FUSARIUM INFECTION AND MYCOTOXIN CONTAMINATION IN PREHARVEST AND STORED MAIZE IN BENIN, WEST AFRICA

By

Pascal Fandohan

Submitted in partial fulfilment of the requirements for the degree of Philosophiae Doctor in the Faculty of Natural and Agricultural Science

University of Pretoria

Pretoria

Republic of South Africa

.....2004

The light shines in the darkness and the darkness has never put it out

John 1.5

DECLARATION

I the undersigned hereby declare that the thesis submitted herewith is result of my own work, and has not been submitted in any form to another University.

Pascal Fandohan

ACKNOWLEDGEMENTS

I am indebted very much to my supervisor Prof. W.F.O. Marasas for all his advices, guidance, and moral and material supports during the course of this research. This also goes to Prof. M.J. Wingfield, my co-supervisor, for his constructive criticism and effort to get me admitted at the University of Pretoria. Their encouragement and keen interest led to satisfactory realisation of this study.

My profound gratitude goes to Dr K. Hell for her constant advices and for having seriously fought to get resources from the International Institute of Tropical Agriculture (IITA) for this research.

I am especially grateful to staff members of PROMEC, Medical Research Council, South Africa, for their technical assistance with mycological and chemical analyses. My special thanks also go to Mr B. Gnonlonfin, Mr D. Kiki, Ms C. Adimou and Mr I. Senou all technicians of the Programme of Agricultural and Food Technology (PTAA), National Institute of Agricultural Research of Benin (INRAB), Porto-Novo, Benin, for laboratory analyses.

To all persons who helped me in one way or another during this work and whom I have not mentioned by nonetheless, I sincerely extend my thanks.

This study has been made possible by the financial support of both the Danish International Development Assistance (DANIDA) and the IITA. Their support is gratefully acknowledged.

My sincere gratitude, finally, goes to my wife Lea and our children for their moral support and encouragement, and for their understanding and patience during my many absences from home. You put up with me for success. I thank you very much.

TABLE OF CONTENTS

PREFACE

Fumonisins are mycotoxins produced mainly in maize by some toxigenic species of the genus *Fusarium*. Since their discovery in 1988, they have become a great challenge for scientists and drawn attention of some government institutions. The interest in these toxins greatly increased since they have been found implicated in animal diseases and associated with oesophageal cancer in humans. Despite the intensive investigations implemented so far, there is still a great need to investigate further, to clarify, to confirm, and new fields remain to be studied. Many factors favouring or disadvantaging fungal infection and fumonisin contamination need to be investigated, mostly in developing countries, where maize is extensively grown and constitutes a staple food for subsistence populations. The present study is a contribution to a great deal of areas still not clarified to date, targeting Benin, a West African country, with the hope that the results provided through the six chapters of the dissertation, will be useful in solving the fumonisin problem in maize.

The first chapter is a general introduction, a review of efforts made so far by many scientists in the world to understand more about infection of maize with *Fusarium* spp. and its contamination by fumonisins. This chapter reviews information on the main species of *Fusarium* producing fumonisins in maize, toxicological effects of fumonisins, and factors (biotic and abiotic) influencing infection of maize with *Fusarium* spp. and its contamination by fumonisins. Strategies developed or still in study to control *Fusarium* infection and to minimise fumonisin contamination in maize have been also reviewed.

With respect to natural occurrence on maize of both *Fusarium* spp. and fumonisin in the world, the general observation is that data are more available for the USA and Europe. There are less for Africa, apart from South Africa. Influence of environmental and agroecological conditions on fumonisin production needs further investigations. Moreover, limited data are available on annual variation in fumonisin levels in maize in consecutive years, although it is clear that considerable variation can occur. Chapter two reports on the results of a 3-year survey of the natural occurrence of *Fusarium* spp. and subsequent fumonisin contamination in different agroecological zones of Benin.

In chapter three, results are reported of a study on the impact of indigenous storage systems on fumonisin contamination in maize. Little information is available regarding the effects of the different storage systems implemented by farmers in developing countries, which in many cases do not guarantee proper storage conditions to minimise fungal infection.

Results of the study on the impact of shelling and dehulling, on fumonisin production in maize, are reported in chapter four. Maize shelling and dehulling are two postharvest operations implemented in Africa, the former by farmers before storing maize, the latter by women as part of maize processing process. Various methods are in use in each case, sometimes involving motorised equipment. The effect of some of these methods on fumonisin production is reported.

One of the approaches explored nowadays to minimise fumonisin concentrations in maize is food processing. Research works carried out so far concern fumonisin fate during the preparation of *tortilla*, a common maize-based food of Central America. Information on Africa is almost non-existent. However, in some regions of this continent, maize undergoes long food processing. Further research, therefore, is urgently needed. With respect to this, chapter five deals with the evaluation of the fate of both aflatoxin, toxin produced by species of *Aspergillus*, and fumonisin during the preparation process of maize-based products in Benin.

Chapter six reports on the effect of essential oils extracted from local plants on *Fusarium* development and fumonisin production in maize. This study aims to propose effective essential oils for treating stored grains as an alternative control approach against *Fusarium* spp. and fumonisin contamination in maize.

All the chapters of this thesis represent interdependent entities encompassing an integrated approach to achieve a better understanding about fumonisins in maize. Consideration is given to both preharvest and postharvest factors that may reduce *Fusarium* infection and fumonisin contamination of maize in Africa.

SUMMARY

Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize were investigated through a three-year survey in four different agroecological zones of Benin, West Africa. *Fusarium* was found to be predominant in maize samples. The two *Fusarium* species most frequently isolated were *F. verticillioides* and *F. proliferatum*. Atypical isolates of *F. verticillioides* were also found. Some *F. verticillioides* strains were extremely high fumonisin producers with total fumonisin levels ranging from 8240 to 16690 mg kg^{-1}. *Fusarium* occurrence was not significantly different from one zone to another, but varied from year to year, and significantly decreased over the six months of storage. Fumonisin occurrence in maize was widespread and levels were significantly higher in the two southern than the two northern zones. Fumonisin levels varied from one year to another, and decreased throughout the storage time, but not significantly every year.

Impact of four storage systems of maize commonly used in Benin was investigated on *Fusarium* infection and fumonisin contamination. *Fusarium* incidence was significantly higher when maize was stored on a cemented floor in a house. The lowest *Fusarium* incidence was recorded when maize was stored in a bamboo granary. In contrast, the storage systems did not have a significant effect on fumonisin contamination. Damage by lepidopterous insects was significantly and positively correlated with both *Fusarium* infection and fumonisin contamination. Conversely, damage by coleopterous insects was significantly and negatively correlated with *Fusarium* infection and fumonisin contamination.

The fate of aflatoxins and fumonisins was studied through the traditional processing of maize into maize-based foods common in Benin. Mycotoxin reduction occurred and was more significant during the preparation of *makume* and *akassa* than that of *owo*. Sorting, winnowing, washing, crushing combined with dehulling of maize grains were the unit operations that appeared very effective in achieving significant mycotoxin removal. Fermentation and cooking showed little effect.

Mechanical shelling and dehulling methods were tested to evaluate their impact on *Fusarium* infection and fumonisin contamination in maize. The mechanical shelling methods were found to damage the grains and motorised sheller type IITA caused the highest level of damage. This could be due to the operation mode of that machine. *Fusarium* populations were higher on damaged grains and highest number of colonies was recorded from grains damaged

by the IITA sheller. Total fumonisin levels were also higher in damaged grains, the highest being in maize shelled by the IITA sheller. On the other hand, the mechanical dehulling methods reduced fumonisin levels in maize.

Eight essential oils extracted from local plants in Benin and oil from seeds of the Neem tree (*Azadirachta indica*) were evaluated *in vitro* and *in vivo* for their efficacy against *F. verticillioides* infection and fumonisin contamination. Oils from *Cymbopogon citratus*, *Ocimum basilicum* and *Ocimum gratissimum* were the most effective *in vitro*. These oils totally inhibited fungal growth in stored maize and affected fumonisin levels in maize stored in closed containers. These oils also significantly reduced grain germination. The oil of Neem seeds showed no inhibitory effect but rather accelerated the growth of *F. verticillioides*.