications. Appropriate sanitary measures need to be taken to ensure that conditions for microbial contamination and toxin production are reduced.

Bandyopadhyay, R., Kumar, M., and Leslie, J.F. 2007. **Relative severity of aflatoxin contamination of cereal crops in West Africa**. *Food Additives and Contaminants* 24: 1109-1114.

Aflatoxins are a common contaminant of cereals that can cause cancer, liver disease, immune suppression, retarded growth and development, and death, depending on the level and duration of exposure. Maize is an introduced crop to Africa and there have been efforts over the last 20 years or so to replace traditional cereal crops, such as sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*), with maize. We found that maize was significantly more heavily colonized by aflatoxin-producing *Aspergillus* spp. than either sorghum or millet, with overall aflatoxin levels being correspondingly higher. On average, Nigerians consume 138 kg cereals annually. If the primary cereal is sorghum instead of maize, then the risk of aflatoxin-related problems is reduced 4-fold; if it is pearl millet, then the risks are reduced 8-fold. Development programs and other ventures to increase maize production in marginal cropping areas of Africa should be reconsidered and, instead, efforts to improve/maintain traditional crops encouraged.

Essono, G., Ayodele, M., Akoa, A., Foko, J., Olembo, S., and Gockowski, J. 2007. ***Aspergillus* species on cassava chips in storage in rural areas of southern Cameroon: their relationship with storage duration, moisture content and processing methods.** African *Journal of Microbiology Research* 1: 1-8.

A survey was carried out to monitor during a two-month period the incidence of *Aspergillus* in samples of stored cassava chips traditionally produced in southern Cameroon. Seventy-two samples associated with two forms of chips (cassava balls and cassava pellets) were collected in two locations (Yaoundé and Ebolowa) and 13 *Aspergillus* species were isolated. In both locations, *Aspergillus versicolor* was seldom isolated, whereas *A. flavus* and *A. clavatus* were most frequently isolated. The level of recovery of isolates obtained was not affected by location and form of chips, but by the duration of storage ($P < 0.01$) and the moisture content ($P < 0.05$). Five core species were identified, which formed more than 70% of the total isolates associated with the samples analyzed. These were *A. clavatus*, *A*. *flavus*, *A*. *fumigatus*, *A*. *niger* and *A*. *ochraceous*. Correlation coefficients computed between pairs of these species based on total isolation figures for the two locations showed that some were significantly associated. *A. clavatus*, *A. niger* and *A. ochraceous* were positively related to one another in a significant way. Similarly, significant correlations, positive or negative, were observed between the moisture content and all core *Aspergillus* species. The larger number of these toxigenic fungi isolated raises concerns on the potential of stored cassava products as a natural substrate liable to mycotoxin formation.

Thomas, D., Vismer, H.F., Rheeder, J.P., Bandyopadhyay, R., Leslie, J.F., and Marasas, W. F. O. 2006. ***Fusarium* species on sorghum grain in Nigeria**. Paper presented at the 14th Biennial Congress of the South African Society for Microbiology, 9 - 12 April 2006, CSIR, Pretoria, South Africa. (Abstract)

Sorghum is the fourth most widely cultivated cereal in Africa and is nutritionally comparable or superior to other major cereals. Sorghum is well adapted to the semi-arid conditions of Africa and constitutes a major part of the diet of many Africans. Sorghum also is used as a raw material in animal feed. Several *Fusarium* species are pathogenic to sorghum and some can produce mycotoxins, which pose a health risk to both human and domesticated animals that consume the contaminated grain. Twenty-seven sorghum samples were collected from 14 locations in the southern and northern guinea savannas of Nigeria and the extent of *Fusarium* contamination in these samples was determined. From each sample, 100 surface-disinfested sorghum kernels were plated on malt extract agar and incubated at 25° C in the dark for 5 days. *Fusarium* isolates were identified to species level based on their morphological characters. Twenty-five of the 27 samples analyzed contained *Fusarium* species. The most frequently isolated *Fusarium* species was *F. equiseti* (16%) followed by the recently described species, *F. andiyazi* (8%). *Fusarium thapsinum* (7%) and *F. semitectum* (6%) also were isolated frequently. The known fumonisin producers, *F. verticillioides* (5%) and *F. proliferatum* (1%) were also isolated from some samples but usually at low frequencies. Other *Fusarium* species also isolated at low frequencies were *F. chlamydosporum* (1.8%), *F. oxysporum* (1.6%), *F. pseudonygamai* (0.6%), *F. compactum* (0.2%), *F. scirpi* (0.2%) and *F. pseudoanthophilum* (0.1%). Further studies will include molecular characterization to confirm the preliminary morphological identifications of the *Fusarium* isolates

Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W.F., and Wingfield, M.J. 2005. **Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa**. *International Journal of Food Microbiology* 99: 173-183.

The natural occurrence of *Fusarium* and fumonisin contamination was evaluated from 1999 to 2003 in both preharvest and stored maize produced by small-scale farmers in four agroecological zones of Benin. Mycological analyses revealed a predominance of both *Fusarium* and *Aspergillus* in maize samples compared to other genera. The two *Fusarium* species most commonly isolated from maize were *Fusarium verticillioides* (68%) and *Fusarium proliferatum* (31%). Atypical isolates of *F. verticillioides* with some characteristics of *Fusarium andiyazi* but apparently closer to *F. verticillioides*, because the isolates were all high fumonisin producers, were also found only on preharvest maize. Study of *F. verticillioides* strains showed the presence of extremely high fumonisin producers in Benin with total fumonisin levels ranging from 8240 to 16690 mg/kg. Apart from 2002–2003, *Fusarium* occurrence was not significantly different from one zone to another, although a slight decrease was observed from south, humid, to north, drier. *Fusarium* occurrence varied somewhat from one season to another. It significantly decreased over the 6 months of storage. Widespread fumonisin occurrence in maize was observed. Most of the maize samples collected were found positive for fumonisin with levels ranging from not detected to 12 mg/kg in 1999–2000, 6.7 mg/kg in 2000–2001 and 6.1 mg/kg in 2002–2003. Fumonisin levels in maize were found to be significantly higher in the two southern zones during all the surveys. The highest mean total fumonisin level was detected in 1999–2000 in maize samples from the southern Guinea Savannah (SGS) (12 mg/kg), whereas in both 2000–2001 and 2002–2003, it was in samples from the forest mosaic savannah (FMS) (6.7 and 6.1 mg/kg, respectively). Fumonisin levels varied from one season to another and, throughout the storage time, showing a decreasing trend in each zone. However, this decrease was not significant every season. An increasing trend was observed during some seasons in the SGS and northern Guinea Savannah (NGS) zones. The results of this study emphasise that farmers and consumers, not only in Benin but also in other West African countries, should be alerted to the danger of fumonisin contamination in maize.

Mestres, C., Bassa, S., Fagbohoun, E., Nago, M., Hell, K., Vernier, P., Champiat, D., Hounhouigan, J., and Cardwell, K.F. 2004. **Yam chip food sub-sector: hazardous practices and presence of aflatoxins in Benin**. *Journal of Stored Products Research* 40: 575-585.

A survey of the sanitary quality, particularly concerning aflatoxin contamination and practices of the dried yam chips food sub-sector was carried out in Benin. Producers and intermediaries of the yam chips food production sub-sector were interviewed and samples collected. Aflatoxin content was assessed by a biochemo-luminescence method on a total of 107 samples. Twenty-three per cent of the samples had aflatoxin contents over the 15 mg kg^{-1} CODEX standard value for total aflatoxin. Moisture content of whole tuber chips was around 20% when producers stopped drying after 3–6 days. Drying was thus not accomplished, but most producers were unaware of this problem. After storage for 7 months, mean moisture content was around 14%, but 41% of the samples stored in rooms had a moisture content over 15%, levels that are still favourable for mould growth. Most producers, wholesalers and retailers complained about storage problems and particularly about insect proliferation, but less than 15% mentioned mould growth as a problem. Mouldy chips are generally washed and dried again. Very rarely are mouldy chips discarded and lack of moulds is not a quality attribute for dried yam chips. Therefore, there is a risk of chronic exposure to aflatoxin for Beninese yam chips consumers.

Hell, K., Cardwell, K.F., and Poehling, H.M. 2003. **Distribution of fungal species and aflatoxin contamination in stored maize in four agroecological zones in Benin, West-Africa**. *Journal of Phytopathology* 151: 690-698.

This study relates pre-harvest and harvest practices to post-harvest quality of maize in Benin, West Africa. Fungal infection and aflatoxin levels were evaluated in 300 farmers' stores in four agro-ecological zones over 2 years (1993–1995), at the beginning of storage (sample A) and 6 months later (sample B). *Aspergillus flavus* infected 10–20% of the kernels in sample A (1993–1994). In sample B, 54–79% of the kernels were infected with *A. flavus*. In 1994–1995, *A. flavus* infection was higher in sample A (27–47%) than B (8–26%). *Fusarium* species were found in 38–58% of the kernels in sample A in both years, but decreased slightly to 29–51% in sample B. Significant agroecozonal effects existed within sampling, but were not consistent between samplings and years. Of the total number of samples collected (744), 38.8% were found to be aflatoxin-positive, with an average of 105 parts per billion (ppb) and 60% of the aflatoxin-positive samples having a contamination approximately 20 ppb, the intervention level recommended by the World Health Organization. Factors associated with increased aflatoxin were: planting local maize varieties in southern Benin, intercropping with cowpea, groundnut, or cassava, use of urea-fertilizer, damage to maize in the field, prolonged harvesting, long drying periods in the field, and winnowing. Practices that reduced aflatoxin contamination were: planting improved varieties in northern Benin, mixed cropping with vegetables, use of NPK-fertilizer, drying of harvested cobs for 60–90 days, drying ears without the husk, sorting out of poor quality ears.

Bassa, S., Mestres, C., Champiat, D., Hell, K., Vernier, P. and Cardwell K.F. 2001. **First Report of Aflatoxin in Dried Yam Chips in Benin**. *Plant Disease* 85: 1032.

In a survey on the sanitary quality of dried yam chips in Bénin during July and August 2000, fifty dried yam chips samples were collected from different points in the marketing chain (producers, wholesalers, retailers, dried yam-based food sellers and consumers). Aflatoxins were detected in all dried yam chip samples, with levels ranging from 2.2 to 200 ppb and a mean value of 14 ppb. An aflatoxin concentration higher than the European Union's maximum residue limit (MRL) of 4 ppb was found in 98% of the samples (n=50), while 6% had an aflatoxin concentration higher than the World Health Organization's MRL of 20 ppb. *Aspergillus* spp. were detected in the inner part of dried yam chips of both samples, with a mean level of 9000 colony forming units/g. *Fusarium* colonies were also present but were not identified to species.

Ngoko, Z., Marasas, W.F.O., Rheeder, J.P., Shephard, G.S., Wingfield, M.J., and Cardwell, K.F. 2001. **Fungal infection and mycotoxin contamination of maize in the humid forest and the western highlands of Cameroon.** *Phytoparasitica* 29: 352-360.

Fungal incidence and mycotoxin contamination of farm-stored maize were assessed and compared in grain samples from three villages each in two agroecological zones over time. Maize samples were collected at 2 and 4 months after stocking from 72 farmers' stores in 1996 and 1997 in the Humid Forest (HF) and Western Highlands (WHL) of Cameroon. Mycological assays of these samples revealed several fungal species. *Nigrospora* spp. were the most prevalent fungi in HF (32%) and WHL (30%) in 1996, *Fusarium verticillioides* (22%) and *F. graminearum* (27%) were also isolated from these samples. In the WHL in 1996, no significant difference in fungal incidence was found among villages for samples collected 2 months after harvest, but at 4 months incidence was significantly higher $P<0.05$). In 1997 the levels of fungal contamination were lower than in 1996. The incidence of *Aspergillus* spp. was low in general, ranging from 0.0 to 5.9% infected kernels. Analysis with thin layer chromatography detected low levels of aflatoxins in a few samples. *F. verticillioides* mycotoxin fumonisin B_1 (300-26,000 ng/g) and *F. graminearum* metabolites deoxynivalenol (<100–1,300 ng/g) and zearalenone (<50–110 ng/g) were determined by means of polyclonal antibody competitive direct enzyme-linked immunosorbent assay. A significant correlation ($r=0.72$; $P=0.0001$) was found between the incidence of *F. graminearum* and the contamination with deoxynivalenol. Storage time (2 *vs* 4 months after stocking) had a significant positive effect ($r=0.39$; $P=0.013$) on the level of fumonisin B_1. This is the first report of the natural occurrence of these mycotoxins in maize in Cameroon.

Sétamou, M., Cardwell, K.F., Schulthess, F., and Hell, K. 1997. ***Aspergillus flavus* infection and aflatoxin contamination of preharvest maize in the Republic of Benin.** *Plant Disease* 81: 1323-1327.

Eighty and sixty maize fields were sampled in 1994 and 1995, respectively, to monitor *Aspergillus* infection and aflatoxin contamination of preharvest maize in Benin. Three *Aspergillus* species were isolated from different agroecological zones, with *A. flavus* being the most prevalent. The countrywide mean percentage of kernel infection was about 20% in both years. Aflatoxin was extracted from maize in at least 30% of the fields sampled. Toxin concentrations exhibited a distinct zonal variation, with relatively high levels in the Guinea Savanna. There was a trend toward higher rate of aflatoxin accumulation per percentage *A. flavus* infection from the south to the north. Damage by the ear borer, *Mussidia nigrivenella,* increased aflatoxin accumulation in maize. Hence, the geographic pattern observed in the occurrence of *A. flavus* and aflatoxin may be related to the incidence of *M. nigrivenella*.

5. Management

Fandohan, P., Hell, K., and Marasas, W.F.O. 2008. **Food processing to reduce mycotoxins in Africa**. Pages 309–316 *in* "Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade". J. F. Leslie, R. Bandyopadhyay and A. Visconti, eds. CABI Publishing, Wallingford, UK.

Mycotoxins have both economic and health impacts because they contaminate human food and animal feeds. A central question is whether there are food processing strategies that reduce mycotoxin levels in food products. In this chapter we critically review food processing methods tested in Africa for their efficacy in reducing mycotoxin contamination in maize and peanut. These methods include cleaning, separation of screenings, washing, steeping, aqueous extraction, dehulling, milling, fermentation, cooking and roasting. Some methods reduced mycotoxin levels significantly while others were less effective. Encouraging the widespread use of the toxin-reducing processing techniques would lower consumption of contaminated food products in Africa, and improve food quality and human health.

Hell, K., Fandohan, P., Bandyopadhyay, R., Cardwell, K., Kiewnick, S., Sikora, R., and Cotty, P. 2008. **Pre- and post-harvest management of aflatoxin in maize**. Pages 413–422 *in* "Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade". J. F. Leslie, R. Bandyopadhyay and A. Visconti, eds. CABI Publishing, Wallingford, UK.

Pre- and post-harvest contamination of aflatoxin in maize is a major health deterrent for people in Africa where maize production has increased dramatically. This chapter highlights management options for pre- and post-harvest toxin contamination in maize. Sound crop management practices are an effective way of avoiding, or at least diminishing, infection by *Aspergillus flavus* and subsequent aflatoxin production. Pre- and post-harvest practices that reduced aflatoxin contamination include: the use of resistant cultivars, harvesting at maturity, rapid drying on platforms to avoid contact with soil, appropriate shelling methods to reduce grain damage, sorting, use of clean and aerated storage structures, controlling insect damage, and avoiding long storage periods. These contamination reducing management practices are being tested in collaboration with farmers. Work continues on food basket surveys, the bio-ecology of aflatoxin production, developing biological control through a competitive exclusion strategy, reducing the impact of post-harvest management practices on human blood toxin levels, and breeding to reduce the impact of mycotoxins on trade.

Kumar, M., Bandyopadhyay, R., Akinbade, S.A. and Kumar, P.L. 2008. **Production of polyclonal antibodies to aflatoxin B_1 and development of an indirect ELISA for estimation of aflatoxin contamination in crops**. Paper presented at the 3rd Annual Conference of Nigerian Mycotoxin Awareness and Study Network: Strategies for Control of Mycotoxin Contamination, 28-30 April 2008, Standards Organization of Nigeria, Lekki, Lagos. Page 7. (Abstract)

Aflatoxins, the carcinogenic secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus*, are common contaminants in several staple crops produced in tropical and sub-tropical conditions around the world. Strict food safety regulations implemented in most countries demand aflatoxin contamination less than 20 ppb. Despite availability of wide variety of diagnostic tools for estimation of aflatoxins, their utilization in most developing countries is limited due to high cost, difficulties with importation and lack of appropriate laboratory facilities and human skills. Antibody-based methods have been reported, and such methods are proven to be simple and easy for adoption in developing countries. Therefore, to enhance diagnostic capacity in sub-Saharan Africa, we developed an indirect enzyme-linked immunosorbent assay (ELISA) for quantitative estimation of aflatoxins. Very high tittered rabbit polyclonal antibodies for aflatoxin B_1 (AFB_1) were produced following an immunization protocol, which include four intramuscular injections with 300 μg AFB_1-BSA conjugate at weekly intervals, and collection of polyclonal serum one week after the last injection. Antibody capture ELISA showed the polyclonal serum has an end-point titer of 1:512,000 when 100 ng ml^{-1} BSA-AFB_1 was used as coating antigen. There was no cross-reaction with BSA or plant extracts indicating very high specificity of these antibodies. An indirect competitive ELISA was established for quantitative estimation of aflatoxins with a detection range of 0.09 to 25 ng ml^{-1}, using antibodies at 1:75,000 dilution. The recovery of AFB_1 spiked to control maize extract by this assay was 98±10%. Results from validation of this assay by analyzing maize samples artificially spiked with known concentration of AFB1 and field grown maize samples demonstrated the reliability and accuracy of the test.

Kumar, P.L., Bandyopadhyay, R., Akinbade, S.A. and Kumar, M. 2008. **Development of an in-house diagnostic test for monitoring aflatoxin contamination in agriculture commodities.** Paper presented in the 21st Annual Conference of Biotechnology Society of Nigeria, themed 'Modern Biotechnology: A gateway to sustainable food production and affordable health care in Nigeria. University of Abeokuta, 15-18 July 2008. A. Poppola, M. Edema, T. Adebamo, and S. Uzochukwu, eds. Nigeria. BSN Publication (ISBN#978-078-799-2), Pages 27-28. (Abstract).

A simple and sensitive enzyme-linked immunosorbent assay (ELISA) test in 96-microwell plate format was developed for the rapid, reliable and high throughput detection of aflatoxins, the carcinogenic metabolites of food invading fungi, *Aspergillus flavus* and *A. parasiticus*, in a variety of commodities. This assay is based on indirect competitive ELISA principle using rabbit polyclonal antibodies produced in-house against aflatoxin B_1. A simple extraction step precedes sample analysis by ELISA. Assay specificity validated by spiking sample matrices (maize) gave a mean recovery of 98% (80-130% range). This test is specific and sensitive enough for measuring of 2 ppb aflatoxin B_1 in maize and other biological matrices. Simplicity, cost-effectiveness, and suitability to the economic and infrastructural needs of the sub-Saharan Africa are positive aspects of this assay. This test has the potential to offer a sustainable solution to meet ever increasing demand for aflatoxin monitoring programs related to food safety and trade in sub-Saharan Africa.

Ortiz, R., Ban, T., Bandyopadhyay, R., Banziger, M., Bergvinson, D., Hell, K., James, B., Jeffers, D., Lava Kumar, P., Menkir, A., Murakami, J., Nigam, S. N., Upadhyaya, H.D., and Waliyar, F. 2008. **CGIAR research-for-development program on mycotoxins**. Pages 413–422 *in* "Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. J. F. Leslie, R. Bandyopadhyay and A. Visconti, eds. CABI Publishing, Wallingford, UK.

The major mycotoxins studied at the Consultative Group on International Agricultural Research (CGIAR) institutes are aflatoxins in maize, peanut, sorghum and cassava, *Fusarium* toxins in maize, wheat and sorghum, and ochratoxin in cocoa and cashew. Genetic enhancement (both through plant breeding and biotechnology), biological control, habitat management, risk assessment, institutional capacity building and public awareness are among the tools in the "CGIAR research-for-development kit" to fight mycotoxins worldwide. A holistic approach should be pursued to deal with mycotoxins that includes the following elements: i) an integrated crop management package that combines mycotoxin-resistant germplasm, biological control, habitat control and soil-amendments; ii) low-cost mycotoxin detection technology for rapid appraisal that also should facilitate trade; iii) a participatory process for mycotoxin assessment in commercially important crops; and iv) a high-level panel composed of scientists, NGOs, farmers, traders, consumers, health officers and policy makers to monitor mycotoxin intervention strategies and to organize awareness campaigns.

Afolabi, C.G., Bandyopadhyay, R., Leslie, J.F., and Ekpo, E.J.A. 2006. **Effect of sorting on incidence and occurrence of fumonisin and *Fusarium verticillioides* on maize**. *Journal of Food Protection* 69: 2019–2023.

Fumonisin mycotoxins are commonly found on maize and pose a health risk to humans and domesticated animals. Visible sorting of grain has been suggested as a simple technique that can be used to reduce exposure to fumonisins. We collected maize samples in 2003 from different farms in the Kaduna state of Nigeria (Northern Guinea Savanna agroecological zone) that had been sorted by farmers as either good quality or poor quality. The amount of fumonisins and the presence of *Fusarium verticillioides* were determined for each sample. All 13 poor quality samples and the 5 good quality samples positive for fumonisins contained *F. verticillioides.* Twelve of 13 poor quality samples contained fumonisins (1.4 to 110 μg/g), as did the five good quality samples that were positive for *F. verticillioides* (0.2 to 3.7 μg of fumonisins per g). Thus, the visible sorting of grain as a technique to reduce the exposure of subsistence farmers to fumonisins could be successful if there were enough good quality grain available to permit the poor quality grain to be used for another purpose or discarded.

Fandohan, P., Ahouansou, R., Houssou, P., Hell, K., Marasas, W.F.O., and Wingfield, M.J. 2006. **Impact of mechanical shelling and dehulling on *Fusarium* infection and fumonisin contamination in maize**. *Food Additives and Contaminants* 23: 415-421.

Mechanical shelling and dehulling methods were tested to evaluate their impact on *Fusarium* infection and fumonisin contamination in maize. All shelling methods which were tested were found to damage the grains. The IITA® sheller caused the highest level (up to 3.5%) of damage. *Fusarium* populations were higher on damaged grains, the highest being recorded from grains damaged by the IITA® sheller (2533.3 cfu g^{-1}). Fumonisin levels were higher in damaged grains, the highest being in maize shelled with the IITA® sheller (2.2 mg kg^{-1}). Fumonisin levels were positively and significantly correlated with the percentage of damage caused by the shelling methods, and with the number of *Fusarium* colonies in maize. Mechanical dehulling methods significantly reduced fumonisin levels in maize, resulting in a mean reduction of 62% for Mini-PRL, 65% for Engelberg, and 57% for the attrition disc mill. It is important for farmers to choose appropriate shelling methods to reduce mycotoxin contamination. Dehulling should be widely promoted for the reduction of mycotoxins in maize.

Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W.F.O., and Wingfield, M.J. 2006. **Impact of indigenous storage systems and insect infestation on the contamination of maize with fumonisin**. *African Journal of Biotechnology* 5: 546-552.

Four storage systems of maize commonly used by farmers in Benin, West Africa, were tested to determine their impact on infection of maize by *Fusarium* and subsequent contamination with fumonisins. The study showed that *Fusarium* incidence was significantly higher when maize was stored on a cemented floor in a house, a non ventilated facility (40.3 ± 17.4%), than in the other tested systems ($p < 0.05$). The lowest *Fusarium* incidence was recorded when maize was stored in a bamboo granary that is a ventilated facility (25.5 ± 13.5%) ($p < 0.05$). All maize samples from the tested storage systems were found to be fumonisin-positive, with levels ranging from 0.6 to 2.4 mg/kg. Fumonisin level, overall, was found to decrease over the storage period, but not significantly in all the tested storage systems. Damage by lepidopterous pests was significantly and positively correlated with both infection of maize with *Fusarium* and contamination by fumonisin. In contrary, damage by coleopterous insects was significantly and negatively correlated with infection of maize with *Fusarium* and contamination by fumonisin. Avoiding the use of non-ventilated systems to store maize and reducing insect infestation in field and during storage are very important recommendations for farmers.

Fandohan, P., Zoumenou, D., Hounhouigan, D.J., Marasas, W.F., Wingfield, M.J., and Hell, K. 2005. **Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin**. *International Journal of Food Microbiology* 98: 249-59.

The fate of aflatoxins and fumonisins, two mycotoxins that co-occur in maize, was studied through the traditional processing of naturally contaminated maize in *mawe*, *makume*, *ogi*, *akassa*, and *owo*, maize-based foods common in Benin, West Africa. Levels of total aflatoxin and fumonisin were measured at the main unit operations of processing, and the unit operations that induce significant reduction of mycotoxin level were identified. Overall reduction of mycotoxin level was more significant during the preparation of *makume* (93% reduction of aflatoxins, 87% reduction of fumonisins) and *akassa* (92% reduction of aflatoxins, 50% reduction of fumonisins) than that of *owo* (40% reduction of aflatoxins, 48% reduction of fumonisins). Sorting, winnowing, washing, crushing combined with dehulling of maize grains were the unit operations that appeared very effective in achieving significant mycotoxin removal. Aflatoxins and fumonisins were significantly recovered in discarded mouldy and damaged grains and in washing water. Fermentation and cooking showed little effect. During the preparation of *ogi* and *akassa*, reduction of fumonisin levels measured in food matrix was lower (50%) compared to *mawe* and *makume*, probably due to significant fumonisin release in *ogi* supernatant. Consequently, the use of *ogi* supernatant for preparing beverages or traditional herbal medicines could be harmful as it is likely to be contaminated with mycotoxin from the raw maize.

Hell, K., Bandyopadhyay, R., Kiewnick, S., Coulibaly, O., Menkir, A., and Cotty, P. 2005. **Optimal Management of Mycotoxins for Improving Food Safety and Trade of Maize in West Africa** "The Global Food & Product Chain- Dynamics, Innovations, Conflicts, Strategies" Deutscher Tropentag, October 11-13, 2005 in Stuttgart-Hohenheim.
http://www.tropentag.de/2005/proceedings/node419.html. (Abstract)

Mycotoxin-producing fungi can infect grains from pre-harvest in the field to post-harvest in the stores. In Benin and Togo (West Africa), aflatoxin levels in maize averaged five times the safe limit in up to 30% of household grain stores. As a result, farmers and consumers are being exposed to high levels of aflatoxins and other mycotoxins. Studies have shown that 99% of fully weaned children had nearly 2-fold higher aflatoxin-albumin adduct levels compared to those breast-fed. The International Institute of Tropical Agriculture (IITA) recognizes mycotoxins as an important constraint to improving human health and well being of African people and enhancing African trade internationally. Based on past work by IITA and its collaborators, several pre- and post-harvest strategies are being developed and tested to reduce risks of aflatoxin and fumonisin contamination. These strategies include the use of resistant and/or tolerant varieties, biological control, appropriate post-harvest handling (sorting, cleaning, drying, good packaging, application of hygiene, use of appropriate storage systems, appropriate transportation means), awareness and sensitization on the impact of mycotoxin contamination on human, animal health and trade, promotion of management practices that reduce mycotoxins in food products and the use of appropriate pesticides on food products during storage. Appropriate technologies for processing food in rural areas and their efficacy in reducing toxin contamination are being evaluated. Work continues to focus on food basket surveys, bio-ecology of aflatoxin production, biological control through competitive exclusion strategy, and resistance breeding. Furthermore, strategies to reduce impact of mycotoxin on regional and international trade need investigations.

Fandohan, P., Gbenou, J.D., Gnonlonfin, B., Hell, K., Marasas, W.F., and Wingfield, M.J. 2004. **Effect of essential oils on the growth of *Fusarium verticillioides* and fumonisin contamination in corn**. *Journal of Agricultural and Food Chemistry* 52: 6824-6829.

Essential oils extracted by hydrodistillation from local plants in Benin, western Africa, and oil from seeds of the neem tree (*Azadirachta indica*) were evaluated in vitro and in vivo for their efficacy against *Fusarium verticillioides* infection and fumonisin contamination. Fumonisin in corn was quantified using a fluorometer and the Vicam method. Oils from *Cymbopogon citratus*, *Ocimum basilicum*, and *Ocimum gratissimum* were the most effective in vitro, completely inhibiting the growth of *F. verticillioides* at lower concentrations over 21 days of incubation. These oils reduced the incidence of *F. verticillioides* in corn and totally inhibited fungal growth at concentrations of 8, 6.4, and 4.8 μL/g, respectively, over 21 days. At the concentration of 4.8 μL/g, these oils did not affect significantly fumonisin production. However, a marked reduction of fumonisin level was observed in corn stored in closed conditions. The oils adversely affected kernel germination at 4.8 μL/g and therefore cannot be recommended for controlling *F. verticillioides* on stored corn used as seeds, when used at this concentration. The oil of neem seeds showed no inhibitory effect but rather accelerated the growth of *F. verticillioides*.

Hell, K., Bandyopadhyay, R., and Sikora, R.A. 2004. **Strategies for the management of mycotoxins in maize in Benin, West Africa**. "Rural Poverty Reduction through Research for Development. Deutscher Tropentag, October 5-7, 2004, Berlin. (Abstract)

According to the FAO, 25% of the world's food crops are affected by mycotoxin, which negatively impact human health, food trade, food availability and consumption. People are primarily exposed to aflatoxin through consumption of contaminated foods. It was shown that 99% of children monitored in Benin and Togo had high aflatoxin levels in the blood, with some of the highest AF-alb levels ever measured in children. The study showed a striking association between aflatoxin exposure and impaired growth. Depending on ecozone and season, up to 57% of stored maize samples were contaminated with aflatoxins. Aflatoxin contamination is influenced by the populations of toxin producing fungi that reside in the soil, cob feeding lepidopteran insects, invading weevils and other beetles; cultural management of the crop, environment and plant stress; and genotype.

In participatory trials in Benin and Togo, crop and store management options were developed to reduce mycotoxin contamination of maize and their economic viability was assessed. Drying and sorting of maize are techniques that can reduce aflatoxin contamination in maize. Similar technologies have been identified for fumonisin contamination in Benin. The study showed that *F. verticillioides* was the predominant *Fusarium* species found in all maize samples. *Fusarium* incidence was significantly higher when maize was stored on a cemented floor in a house (40.3±17.4%) than in the other systems. The lowest *Fusarium* incidence was recorded when maize was stored in a bamboo granary (25.5±13.5%) ($p = 0.04$). This suggests that storage systems used by farmers may affect *Fusarium* and *Aspergillus* infection on maize, if these systems create conditions favourable for fungal growth. Damage by lepidopterous pests was significantly and positively correlated with both *Fusarium* infection ($r = 0.802$, $p < 0.01$) and fumonisin contamination ($r = 0.852$, $p < 0.01$). Insect damage was positively correlated to aflatoxin content ($r = 0.20$, $p < 0.01$). The control of mycotoxigenic fungi with management practices easily accessible and affordable to farmers' will lower the risk that mycotoxins pose to human health and improve health and human well being.

Fandohan, P., Hell, K., Marasas, W.F.O., and Wingfield, M.J. 2003. **Infection of maize by *Fusarium* species and contamination with fumonisin in Africa**. *African Journal of Biotechnology* 2: 570-579.

Fusarium is one of the major fungal genera associated with maize in Africa. This genus comprises several toxigenic species including *F. verticillioides* and *F. proliferatum*, which are the most prolific producers of fumonisins. The fumonisins are a group of economically important mycotoxins and very common contaminants of maize-based foods and feeds throughout the world. They have been found to be associated with several animal diseases such as leukoencephalomalacia in horses and pulmonary oedema in pigs. Effects of fumonisins on humans are not yet well understood. However, their occurrence in maize has been associated with high incidences of oesophageal and liver cancer. Infection of maize by *Fusarium* species and contamination with fumonisins are generally influenced by many factors including environmental conditions (climate, temperature, humidity), insect infestation and pre- and post-harvest handling. Attempts to control *F. verticillioides* and to detoxify or reduce fumonisin levels in maize have been undertaken. However, more research studies are urgently needed in order to understand more about this toxin. Fumonisins are less documented because they are recently discovered mycotoxins compared to aflatoxins. To date in Africa, apart from South Africa, very little information is available on *Fusarium* infection and fumonisin contamination in maize. It is a matter of great concern that on this continent, millions of people are consuming contaminated maize and maize-based foods daily without being aware of the danger.

Bassa, S., Mestres, C., Champiat, D., Hell, K., Vernier, P., and Cardwell K.F. 2001. **First report of aflatoxin in dried yam chips in Benin**. *Plant Disease* 85: 1032.

In a survey on the sanitary quality of dried yam chips in Bénin during July and August 2000, fifty dried yam chips samples were collected from different points in the marketing chain (producers, wholesalers, retailers, dried yam-based food sellers and consumers). Aflatoxins were detected in all dried yam chip samples, with levels ranging from 2.2 to 200 ppb and a mean value of 14 ppb. An aflatoxin concentration higher than the European Union's maximum residue limit (MRL) of 4 ppb was found in 98% of the samples (n=50), while 6% had an aflatoxin concentration higher than the World Health Organization's MRL of 20 ppb. *Aspergillus* spp. were detected in the inner part of dried yam chips of both samples, with a mean level of 9000 colony forming units/g. *Fusarium* colonies were also present but were not identified to species.

Hell, K., Sétamou, M., Cardwell, K.F., and Poehling, H.M. 2000. **The influence of storage practices on aflatoxin contamination in maize in four agroecological zones in Benin, West Africa**. *Journal of Stored Products Research* 36: 365-382.

Aflatoxin level in 300 farmers' stores in four agro-ecological zones in Benin, a west African coastal country, were determined over a period of 2 years. At sampling a questionnaire was used to evaluate maize storage practices. Farmers were asked what storage structure they used, their storage form, storage period, pest problems in storage and what was done against them. Beninese farmers often changed their storage structures during the storage period, transferring the maize from a drying or temporary store to a more durable one. Most of the farmers complained about insects damaging stored maize. Often, storage or cotton insecticides were utilized against these pests. Regression analysis identified those factors that were associated with increased or reduced aflatoxin. Maize samples in the southern Guinea and Sudan savannas were associated with higher aflatoxin levels and the forest/savanna mosaic was related to lower toxin levels. Factors associated with higher aflatoxin were: storage for 3-5 months, insect damage and use of *Khaya senegalensis*-bark or other local plants as storage protectants. Depending on the agroecological zone, storage structures that had a higher risk of aflatoxin development were the "*Ago*", the "*Secco*", the "*Zingo*" or storing under or on top of the roof of the house. Lower aflatoxin levels were related to the use of storage or cotton insecticides, mechanical means or smoke to protect against pests or cleaning of stores before loading them with the new harvest. Fewer aflatoxins were found when maize was stored in the "*Ago*" made from bamboo or when bags were used as secondary storage containers.

Udoh, J.M., Cardwell, K.F., and Ikotun, T. 2000. **Storage structures and aflatoxin content of maize in five agro-ecological zones of Nigeria**. *Journal of Stored Products Research* 36: 187-201.

A survey was conducted in 1994 to describe the maize storage systems, quantify the aflatoxin levels in these storage systems, and identify the main problems of maize storage recognized by both men and women farmers in five agroecological zones in Nigeria. Maize storage in bags was the most common among all farmers. The clay "*rhumbu*" was used in 4 out of 5 agroecological zones by both male and female farmers. The woven "*oba*" was found only in the southern Guinea savanna and was used predominantly by women. Only 13% of the male farmers in the southern Guinea savanna and none in the other zones stored in an improved crib while no female farmers across all the zones used the crib system of storage. Male and female farmers across all the zones identified insect infestation, and fungal and rodent attack as the main problems in their stored maize. Insect infestation was reported by 83% of the female farmers in the southern Guinea savanna zone who stored maize in bags. The highest fungal attack on stored maize was reported by 71% of the male farmers who stored maize in bags in the humid forest zone, while 75% of the male farmers who stored in bags in the Sudan savanna zone complained of rodent attack. Across all zones, farmers of both genders identified insects as the most common storage problem. Farmers who reported insect problems were significantly more likely to have aflatoxin in their stores. The highest zonal mean aflatoxin level of 125.6 µg/kg was obtained from maize samples provided by male farmers in the Sudan savanna zone who stored maize in bags or in a "*rhumbu*". Across the storage systems, 33% were contaminated with detectable levels of aflatoxin. No aflatoxin was detected in the storage systems of male or female farmers in the northern Guinea savanna zone in 1994.

6. Awareness

Bandyopadhyay, R., Leslie, J.F., and Frederiksen, R.A. 2008. **Nominal Group Discussion Technique: Questions and Responses.** Pages 19–25 *in* "Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. J. F. Leslie, R. Bandyopadhyay and A. Visconti, eds. CABI Publishing, Wallingford, UK.

The Nominal Group technique was used as the discussion format for small group discussions during a conference in Accra sponsored by the MycoGlobe project of the European Union, the International Institute of Tropical Agriculture, INTSORMIL and the Council of Scientific and Industrial Research (Ghana). This chapter is based on these group discussions. The meeting participants identified several key areas. The foremost area was that of communication – with the farming and consumer constituency, with government and international aid officers, and with one another. Both farmers and consumers need to understand that there is a real or potential problem with mycotoxins in their food, and that the chronic long-term effects of exposure to low levels of mycotoxins may be either debilitating or lethal. Mitigation of the worst of these effects is possible with some relatively simple technologies that need to be better publicized. Government and international aid officials need to hear a similar message and to participate in the notification process. As the mycotoxin problem is interdisciplinary in nature, this communication will be difficult since many of these officials have a mandated area that will be touched by mycotoxins, e.g., health, trade or agriculture, but will be dominated by other problems with a narrower focus. A second key area was to develop the protocols and infrastructure to remediate and/or quantify toxin problems. Some of this work needs more research and development, but much, including some dietary interventions and biocontrol strategies are essentially ready to implement. Development of testing laboratories is a matter of building and equipping the necessary laboratories and recruiting/training qualified personnel to staff them. Action in these areas is for implementation on a relatively broad scale, as the techniques needed are already available. Finally there is a need for further research. The medical impacts of chronic, low-level exposure to mycotoxins are poorly understood for aflatoxins and not understood at all for any other mycotoxins. Differences between pathogens from tropical and temperate areas are poorly described, even though temperate zone models and hypotheses often fare poorly when applied to tropical conditions. The identification and development of technologies aimed at low-input, subsistence agriculture is a continuing challenge with unique attributes that receives, at least relatively speaking, little specific attention. The central theme running through all of the meeting and the discussion groups was that food quality, food security, food safety and mycotoxins are all facets of a common problem that will require a multi-disciplinary, cross-sector effort to characterize and remediate. Single sector approaches will help, but any sustainable solution will go far beyond what any single sector approach can be expected to supply.

Coulibaly, O., Hell, K., Bandyopadhyay, R., Hounkponou, S., and Leslie, J.F. 2008. **Economic impact of aflatoxin contamination in Sub-Saharan Africa**. Pages 67–76 *in* “Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade”. J. F. Leslie, R. Bandyopadhyay and A. Visconti, eds. CABI Publishing, Wallingford, UK.

Globalization, including new international trade standards and regulations, has placed significant constraints on competitiveness in and access to international markets for developing countries, especially those in Sub-Saharan Africa. Agricultural commodities from these regions must overcome problems ranging from lower productivity and product quality to higher per unit transportation costs, and lower capacity to manage product flow from suppliers to end-users (value chain management). The competitiveness of African commodities also is impeded by poor policy and institutional environments that result in high transaction costs. One factor reducing African agricultural commodity competitiveness for export is aflatoxin contamination. Aflatoxin lowers product quality and discounts export values, which may lead to significant economic losses for the countries and the agents in commodity value chains. Losses from rejected export shipments and lower prices due to poor quality may exceed 100% if the product is destroyed and the exporter is paying for the shipping. Negative impacts on human health and household include mortality, loss of productivity and reduced income due to lower productive capacity, and related health costs. In this context, the cost of complying with food safety and agricultural health standards has been a major source of concern in the international development community and for African economies, but without increased food quality, neither competitiveness nor more revenue from exports will result. We address the problem of access of African commodities to international markets by recommending an increase in public awareness of the costs and ill effects incurred due to mycotoxins, and the diffusion of aflatoxin control technology and related capacity building to improve food quality in Sub-Saharan Africa. We also recommend strengthening the capacity for conducting impact assessments and collecting the data needed to make optimal decisions amongst possible aflatoxin control measures.

Hughes, J., Bandyopadhyay, R., Makinde, K., and Olembo, S. 2008. **Institutional aspects of sanitary and phytosanitary issues in ECOWAS trade**. Pages 335–348 *in* "Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade". J. F. Leslie, R. Bandyopadhyay and A. Visconti, eds. CABI Publishing, Wallingford, UK.

The Economic Community of West African States (ECOWAS) has accepted trade liberalization and globalization as important policy directions. West African trade with Europe and the United States is already much greater than trade with other developed countries or intraregional trade, although trading with developed countries may entail considerable difficulties due to trade regulations and the need to conform to Sanitary and Phytosanitary (SPS) standards. There is generally a low level of awareness of quality standards among produce exporters in West Africa. SPS focal points are not established in all countries, which make it difficult for exporters to check on standards and requirements. Frequent changes to standards, excessive procedural requirements, high costs for testing and certification, and a lack of transparency in the application of standards combine to compromise the ability of many countries to comply effectively with SPS. Many West African countries have not upgraded their national SPS systems in response to the introduction of the SPS Agreement, leading to differences between local and international standards that make meeting standards difficult for firms that do business in multiple markets. There also is insufficient testing capability to meet the needs for international trade and a lack of regional coordination. Standards application is not enforced in a number of countries, but others have set up institutions for testing, certification, and quality control of both domestic products and imported goods. The effectiveness of these agencies often is weak due to inadequate equipment, a dearth of skilled technical personnel, inability to assess risks, inadequate laboratory accreditation, and a lack of enforcement.

Leslie, J. F., Bandyopadhyay, R., Visconti, A. (eds.). 2008. **Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade**. CABI Publishing. Wallingford, UK. 476 pp.

An International conference "Learning from EC: Reducing Impact of Mycotoxins in Tropical Agriculture with Emphasis on Health and Trade in Africa" was organized in Accra, Ghana, 13-16 September 2005 as a part of the MycoGlobe Specific Support Action project of the European Commission. Representatives from various development organizations, international research and development organizations, advanced research institutes in Europe and the United States, national research organizations in Africa and the Mediterranean, public health ministries, regulatory agencies, and farmers' organizations explored challenges and opportunities to mitigate health and trade problems associated with mycotoxins, and sensitized policy makers and opinion-leaders to the urgency of reducing mycotoxin-related problems. The goal of the conference was to recommend priority actions in the areas of technical, institutional and policy options for improving public health and trade through management of mycotoxins from "field to fork". This book is an outcome of the MycoGlobe conference in Accra. The chapters in this book touch on issues including health, trade, ecology, epidemiology, occurrence, detection, management, awareness and policy. A chapter based on structured discussions at this conference identified priorities for mycotoxin management from technical, institutional and policy perspectives that can serve as a guide for development of inter-institutional, cross-disciplinary programs to reduce the severity of the current problems.

Bandyopadhyay, R., Williams, J.H., and Ojiambo, P.S. 2007. **Social and political challenges in the regulation of mycotoxin contamination**. *Phytopathology* 97: S148. (Abstract)

Mycotoxins are toxic to humans and animals when present in food and feed above tolerance limits. Reduction in mycotoxin exposure of humans and animals has necessitated regulatory interventions by some national governments and international agencies. Conservative estimates of lost crop revenues, and the cost of research and monitoring activities, range from $0.5 to $1.5 billion annually. To achieve current global regulatory compliance in mycotoxin safety levels farmers also have to spend more effort and assume higher risk. Regulatory levels vary between countries and, as a result, mycotoxins act as Technical Barrier to Trade and have led to trade disputes between nations. While food production, processing and marketing systems of developed economies have been largely successful in delivering mycotoxin safe products, regulations have been ineffective in most less-developed countries due to socio-economic, technical, institutional and policy factors. Agricultural products of the best quality are exported from less developed countries to augment export earnings, leaving poorer quality foods to be consumed locally, compromising the health of local populations. Wherever regulations cannot be implemented, the key management options for consumers and farmers are awareness and adoption of simple mycotoxin management practices.

James, B., Adda, C., Cardwell, K., Annang, D., Hell, K., Korie, S., Edorh, M., Gbeassor, F., Nagatey, K., and Houenou, G. 2007. **Public information campaign on aflatoxin contamination of maize grains in market stores in Benin, Ghana and Togo**. *Food Additives and Contaminants* 24: 1283-1291.

Rotary International with the International Institute of Tropical Agriculture (IITA) conducted an information campaign from 2000 to 2004 to increase public awareness of aflatoxin in Benin, Ghana and Togo. Key informant interviews with 2416 respondents showed poor baseline knowledge of aflatoxin and its health risks. The campaign included monitoring of aflatoxin contamination in maize grains from market stores in 38 cities and towns. Aflatoxin concentration in contaminated samples ranged from 24 to 117.5 ng g^{-1} in Benin, from 0.4 to 490.6 ng g^{-1} in Ghana, and from 0.7 to 108.8 ng g^{-1} in Togo. The campaign significantly increased public awareness that populations were exposed to high levels of aflatoxin. The number of maize traders who were informed about the toxin increased 10.3 and 3.2 times in Togo and Benin, respectively; at least 33% more traders believed the information in each of Benin and Togo; 11.4 and 28.4% more consumers sorted out and discarded bad grains in Benin and Ghana, respectively. This paper concludes that sustained public education can help reduce aflatoxin contamination.

James, B. 2005. Rotary Project 3H #99 – 17: **Food Quality Control in Ghana, Togo and Benin**. Terminal report: Public Awareness of Aflatoxin and Food Quality Control. IITA, Cotonou, Benin. pp 1-60.

The project set out to a) develop/integrate aflatoxin monitoring schemes in activities of national food standards boards; b) increase public awareness of aflatoxin and its health risks; and c) conduct feasibility study to de-contaminate poultry feed of aflatoxin and thereby create alternative market for poor quality grains.

Aflatoxin Monitoring: Aflatoxin contamination levels in maize sold to the public were high and ranged from 0.4 to 491 ppb in Ghana, 0.7 to 109 ppb in Togo, and 0.2 to 118 ppb in Benin. The percentage of maize samples with more than 20 ppb aflatoxin contamination ranged from 10% to 40% in Ghana, 2 to 16% in Benin, and approximately 5% in in Togo. The project strengthened national capacity for aflatoxin monitoring (trained manpower, infrastructure, and resources) by food standards boards in the three countries. Also, by fostering collaborative linkages between the food standards board and IITA, the project provided for technical backstopping by IITA, especially in the area of quantifying contamination levels.

Public Awareness Campaign: The project adopted non-formal education methods to convince maize farmers, traders, consumers, poultry farmers and feed mill operators that moldy maize can contain poisonous toxins. Results of beneficiary impact assessment of the campaign showed that significantly more farmers, traders, consumers, poultry farmers and feed mill operators in Ghana, Togo and Benin were better informed and believed in the information than prior to the campaign. By integrating results of aflatoxin monitoring and of farmer participatory field trials on the effect of the toxin in poultry into campaign messages, the project used scientific data to convince the public that the populations were chronically exposed to unacceptably high levels of aflatoxin in the staple food and feed ingredients. All the target groups were willing to transform the acquired knowledge and belief into better grain handling practices. This contributed to observed increases in revenues in the countries. In Ghana, for example, the economic surplus model estimated a net economic gain of ¢24.5 billion resulting from better grain handling by farmers and traders by the year 2010. In the three target countries, radio and TV appeared to be the most reliable channels to scale-out the gains of the campaign.

Use o Bad Grain: About 60% of respondents indicated that they discarded bad grain, and the use of bad grain was grouped into three main categories: 25% of the population fed bad grain to animals (mainly poultry and pigs), 9% mixed bad grains with good grain sold to the public, and 6% used bad grain as food ingredients at home. Through farmer participatory field trials, the project convinced poultry farmers that aflatoxin-contaminated feed reduces body weight and egg laying in poultry.

End-Users’ Appreciation of the Project**:** the percentage of target groups who rated the campaign as good was 53.3% in Ghana, 50.3% in Togo, and 49.1% in Benin.

Cardwell, K.F. (ed.). 1996. **Proceedings of the Workshop on Mycotoxins in Food in Africa**. November 6-10, 1995, Cotonou, Benin. IITA, Ibadan, Nigeria. 77 pp.

Evidence is fast accumulating that people in much of sub-Saharan Africa are experiencing heavy dietary exposure to numerous foodborne mycotoxins. The high exposure levels appear to be having a profound effect on sociological and economic development in the continent. Reports from medical and agricultural research across the continent during the workshop painted a picture so alarming that it was resolved that the issue of mycotoxins in foods in Africa must become a primary research and development target in the immediate future. This proceedings summarizes the information that was brought to the workshop and the conclusions that were drawn by the participants. Ten discussion papers were presented. Half of them reviewed medical case studies in Africa and Asia and detection methods for mycotoxin exposure. The other half assessed available or potential agricultural technologies for the reduction of mycotoxins in foods. As a prelude to regional research planning, participants from many of the African countries completed questionnaires about medical and agricultural research infrastructure in their countries. Three working groups developed the concepts for action in the areas of monitoring toxin levels in foods and human exposure, public health risk assessment and monitoring, and agricultural research and extension. Comparative and interactive research is envisaged to take place in various countries across Africa. Means to facilitate communication and research logistics among the medical and agricultural research is the first requirement. The concept notes and action frameworks are compiled at the end of the proceedings. The general consensus was that an organized effort on a pan-African scale is needed to address the mycotoxin problem.

7. Fungus-Toxin-Insect-Interactions

Lamboni, Y., and Kerstin, H. 2009. **Propagation of mycotoxigenic fungi in maize stores by post-harvest insects.** *International Journal of Tropical Insect Science* (in press)

Maize pests transmit with their movement moulds harmful to human and animal health. The aim of the present work was to study the immigration and the dynamics of storage pests in traditional African maize granaries and the fungal spectrum associated with these insects. Maize ears were protected in the field, just after pollination with gauze; after harvest they were stored, with non-protected ears being the control. Eight different species of insects were identified in stores. No *Prostephanus truncatus* was found in “protected” maize during the six months of storage but their population builds up after three months of storage in the “non-protected” maize to up to 239 insects per kg. The density of *Sitophilus zeamais* on “protected” maize was lower than that on “non-protected” maize. Nine fungal species were found to be associated with the storage insects. On “non-protected” ears the genus *Fusarium* (36%) was the most frequently identified, followed by *Penicillium* (23%), *Rhizoctonia* (6%) and *Aspergillus* (4%). On “protected” ears, *Rhizoctonia* sp. was most frequent (17%), followed by *Fusarium* spp. (17%), *Penicillium* spp. (8%) and *Aspergillus* spp. (2%). The toxigenic species encountered were *Aspergillus flavus*, *A. parasiticus* and *Fusarium verticillioides*. *Cathartus quadricollis* appeared to carry more fungi towards the store, mainly *Penicillium* spp. (51%), *Aspergillus* spp. (47%) and *Fusarium* spp. (32%). Storage pests, in particular *C. quadricollis* and *S. zeamais*, play an important role in the contamination of foods with moulds, especially those that produce toxins.

Gnonlonfin, G.J.B., Hell, K., Siame A.B., and Fandohan, P. 2008. **Infestation and Population Dynamics of Insects on Stored Cassava and Yams Chips in Benin, West Africa.** *Journal of Economic Entomology* 101: 1967-1973.

Natural insect infestation in cassava (*Manihot esculenta* Crantz subspecies *esculenta*) and yam (*Dioscorea* spp.) chips was evaluated during two consecutive storage seasons (2003-2004 and 2004-2005) in two agroecological zones of Benin (Northern Guinea Savanna [NGS] and Sudan Savanna [SS]). The insects infesting chips were collected, identified, and counted, they included *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), *Cathartus quadricollis* (Guerin) (Coleoptera: Silvanidae), *Carpophilus dimidiatus* (F.) (Coleoptera: Nitidulidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *P. truncatus* and *C. quadricollis* were observed with a higher prevalence on cassava than on yam chips. During both seasons after 3 mo of storage, all (100%) cassava chip samples were infested with *P. truncatus* and *C. quadricollis* in both agroecological zones, whereas yam chips only showed lower infestation rates of 59.5 and 19.1% for *P. truncatus* and *C. quadricollis*, respectively, at the end of storage in 2003-2004. During the 2004Ð2005 season after 3 mo of storage infestation rate in yam chips was 66 and 24% in NGS and 100 and 0% in SS for *P. truncatus* and *C. quadricollis*, respectively, showing that insect infestation levels vary significantly with commodity, year, and fluctuate during the storage season.

Mouhoubè, A.K.O., Schulthess, F., Gumedzoe, M., Mawuena, Y.D., and Cardwell. K.F. 2003. **The effect of *Fusarium verticillioides* on oviposition behaviour and bionomics of lepidopteran and coleopteran pests attacking the stem and cobs of maize in West Africa.** *Entomologia experimentalis et applicata* 106: 201-210.

The effect of *Fusarium verticillioides* in maize stem and grain on the oviposition behaviour and bionomics of lepidopteran and coleopteran pests in West Africa was studied in olfactometer, greenhouse, and field trials in Benin. In a choice experiment, the pyralid *Eldana saccharina* laid on average 31.9 eggs on inoculated maize stems vs. 9.2 and 7.8 on stems from plants, grown from hot-water or fungicide treated seeds, respectively. For the pyralid *Mussidia nigrivenella* the values were 42.1 in the inoculation and 7.8 eggs in the fungicide treatment. The survival of *E. saccharina* larvae was significantly higher from the inoculation than the hot-water or fungicide treatments. Fecundity in the three treatments was 494, 307, and 268 eggs per female, respectively. In an olfactometer experiment, no significant differences were found in the time spent by the curculionid *Sitophilus zeamais* between the odor field with *Fusarium*-infected grain and with uninfected grain, but both were significantly different from the controls. Significant differences were obtained for the nitidulid *Carpophilus dimidiatus* with 50.5 and 35.8%, respectively, of the time spent in the *Fusarium*-infected and non-infected odor field. For both species, no differences were found between sexes. For *S. zeamais*, development time was significantly higher when reared on infected (37.2 days) than on non-infected grain (34.4 days), whereas fecundity, expressed as number of progenies during the first 4 weeks, was lower on the inoculated grain (11 vs. 16 offspring per female). For *C. dimidiatus*, the situation was the reverse: development time was shorter and fecundity was higher on infected grain (32.4 vs. 34.4 days, and 18 vs. 13 offspring per female, respectively). It was concluded that the higher pest densities found in the stem and ear of field grown maize was due to a higher attraction to and higher immature survival and adult fecundity on *F. verticillioides* infected plants.

Schulthess, F., Cardwell, K.F., and Gounou, S. 2002. **The effect of endophytic *Fusarium verticillioides* on infestation of two maize varieties by *lepidopterous* stemborers and coleopteran grain feeders.** *Phytopathology* 92: 120-128.

A series of experiments were conducted to test the effect of the presence of *Fusarium verticillioides* in the maize plant on subsequent infestation by coleopteran and lepidopteran pests. The effect of percent internodes 1 to 5 infected with *F. verticillioides*, time after planting, and maize variety on attacks of stem and ears by lepidopterous and coleopteran pests was assessed in field experiments in early and late season 1998 and early season 1999 in Benin Republic. Artificial inoculation of the first internode with fungal-treated toothpicks was compared with a hot-water-fungicide seed treatment and a control. In 1998, two varieties that differed in husk tightness, the improved DMRLSR-W and the local Gbogbe, were used. Percentage of node 1 to 5 and plants infected was highest with the inoculation treatment but tended to be similar in the seed treatment and the control. The infection rate tended to increase with time and, within sampling date, decreased with node level. Ear infection was strongly correlated with percent infected nodes, indicating that *F. verticillioides* in the stem predisposed kernel infection. *F. verticillioides* incidence was higher in Gbogbe than in DMRLSR-W. Stem and ear infestations by the pyralid *Eldana saccharina*, the major pest in the area, tended to be highest in inoculation and lowest in the protection treatment. The same trends were found for the pyralid *Chilo* spp., the tortricid *Cryptophlebia leucotreta*, and beetles pooled across species. Significant positive correlations were found between ear/stem *F. verticillioides* infection and *E. saccharina*, *Cryptophlebia leucotreta*, *Mussidia nigrivenella*, and the noctuid *Sesamia calamistis*, but the latter three pest species were only significantly correlated with fungal infection of the upper nodes of the plant. Similar to disease incidence, *E. saccharina* numbers in stem and ear were higher in Gbogbe than DMRLSR-W in late 1998, whereas for the pyralid ear feeder *M. nigrivenella*, it was reversed. It was suggested that some lepidopterous and coleopteran pests are attracted by and survive longer (or have lower mortality) on plants infected with *F. verticillioides*.

Cardwell, K. F., Kling, J. G., Maziya-Dixon, B., and Bosque-Pérez, N. A. 2000. **Interactions between *Fusarium verticillioides*, *Aspergillus flavus*, and insect infestation in four maize genotypes in lowland Africa**. *Phytopathology* 90: 276-284.

An experiment was designed to compare cycles of selection of four maize genotypes for ear- and grain-quality characteristics, interactions with *Aspergillus flavus* and *Fusarium verticillioides* infection, and insect ear infestation in two seasons. Mean infection levels by *A. flavus* and *F. verticillioides* were significantly higher in inoculated rows than in the controls. The *F. verticillioides*-inoculated rows had significantly more coleopteran beetles and lepidopteran borers per ear than the controls and *A. flavus*-inoculated rows. Genotypes and cycles of selection within genotype were not different with respect to number of insects or percent fungal incidence in the ear, but they were different for husk extension, field weight, 100-grain weight, and grain density. Inoculation with either fungus resulted in significantly higher percentage of floaters (i.e., loss of grain density) and lower grain weight than the controls. Aflatoxin (B_1 and B_2) in *A. flavus*-inoculated rows averaged 327 ppb in the first season and 589 ppb in the second (dryer) season. Fumonisin levels in *F. verticillioides*-inoculated rows did not differ between seasons, with an average of 6.2 ppm across seasons. In the noninoculated control rows, fumonisin was significantly higher in the first (5.3 ppm) than in the second (3.1 ppm) season. For all genotypes, husk extension and yield parameters decreased in the fungal-inoculated treatments. General ear-rot scoring was significantly correlated with incidence of *F. verticillioides* in kernels and grain-weight loss but not with *A. flavus* in the grain.

Hell, K., Cardwell, K.F., Sétamou, M., and Schulthess, F. 2000. **Influence of insect infestation on aflatoxin contamination of stored maize in four agroecological regions in Benin**. *African Entomology* 8: 169-177.

Insect species and damage levels were evaluated and related to aflatoxin content in maize sampled from farmers' stores in four agroecological zones over a two-year period in Benin, West-Africa. In 1993, no aflatoxin was detected in maize that was free of insect damage. In the same year, in maize with more than 70% of cobs damaged by insects 30.3% were aflatoxin positive, with a mean aflatoxin contamination of 77.8 ppb (parts per billion or µg/kg). Grain moisture increased with damage levels. The mean aflatoxin content of maize infested with *Carpophilus dimidiatus* (Coleoptera: Nitidulidae) was significantly higher than maize free of this pest (F = 5.05*, $P \leq 0.05$). In 1994/95, the density of *Mussidia nigrivinella* (Lepidoptera: Pyralidae), was significantly higher in the Northern Guinea Savanna than in the other zones, and the presence of this pest was positively correlated with the cob area visibly infected with *Aspergillus flavus* (Deutoremycetes: Monoliales) (r = 0.239*) early in storage. Six months later, damage levels due to insects were significantly lower in the Sudan Savanna than in the other ecozones. The infestation level of the most common storage pest, *Sitophilus zeamais* (Coleoptera: Curcilionidae) decreased from the south to the north. After six months of storage aflatoxin level was positively correlated with the cob area damaged by *Sesamia calamistis* (Lepidoptera: Noctuidae) (r = 0.25*), the number of *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae) observed on maize (r = 0.26*) and cob area damaged by *S. zeamais* (r = 0.22*).

Borgemeister, C., Adda, C., Sétamou, M., Hell, K., Djomamou, B., Markham, R.H., and Cardwell, K.F. 1998. **Timing of harvest in maize: Effects on post harvest losses due to insects and fungi in central Benin, with particular reference to *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)**. *Agriculture, Ecosystems & Environment* 69: 233-242.

A storage experiment was conducted in Banté, central Benin between autumn 1994 and spring 1995. The maize was harvested 1, 3, and 7 weeks after physiological maturity and stored for up to eight months. The main results were: (a) Leaving the maize in the field for extended periods after physiological maturity resulted in severe grain losses after eight months of storage; (b) Most of the grain losses were attributed to *Prostephanus truncatus*; (c) Early harvested maize had a higher proportion of moldy grain: (d) Harvest date had no consistent effect on the level of aflatoxin contamination; (e) Based on a participatory evaluation of maize quality by local farmers, the economic value of maize stored for eight months was highest in maize harvested three weeks after physiological maturity.

Sétamou, M., Cardwell, K.F., Schulthess, F. and Hell, K. 1998. **Effect of insect damage to maize ears, with special reference to *Mussidia nigrivenella* (Lepidoptera; Pyralidae), on *Aspergillus flavus* (Deuteromycetes; Monoliales) infection and alfatoxin production in maize before harvest in the Republic of Benin**. *Journal of Economic Entomology* 91: 433-438.

Maize infection by *Aspergillus flavus* Link and subsequent aflatoxin contamination as affected by insect damage to maize ears before harvest was studied with surveys in farmers' fields and in a field trial in the Republic of Benin, West Africa. The most important pest species was the lepidopteran earborer *Mussidia nigrivenella*. Percentage of grain infected by *A. flavus* and of samples contaminated with aflatoxin, as well as the mean aflatoxin content of samples, increased with increasing borer damage. Ears with <2% insect damage had an average of 11.7 and 43.6 ppb of aflatoxin in 1994 and 1995, respectively. Ears in the highest damage class (i.e., > 10% damage) had an average aflatoxin of 514.6 and 388.2 ppb in 1994 and 1995, respectively. In 1994 only, coleopteran species such as *Sitophilus zeamais* and *Carpophilus* sp. significantly increased levels of aflatoxin in grain samples. In a field trial using *M. nigrivenella* infestation and *A. flavus* inoculation treatments, the presence of the insect feeding resulted in increased kernel infection and aflatoxin contamination. Artificial infestation with *M. nigrivenella* larvae increased aflatoxin content of maize by an average of 45 ppb, whereas inoculation with *A. flavus* spores increased the toxin level by 517 ppb. The significant interaction between infestation and inoculation indicated that higher levels of aflatoxin B1 were found when the fungus was associated with borers than with the fungus alone. *M. nigrivenella* was the major field pest connected with *A. flavus* infection and subsequent aflatoxin production in preharvest maize in Benin.

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The International Institute of Tropical Agriculture (IITA) is an international non-profit R4D organization founded in 1967 as a research institute with a mandate to develop sustainable food production systems in tropical Africa. It became the first African link in the worldwide network of agricultural research centers supported by the Consultative Group on International Agricultural Research (CGIAR) formed in 1971. Mandate crops of IITA include banana & plantain, cassava, cowpea, maize, soybean and yam. IITA operates throughout sub-Saharan Africa and has headquarters in Ibadan, Nigeria. IITA's mission is to enhance food security and improve livelihoods in Africa through research for development (R4D), a process where science is employed to identify problems and to create development solutions which result in local production, wealth creation, and the reduction of risk. IITA works with the partners within Africa and beyond. For further details about the organization visit: www.iita.org and www.cgiar.org.

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