MYCOTOXIN CONTAMINATION OF MAIZE IN RELATION TO INSECT INFESTATION, AGRICULTURAL PRACTICES AND AGROECOLOGY IN THE REPUBLIC OF CAMEROON

A thesis submitted in fulfilment of the requirements for the degree of Philosophiae Doctor in the Faculty of Natural Sciences, Department of Microbiology and Biochemistry of the University of the Orange Free State, Bloemfontein, Republic of South Africa

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DECLARATION

I the undersigned hereby declare that the dissertation submitted herewith for the degree of Doctor of Philosophy to the University of the Orange Free State, contains my own independent work and has hitherto not been submitted for any degree at any other university.

Zachee Ngoko

6 May 1999

DEDICATED TO

My wife and friend Colette Ngoko, born PUEMI

Our children, Krystele, Corine, Brice and Isere

My mother Wandji Marie

ALWAYS

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PREFACE

This dissertation is a compilation of five manuscripts in preparation for publication. Each manuscript constitutes an independent entity. Because the studies were conducted in the same agroecological zones in Cameroon, some duplication was unavoidable.

Chapter 1 presents farmers' knowledge, and perceptions of pests and diseases as constraints to maize production. It also gives their approach and viewpoint for improved integrated pest management (IMP) technologies. Though they can recognize some insects in the fields and stores, their knowledge of maize diseases is very limited and calls for sustainable education campaigns through farmers' field schools. Their indigenous solutions to diseases and pests are limited and they are ready to try new IPM technologies.

Chapter 2 presents the results of two maize disease surveys conducted in two agroecological zones of Cameroon. The survey records the major diseases in the Humid Forest (HF) and in the Western Highlands (WHL). Areas of high incidence and high inoculum density are identified and it is recommended that screening for resistance to major pathogens should be initiated. Furthermore, because of the fragility of the HF ecosystem, maize diseases could make the crop unproductive unless high levels of inputs are used. Therefore other food crops such as cassava, bananas and plantains should be encouraged.

Chapter 3 deals with the identification of the biological and physical factors affecting maize production in the same locations. These factors vary from the HF to the WHL. While *Bipolaris maydis*, soil fertility and texture, and organic matter appear to be the factors that decrease production in the HF, insect damage, specifically the stem borer *Busseola fusca*, as well as fungal pathogens including *Cercospora zeae-maydis* and *E. turcicum* are the major constraints in the WHL.

Chapter 4 reports on the fungal infection of maize kernels collected from 72 farmers' stores. It reveals that numerous fungi occur on maize in both ecological zones. *Fusarium moniliforme* and *F. graminearum* are the most frequently isolated pathogens. Associated mycotoxins detected by ELISA include the fumonisins which are known carcinogenic metabolites, deoxynivalenol and zearalenone. This is the first report of the natural occurrence of fumonisin in maize in Cameroon.

Chapter 5 reveals post-harvest factors that affect maize grain quality through a questionnaire submitted to 108 farmers in the HF and in WHL in 1996 and 1997. High fumonisin levels occur in maize in both areas. The questionnaire reveals several farming practices that affect the infection of kernels by fungi and subsequent contamination by fumonisin. Delayed harvest and especially harvesting on wet days as well as the storage weevil *Sitophilus zeamais* are factors associated with increased fumonisin contamination. Drying over the fireplace and sorting are identified as factors associated with reduced fumonisin contamination.

CHAPTER 1

FARMERS' KNOWLEDGE, PERCEPTIONS AND CONTROL METHODS OF MAIZE PESTS AND DISEASES IN CAMEROON

Farmers' knowledge, perceptions and control methods of maize pests and diseases in Cameroon

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Farmers' knowledge and perceptions of pests, diseases and control strategies play a key role in the acceptance of integrated pest management technologies. A multidisciplinary team including an entomologist, a plant pathologist, extension agents and social scientists carried out a survey to assess such perceptions on maize health management in three agroecological zones, the Humid Forest, the Moist Savanna and the Highland Savanna of Cameroon. The survey was focused on benchmark sites or representative recommendation domains delineated in each agroecological zone. A sample of 178 farm households was selected for formal interviews. Questions were related to farmers' perceptions of the main pests and diseases, farm resource endowments, different pest control strategies and their effectiveness. Farmers reported that borers (all types) are the main constraints to maize production in all three agroecological zones, followed by grasshoppers and rodents in the Humid Forest. Diseases are not known to most farmers, who however do report signs like spots on leaves. The storage weevil (*Sitophilus zeamais*) is the main damaging insect in storage reported by all farmers. Pest control techniques are applied in storage with the use of chemicals by some farmers in the Highlands. Control in the field is rare and some farmers practice the use of local plant materials in crop protection. The information on farmers' perceptions on pests, diseases and control strategies guide biological scientists in the design and recommendations of cost effective and adapted integrated pest management technologies.

Keywords: Cameroon, farmers' traditional knowledge, maize, pest management

Maize (*Zea mays* L.) is considered the most important staple in western regions of Cameroon, which also have the highest population density with a 3.2% mean annual growth rate. Maize pests along with land scarcity and decreasing soil fertility are reported to be the main constraints to maize production by farmers (Bosque-Perez, 1995; Conte & Fusillier, 1993; Kotto-Sama et al., 1997). While most of the studies of maize biological factors have concentrated on inventories of pests diseases and storage techniques (Cardwell et al., 1997; McHugh & Kikafunda-Twine, 1995; Ngoko et al., 1999 Chapter 2), information about farmers' knowledge of pests, their perceptions of crop losses and the indigenous control techniques is not well documented in Cameroon. Understanding these factors is a starting point for the design of appropriate and cost-effective integrated pest management (IPM) strategies (Adesina et al., 1994; Bottenberg, 1995) which will make it possible for alert farmers to be informed and independent decision makers (Ochou, 1998).

Farmers' resource endowment and knowledge of pest control strategies are important factors as pest control recommendations are shifting toward crop and habitat management within the IPM framework. Furthermore, many IPM technologies and strategies are based on farm labor and are affected by land and capital constraints and allocation, access to input and output markets and extension services. Farm surveys are therefore required to provide such information if the new technologies have to be adapted to farmers' resource endowment and constraints.

This paper reports the results of a multi-disciplinary survey on farmers' knowledge and perceptions of maize pests, diseases and crop damage, and the control strategies based on modern and indigenous techniques. The survey compares and contrasts farmers' knowledge and perceptions of pests and pest control techniques across three agroecological zones in Cameroon and formulates recommendations for pest control specialists, extension agents and policy makers for the adequate design of sustainable IPM strategies.

MATERIALS AND METHODS

Sampling

Farm surveys were carried out in three agroecological zones in Cameroon: the Highland Savanna (HS), the Moist Savanna (MS) and the Humid Forest (HF). In the HF, dominated by secondary and primary forest, maize is produced in newly opened fields with shifting cultivation where farmers have to cut down several trees for farmland. Maize fields are therefore very small

(<0.25 ha) as the creation of new farmland requires considerable labor and capital. The rainfall is bimodal starting from March to June and again from mid-August to mid-November. The Western Highlands (WHL) which encompass the HS and MS, are mostly grassland with altitude ranging from 400m to 2300 m above sea level. Two seasons are dominant, with a long rainy season from mid-March to mid-November, the main maize cropping season. Continuous cropping is the common farming practice.

A preliminary survey was conducted in each agroecological zone. Each zone was subdivided into villages. Six villages were chosen from the HF and four villages were selected from each of the HS and MS. The HF agroecological zone was categorized into three blocks according to gradients of population pressure on land (population density used as proxy) and intensification of cropping systems. The three blocks were high population density/high land pressure and intensive cropping system, a medium population density/high land pressure, and a low population pressure/low land pressure system. The WHL agroecological zone was subdivided into sub-zones according to the altitude (high, medium and low). Two villages were randomly chosen in each block. A stratified random sample of farm households was then drawn from each village for the structured data collection with a total of 178 farm households in the three agroecological zones. A plant pathologist, an entomologist and a social scientist were involved in the sampling of agroecological zones and sub-zones as well as villages and farm households to focus the study on both biological and socio-economic issues related to farmers' knowledge and management of maize pests and diseases.

Survey

The survey was carried out in two steps. The first step consisted of an extensive survey in the sample villages through a rapid appraisal. This technique helped focus the data collection on specific relevant issues and therefore substantially decreased the cost of data collection. The data were collected from focus groups, key informants, village leaders, extension officers and marketing agents. The second step involved the completion of questionnaires (Appendix 1) related to key issues on farmers' knowledge and perceptions of maize pests and diseases, local and modern pest and disease control techniques and other constraints related to maize production. In the HF, questions were either asked in the local language by a local guide/ member of the household or in French. In the HS and MS, the questionnaire was administered in "Pidgin English", a vernacular English dialect spoken by the population of the area or in French.

Frequencies of the responses were computed using the SYSTAT for Windows 7.0 statistical package (SPSS Inc., Detroit, USA).

RESULTS

Maize in the farming systems across three agroecological zones

Maize sole cropping is rare for small-scale farmers (average field's size of 0.6 ha), except in small pockets with low population density. In the HF, maize is cultivated in association with other food crops, whereas sole cropping was more frequently observed in middle (area >1ha) to large scale (>5 ha) maize farms in the HS and MS. Depending on the crop, harvest was spread from June to August in the three zones. August is the peak of the rainy season in the HS and MS which makes harvesting, transportation and drying very difficult. During the second cropping season, legumes are planted as sole crops in this area. Soybeans are planted in July, while cowpeas and beans are planted in August/September. All these legumes are harvested in November/ December in the MS. In the HS, the maize cropping cycle lasts about 6 months, which makes it impossible for farmers to have a second cycle of maize as in other ecological zones.

Farmers' knowledge and perceptions of maize field insects and diseases, storage insects, rodents and birds

When asked about the outbreaks of insect infestation of maize in the field 70%, 31% and 100% of farmers reported field insects in general as a major constraint in the HF, HS, and MS, respectively (Table 1). Though the percentage of respondents varied across ecological zones, 54%, 98%, and 74%, in the HF, MS and HS, respectively, reported borers, (known as "fouasse" in local language in the HF, "toubou" and "boueoõc" in the MS) as an important damaging constraint of maize stalks and ears. However, farmers could rarely identify specific insects in the field. Insects such as grasshoppers (*Zonecerus variegatus*) were easy to identify compared to borers because they destroyed most of the leaves of plants, were easily observed and because of their strong body odor. Rodents (rats) (75 %) and birds (30 %) were also reported as causes of damage in the field in the HF.

The survey showed that farmers' knowledge of maize field diseases was limited (Table 2). They recognized symptoms on the leaves but most of them did not associate them with diseases except for smuts and ear rots, which are manifested by visible damage on the ear or on the tassels. On the basis of the descriptions of disease symptoms given by them, we could report 30% for lowland blight [*Bipolaris maydis* (Nisikado & Miyake) Shoemaker] in the HF, 17% for highland blight [*Exserohilum turcicum* (Passerini) Leonard & Suggs] in the MS and 4 % in the HS; for head smut [*Sporisorium reilianum* (Kühn) Langdon & Fullerton=*Sphacelotheca reiliana* (Kühn) Clint], 20% and 34% in the HS and MS, respectively, and 24% for common smut (*Ustilago maydis* DC) in the HF. Farmers also believed that the occurrence of these pathogens in a field was a witchcraft phenomenon and this perception could lead to abandonment of the field for many years. The majority of diseases were reported in the HF, though 88% of the farmers reported diseases as a major constraint in the HS. In all the ecological zones, farmers reported damage due to blights and smuts with no specific knowledge neither between lowland and highland blight, nor between common and head smut. Diplodia leaf spot [*Stenocarpella macrospora* (Earle) Sutton =*Diplodia macrospora* Earle] was mentioned by 11% of the farmers in the HF, while 23 and 12 % of the farmers in the MS and HS reported grey leaf spot (*Cercospora zeae-maydis* Tehon & Daniels). Maize streak virus was reported by 16 % of the farmers in the HF. Ear rots (*Fusarium* spp. and *Stenocarpella* spp.) (44 %), sheath blight (*Rhizoctonia solani* Kühn) (11%) and brown spot (*Physoderma maydis* Miyabe) were also reported only in the HF.

Storage pests and the damage caused by them were the best known and most noticeable as storage length increased. The common insect cited by all the farmers, as damaging maize in storage during the period of the survey was the storage weevil [*Sitophilus zeamais* Mostschultsky (*Coleoptera:Curculiomidae*)]. Rats were reported rarely in the MS and HS. No disease problem was reported on maize grain and seeds in storage. None of them accepted that maize grain could be affected by diseases; some believed that rotten grains were the results of rain during harvest or senescence.

Farmers' pest control strategies across three agroecological zones

Farmers reported several local and chemical strategies to control insects (Table 3). Twenty two percent of the farmers in the MS, and 5% in the HS reported that the common insecticide used to control weevils was actellic powder (perimiphos-methyl), while 4% of farmers in the HF used methyl-paraphen (pyrimiphos-ethyl) (Table 3). In the HF, methyl-paraphen, Kocid 105 (a common cupric derivative provided by the extension service to control cocoa diseases), and 'Cypercal M' (deltametrine) were the chemicals reported by farmers. A few farmers also used other techniques such as wood ash and local plants to protect their grain from insect and rodent damage. No farmer was aware of seed treatment against diseases. Some reasons for this included the ignorance of maize grain diseases, the non-availability of recommended fungicides for seed treatment and the lack of knowledge on their utilisation. The indigenous control techniques included the use of a local shrub plant (*Kezem*) (5% in the MS), the use of the leaves of a common local tree (Cypress) in protecting maize grain against insects in the MS, and wood ash was used in all the localities. In the HF heat and smoke from the fireplace (10%) and frequent sun drying (6%) were the most commonly used practices reported by farmers for seed storage and preservation against weevils. The estimated maize crop losses due to insects ranged between 2% and 64%.

Maize seeds were not as frequently damaged as food grains because they were in small quantity and better quality (only high quality cobs are selected for seeds). In the HF, 10 % of the farmers stored seeds in the kitchen (Table 3). In the MS and HS, 2 and 8% of farmers, respectively, stored maize with wood ash in small containers while 5% used this practice in the HF; the frequent drying of seeds by the sun is common in the HF where the level of moisture is high due to higher and more frequent rainfall. Dried cobs are stored in bamboo granaries over the fireplace. The control of insects and rodents is done by the smoke and heat from the fire during the cooking of meals. In the HS and MS, maize was stored in different ways ranging from hanging cobs from the ceiling to the use of modern bags. Insecticides were often used in storage in different ways. The chemical compounds used are actellic powder, methyl paraphen and 'Cypercal M'. The insecticides were sprayed in the storage structure before storage.

The pest control techniques for maize were mainly carried out for insects in the field and in storage (Table 3). Traditional control practices included dusting ash on the plant mainly in the MS (15% of sampled farmers) or covering-up the infected part of the plant with mud in the HF (5%). Other minor techniques included tying the cob or mechanical control through removal of the infested plant. Most of the traditional practices were reported in the HF where maize was not the main crop (0.10-0.25 ha /household). The intensive labor required by the traditional practices would not be possible in the two other agroecological zones where maize areas averaged 0.75 ha per household. In the HF, two tablespoons of Decis (deltametrine) or methyl-paraphen were mixed with 13 liters of water using Solo or Berthoud brand sprayers. Only 10% of farmers used insecticide to control pests during the second season. Most farmers who grew maize during this period declared that the harvest was usually sold as green maize or dried and sold as seeds.

Farmers' perception of the period of insect, rodent and bird infestations in the field

Damage to maize in the field by insects was reported by 78% of farmers as important at the beginning of the rainy season in the HS (Table 4). The mid-season and end-season were the most critical periods for insect infestation of maize in the MS, while no significant difference between periods was reported in the HF.

Farmers reported that birds dug out late-planted maize. Attacks by small rodents were also reported to occur at the beginning and during mid-season, and some at the beginning of the rainy season. This usually occurs during the night.

Farmers' perception of the period of disease outbreaks in the field

Diseases were less noticed by farmers compared to the physical presence of insects on maize in the field. However, most of the spots observed on leaves and on other parts of plants were important at mid-season in all three agroecological zones (Table 4). In the MS, no disease damage was reported at the end of season, in contrast to the HF and HS where 50% and 34%, respectively, of farmers reported disease outbreaks at the end of the season.

DISCUSSION

In the HF, Kotto-Sama et al. (1997) reported that shifting cultivation was the most common cropping practice. Farmland is opened by cutting down several trees. During the fallow period, the farm is covered by some stands of cassava but mainly by a dominant weed *Chromolina odorata* or *Imperata cylindrica.* Groundnuts, "egusi", melon, cassava, cocoyams (*Xanthosoma mafafa*), "okra" and banana are often associated with maize. Local varieties and an improved open pollinated variety (CMS8704) from IRAD are popularly used mostly as green maize.

In the HS and MS maize is mainly associated with red beans, potatoes or cocoyams. The cool weather is not favorable to maize varieties available in the areas and the growing cycle lasts 6 to 7 months. Maize can be continuously cropped for an average of 9 years without fallow. The most popular open pollinated varieties, COCA and Kitale II, a long cycle (7 months) and stable, tall with big stalks and white grain, adopted by farmers in the area are still highly demanded. The main constraint is the shortage of land with an average per capita of 0.6 ha; but also soil erosion, and soil fertility decline, are among key problems reported by farmers. McHugh & Kikafunda-Twine (1995) reported that the average value of storage losses to a Ndop plain farmer storing 2.5 tons of maize was equivalent to 15% of total revenue from the farm. Drying and storing were the main post-harvest constraints reported by farmers. Weevil outbreaks ranked as the major constraint among the damage caused by insects in the stores. The lack of cash to purchase inputs especially insecticides constituted another cause of the destruction of grain by insects. Treating seeds with pesticides (fungicides or insecticides) or with local products was not a common practice, however, 15% and 8% of farmers most of whom owned more than 1 ha of land in the MS and the HF zones, respectively, used fungicides for seed treatment. They also stored more than 100 kg of maize seeds for self-use and for selling. All farmers reported that fungicides were expensive, especially after the removal of subsidies from inputs in 1986 and the devaluation of the CFA"Communaute Financiere Africaine" currency in 1994.

The present survey across three agroecological zones showed that farmers do not have much knowledge about pests and diseases of maize. While maize is the staple food for the population of the HS and of the MS, this crop is produced to a limited extent in newly open fields in the HF and is eaten as fresh maize; the staple food in this region being tuber crops and plantain. Therefore, maize pests and diseases are more frequently perceived as constraints in the WHL than in the HF though confusion occurred sometimes in both regions because diseases were misidentified as insects. Consequently, pest management becomes a critical issue. Borers are the best known maize pests to farmers. As in other African countries, farmers have a poor knowledge of foliar and stem diseases. They do not know that lesions on the plant could have an impact on production. However, they report symptoms on leaves and recognize smut and ear rots, which are visible.

Very few pest control practices are used in the field and the use of ash on plants to control borers was judged to be non-effective by farmers themselves. Stem borer control by cultural practices or chemicals was not done by any farmer in any of the three agroecological zones. Lack of knowledge (period of outbreak, dynamics of the population) and labor are reported as the main constraints to the use of cultural practices in controlling borers in the field. The cultural practices were mainly constrained by labor availability in the household and the scarcity of cash resources to hire labor. Pesticide utilization to control storage insects is limited to actellic powder (and to a limited extent to some local practices) by farmers despite complaints about its high price following the removal of input subsidies and the devaluation of the CFA currency, which doubled the prices of imported products. Algali (1991) and Bosque-Perez (1995) reported similar results from a survey carried out in Kano, Nigeria. Similar to the situation in Uganda and Ghana (Coulibaly, unpublished data) very few farmers could afford to store maize until prices increased because of the cash pressure for family needs such as paying for emergency health costs and children's school fees.

To be successful, the new IPM technologies should take into account these perceptions about pests and diseases, their importance and the constraints for the use of the resources at the farm level. Pest and disease awareness was not well developed among farmers and most of the farmers did not have access to extension services. To generate substantial cash, through good quality grain, more attention needs to be focused on the pest and disease control practices, both pre-and post-harvest; this should take into consideration farmers' knowledge of pests and diseases. This can be achieved through farmers' field schools and also farm level resource constraints and the economic profitability of the new technologies. Such pest control packages should be designed to target specific agroecological and socio-economic circumstances of farmers. This survey of farmers' knowledge, perceptions, and control methods of maize pests and diseases, provided baseline information in three agroecological zones of Cameroon. Emphasis should be placed on the training of village extension workers and farmers on the methods of identification of the major maize pests and diseases. A field manual of identification of pests and diseases should be developed and the proper and safe use of chemicals as grain protectants should be encouraged.

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Table 1. Farmers' perceptions of outbreaks of insects, rodents and bird in maize in the field in three agroecological zones of Cameroon in 1996

Field insects, rodents and birds	Humid Forest n=80 (%)	Moist Savanna n=48 (%)	Highland Savanna n=50 (%)
A major constraint*	70	31	100
Grasshoppers	56	4	0
Borers (all types)	54	98	74
Ants	9	2	0
Rodents	75	0	0
Birds	30	2	0

n= Number of farmers interviewed

* Percentage of farmers who reported field insects as a major constraint to maize production in each ecological zone.

Table 2. Farmers' knowledge and perceptions of field diseases of maize in three agroecological zones of Cameroon in 1996

Field diseases	Humid Forest n=80 (%)	Moist Savanna n=48 (%)	Highland Savanna n=50 (%)
A major constraint*	70	31	88
Brown spot	7	0	0
Common smut	24	0	0
Diplodia leaf spot	11	0	0
Ear rots[τ]	44	6	12
Grey leaf spot	0	23	12
Head smut	0	20	34
Highland blight	0	17	4
Lowland blight	30	0	0
Maize streak virus[Ψ]	16	0	0
Sheath blight	11	0	0

n= Number of farmers interviewed

*Percentage of farmers who reported field diseases as a major constraint to maize production in each ecological zone

[τ]Ear rots caused by *Fusarium* spp and *Stenocarpella* spp.

[Ψ] Maize streak virus vectored by *Cicadulina mbila*

Table 3. Farmers' maize treatment conservation practices in three agroecological zones of Cameroon in 1996

Seed and product treatment	Humid Forest n=80 (%)	Moist Savanna n=48 (%)	Highland Savanna n=50 (%)
Fungicide (Kocid 105)	8	15	0
Insecticides			
Methyl paraphen	4	0	0
Cypercal M	1	0	0
Actellic powder(2%)	0	22	5
Decis	0	5	0
Indigenous techniques in storage			
store seeds in the kitchen	10	0	2
with wood ash	5	2	8
with local plant	0	5	nd
frequent sun drying	6	0	nd
Traditional control practices			
Dust ash on the plant	1	15	1
Rouging	6	0	0
Tie-up the cob	3	1	2
Cover the infested part with mud	5	0	0
Other	5	0	0
Store in bags	na	na	10

n= Number of farmers interviewed
nd=not determined
na = not applicable

Table 4. Farmers' perceptions of the periods of outbreaks of maize insects and diseases in the field in three ecological zones in Cameroon in 1996

Periods of outbreak	Humid Forest n=80 (%)	Moist Savanna n=48 (%)	Highland Savanna n=50 (%)
Insects			
Beginning of the season	61	66	78
Mid-season	67	91	42
End of season	62	85	22
Diseases			
Beginning of the season	32	20	2
Mid-season	87	92	42
End of season	50	0	34

n = Number of farmers interviewed

CHAPTER 2

DISTRIBUTION AND RELATIVE IMPORTANCE OF MAIZE DISEASES IN THE HUMID FOREST AND WESTERN HIGHLANDS OF CAMEROON

Distribution and relative importance of maize diseases in the Humid Forest and Western Highlands of Cameroon

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Surveys for disease diagnostics were conducted during the major maize growing seasons in each of two ecological zones in Cameroon, the Humid Forest (HF) and the Western Highlands (WHL). A total of 108 farms were visited in three to four villages in each zone. In the HF, five and seven major diseases were recorded in 1995 and 1996, respectively. *Bipolaris maydis* and *Puccinia polysora* were the most prevalent pathogenic fungi with a mean incidence of 70 and 44% in 1996, respectively. *Rhizoctonia solani, Stenocarpella macrospora,* and *Physoderma maydis* were recorded in all villages at low incidence. In the WHL, *Exserohilum turcicum* and *Cercospora zeae-maydis* were the most prevalent pathogens, with incidence ranging from 16 % to 100 %. *Phaeosphaeria maydis*, which was the most severe disease following *E. turcicum* in 1993, has been displaced by *Cercospora zeae-maydis* which increased between 1995 and 1997. The incidence of *Sporisorium reilianum* and *Puccinia sorghi* was about 5 %. Most of these diseases occurred in association in many fields. There was a positive relationship between *Puccinia polysora* and *Bipolaris maydis* in the HF. Phaeosphaeria leaf spot was negatively correlated to *C. zeae-maydis* and *E. turcicum* in the WHL. Though some diseases were specific to their ecological areas, *E. turcicum* was found in both ecological zones but with low incidence in the HF.

Keywords: *Bipolaris maydis,* Cameroon, C*ercospora zeae-maydis*, Exs*erohilum turcicum*, maize, P*haeosphaeria maydis*

Maize (*Zea mays* L.) is one of the most important food crops grown in Cameroon. When the Communauté Financiere Africaine (CFA) currency was devaluated in 1994, the area under maize cultivation increased to 700,000 ha in 1997, up from 320,000 ha in 1991 (FAO, 1994; MINAGRI, 1996). This commodity is cultivated in all five ecological zones of the country (Fig.1) but mostly in the Western Highlands (WHL) where it constitutes the staple diet for about 80 % of the population. From its original subsistence status, maize has become as commercially important as cocoa and coffee, with local industries as the main consumers. Despite the fact that the average yield (1.8 t/ha) has remained constant for many years (NCRE, 1994), Ayuk-Takem et al. (1982) reported that the production has increased from 500,000 MT in 1991 to over 800,000 MT in 1997 due to the expanded area under cultivation. The production would be higher if many factors such as soil fertility, poor managerial skills, the costs of the inputs, environmental stresses, pests and diseases were not limiting the actual potential yield of the improved varieties released so far. Cardwell et al. (1997) showed that losses due to pests and diseases ranged from 20 % to 50 % but MINAGRI (1996) reported losses up to 90 % in some fields in Bali and other parts of the country due to grey leaf spot (*Cercospora zeae-maydis* Tehon & Daniels) in the 1995-1996 cropping season.

In 1993, three countrywide field surveys were conducted to provide a basic data set on the time, the locations and field conditions under which maize losses occurred due to pests and diseases. This rapid appraisal provided an overview of the status of diseases and pests of maize in the country. The objectives of the present study were to provide a more detailed assessment of the relative importance of the most prevalent diseases in ecological zones with high disease pressures identified during the 1993 surveys, i.e., in the HF and the WHL. Locations with reliably high disease pressure will also be used later for researching and testing of Integrated Pest Management (IPM) technologies appropriate for the specific socio-economical and ecological requirements of these zones.

MATERIALS AND METHODS

The agroecological zones

In the HF, which is dominated by secondary and primary forest, maize is produced in newly opened fields in shifting cultivation where farmers cut forest for farmland. Maize fields are therefore very small (<0.25 ha) and maize does not constitute the staple food of the population of the zone because the creation of new farmland requires substantial effort and investment. There are two cropping seasons interrupted by a dry period in August; a major from March to July, and a minor from September to November.

The WHL is mostly grassland with the altitude ranging from 800 to 2,300 m above sea level. Two maize crops are possible during the long uninterrupted cropping seasons lasting from March to November, giving rise to soil fertility decline and disease inoculum build-up. Maize production is intensive in these areas because the population is very dense (230 inhabitants/km ^{2}) and is increasing at a rate of 3.2 % /year. Farm size is dwindling, and continuous cropping is a common farming practice.

Field surveys

In June 1995, a survey was conducted in four villages in the HF, i.e., Kometou, Ngat, Etoud, and Mvoutessi III (Fig.1). In June 1996, two surveys were conducted in three villages of each of the HF (Kometou, Ngat and Etoud) and the WHL (Bamunka, Bali, and Njinikom). In June 1997, a survey was conducted in the same villages in the WHL. In all surveys, twelve farmers were selected at random per village with the collaboration of the extension agent of the locality. Where possible, a notice was sent to the village prior to the evaluation date in order to have the farmer present at the time of the investigation. A plant pathologist and an entomologist visited a total of 108 farmers. At each farm, 15 plants (growth stage 6 to 9) (Hanway, 1966) were chosen at random and bought from the farmer. Each plant was assessed for growth and damage variables. The fields were generally at the grain filling to maturity growth stage. In fields, data on the following variables were recorded: crop system; organic matter (1-3 with 1= low, 3= high); soil texture (1-3 scale: 1= sandy and 3= clay); weediness (1-3 scale: 1= clean and 3= very weedy field); crop stage 1-9 scale: 1= emergence, 5= silking, 7= milk stage, 8 = hard dough and 9=physiological maturity; mm stem diameter (second internode above the ground); % grain fill; % cob damage by fungi; width and length of cob; weight of cobs without husk. The disease data included the aetiology and severity of infection (1-5 scale: 1= clean and 5= heavily infected plant) of at least the two most important diseases per plant.

Leaf samples infected with highland blight [*Exserohilum turcicum* (Passerini) Leonard & Suggs], and lowland blight [*Bipolaris maydis* (Nisikado & Miyake) Shoemaker], were incubated on PDA for confirmation of the identity of the causative fungi according to

Shurtleff (1986). Symptoms of Diplodia leaf spot [*Stenocarpella macrospora* (Earle) Sutton = *Diplodia macrospora* Earle], common smut (*Ustilago maydis* DC), head smut [*Sporisorium reilianum* (Kühn) Langdon & Fullerton = *Sphacelotheca reiliana* (Kühn) Clint], Phaeosphaeria leaf spot [*Phaeosphaeria maydis* (P. Henn) Rane, Payak & Renfro], highland rust (*Puccinia sorghi* Schw.), lowland rust (*Puccinia polysora* Underw.), brown spot (*Physoderma maydis* Miyabe) and sheath blight (*Rhizoctonia solani* Kühn) were identified according to the Compendium of Corn Diseases (Shurtleff, 1986). Grey leaf spot was identified according to Latterell & Rossi (1983).

The disease incidence of each location consisted of the total number of infected plants per location over 180 plants. Fungal stem infections and % total leaf area infected (LAI) with diseases were calculated according to Clive (1971). The general phytosanitary condition of the field was also assessed and incidence of diseases that prevailed on maize plants other than the samples collected was recorded. In this paper we report only disease distribution and the relationships between them.

Data analysis

Analysis was carried out with SYSTAT 3.22 for windows. Mean disease severity expressed as % LAI and individual disease frequency expressed as number of infected plants/ total number of plants (180) investigated were computed per village. Correlation coefficients were computed to single out the relationships between disease variables. Prior to analysis all percentage data were Arcsine transformed. Data were analyzed separately for each year and per location; locations and years were then compared.

RESULTS

Humid Forest

In general, disease incidence (Table 1) varied with location and year. Five major leaf diseases were recorded on maize in 1995 and seven in 1996 in the HF. *Bipolaris maydis* (Fig. 2) and *P. polysora* (Fig.5) were the most frequent in all the villages in both years. In 1996, 81.8 % incidence of disease caused by *B. maydis* was recorded in Etoud, up from 38 % in 1995. This pattern was also noticed in Ngat where 80 % incidence of the pathogen was recorded in 1996 up from 65 % in 1995. No significant change in disease incidence was found in Nkometou and the levels were high in both years (>70 %). *Puccinia polysora* was recorded mostly in the

HF with the highest incidence of 59 % recorded in Etoud in 1996 up from 49 % in 1995. *Exserohilum turcicum* (Fig.3) was recorded at low incidences ranging from 0 to 18% in all the villages in both years (Table 1). The incidence of sheath blight caused by *Rhizoctonia solani* was moderate across the locations, with highest incidence (21%) recorded in Ngat in 1996. *Stenocarpella macrospora* was found in all the locations. The highest incidence (21 %) was recorded in Etoud in 1996. *Physoderma maydis* (Fig.8) was recorded sporadically in less than 30 % of the sites, and incidence in farmers' fields outside the survey area was about 10 % (data not shown). Likewise, *Ustilago maydis* (Fig.6) was recorded rarely in some newly opened fields with incidence below 2%.

Disease severity was moderate in all the locations, with approximately 10% LAI at most locations (Table 2). The highest LAI of 17.7% was recorded in Mvoutessi in 1995. Significant differences in LAI were found between years in Nkometou, Etoud and Ngat.

Rhizoctonia solani had the highest disease severity (1.2-2.7) in all locations in 1995 (Table 3) and 1996 and a significant difference between years was recorded only in Etoud (Table 2). The highest *P. polysora* severity (2.2) was recorded in Etoud in 1995, with no difference between years except in Nkometou. *Stenocarpella macrospora* was recorded at all the locations, with the highest severity (1.4) recorded in Etoud in 1995.

Significant relationships were found between individual pathogens and the LAI in the HF in 1995. *Exserohilum turcicum* (p=0.001), *S. macrospora* (p=0.01), *and P. polysora* (p=0.01) were significantly positively correlated to LAI (Table 3). A weak positive relationship was found between *P. polysora* and *B. maydis* whereas *B. maydis* and *R. solani* were significantly (p=0.001) negatively correlated with LAI and to *E. turcicum* (Table 3). In 1996 in the HF, all the pathogens were significantly and positively correlated with LAI (p=0.001) (Table 4). *Bipolaris maydis* and *S. macrospora* were significantly and negatively correlated with *E. turcicum* (p=0.01). The physiological spots, *B. maydis*, and *P. polysora* were positively correlated with maize streak virus (MSV). *Exserohilum turcicum* was negatively correlated with *B. maydis, S. macrospora* and *P. polysora* (Table 4).

Western Highlands

In the WHL, six important diseases were recorded in 1996 and 1997 (Table 5). Highland blight caused by *E. turcicum* was found in all the fields. The incidence varied from 16 % in Bali to 100 % in Bamunka in 1997. Disease severity was high in 1996 (severity>3.5 on a 1-5 scale) in Bali and Njinikom (Table 6). No difference in disease severity between years was found in Njinikom, but disease severity declined significantly ($p<0.05$) between 1996 and 1997 in Bali (Table 6).

Grey leaf spot caused by *C. zeae-maydis* occurred only in the WHL. The incidence and severity were very high in both years. Over 33 % was recorded in all the villages with 90 % noted in Njinikom in 1997 (Table 5) with a mean severity of 3.4 across villages and years (Table 6). *Physoderma maydis* occurred in all locations with highest severity in Bali in both years. *Stenocarpella macrospora* was rarely observed with low incidence in Bali in 1996 and Bamunka in 1997. *Phaeosphaeria maydis* (Fig.4) was found in >30 % of the fields in Bali and Bamunka in 1997. It was recorded in Njinikom in both years at a low incidence (Table 5). In all locations, the severity was significantly ($p<0.05$) higher in 1997 than in 1996 (Table 6).

Common smut, incited by *U. maydis* occurred at a very low incidence (1%) in all locations (data not shown), though higher incidences were observed in some fields along the roads. Head smut caused by *S. reilianum* (Fig.7) was found at approximately 5% incidence in Njinikom and Bali (data not shown). The highland rust *P. sorghi* was found at a very low level of infection mainly in Bamunka, and Njinikom (data not shown).

In 1996, only *C. zeae-maydis* and *E. turcicum* prevailed in the fields in the WHL and both were significantly ($p=0.001$) positively correlated with LAI (Table 7). *Cercospora zeae-maydis* was also positively and significantly correlated with *E. turcicum.*

In 1997, in the WHL, *E. turcicum*, *Pha. maydis*, and *C. zeae-maydis* were significantly ($p=0.001$) positively and *S. macrospora* was significantly ($p=0.01$) negatively correlated with LAI (Table 8). Negative correlations existed between *E. turcicum* and *S. macrospora* and *C. zeae-maydis*, as well as between *C. zeae-maydis* and physiological spot and *Phy. maydis*, but the former increased with *Pha. maydis*. Ear rots caused by *Fusarium* spp. (Fig.9) and *Stenocarpella* spp. (Fig.10) were a serious threat to maize harvested late in the WHL in 1996 and 1997 (data not shown).

DISCUSSION

Detailed disease surveys conducted in two agrocrological zones of Cameroon in 1995, 1996, and 1997 showed that *P. polysora*, *B. maydis* and *S. macrospora* were the most prevalent maize pathogens in the HF and *C. zeae-maydis* and *E. turcicum* were the predominant maize pathogens in the WHL. Generally, *P. polysora* was found more frequently on mature leaves in all the locations of the HF. Previously Cardwell et al. (1997), Foko (1973), Ngoko (1994), Praquin (1976) and Timti (1980) reported several maize diseases in Cameroon, but only a few were found at high incidence and severity in localised areas. The severity of these diseases varied between years and areas. *Phaeosphaeria maydis* which was reported as important in 1993 with 80 % prevalence in previous maize disease surveys (Ngoko, 1994) is decreasing in severity. This leaf spot was displaced almost entirely by grey leaf spot in the WHL. Though many diseases remained zone specific, highland blight was also found in the HF where it has been noted previously (Buddenhagen, 1985). It still occurs at a low incidence and severity and is certainly out of its optimum environment.

In the past, several maize disease surveys have been conducted in Cameroon with different survey techniques (Cardwell et al., 1997; Foko, 1973; Ngoko, 1994; Praquin, 1976; Timti, 1980). Some were systematic with stops at regular intervals. Others were carried out in randomly selected fields, or in localised fields and experimental research sites. Surveys carried out in the 1970's and 1980's were on a very small scale, while those carried out in the 1990's covered almost the entire country.

From 1970 to 1997, several leaf, stem and ear diseases of maize were identified in Cameroon. The number of diseases and their incidence and severity have not been constant throughout this period. Though *E. turcicum*, *S. reilianum*, and *P. polysora* have been reported consistently, their importance has varied. In the WHL, *E. turcicum* was displaced by *Pha. maydis* in the early-to-mid nineties (Ngoko, 1991). After that period, Ngoko (1994) noted that *Pha. maydis* was displaced by grey leaf spot. *Helminthosporium carbonum* Ullstrupp reported by Foko (1973) was not observed in the nineties by Cardwell et al. (1997), Ngoko (1991) and Ngoko (1994). While the incidence of highland blight is decreasing in the WHL, Ngoko (1994) found that head smut is becoming more important in the region. These phenomena could be the results of the existence of resistant maize varieties to blight whereas breeding for resistance to *S. reilianum* has not been carried out since the disappearance of Z155 which was the only resistant genotype available in the mid-seventies (Ayuk-Takem et al., 1982).

Grey leaf spot was one of the most important maize diseases in the WHL in both years. In their earlier findings, Foko (1973) and Timti (1980) did not record it as a maize disease in the WHL. Ngoko (1994) reported a low incidence, while Cardwell et al. (1997) noted that in western areas, *C. zeae-maydis* became more prevalent as organic matter decreased. This disease is displacing other foliar diseases such as *E. turcicum* and *Pha. maydis* in the areas. Considering the increasing importance of the disease, it has become a necessity to initiate a screening program for grey leaf spot in WHL.

Phaeosphaeria maydis, known in 1985 as a minor pathogen, was very serious in 1992 – 1993 (NCRE, 1994). This disease which was first recorded in 1963 in the highlands of Mexico (Rane et al., 1966), is now observed in Cameroon both in the highlands of Donga-Mantung Division (altitude > 1800 m.a.s.l) and in the low altitude in Bali (<1000 m a.s.l). Rane et al. (1966) reported that this pathogen is prevalent in highland zones with altitudes of 2000 m a.s.l. Our observations apparently contradict previous findings. This may be due to genotypes or new races of the pathogen which are adapted to different environmental conditions. More epidemiological and etiological investigations need to be conducted in the WHL of Cameroon.

Significant correlations were found between some diseases, and between some diseases and LAI. In 1995 in the HF and 1997 in the WHL, the negative correlation between *R. solani* and *B. maydis, S. macrospora* and physiological spot, and LAI, may suggest that despite their presence on the leaf surface, their effects may have been diluted by *E. turcicum* which was negatively correlated with both pathogens. *Exserohilum turcicum* and *P. polysora* were associated with leaf destruction. This pattern was not apparent in 1997, but *B. maydis*, *S. macrospora* and *P. polysora* were inversely correlated to *E. turcicum.* This relationship suggests that while some diseases coexist in the same ecological areas, others are displaced by the better adapted ones. Understanding the type of relationship may be beneficial for the establishment of specific control strategies.

Annual maize production in Cameroon has not increased as it should have, taking into consideration the technological packages released so far (Fajemisin, 1985; NCRE, 1994). Intensified food production has led to ineffective crop rotation or fallowing. Continuous maize cultivation in areas where several diseases are endemic contributes to pathogen build-up. Although the type of synergy that occurs among the different maize pathogens is not yet well elucidated, individual pathogens may cause less damage to plants and plant parts compared to the association of several fungi (Cardwell et al., 1997). The association of several maize pathogens in the fields needs to be thoroughly investigated to determine whether these fungi cause disease in succession and to what extent synergism occurs. Our data, similar to earlier findings (Cardwell et al., 1997), have identified hot spots for major diseases where screening for resistance to diseases should take place without having to apply artificial inoculation. The use of resistant varieties is at the moment the most appropriate control strategy for small farmers with limited resources.

The HF is a fragile ecosystem where farmers have to cut down several trees for a small area of farmland. Maize in this ecosystem is less important compared to cassava, bananas and plantains. The combination of these factors with maize diseases could make the crop unproductive without the use of high level inputs that the low-income farmers can not afford. Therefore, more emphasis should be placed on indigenous staple foods of the population of the HF. With the high incidence and outbreaks of both *Pha. maydis* and *C. zeae-maydis*, it has become a necessity for plant breeders in collaboration with plant pathologists to initiate screening programmes for resistance to these two pathogens in the WHL, the area of intensification of maize production in Cameroon.

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Table 1. Mean incidence* of individual maize pathogens in the Humid Forest of Cameroon in 1995-1996

Pathogens	Locations								
	Nkometou		Etoud		Ngat		Mvoutessi		
	95	96	95	96	95	96	95	96	Mean
B. maydis	74.5	71.9	38.0	81.8	65.2	80.0	77.0	nv†	69.8
P. polysora	12.7	51.1	49.3	58.8	39.3	50.3	49.1	nv	44.4
R. solani	11.5	2.2	4.7	17.0	11.9	21.2	13.3	nv	11.7
E. turcicum	10.9	0.0	18.0	0.0	0.0	17.6	0.0	nv	6.9
S. macrospora	3.0	18.5	4.7	21.2	13.3	10.9	13.3	nv	12.1

*Mean incidence = total number of plants infected of 180 plants per location.
Physoderma maydis and *Ustilago maydis* not shown because they were rarely recorded.
B = *Bipolaris*; *P* = *Puccinia*; *R* = *Rhizoctonia*; *E* = *Exserohilum;* *S* = *Stenocarpella*
† = not visited

Table 2. Mean disease severity ratings (1-5) * and leaf area infected (LAI) of maize pathogens in the Humid Forest of Cameroon in 1995-1996

Pathogens	Locations								
	Nkometou		Etoud		Ngat		Mvoutessi		
	95	96	95	96	95	96	95	96	Mean
B. maydis	1.2a	1.0b	1.4a	1.2b	1.0a	1.3b	1.0	nv[†]	1.2
P. polysora	1.3a	1.9b	2.2a	1.9a	1.9a	1.8a	1.9	nv	1.8
R. solani	2.6a	2.3a	1.7a	2.7b	2.4a	2.5a	2.8	nv	2.4
E. turcicum	1.3a	1.0a	1.4a	1.1b	1.0a	1.3b	1.0	nv	1.2
S. macrospora	1.1a	1.3b	1.1a	1.4a	1.2a	1.2a	1.3	nv	1.2
LAI	11.7a	10.6b	11.0a	10.1b	7.4a	11.3b	17.7	nv	11.4

*Means followed by the same letter in rows within locations are not significantly different ($p > 0.05$)
B=Bipolaris; P=Puccinia;R=Rhizoctonia;E=Exserohilum;S=Stenocarpella
†= not visited

Table 3. Correlation matrix between leaf area infected (LAI) and individual pathogens in the Humid Forest of Cameroon in 1995

	LAI	Et	Rs	Bm	Sm	Pp
LAI	1.00					
E. turcicum	0.21***	1.00				
R. solani	-0.40***	-0.04	1.00			
B. maydis	-0.41***	-0.21**	-0.15**	1.00		
S. macrospora	0.02**	-0.03**	-0.07	-0.06**	1.00	
P. polysora	0.25**	0.03	-0.03*	0.22*	-0.14**	1.00

Et= *Exserohilum turcicum;* Rs =*Rhizoctonia solani*; Bm =*Bipolaris maydis*; Sm= *Stenocarpella macrospora;* Pp = *Puccinia polysora*
E= Exserohilum; R= Rhizoctonia; B= Bipolaris; S= Stenocarpella; P= Puccinia
*significant at p=0 .05; ** significant at p=0.01, *** significant at p=0.001

Table 4. Correlation matrix between leaf area infected (LAI) and individual pathogens in the Humid Forest zone of Cameroon in 1996

	LAI	Et	Rs	Bm	Sm	Pp	MVS	P	Pspt
LAI	1.00								
E. turcicum	0.08***	1.00							
R. solani	0.26***	-0.06	1.00						
B. maydis	0.39***	-0.18**	-0.04***	1.00					
S.macrospora	0.12***	-0.03**	0.01*	-0.15	1.00				
P. polysora	0.52***	-0.04	0.06***	-0.06	-0.13***	1.00			
MSV	0.42***	0.12**	0.08	0.11***	0.11**	0.19***	1.00		
Phy. maydis	0.03**	-0.04	-0.02***	0.06***	-0.09***	-0.04**	-0.07**	1.00	
Pspt	0.28**	-0.03**	0.06***	0.09***	-0.05**	0.13	0.09***	0.07**	1.0

Et =Exserohilum turcicum; Rs =Rhizoctonia solani; *Bm =Bipolaris maydis*; *Sm =Stenocarpella macrospora; Pp = Puccinia polysora*; MSV= maize streak virus; Pspt =physiological spot.

E= Exserohilum; R= Rhizoctonia; B= Bipolaris; S= Stenocarpella

P= Puccinia; Phy= Physoderma

*significant at p=0.05; ** significant at p = 0.01, *** significant at p = 0.001

Table 5. Mean incidence* of individual maize pathogens in the Western Highlands of Cameroon in 1996-1997

Pathogens	Locations						
	Bali		Njinikom		Bamunka		
	96	97	96	97	96	97	Mean
E. turcicum	3.8	16.7	64.3	65.0	51.4	100.00	50.2
C. zeae-maydis	4.3	33.6	71.4	90.5	68.6	84.0	58.7
Phy. maydis	0.0	27.8	0.0	27.8	0.0	33.00	14.8
Stem disease	3.8	1.5	4.3	7.5	10.0	5.00	5.4
Pha. maydis	0.0	33.3	1.6	3.6	0.0	33.00	11.9
S. maydis	0.0	0.0	0.0	0.0	0.0	13.90	2.3

*Mean incidence = total number of plants infected of 180 plants per location
E= Exserohilum; C= Cercospora ; Phy. = Physoderma; Pha. = Phaeosphaeria; S= Stenocarpella

Table 6. Mean disease severity ratings (1-5)* and leaf area infected (LAI)of maize pathogens in the Western Highlands of Cameroon in 1996-1997

Pathogens	Locations						
	Bali		Njinikom		Bamunka		
	96	97	96	97	96	97	Mean
E. turcicum	3.5a	2.3b	3.6a	3.3a	3.0a	2.8a	3.1
C.zeae-maydis	3.5a	3.5a	3.1a	3.9b	3.6a	2.6b	3.4
Phy. maydis	1.9a	2.9b	1.0a	2.2b	1.0a	2.6a	1.9
Stem disease	1.0a	1.6b	1.1a	1.6b	1.2a	1.9a	1.4
Pha.maydis	1.0a	2.1b	1.0a	2.5b	1.0a	2.1a	1.6
S.macrospora	0.7	-	-	-	-	0.6	-
LAI	18.9a	3.5b	18.2a	33.2b	12.8a	8.3b	15.8

*Means followed by the same letter in rows within location are not significantly different (p> 0.05).

E= Exserohilum; C= Cercospora; Phy. = Physoderma; Pha. = Phaeosphaeria
S= Stenocarpella.
\- = not recorded

Table 7. Correlation matrix between leaf area infected (LAI) and individual pathogens in the Western Highlands of Cameroon in 1996

	LAI	Cz	Et
LAI	1.00		
C. zeae-maydis	0.65***	1.00	
E. turcicum	0.39***	0.2***	1.00

Cz= *Cercospora zeae-maydis*; Et = *Exserohilum turcicum;*
C= Cercospora; *E= Exserohilum*
*significant at p=0.05; ** significant at p=0.01, *** significant at p=0.001

Table 8. Correlation matrix between leaf area infected (LAI) and individual pathogens in the Western Highlands of Cameroon in 1997

	LAI	*Et*	*Pha*	*Cz*	*Phy*	*Pspt*	*Sm*
LAI	1.00						
E. turcicum	0.63***	1.00					
Pha.maydis	0.44***	0.27***	1.00				
C.zeae-maydis	0.28***	-0.22*	0.16*	1.00			
Phy. maydis	0.11*	0.17	0.21	-0.34**	1.00		
Pspt	-0.08*	0.08	-0.11	-0.34**	0.23	1.00	
S.macrospora	-0.34**	-0.33***	-0.16	0.14	0.15	0.36	1.00

Et= Exserohilum turcicum; Pha = Phaeosphaeria maydis; Cz= *Cercospora zeae-maydis*
Phy =Physoderma maydis; Pspt =physiological spot; *Sm =Stenocarpella macrospora*
E= Exserohilum; Pha= Phaeosphaeria; C= Cercospora; Phy= Physoderma
S= Stenocarpella
* significant at p=0.05, **significant at p=0.01, and ***significant at p=0.001

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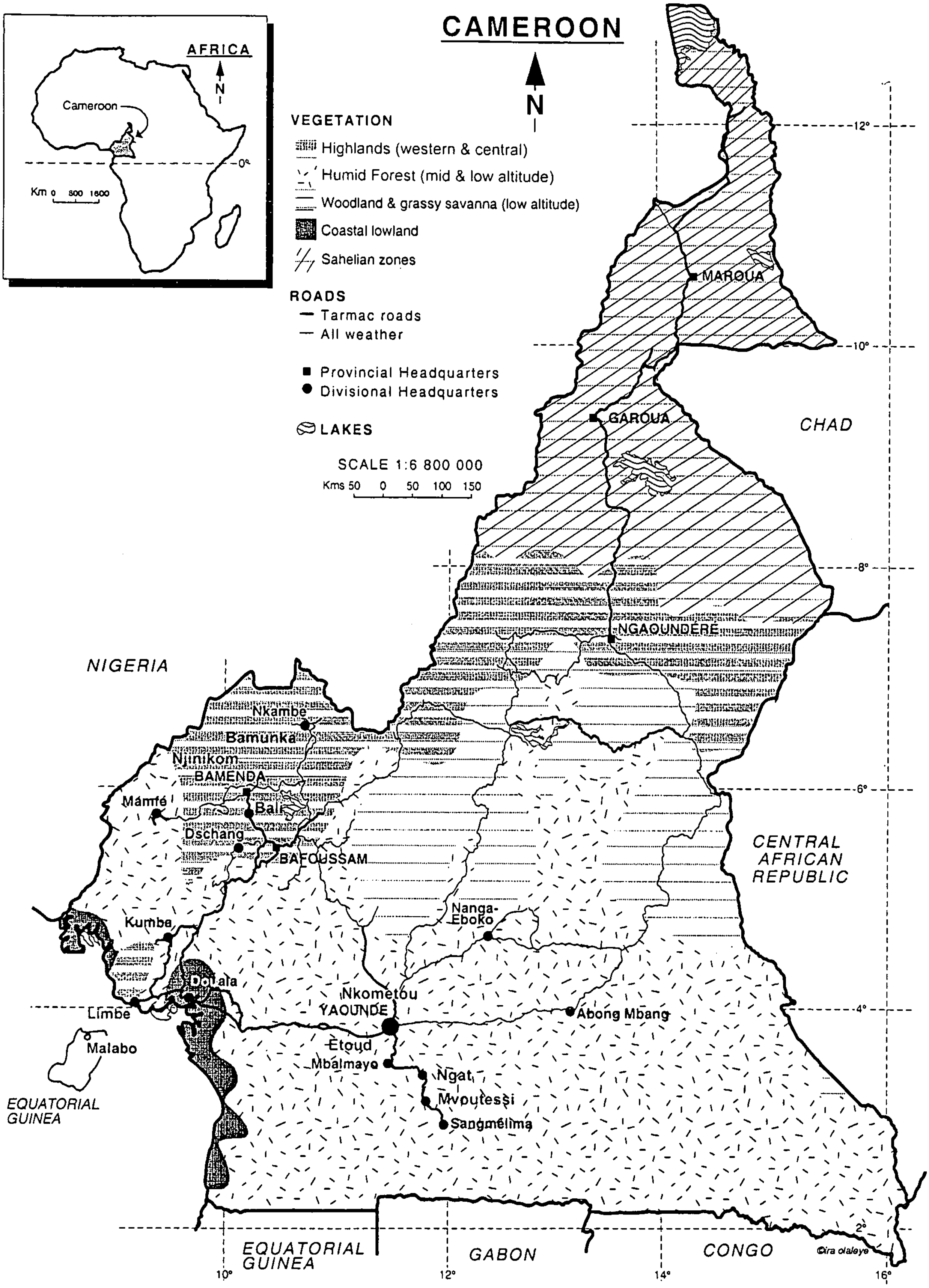
Figure 10. Ear rot *(Stenocarpella spp.)* Bamunka , Western Highlands, 1996

Fig 1. Map of Cameroon showing the agroecological zones, including the Humid Forest and Western Highlands.



Designer: Chuks Onianwa

Figs. 2-4. Maize diseases in Cameroon

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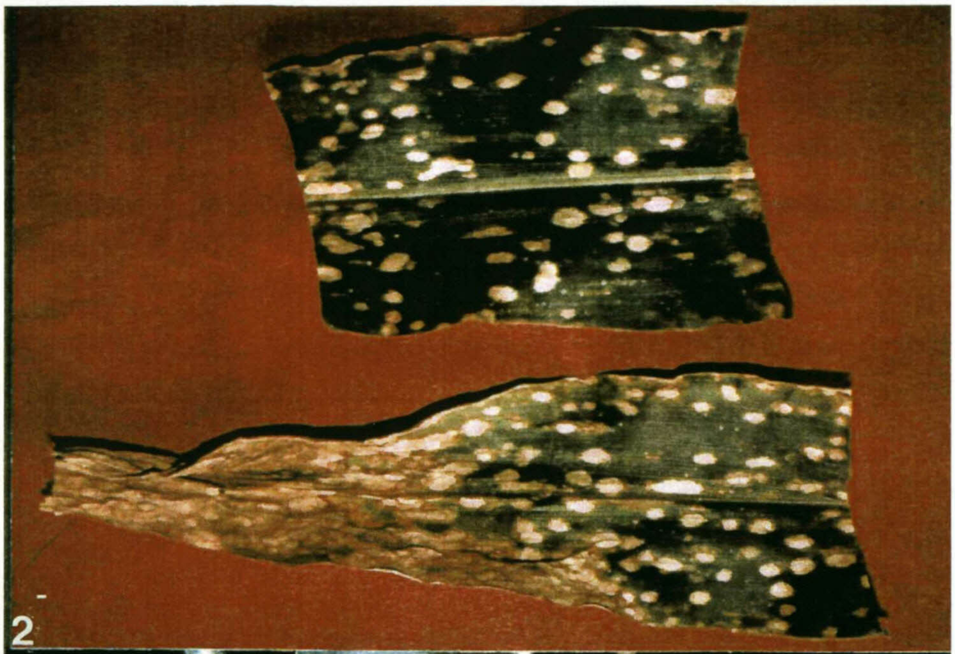
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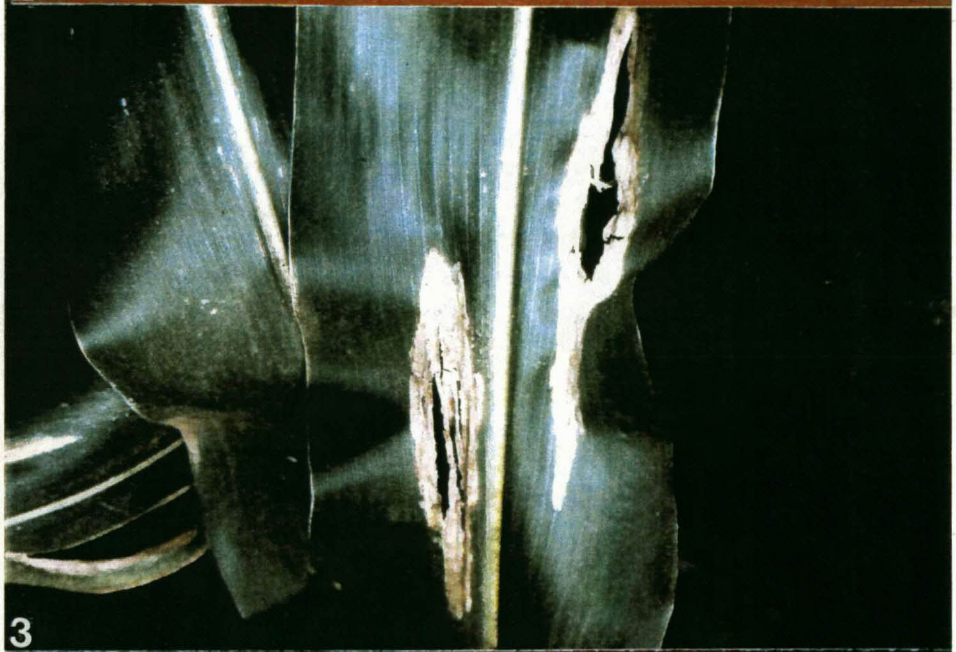
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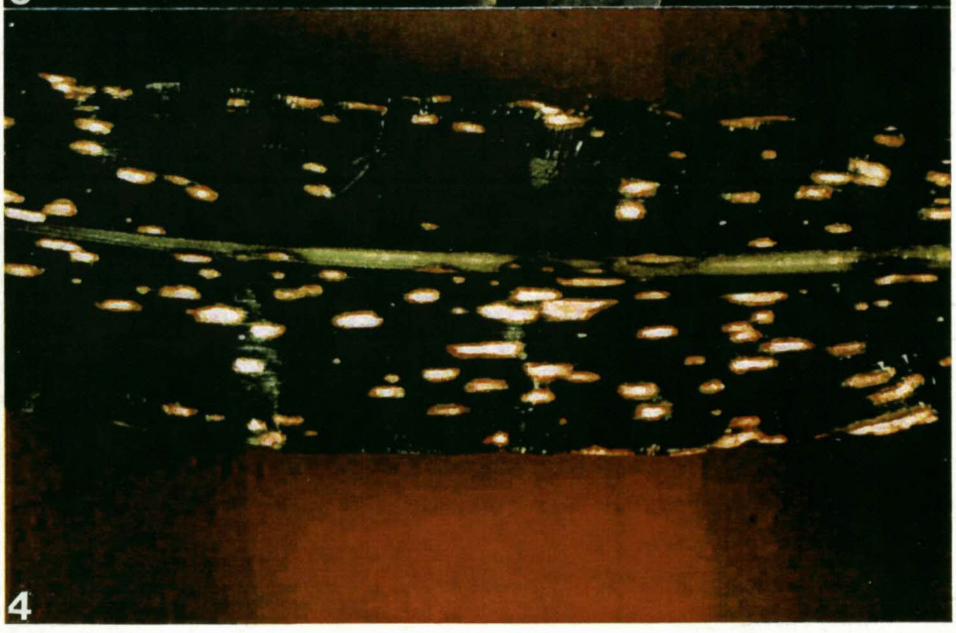
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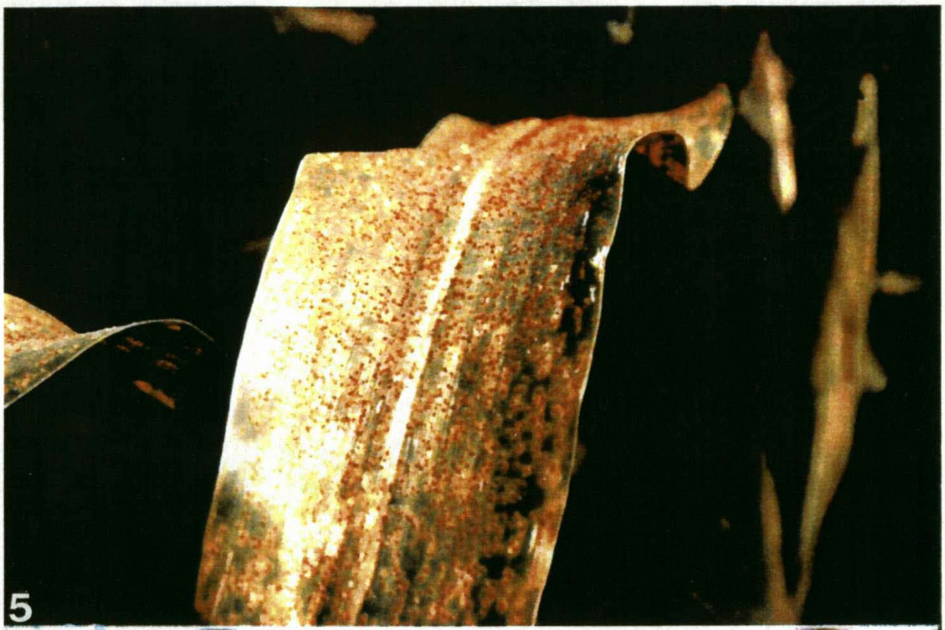
Figs. 5-7. Maize diseases in Cameroon

Figure 5.	Lowland rust (*Puccinia polysora*)	Etoud, Humid Forest, 1995
Figure 6.	Common smut (*Ustilago maydis*)	Mvoutessi, Humid Forest, 1995
Figure 7.	Head smut (*Sporisorium reilianum*)	Njinikom, Western Highlands, 1996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Figs. 8-10. Maize diseases in Cameroon

Figure 8. Brown spot (*Physoderma maydis*) Bali, Western Highlands, 1996

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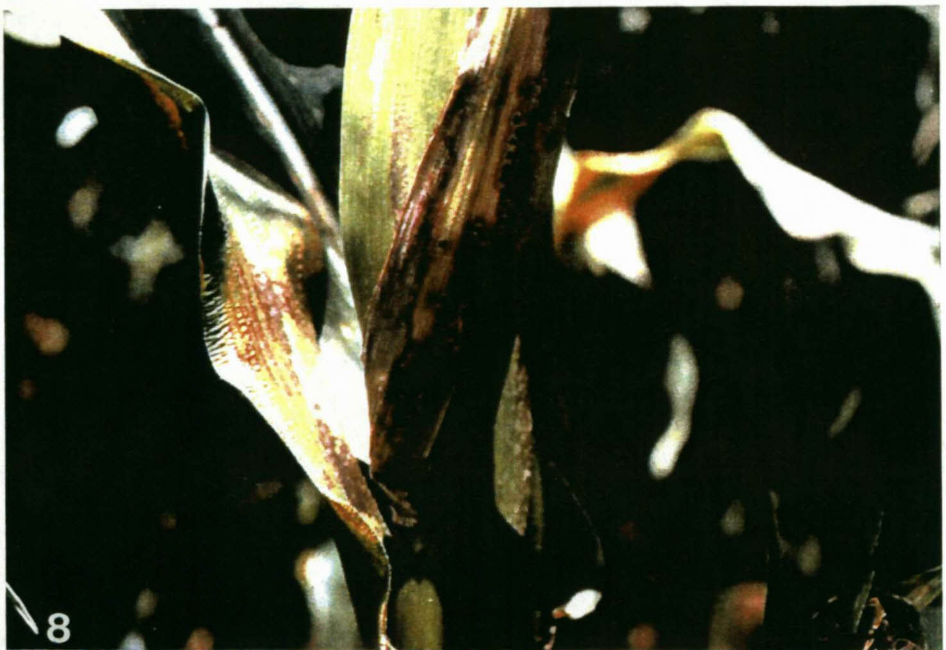

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CHAPTER 3

BIOLOGICAL AND PHYSICAL CONSTRAINTS ON MAIZE PRODUCTION IN THE HUMID FOREST AND WESTERN HIGHLANDS OF CAMEROON

Biological and physical constraints on maize production in the Humid Forest and Western Highlands of Cameroon

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A study was carried out to identify biological and physical factors responsible for the reductions in maize production in Cameroon. Two surveys were conducted in 72 fields in two of the five agroecological zones in the country in 1995, 1996, and 1997. In the Humid Forest (HF), *Bipolaris maydis, Stenocarpella macrospora, Puccinia polysora, Rhizoctonia solani* and soil fertility were factors that reduced maize production in villages in 1995 and 1996. In the Western Highlands (WHL), *Cercospora zeae-maydis*, and the interaction between soil fertility and maize variety were the most important constraints to maize production in 1996. In 1997, in addition to *C. zeae-maydis, S. macrospora*, physiological spot and insect damage expressed as the number of holes bored in the stem by *Busseola fusca* were negatively related to ear weight. The combination of these biological factors (diseases and insects), and the physical parameter soil fertility were responsible for reducing maize yield in these selected benchmarks of Cameroon. Average yield reductions were 68.2% due to *B. maydis* and 9.2% due to *S. macrospora,* respectively, in the HF in 1995. In 1996, yield reductions were estimated at 34.3%, 41.4% and 29.6% due to *S. macrospora*, *P. polysora*, and *R. solani,* respectively, in the HF. In the WHL, *Cercospora zeae-maydis* caused a yield reduction of 78.5% in 1996. The interaction between *C. zeae-maydis* and *B. fusca*, the stem diseases and the physiological spot caused reductions of 37.9%, 33.6% and 39% respectively, in the WHL in 1997.

Keywords: Cameroon, field pathogens, maize, regression analysis, yield loss

Maize (*Zea mays* L.) production has become a lucrative activity in Cameroon since the devaluation of the "Communauté Financiere Africaine" (CFA) currency. The demand for maize from the feed industries, breweries, small livestock producers, and local consumers has increased markedly. The hardship due to the poor economic situation has prompted civil servants, and many unemployed youths into agricultural activities, with maize as the commodity of interest. This crop is cultivated in all five ecological zones of the country varying from Humid Forest (HF) to Soudano-Sahelian zones. In the Western Highlands (WHL) zone, maize production is intensive because the population is very dense (230 inhabitants/km ^{2}) and is increasing at a rate of 3.2 % annually, resulting in dwindling farm size (Ayuk-Takem, 1996; Ayuk-Takem et al.,1982). In these areas, continuous cropping over time without a fallow period results in a decline in soil fertility and disease inoculum build-up. The area under maize cultivation has increased from 320,000 ha in 1991 to 700,000 ha in 1997 (MINAGRI, 1996; NCRE, 1994; Ngoko,1994). The average yield (1.8 t/ha) has remained constant for many years and production has increased from 500,000 MT in 1991 to over 800,000 MT in 1997 (Ayuk-Takem, 1996; Ayuk-Takem et al., 1982; MINAGRI, 1996). Production could be higher if factors such as soil infertility, poor managerial skills, the high costs of supplies, environmental stresses, pests and diseases were not disrupting the actual potential yield of the improved varieties released so far (NCRE, 1994).

Losses reported due to pests and diseases range from 20 % to 50 % (Cardwell et al., 1997). Several maize diseases have been reported in the country though the findings did not relate the prevalence of diseases to yield loss (Foko, 1973; Ngoko, 1994; Timti, 1980). However, losses up to 90 % were reported by the extension service in some fields in Bali and other parts of the country due to grey leaf spot (*Cercospora zeae-maydis* Tehon & Daniels) in the 1995-1996 cropping season (MINAGRI, 1996). Timti (1980) reported that foliar diseases were responsible for the reduction of maize production though the loss was not computed. Cardwell et al. (1997), in a country-wide survey demonstrated about 600 kg/ha yield loss attributed to the combined effect of foliar, stem diseases and insect damage. Detailed information on factors affecting the yield and the relations between these factors are required in specific areas.

The objectives of the study were to identify and quantify the biological and physical production constraints affecting maize yield in two ecological zones of Cameroon.

MATERIALS AND METHODS

Field surveys

Two field surveys were conducted in four villages (Kometou, Ngat, Etoud, and Mvoutessi) in the Humid Forest (HF) in 1995 and in three villages (Kometou, Ngat, and Etoud) in 1996. In 1996 and 1997, two surveys were conducted in three villages (Bamunka, Bali, and Njinikom) of the Western Highlands (WHL). In all surveys, twelve farmers were selected per village with the collaboration of the extension agent of the locality. Where possible, a notice was sent to the village prior to the evaluation date in order to have the farmer present at the time of the investigation. A plant pathologist and an entomologist carried out the surveys.

A total of 72 farmers were visited. On each farm, 15 plants were chosen at random and bought from the farmers. Each plant was assessed for growth and damage variables. The fields were generally at the grain filling to maturity growth stage. In all fields, data were recorded on the following parameters: cropping system; organic matter (1-3 with 1= low, 3= high); soil texture (1-3 scale: 1= sandy and 3= clay); soil fertility (on a 1-3 scale: 1= poor soil with weak plants and 3= rich soil with vigorous plants); weediness (1-3 scale: 1= clean and 3= very weedy field); crop stage on a 1-9 scale: (1= emergence, 5= silking, 7= milk stage, and 8 = hard dough and 9 = physiological maturity); variety used (local = 0 and improved =1); weight of cobs without husk; ear length and width; and % grain fill on a 0-100 % scale (0=cob without grain, 100=cob full of grain to the tip).

The disease data included the identification of the most important diseases and severity rating on a 0-4 scale: (0=no symptoms and 4=severe plant damage due to presence of the pathogen); stem infections on a 0-4 scale: (0 = no symptoms and 4= > 90 % of the stem damaged by fungi; and the percent of total leaf area infected (LAI) with diseases calculated according to Clive (1971). For the identification of the causative agents, leaf samples infected with highland blight [*Exserohilum turcicum* (Passerini) Leonard & Suggs], and lowland blight [*Bipolaris maydis* (Nisikado & Miyake) Shoemaker], were incubated on PDA for confirmation of the identity of the causative fungi according to Shurtleff (1986). Symptoms of Diplodia leaf spot (*Stenocarpella macrospora* (Earle) Sutton = *Diplodia macrospora* Earle), common smut (*Ustilago maydis* DC), head smut [*Sporisorium reilianum* (Kühn) Langdon & Fullerton = *Sphacelotheca reiliana* (Kühn) Clint], Phaeosphaeria leaf spot [*Phaeosphaeria maydis* (P. Henn) Rane, Payak & Renfro], highland rust (*Puccinia sorghi*

Schw.) , lowland rust (*Puccinia polysora* Underw.), brown spot (*Physoderma maydis* Miyabe) and sheath blight (*Rhizoctonia solani* Kühn) were identified according to the Compendium of Corn Diseases (Shurtleff, 1986). Grey leaf spot was identified according to Latterell & Rossi (1983) and Diplodia leaf spot according to Marasas et al. (1979).

Entomological data were collected only in 1997 and included identification of the stem borer [*Busseola fusca* Fuller (Lepidoptera: Noctuidae)] according to Van Rensburg (1987), the tunneling in cm and the number of holes and internodes bored on the stem.

Statistical analysis

Analysis was carried out with SAS 6.12 for Windows. Means of each variable were computed by field on the basis of 15 plants per field. Pearson correlation analysis was conducted to find the significant interactions among variables. Backward linear regression was performed using the variables that were significantly related in the correlation analysis. Ear weight (in all years and zones) and % grain fill (in the WHL in 1996 and 1997) were used respectively as the dependant variables and physical and biological factors as the independent variables. Factors that were removed by the regression equation were not used for the analysis of the results though they were recorded.

Maximum potential yield (Y_{max}) was calculated using the maxima of significant positive variables ($p <= .10$) while holding disease variables at their minima. Regional yield losses were then estimated by substituting the mean value of each significant variable into the regression equation per ecological zone and per year (Table 1). Percentage regional loss in ear weight was calculated using the following formula: $Y_R=1-(Y_{mean}/Y_{max}) \times 100$. The potential loss per disease causal agent was calculated by the formula $Y_P = 1-(Y_{min}/Y_{max}) \times 100$, where Y_{min} is the yield obtained under the effect of a given disease at its maximum interference, the other factors being held at their mean level, or in the case of other disease agents, being held at the minima (modified from Cardwell et al., 1997). Data were analyzed separately for each year and each zone.

RESULTS

Humid Forest 1995

Pearson correlation analysis (data not shown) showed that several biotic and abiotic factors were significantly related. Stem diameter and *B. maydis* were negatively related to *E.*

turcicum (p=0.0001 for both). *Bipolaris maydis* and *P. polysora* were strongly negatively related (p=0.0001) while *S. macrospora* was inversely related with *R. solani* (p=0.04), but positively with stem diameter (p=0.001). Increased organic matter content of the soil had a negative effect on *E. turcicum* (p=0.005) and *R. solani* (p=0.004), and increased stem diameter (p=0.0001). In all, there were 31 significant interactions among 14 variables on 615 plants. These interactions were used as variables in the stepwise backward regression analysis along with the primary factors.

Backward regression analysis demonstrated which factors had a significant effect on ear weight (Table 1). Ear weight increased with crop stage, but there was a crop stage by soil texture interaction during this season that reduced ear weight. Among the pathogens present only *B. maydis* (p=0.001) and *S. macrospora* (p=0.084) had significant negative relationships with ear weight. When stem diameter and organic matter content increased together, the ear weight also increased significantly (p=0.001). Otherwise, in these data, increase in stem diameter had a negative effect on ear weight. Although *B. maydis* alone caused significant loss in ear weight, there were interactions of *B. maydis* with weediness and soil texture for which ear weight increased.

Maximum potential yield that can be calculated from Table 1 would be 222.8 g/ear. The mean yield estimate using the significant variables that entered the equation was 156.4 g/ear. At maximum disease expression of *B. maydis*, the ear weight would decrease to 70.9 g/ear, while the maximum level of *S. macrospora* would reduce ear weight to 120.2 g/ear.

Humid Forest 1996

Pearson correlation analysis (data not shown) showed 33 significant interactions among 15 biotic and abiotic factors. *Bipolaris maydis* was negatively correlated with *S. macrospora* (p=0.005) and organic matter content (p=0.0001). *Puccinia polysora* was positively correlated with maize streak virus (p=0.0001). *Bipolaris maydis* and *R. solani* decreased with increasing organic matter and heavier soil texture (p=0.01 for both) and *E. turcicum* was also inversely related to soil organic matter (p=0.05). The significant interactions and principal variables were used in the regression against ear weight (Table 2).

From the regression analysis against ear weight, three disease variables entered alone, i. e. *P. polysora* (p=0.0001), *R. solani* (p=0.031) and *S. macrospora* (p=0.0003) significantly decreased ear weight. Interaction between *E. turcicum* and maize streak virus exerted a highly significant (p=0.0001) negative effect on ear weight, but *E. turcicum* and *B. maydis* cancelled each other resulting in a slight increase (Table 2). The interaction between stem diameter and soil fertility was positively related to ear weight. The interaction between the crop stage and *B. maydis* had a negative impact on ear weight, indicating the effect of early infection.

Maximum potential yield for this zone in this year can be calculated from the equation in Table 2 to be 183.0 g/ear. The mean yield estimate using the significant variables that entered the equation was 121.6 g/ear. At maximum disease expression of *P. polysora*, the ear weight would decrease to 107.2 g/ear, while the maximum level of *S. macrospora* would reduce ear weight to 120.0 g/ear and *R. solani* to 128.7 g/ear.

Western Highlands 1996

Pearson correlation analysis (data not shown) produced 14 significant interactions among 11 biotic and abiotic factors. In this survey, variety was assessed as local or improved. The improved variety was significantly (p=0.0001) negatively correlated with *E. turcicum* and *C. zeae-maydis*, indicating better resistance than the local variety. *Cercospora zeae-maydis* and *E. turcicum* were significantly related to each other (p=0.0001) and both increased significantly as soil fertility increased (p=0.01 and p=0.0001, respectively). Stem lesions interacted significantly with soil texture (p=0.004), indicating more stem disease in lighter textured soils.

The significant interactions of the correlation matrix and the principal variables were used in the regression against ear weight (Table 3) and against %grain fill (Table 4). As expected, crop stage, soil texture and soil fertility were positively correlated with ear weight (Table 3). Likewise, variety by fertility interaction was positively related to ear-weight, indicating a strong yield advantage from using both. The interactions between variety and *C. zeae-maydis* and soil fertility and *C. zeae-maydis* were the main factors associated with ear weight lost. Since the correlation between *C. zeae-maydis* and variety was negative, it can be inferred that most yield loss occurred because of the disease on susceptible local cultivars. As *C. zeae-maydis* increased with soil fertility, the negative yield effect of the interaction of these two variables indicates that there is a point at which increased fertility will begin to be counterproductive.

Maximum potential yield for the WHL during this year can be calculated from the equation in Table 3 to be 203.2 g/ear. The mean yield estimate using the significant variables that entered the equation was 99.2 g/ear, while with maximum disease expression of *C. zeae-maydis*, the ear weight would be reduced to 43.6 g/ear.

When the biotic and abiotic variables were regressed against % grain fill, a different set of variables entered than for ear weight (Table 4). Weediness of the field, *C. zeae-maydis* on the local variety, and stem disease reduced grain filling. Soil fertility and soil texture had a positive effect on in grain filling.

Western Highlands 1997

Pearson correlation analysis (data not shown) produced 14 significant interactions among 16 biotic and abiotic variables. In this survey, variety was strongly negatively related to *C. zeae-maydis* and stem borer parameters (p=0.0001 for both), again indicating that the improved variety is significantly more resistant to these biotic factors. As *C. zeae-maydis* increased, *Phy. maydis* (p=0.05), *S. macrospora* (p=0.04), and the physiological spot (p=0.05) significantly decreased. *Exserohilum turcicum* and S. *macrospora* were also inversely related (p=0.05). *Cercospora zeae-maydis* was significantly correlated with all stem borer damage parameters (p=0 .0001), indicating that plants infested with the insect are more susceptible to the pathogen, or vice versa. Fertility and organic matter were significantly correlated in this survey (p=0.0001).

The significant interactions of the correlation matrix and the principal variables were used in the regression against ear weight (Table 5) and against %grain fill (Table 6). Once again, crop stage and soil fertility by organic matter interaction were positively correlated with ear weight. *S. macrospora* was also positively related to ear weight, but this pathogen increased with fertility (p<0.05), thus the positive slope may reflect infection on larger plants. The interaction between the number of internodes bored by the stem borer and *C. zeae-maydis* was the main factor associated with ear weight lost. Since the correlation between stem borer variables, *C. zeae-maydis* and variety were negative, it can be inferred that most yield loss occurs because of the damage to susceptible local cultivars. Stem disease and the physiological spot also had a negative effect on ear weight.

Maximum potential yield for the WHL during this year can be calculated from the equation in Table 5 to be 278.9 g/ear. The mean yield estimate using the significant variables that entered the equation was 173.5 g/ear, while with maximum expression of disease caused by *C. zeae-maydis* and internodes bored by *B. fusca*, the ear weight would decrease to 134.9 g/ear.

When grain filling was used as dependant variable, stem disease and *C. zeae-maydis* on susceptible varieties were seen to reduce grain filling. The improved variety had significantly ($p=0.009$) better grain filling (Table 6).

Actual yield losses due to the combination of factors were 30 % and 33.6% in the HF in 1995 and 1996, respectively (Table 7). In the WHL, *C. zeae-maydis* alone caused a yield reduction of 78.5% in 1996, while the combined effects of the same pathogen and *Busseola fusca*, the stem disease and the physiological spot reduced the yield by 37.9 % in 1997 (Table 7).

DISCUSSION

The assessment of biological and physical constraints to maize production in the HF zone of Cameroon in 1995, revealed that individual pathogens such as *B. maydis, S. macrospora*, and physical factors such as soil texture were the most important factors that affected the yield. Many diseases with high incidence may have been diluted by diseases with a low prevalence. The main abiotic factor that was measured and that was responsible for the difference between potential yield and actual yield was soil fertility. In general, the reduction of the ear weight was a result of the interaction between factors. The negative effects of the soil fertility are explained by the acidic soils, that are more predominant in the HF though not uniformly distributed across the region (Embrechts, 1978; FAO, 1986; Kotto-Same et al., 1997).

Contrary to the 1995 observations, diseases that contributed to the reduction of maize production in 1996 in the HF were *S. macrospora, R. solani,* and *P. polysora.*. Though *E. turcicum* was not retained by the regression analysis as an individual factor causing yield loss, its interaction with the organic matter and maize streak virus reduced yield significantly ($p=0.0001$). This is in accordance with Cardwell et al. (1997) who found that individual diseases often have no direct impact on ear weight. In the field environment, yield is usually affected by a combination of several factors, some of which can also be inversely related. The inverse significant correlation between *S. macrospora*, *E. turcicum* and *B. maydis* suggests that they are not favored by the same agroecological conditions. The presence of *E. turcicum* in the HF is contrary to its described ecological niche, which is considered to be in cooler, mid-altitude climates. Losses from *S. macrospora* averaged about 20% in the HF over the two years. This disease has never been considered as a major problem in the IRAD maize improvement program. A policy decision needs to be made about investing in screening for resistance to *S. macrospora.* At its maximum occurrence, *B. maydis* was estimated to cause up to 70% ear weight loss. It is clear that work needs to be done on host plant resistance to these foliar pathogens. Losses from *P. polysora* in the HF were estimated at 41 % in 1996. This is contrary to a previous report in which Cardwell et al. (1997) reported no yield loss due to *P. polysora* because the disease rating was positively correlated with yield. This difference could be the result of environmental factors, a breakdown in resistance or a consequence of newly introduced susceptible maize cultivars at farmers' level by the extension service

Maize is the primary crop in the WHL and more emphasis is also being placed on maize production in the HF for commercial purposes. Therefore, maize pathogens may become more important due to the intensification the production of this commodity. Disease factors should be considered as important constraints to maize production in both agroecological zones. Though *E. turcicum*, *Pha. maydis*, and maize streak virus were not associated with the estimation of the yield losses in this study, this does not imply that they do not cause yield lost. Research programmes should be aimed at elucidating these aspects, especially as maize is becoming a cash crop in areas where agricultural practices are still traditional for most farmers. In the HF shifting cultivation, slash-and-burn (Kotto-Same et al., 1997), is among the most used farming practices. This farming practice in the long term contributes to the rapid loss of soil fertility due to leaching of mineral and degradation of organic matter at a much faster rate (Kotto-Same et al., 1997).

In the WHL, *B. fusca*, the most important insect pest in the region, together with *C. zeae-maydis* and stem diseases, and the interaction between maize streak virus and *E. turcicum* were the most important constraints to maize production in 1996 and 1997. Although the number of insects collected is not mentioned in this paper, the effect of *B. fusca* is expressed by the interaction between *C. zeae-maydis* and number of internodes bored. The length of the tunnel and the number of holes bored on the stem, though recorded, were not retained by the regression analysis. The tunneling was more extensive in plants growing in fertile soil than in poor sandy soils and it appears that *B. fusca* prefers softer stems found in more fertile soil (data not shown). This suggests that this insect is also a major constraint to maize production in the WHL which needs to be addressed. These associations between stems, insects and pathogens may lead to stalk rots and lodging. Cardwell et al. (1997) noted a positive relationship between soil organic matter and the stem borers *B. fusca* and *Eldana saccharina* Walker (Lepidoptera: Pyralidae) and *B. maydis*.

Stenocarpella macrospora was positively related to soil fertility, i.e. fertility increased the severity of this disease. High levels of nitrogen have been shown to increase some leaf diseases of maize (Fajemisin, 1985). Farmers in the WHL used mostly organic fertilizer such as chicken manure, cow manure and/or compost. Chicken manure is known to contain a high level of nitrogen and phosphorous. How the natural fertilization relates to disease incidence and severity is still not well elucidated. No relationship was found between the incidence *of S. macrospora, E. turcicum, B. maydis* and the effects of *B. fusca*. However, Flett & Van Rensburg (1992) found that *B. fusca* increased the incidence of *Fusarium moniliforme* Sheldon ear rot though this did not result in a increase in kernels rotted by *F. moniliforme* . More investigations are needed to better understand the relationship between insect infestations and stem rotting fungi. Livestock farmers are using more maize stalks to feed their animals, but there is no information on the effects of the complex of fungi found in Cameroonian maize on livestock production. Diplodiosis has been reported in cows fed with stalk and leaves infected by *Stenocarpella maydis* (Berk.) Sutton (Marasas, 1977).

Although this study was carried out in only 72 fields, the results indicated that the combination of biological factors such as diseases and insects, and physical parameters such as soil fertility and organic matter are responsible for the significant yield reduction of maize in Cameroon. Although yield reduction due to a number of diseases was shown, it is probable that all the other maize pathogens also cause some losses (Zadocks & Schein, 1979). Future investigations may highlight the importance of other plant pathogens. On-station trials should be conducted to fully understand how these factors should be ameliorated to enhance maize production in Cameroon. Efforts should be geared towards strategies that increase the levels and/ or value of production translated into the improvement of the quality of life of the rural population and the poor citizen.

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Table 1. Significant factors affecting ear weight as calculated by a stepwise backward regression analysis, maxima (Max), mean, and minima (Min) in the Humid Forest zone of Cameroon in 1995 (R^2=0.10; p=0.0001; N=615)

Variable			Slope Mean	F Min	P>t	Max
Intercept	109.34	12.86	0.004			
CSTG	9.09	5.28	0.021	9.0	7.4	6.0
B.maydis	-56.47	15.37	0.001	4.0	1.4	0.0
CSTG x soil	-2.75	22.67	0.001	27.0	15.9	7.0
Weed x Bm	3.18	11.26	0.000	8.0	2.8	0.0
Sdia x OM	12.52	16.78	0.001	6.0	3.8	1.0
Sdia	-23.77	7.66	0.006	2.5	1.8	1.0
Soil x Bm	3.97	3.03	0.008	12.0	2.8	0.0
Sm	-5.88	2.79	0.084	3.0	0.2	0.0

CSTG=maize crop growth stage (1-9); *B.=Bipolaris* Cstg x soil = interaction between the growth stage and the soil texture (1-3 with increasing clay content); Weed x Bm = weediness (1-3 scale) by *B. maydis* interaction; Sdia x OM = Stem diameter by organic matter content (1-3 scale); Sdia = stem diameter (cm); Sm = *Stenocarpella macrospora;* Soil texture (1-3 with 1=sandy 3=clay).

$$Y_{max} = 109.34 + (9.1CSTG_{max}) - (56.47xBm_{min}) - (2.75CSTGxSoil_{min}) + (3.2WeedxBm_{min}) + (12.52SdiaxOM_{max}) - (23.7Sdia_{min}) + (4\ SoilxBm_{min}) - (5.9\ Sm_{min}) = 222.84 \text{ g/ear.}$$

$$Y_{mean} = 109.34 + (9.1CSTG_{mean}) - (56.47xBm_{mean}) - (2.75CSTGxSoil_{mean}) + (3.2WeedxBm_{mean}) + (12.52SdiaxOM_{mean}) - (23.7Sdia_{mean}) + (4\ SoilxBm_{mean}) - (5.9\ Sm_{mean}) = 156.45 \text{ g/ear.}$$

$$Y_{Bm} = 109.34 + (9.1CSTG_{mean}) - (56.47xBm_{max}) - (2.75CSTGxSoil_{mean}) + (3.2WeedxBm_{max}) + (12.52SdiaxOM_{mean}) - (23.7Sdia_{mean}) + (4\ SoilxBm_{max}) - (5.9\ Sm_{min}) = 70.76 \text{ g/ear.}$$

$$Y_{Sm} = 109.34 + (9.1CSTG_{mean}) - (56.47xBm_{min}) - (2.75CSTGxSoil_{mean}) + (3.2WeedxBm_{min}) + (12.52SdiaxOM_{mean}) - (23.7Sdia_{mean}) + (4\ SoilxBm_{min}) - (5.9\ Sm_{min}) = 120.20 \text{ g/ear.}$$

Table 2. Significant factors affecting ear weight as calculated by a stepwise backward regression analysis, maxima (Max), mean, and minima (Min) in the Humid Forest zone of Cameroon in 1996 (R^2=0.20; p=0.0001; N=465)

Variable			Slope Mean	F Min	P>t	Max
Intercept	133.34	6.93	0.0001			
CSTG	2.03	0.35	0.055	9.0	7.5	6.0
P. polysora	-8.35	73.40	0.0001	4.0	0.9	0.0
Et x Bm	7.65	11.55	0.0001	4.0	0.1	0.0
Et x MSV	-13.87	12.21	0.0001	2.0	0.04	0.0
CSTG x Bm	-0.84	39.70	0.0001	32.0	11.6	0.0
CSTG x Soil	-0.55	11.51	0.0001	25.5	14.4	6.0
S macrospora	-5.09	13.43	0.0003	4.0	0.3	0.0
R. solani	-3.93	4.64	0.031	3.0	0.2	0.0
Sdia x Fertility	2.17	3.51	0.061	4.0	0.4	1.0

CSTG= crop growth stage (1-9); Et = *Exserohilum turcicum*; *P.* = *Puccinia*
R = *Rhizoctonia*; Sdia = stem diameter (cm); *B.*= *Bipolaris*; MSV= maize streak virus; Et x MSV= the interaction between *E. turcicum* and maize streak virus; CSTG x Soil = interaction between the growth stage and the soil texture (1-3 with increasing clay content); Sm = *Stenocarpella macrospora;* Rs=*Rhizoctonia solani*; Sdia x Fertility = Stem diameter by field fertility (1-3 scale);

Y_{max} = 133.34 + ($2.0CSTG_{max}$) – ($8.4xPp_{min}$) + ($7.7EtxBm_{min}$) - ($13.9EtxMSV_{min}$) - ($0.8CSTGxBm_{min}$) – ($CSTGxSoil_{min}$) - ($5.1\ Sm_{min}$) – ($3.9Rs_{min}$) + ($2.2SdiaxFertility_{max}$) = 183.00 g/ear.

Y_{mean} = 133.34 + ($2.0CSTG_{mean}$) – ($8.4xPp_{mean}$) + ($7.7EtxBm_{mean}$) - ($13.9EtxMSV_{mean}$) - ($0.8CSTGxBm_{min}$) – ($CSTGxSoil_{mean}$) - ($5.1\ Sm_{mean}$) – ($3.9Rs_{mean}$) + ($2.2SdiaxFertility_{mean}$) = 121.60 g/ear.

Y_{Sm} = 133.34 + ($2.0CSTG_{mean}$) – ($8.4xPp_{min}$) + ($7.7EtxBm_{min}$) - ($13.9EtxMSV_{min}$) - ($0.8CSTGxBm_{min}$) – ($CSTGxSoil_{mean}$) - ($5.1\ Sm_{max}$) – ($3.9Rs_{min}$) + ($2.2SdiaxFertility_{mean}$) = 120.03 g/ear.

Y_{Pp} = 133.34 + ($2.0CSTG_{mean}$) – ($8.4xPp_{max}$) + ($7.7EtxBm_{min}$) - ($13.9EtxMSV_{min}$) - ($0.8CSTGxBm_{min}$) – ($CSTGxSoil_{mean}$) - ($5.1\ Sm_{min}$) – ($3.9Rs_{min}$) + ($2.2SdiaxFertility_{mean}$) = 107.23 g/ear.

Y_{Rs} = 133.34 + ($2.0CSTG_{mean}$) – ($8.4xPp_{min}$) + ($7.7EtxBm_{min}$) - ($13.9EtxMSV_{min}$) - ($0.8CSTGxBm_{min}$) – ($CSTGxSoil_{mean}$) - ($5.1\ Sm_{min}$) – ($3.9Rs_{max}$) + ($2.2SdiaxFertility_{mean}$) = 128.73 g/ear.

Table 3. Significant factors affecting ear weight as calculated by a stepwise backward regression analysis, maxima (Max), mean, and minima (Min) in the Western Highlands zone of Cameroon in 1996 (R^2=0.10; p=0.0001; N=435)

Variable			Slope Mean	F Min	P>t	Max
Intercept	-41.09	17.17	0.076			
CSTG	11.77	20.56	0.0001	9.0	6.9	6.0
Fertility	21.30	13.27	0.0003	3.0	2.1	1.0
Var x Fertility	15.81	6.08	0.014	3.0	0.3	0.0
Soil texture	8.84	5.71	0.017	3.0	2.3	1.0
Var x Cz	-16.55	4.59	0.032	3.0	0.2	0.0
Fertility x Cz	-1.98	3.06	0.081	9.0	4.3	0.0

CSTG= crop growth stage (1-9); field fertility (0-2 scale); Var= maize variety (0 = local, 1 = improved); Soil texture= soil texture (1-3 with increasing clay content); Cz=*Cercospora zeae-maydis*; Var x Cz= interaction between variety and *C. zeae-maydis*

$Y_{max} = -41.1 + (11.7CSTG_{max}) + (21.3Fertility_{max}) + (15.81VarxFertility_{max}) + (8.8Soil_{max}) - (16.5VarxCz_{min}) - (1.98\ FertilityxCz_{min}) = 203.2$ g/ear

$Y_{mean} = -41.1 + (11.7CSTG_{mean}) + (21.3Fertility_{mean}) + (15.81VarxFertility_{mean}) + (8.8Soil_{mean}) - (16.5VarxCz_{mean}) - (1.98\ FertilityxCz_{mean}) = 99.2$ g/ear.

$Y_{Cz} = -41.1 + (11.7CSTG_{mean}) + (21.3Fertility_{mean}) + (15.81VarxFertility_{mean}) + (8.8Soil_{mean}) - (16.5VarxCz_{max}) - (1.98\ FertilityxCz_{max}) = 43.6$ g/ear.

Table 4. Significant factors affecting % grain fill (arcsine transformed) as calculated by a stepwise backward regression analysis, maxima (Max), mean, and minima (Min) in the Western Highlands zone of Cameroon in 1996 (R^2=0.23; p=0.0001; N=435)

Variable			Slope	t	P>t	Max
			Mean	Min		
Intercept	40.10	3.7	0.0003			
CSTG	11.77	0.9	0.3792	9	6.9	6
Weed	-11.44	1.9	0.0507	2	1.6	0
Soil	2.80	2.3	0.0222	3	2.3	1
Fertility	7.08	1.7	0.0886	3	2.1	1
Variety x Cz	-2.28	2:37	0.0183	3	0.2	0
Soil x Stem disease	-1.59	2.08	0.0375	9	2.5	0
Fertility x Weed	4.37	5.50	0.1105	6	3.3	1

CSTG= crop growth stage (1-9); Stem disease (0-4 scale); Weeds (0-2 scale); Fertility (1-3 scale); Variety= maize variety (0 = local, 1 = improved); Soil = soil texture (1-3 with increasing clay content); Cz= *Cercospora zeae-maydis*; Var x Cz= interaction between variety and *C. zeae-maydis*

Table 5. Significant factors affecting ear weight as calculated by a stepwise backward regression analysis, maxima (Max), mean, and minima (Min) in the Western Highlands zone of Cameroon in 1997 (R^2=0.79; p=0.0001; N=540)

Variable			Slope	F	P>t	Max
			Mean	Min		
Intercept	-122.63	4.52	0.0422			
CSTG	39.36	28.32	0.0001	8.5	7.8	6.7
Cz x IB	-14.36	62.29	0.0001	6.3	2.5	0.14
Fertility x OM	13.69	9.88	0.0038	5.0	3.0	2.2
Stem disease	-20.06	8.71	0.0063	2.0	0.9	0.0
PSPT	-36.83	7.36	0.0111	1.5	0.1	0.0
Sm	11.44	3.48	0.0721	1.6	0.5	0.0

CSTG=crop growth stage (1-9); Fertility=field fertility (1-3 scale); OM=organic matter (1-3 scale)Cz=*Cercospora zeae-maydis*; IB= square root of number of internodes bored by *Busseola fusca* ; PSPT=physiological spot; Sm=*Stenocarpella macrospora*

Y_{max} = -122.63 + (39.36$CSTG_{max}$) – (14.36 $CzxIB_{min}$) + (13.69$FertilityxOM_{max}$) – (20.06$Stem_{min}$) - (36.83$PSPT_{min}$) + (11.44Sm_{min}) = 278.94 g/ear

Y_{mean} = -122.63 + (39.36$CSTG_{mean}$) – (14.36 $CzxIB_{mean}$) + (13.69$FertilityxOM_{mean}$) – (20.06$Stem_{mean}$) - (36.83$PSPT_{mean}$) + (11.44Sm_{mean}) = 173.53 g/ear

Y_{CzxIb} = -122.63 + (39.36$CSTG_{mean}$) – (14.36 $CzxIB_{max}$) + (13.69$FertilityxOM_{mean}$) – (20.06$Stem_{min}$) - (36.83$PSPT_{min}$) + (11.44Sm_{min}) = 134.98 g/ear

Y_{Stem} = -122.63 + (39.36$CSTG_{mean}$) – (14.36 $CzxIB_{min}$) + (13.69$FertilityxOM_{mean}$) – (20.06$Stem_{max}$) - (36.83$PSPT_{min}$) + (11.44Sm_{min}) = 185.33 g/ear

Y_{Pspt} = -122.63 + (39.36$CSTG_{mean}$) – (14.36 $CzxIB_{min}$) + (13.69$FertilityxOM_{mean}$) – (20.06$Stem_{min}$) - (36.83$PSPT_{max}$) + (11.44Sm_{min}) = 170.20 g/ear

Table 6. Significant factors affecting % grain fill (arcsine transformed) as calculated by a stepwise backward regression analysis, maxima (Max), mean, and minima (Min) in the Western Highlands zone of Cameroon in 1997 (R^2=0.29; p=0.0102; N=540)

Variable			Slope Mean	F Min	P>F	Max
Intercept	69.31	581.89	0.0001			
Stem disease	-1.63	3.17	0.0843	2.0	0.9	0.0
Variety	3.67	7.57	0.0097	1.0	0.5	0.0
CzxVariety	-2.10	10.36	0.0029	4.0	0.6	0.0

Variety=local (0) or improved (1) variety; Cz=*Cercospora zeae-maydis*

Table 7. Summary of ear weight reduction due to maize diseases and pests in the Humid Forest and Western Highlands of Cameroon in1995-1997

Ecological zone	Yield (g/ear)	% Reduction*
Humid Forest 95		
Potential	222.8	
Actual	156.5	30.0
Bipolaris maydis	70.8	68.2
Stenocarpella macrospora	202.2	9.2
Humid Forest 96		
Potential	183.0	
Actual	121.6	33.6
Stenocarpella macrospora	120.3	34.3
Puccinia polysora	107.2	41.4
Rhizoctonia solani	128.7	29.6
Western Highlands 96		
Potential	203.2	
Actual	99.2	51.2
Cercospora zeae-maydis	43.6	78.5
Western Highlands 97		
Potential	278.9	
Actual	173.5	37.9
Cercospora zeae-maydis & *Busseola fusca*	134.9	51.6
Stem disease	185.3	33.6
Physiological spot	170.2	39.0

* Reduction over potential yield

CHAPTER 4

FUNGAL INFECTION AND MYCOTOXIN CONTAMINATION OF MAIZE IN THE HUMID FOREST AND THE WESTERN HIGHLANDS OF CAMEROON

Fungal infection and mycotoxin contamination of maize in the Humid Forest and the Western Highlands of Cameroon

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Maize samples were collected from 72 farmers' stores in 1996 and 1997 in the Humid Forest (HF) and Western Highlands (WHL) of Cameroon. Mycological investigations on these samples revealed that several fungi developed on maize grain. *Nigrospora* spp. were the most prevalent fungi in both the HF (32.1%) and the WHL (30.2%) in 1996. *Fusarium moniliforme* and *F. graminearum* were isolated from most of the samples analyzed. No significant difference ($p>0.05$) was found between the incidence of *Fusarium* spp. in Bali and Bamunka in samples collected two and four months after harvest. In Njinikom, high levels of *Fusarium* contamination were recorded in 1997. The incidence of *Aspergillus* spp. remained low, but was higher in samples collected four months rather than two months after harvest. Chemical analysis with thin layer chromatography did not detect quantifiable levels of aflatoxins in most samples. The *F. moniliforme* mycotoxin fumonisin B_1 (300-26,000 ng/g) and the *F. graminearum* metabolites deoxynivalenol (<100 –1,300ng/g) and zearalenone (<50 -110ng/g) were determined by means of polyclonal antibody (PAb-based) 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 was positively ($r=0.39$; $p=0.013$) correlated with the level of fumonisin B_1, but the location had no effect. This is the first report of the natural occurrence of fumonisins, deoxynivalenol and zearalenone in maize in Cameroon.

Keywords: aflatoxin, deoxynivalenol, fumonisin, *Fusarium graminearum, Fusarium moniliforme,* maize, zearalenone

Maize (*Zea mays* L.) is one of the most important food commodities produced in Cameroon. Ayuk-Takem (1996) reported that maize is cultivated on about 600,000 ha for a total production of 800,000 metric tons. This production could be higher if many factors such as soil infertility, poor managerial skills of farmers, high cost of inputs, environmental stresses, pests and diseases from the fields to the stores were not disrupting the actual yield potential of the available local and improved maize varieties released (Ayuk-Takem et al., 1982).

The presence of leaf, stem and ear diseases in most maize production areas of Cameroon were reported by Ngoko, (1994) and Cardwell et al. (1997). Leaf and stem diseases reduce production through lowering photosynthesis, reducing nutrient transport and facilitating lodging. Ear rots in the field and/or in the store affect grain quantity and quality. Since the devaluation of the CFA (Comminute Financier African) currency, maize production has increased markedly and most farmers are involved in livestock production, especially poultry and swine, which demands large amounts of maize for feed. Agricultural production techniques, however, have not kept pace with the demand for maize which is harvested manually, dried, shelled and stored using traditional methods. In a farmer survey, McHugh (1994) found that about 40 % of the farmers sort the grains before selling and /or consumption. Therefore, in about 60% of the cases good and bad grain are mixed at shelling and storing. In most cases bad grain will end up in local breweries or animal feeds but during hardship conditions it is consumed directly as human food.

Maize grain is usually contaminated with fungi such as *Fusarium* spp, *Aspergillus* spp., *Penicillium* spp., *Acremonium* spp. and *Diplodia* spp. (Marasas, 1995). The contamination of maize by these fungi and their toxic metabolites has been associated with several human and animal diseases including liver and oesophageal cancer in many parts of the world, particularly in Africa (Adhikari et al., 1994; Allen et al., 1992; Cova et al., 1990; Gelderblom et al., 1988; 1994; Marasas, 1995; Marasas et al., 1988; Rheeder et al., 1992; Shephard et al. 1992; 1995; Thiel et al., 1991; Yang, 1980). No information on aflatoxin and fumonisin related cancers in humans is presently available in Cameroon.

The objectives of this study were to identify the fungi associated with maize kernels in two agroecogical zones of Cameroon, and to detect the associated mycotoxins.

MATERIALS AND METHODS

Sample collection

Two surveys of maize storage were carried out in 1996 and 1997 in the Humid Forest (HF) and Western Highlands (WHL) zones of the Republic of Cameroon. Twelve farms were selected in each of three villages of each zone.

In the WHL the villages were Bamunka, Bali and Njinikom. Bamunka is located at 1,100 m above sea level (a.s.l.) in the Ndop valley and is surrounded by mountains with peaks at 2,000 m a.s.l. The rainfall distribution is bimodal and ranges from 1,000 mm to 1,500 mm per annum with temperatures ranging from 18^0 C to 35^0 C. Bali (1000 m a.sl.) receives 1500 mm to 2000 mm rainfall a year, and has two cropping seasons. It is located on the Guemba plateau. Njinikom, one of the most important maize production and consumption areas in the province, is situated at about 1,500 m a.s.l. The rainfall is also bimodal and the temperature varies between 18^0 C to 30^0 C. In each village, two sets of 1 kg samples were collected from each of the 12 farmers per village. The first set was collected two months after harvest and the second set was collected two months later from the same stores.

The villages in the HF areas were Ngat, Nkometou, and Etoud. They have approximately the same climatic pattern and Nkometou has a forest/savanna mosaic vegetation. The rainfall varies between 1,200 mm to 2,000 mm per year, distributed between two seasons, i. e. March to June and September to November. The maximum temperature is about 32^0 C. In this zone, one set of samples (1kg) of maize was collected from each of 12 farmers per village two months after harvest.

An equal quantity of improved maize variety was given to farmers in exchange for samples received from them. The cobs of all samples were shelled and grains were divided into two equal sub-sets. The first sub-set was kept as kernels in a freezer for mycological analysis, and the second sub-set was milled and stored in a freezer pending mycotoxin analyses.

Mycological analysis

Kernels were surface disinfested for one minute in 3.5 % NaOCl and rinsed twice in sterile water. In 1996, fifty kernels (5 kernels /plate, 90 mm) from each sample (=1800 kernels) were transferred to 1.5 % malt extract agar (MEA) containing 150 mg novobiocin/liter and plates were incubated at 25°C in the dark for 5 to 7 days at the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC) in South Africa. In 1997, after surface disinfesting, 100 kernels (5 kernels/plate, 90 mm) were plated twice per sample (=7200 kernels) on sterile filter paper and incubated at room temperature (24^0C) for 5 to 7 days at IRAD Bamenda, Bamenda, Cameroon. All the isolated fungi were recorded and their frequencies determined directly on the plates using a dissecting and /or compound microscope. *Fusarium* species were identified according to Nelson et al. (1983) and *Stenocarpella macrospora* (Earle) Sutton (= *Diplodia macrospora* Earle) according to Marasas & Van der Westhuizen (1979) and Sutton & Waterston (1966). Other fungi were identified to the level of genus on the basis of their cultural and morphological characteristics, i.e. *Acremonium* spp., *Aspergillus* spp., *Nigrospora* spp. and *Penicillium* spp. The fungi of primary interest were *Fusarium* spp. and *Aspergillus* spp. because of their known relationship with human and animal diseases.

Aflatoxin analysis

Aflatoxin analyses were performed by the method of Thomas et al. (1975) only on samples collected in 1997. A 50 g sub-sample was homogenized with 250 ml of methanol and water (60:40 vol./vol.) with a mechanical shaker for 30 min. The mixture was filtered and the filtrate was separated with a saturated solution of chloride and hexane (1:1); chloroform was used to perform a second separation and the solution drained in a flask containing 5 g of cupric carbonate. After a rapid shaking, the cupric carbonate was allowed to settle for about 5 min. The extract was filtered through a Whatman filter paper No. 42 with a bed of anhydrous sodium sulfate into a beaker. The chloroform was allowed to evaporate. The residue was dissolved in 1 ml of chloroform, transferred into a screw-cap vial, and kept in the refrigerator at 4^O C pending analysis. The analysis was done by thin layer chromatography (TLC). The level obtained was compared with a standard containing a mixture of aflatoxins B_1, B_2, and G_1:G_2 at 2.5, 0.75, 2.5:0.75 ppb. Three spots of 5, 10, and 15 μl, respectively, of the extract and of the standard were deposited at the same base of the precoated silica gel plates. Spots were placed 1 cm apart. The plates were introduced in a saturated solution of chloroform-acetone (94:6 vol/vol) solvent system for 20 to 30 min with the spotted side downwards in the solution for aflatoxin migration. After saturation, the plates were removed and allowed to dry. The plates were read under long wave (365 nm) UV light in a black cabinet. The intensity of fluorescence produced by each sample-spot was compared with that of the standard. The concentration of aflatoxin (ng/g) in a maize sample was calculated using the formula (S x Y x V)/(O x E), where S is the volume of the standard of equivalent intensity to E, Y is the concentration of the standard, and V is the volume of the solvent required to dilute the final extract, and O is the weight to the original sample, E is volume of sample extract.

Fumonisin, deoxynivalenol and zearalenone analyses

These analyses were carried out only on the samples collected in 1996 and 1997. The samples collected in 1996 were analyzed for fumonisin B_1 (FB_1), deoxynivalenol, (DON) and zearalenone (ZEA) at PROMEC in Cape Town, South Africa as described by Sydenham et al (1996). Samples collected in 1997 were analysed for FB_1 at the mycotoxin laboratory at the IITA Biological Control Center for Africa in Cotonou, Benin. Fumonisin, DON and ZEA concentrations were determined by polyclonalantibody (PAb)-Based competitive direct enzyme-linked immunosorbent assay (Agri-Screening kit catalogue no. 70/8830, Neogen Corp.; Lansing, MI) as described by the manufacturer. Aliquots of 50 g of corn were extracted with CH_3OH/H_2O (70:30). Aliquots of each extract were applied to individual coated microtiter wells which were then incubated for 10 minutes to allow any free toxin and the toxin-peroxydase conjugate to compete for binding to the available antibody sites. Reagents were washed from the wells with distilled water. Bound toxin-conjugate levels were measured colorimetrically, following the addition of an enzyme substrate, incubation for 10 min and a stop reagent was added. Fumonisin, DON, and ZEA concentrations were measured by recording optical density (OD) readings at 650 nm, using a Bio-Tek EL301 microwell strip reader. Sample toxin levels were compared to the standards received from Veratox, Neogen Corporation, Lansing, MI.

Data analysis

The statistical analyses were performed with the SAS package using the general linear model (GLM) on log (y+1) transformed data on village and zone. Mean comparisons (LSD) were made on fungal incidence and mycotoxins per zone. Pearson correlation analysis was conducted for relationships between fungal infection and levels of mycotoxin contamination in the samples.

RESULTS

Mycological analysis, 1996

Six fungal genera were isolated from maize kernels in both zones in 1996 (Table 1). Five genera, i.e. *Aspergillus*, *Fusarium*, (3 species), *Nigrospora*, *Penicillium* and *Stenocarpella* were identified and one unidentified fungus is still under investigation. *Nigrospora* spp. were the most prevalent fungi that occurred at high incidence in all villages, with the highest at Etoud (52 %) (data not shown). Of the mycotoxigenic fungi, *Fusarium moniliforme* Sheldon was the most commonly isolated with a maximum of 27.3% at Bamunka, 24.9% at Nkometou and the lowest incidence of 2.4% at Bambui. *Fusarium graminearum* Schwabe was mostly isolated at Bamunka (17.6%) and Njinikom (25%). *Fusarium subglutinans* (Wollenweber & Reinking) Nelson, Toussoun & Marasas was rare at all the villages. A high incidence *of Penicillium* spp. was recorded in Bamunka (15.6%). *Stenocarpella macrospora* and *Aspergillus flavus* Link: Fr. were rarely isolated. An unidentified fungus was frequently isolated at incidences from 2 % at Bamunka to 21 % at Bambui (data not shown). *Fusarium proliferatum* (Matsushima) Nirenberg was isolated at only one location (Bamunka, 4.66%) and the incidence was so low that it was not included in the analysis.

Analysis of variance showed significant ($p<0.05$) differences in incidence among the fungi within each zone (Table 1). The interaction between location and fungi was not significant ($p>0.05$).

Chemical analysis, 1996

No aflatoxin analyses were conducted because the incidence of *A. flavus* was low in all the samples from the HF and WHL (Table 1). Three *Fusarium* mycotoxins, FB_1, DON and ZEA were detected in the maize samples (Table 2). Fumonisin was the most prevalent toxin identified. Three samples (improved maize varieties) were fumonisin-free, meaning that 83.33 % of the samples analyzed were contaminated. The highest levels of contamination were in Ngat (26,000 ng/g) and Nkometou (11,600 ng/g). Deoxynivalenol was detected in 77.7 % (14 out of 18) of the samples. The highest levels were detected in Bambui (600 ng/g) and Bamunka (1,300 ng/g). Zearalenone was detected in 77.7 % (14 out of 18) of the samples with the highest concentrations recorded in Bamunka (1,100 ng/g) and Bambui (220 ng/g). All samples that were free of DON were also free of ZEA. In some cases these three toxins were found to co-occur in the same maize samples (data not shown).

Fusarium graminearum was significantly related to DON concentration ($r=0.8$; $p=0.001$), but not at all to ZEA (Table 3). No functional relationship was found between the incidence of *F. moniliforme* and the fumonisin concentration ($r=0.8$; $p=0.77$) (Table 4).

Mycological analysis, 1997

In 1997, the most important fungal genera found in maize samples collected from farmers' stores in the WHL were *Fusarium, Aspergillus and Penicillium* (Table 5). *Penicillium* spp were the most frequently isolated at all villages for both sampling periods, i. e. after 2 and 4 months in storage. *Nigrospora* spp. and *Acremonium* spp. were isolated at very low incidence. Although the incidence of *Fusarium* spp. remained below 5% two months after harvest, it decreased significantly in Bamunka and increased significantly in Njinikom after 4 months.

Chemical analysis, 1997

In all villages, aflatoxin was detected in very few samples collected two and four months after harvest (data not shown). In Njinikom, 1.3% of the samples were contaminated with aflatoxin B_1. Four months after harvest the level was 31.3 ppb, up from 16.7 ppb two months earlier (data not shown). Aflatoxin G_1 was detected at trace amounts in 16.6 % of the samples analyzed four months after harvest. Uncharacterized fluorescing compounds were found in about 58.3 % of the samples (data not shown).

In Bali, FB_1 levels varied from 306.3 ng/g to 1195.7 ng/g in 100% of maize samples collected two months after storage (Table 6). Four months after harvest, FB_1 was detected in 25% of the samples with a mean FB_1 concentration in positive samples of 1,742.5 ng/g, up from 594.5 ng/g two months earlier, i. e. a mean FB_1 increase of 70%. In Bamunka, FB_1 was detected in 66.6 % and 100 % of the samples collected two and four months after harvest, respectively. The mean FB_1 concentration increased from 2,717.7 ng/g two months after harvest to 5,794.1 ng/g two months later, corresponding to a 50% increase (Table 6). In Njinikom, FB_1 was detected in all the samples collected two months after harvest with levels ranging from 99.8 to 4,719.2 ng/g. In the samples collected four months after harvest, FB_1 levels ranged from 405.0 to 4235.0 ng/g in 100% of the samples analyzed. The mean contamination levels four months after harvest increased from 1,193.2 ng/g after to months 1,607.9 ng/g, resulting in a 30% increase.

There was a significant difference between the storage period and the level of fumonisin B_1 in Bamunka ($p<0.05$), but the levels did not change significantly either in Bali, or Njinikom (Table 7).

DISCUSSION

The predominant fungi associated with maize from rural areas in the HF and WHL of Cameroon in 1996, were *Acremonium* spp, *F. graminearum, F. moniliforme*, *Nigrospora* spp. and *Penicillium* spp. Other fungi observed more rarely were *F. proliferatum*, *F. subglutinans,* and *Aspergillus flavus.* These findings are in accordance with worldwide reports on the presence of these fungi in most maize growing areas (Marasas, 1996). Infection of maize by *F. moniliforme* is less visible than by *F. graminearum,* which colors the kernels from brown to dark-red. Zummo & Scott (1990) and Thomas & Buddenhagen (1980) have shown that *F. moniliforme* can be isolated from apparently healthy maize grain. A survey on maize storage in Cameroon (Ngoko et al. unpublished data, Chapter 1) revealed that 10%, of the farmers consumed their moldy maize, and 20 % sold it on the market for various uses including human consumption.

High levels of FB_1, which can cause diseases in animals and humans, are reported throughout the world. Ross et al. (1993) reported field outbreaks of leukoencephalomalacia in horses associated with corn naturally contaminated with fumonisins. Motelin et al. (1994) reported that pulmonary edema syndrome and hydrothorax symptoms were associated with maize contaminated with 155 mg/kg of FB_1 fed to pigs. Rheeder et al. (1992) reported that, exposure to *F. moniliforme*-infected maize contaminated with FB_1 is associated with oesophageal cancer in the Transkei, South Africa. Gelderblom et al. (1988, 1991) demonstrated that FB_1 is hepatotoxic and hepatocarcinogenic in rats fed 50 mg/kg. Marasas (1997) considered risk assessment parameters for safe levels of FB_1 in foods and feeds. In South Africa, the mean FB_1 levels in maize varies from 0.3 mg/kg in commercial maize products to 54 mg/kg in moldy, home-grown maize. Therefore, Marasas (1997) suggested that levels of 100 to 200 μg/kg should be safe for human health. In addition to FB_1, DON and ZEA were also detected in Cameroonian maize . Beardall & Miller (1994) and Miller (1995) showed that DON was associated with several animal diseases. Prelusky et al. (1994) showed that ZEA, produced by *F. graminearum,* is associated mainly with swine diseases. The potential importance of these metabolites to human health should not be underestimated.

In the WHL, only a few samples had detectable levels of aflatoxins. This may be explained by the fact that the climate (generally cool) is not suitable for the development of *A. flavus* and *A. parasiticus* Speare and aflatoxin production. Udoh (1997) and Hell (1998) reported high contamination levels in warm areas in Nigeria and Benin. Since *F. moniliforme* and *A. flavus* were sometimes isolated from the same maize grain, it is possible that FB_1 and aflatoxin co-occur in Cameroon. Miller (1993) investigated the combined effects of the metabolites produced by these fungi and reported that *A. flavus* and *F. moniliforme* co-occurred on maize. The phenomena of antagonism between fungi and synergism between mycotoxins also need to be considered.

This study showed that several fungi infected maize produced in rural areas in Cameroon. *Fusarium moniliforme* was the most frequently isolated fungus whereas *A. flavus* was rarely isolated. *Nigrospora* spp., *Acremonium* spp. and *Penicillium* spp. had high incidence levels. Although these fungi were not the focal points of this study, their effects on maize quality should not be ignored. Chemical analyses showed that FB_1 was the most important contaminant of maize in both ecological zones. DON and ZEA were also detected in both zones. The levels of DON and ZEA found in this study were similar to those detected in corn in Italy (Logrieco et al., 1988). Bottalico et al. (1989) reported that maize isolates of *F. graminearum* produced only DON whereas Sydenham et al. (1991) found that maize isolates produced simultaneously the oestrogenic metabolite ZEA and the trichothecene DON. Aflatoxin was rarely detected, but it should not be concluded that maize produced in these zones of Cameroon is aflatoxin-free. The climatic conditions such as rainfall and temperature, and/or the drying and storing techniques may have suppressed the development of *Aspergillus spp.* and subsequent aflatoxin production.

In summary, FB_1, DON and ZEA occur in maize in Cameroon. Given the fact that most samples were collected from maize intended for human consumption, it might be assumed that the rural and urban population is exposed to FB_1 and other mycotoxins such as DON and ZEA. Considering the fact that *Fusarium* spp. are isolated from a wide range of commodities, these metabolites may also occur in other important food crops in Cameroon. Therefore *Fusarium* mycotoxins should be considered as important economic and public health concerns. Consequently, it is very important that farmers, feed manufacturers, agronomists, plant pathologists, social scientists, extension agents, biological scientists and health professionals in Cameroon should be informed about the risks of consuming moldy maize and maize products. Emphasis should be placed on designing strategies that will create awareness about the undesirable effects of contaminated grain in human and animal foods and feeds.

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Table 1. Mean incidence (%) of fungi associated with maize kernels from farmers' stores in the Humid Forest (HF) and Western Highlands (WHL) of Cameroon in1996

Fungi	HF	WHL
F. graminearum	27.2b	10.5d
F. moniliforme	22.1c	28.3b
F. subglutinans	9.3e	0.0g
A. flavus	4.7f	5.9e
Nigrospora spp.	32.1a	30.2a
Penicillium spp.	14.3d	18.8c
S. macrospora	8.4e	5.6e
Unidentified fungus	21.6c	1.2f

Means in columns followed by different letters differ significantly ($p<0.05$)
Means based on 7200 kernnels from each ecological zone.
F= Fusarium; A=Aspergillus;S=Stenocarpella

Table 2. Incidence of *Fusarium moniliforme* and *Fusarium graminearum* and mycotoxin contamination of maize samples in the Humid Forest and Western Highlands of Cameroon in 1996

Maize samples	Incidence of *Fusarium* spp (%)		Mycotoxin level (ng/g)		
	Fmon	Fgram	FB1	DON	ZEA
Humid Forest					
NGT3	6	2	1,900	100	<50
NGT7	4	nd	3,200	nd	nd
NGT8	12	nd	5.400	nd	nd
NGT10	14	4	26,000	200	<50
NKT11	82	nd	11.600	nd	50
NKT12	20	2	2.800	500	50
ETD5	20	6	3.800	100	50
ETD9	4	16	5.800	100	50
ETD11	30	nd	6.800	nd	nd
Western Highlands					
BL1	6	6	1.700	200	<50
BL2	nd	10	nd	100	<50
BL3	16	18	300	600	60
BL4	nd	22	nd	100	220
BL5	nd	2	1.900	100	<50
NJK6	10	16	600	300	50
BKA2	14	40	2.000	1.300	180
BKA3	48	18	1.100	600	1.100
BKA4	14	14	900	600	140

Fmon = *Fusarium moniliforme*
Fgram = *Fusarium graminearum*
BL = BALI; BKA = BAMUNKA; ETD = ETOUD; NJK = NJINIKOM NGT = NGAT; NKT = NKOMETOU
nd = not detected
Number following acronym is the farmer's identification number.

Table 3. Relationships between *Fusarium graminearum,* Deoxynivalenol (DON) and Zearaleone (ZEA) from selected maize samples from the Western Highlands in Cameroon in 1996 as determined by regression analysis

Variables estimate	Parameter	SE	T	Prob>TR^2	
Intercept	1.94	1.78	1.08	0.295	0.72
DON	0.02	0.01	5.96	0.001	
ZEA	0.01	0.01	0.05	0.957	

Table 4. Relationships between *Fusarium moniliforme* and fumonisin B_1 (FB_1) from selected maize samples from the Western Highlands in Cameroon in 1996 as detemined by regression analysis

Variables estimate	Parameter	SE	T	Prob>T	R^2
Intercept	11.98	3.49	3.43	0.003	
FB_1	0.01	0.01	0.29	0.775	0.72

Table 5. Mean incidence (%) of mycotoxigenic fungi associated with maize kernels from three villages of the Western Highlands of Cameroon in 1997 collected from farmers' stores two (sample A) and four (sample B) months after harvest

Mycotoxigenic fungi	Bali		Bamunka		Njinikom	
	Incidence (%)					
	A	B	A	B	A	B
Fusarium spp.	3.3a	3.2a	3.3a	2.8b	3.2a	7.0b
Aspergillus spp.	0.7a	0.0b	0.1a	1.2b	0.2a	3.3b
Penicillium spp.	6.5a	9.2b	3.8a	10.9b	2.8a	8.4b

A = samples collected two months after harvest
B = samples collected four months after harvest
Mean based on 1200 kernels at each sampling time from each village (100 kernels analyzed for each of 12 farmers)
Means in rows followed by different letters within villages are significantly different ($P<0.05$)

Table 6. Fumonisin contamination of maize samples from three villages of the Western Highlands of Cameroon in 1997 collected from farmers' stores two (sample A) and four (sample B) months after harvest

	Fumonisin B_1 level (ng/g)					
Maize	Bali		Bamunka		Njinikom	
Samples	A	B	A	B	A	B
1	1 195.7	1 985.7	4 269.6	10 453.5	1 222.8	1 978.9
2	306.2	-	2 472.2	623.5	799.3	2 714.2
3	1 005.1	-	4 762.9	2 748.9	365.6	564.8
4	407.6	1 437.1	1 860.9	3 694.3	728.6	497.1
5	506.8	804.7	2 528.8	6 271.7	99.8	1 202.7
6	316.1	0.0	0.0	3 799.6	2 427.4	-
7	312.4	0.0	0.0	3 603.7	1 104.2	2 573.1
8	395.8	0.0	0.0	1 114.5	611.8	1 363.7
9	385.9	0.0	8 292.1	15 134.4	1 207.8	3 337.5
10	393.9	0.0	6 926.3	8 295.3	802.7	4 234.9
11	985.8	0.0	0.0	1 472.4	229.7	422.8
12	725.9	0.0	1 499.7	12 316.8	4 719.2	404.9
Mean*	594.5	1 742.5	2 717.7	5 794.1	1 193.2	1 607.9

A = samples collected two months after harvest
B = samples collected four months after harvest
\- = stored maize consumed by farmers prior to the date of sample collection
* = Mean of positive samples

Table 7. Relationship between storage period and fumonisin B_1 contamination of maize samples collected from farmers' stores in the Western Highlands of Cameroon in 1997

Variable	Mean differences	Std error	t	Prob>t	R^2
Village	2048.11	376	16.29	0.001	0.39
Time (Village)	978	316	3.64	0.061	
Time(Bali)	225.80a	183	-1.23	0.243	
Time(BKA)	3083.14b	1052	2.93	0.013	
Time(NJK)	376.0	620	0.61	0.557	

BKA= Bamunka, NJK=Njinikom, Time= difference between 2 and 4 months in store.

Means followed by different letters are significantly different ($p<0.05$)

CHAPTER 5

BIOLOGICAL AND PHYSICAL CONSTRAINTS ON MAIZE GRAIN QUALITY IN THE HUMID FOREST AND WESTERN HIGHLANDS OF CAMEROON

Biological and physical constraints on maize grain quality in the Humid Forest and Western Highlands of Cameroon

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A survey was conducted in the Western Highlands (WHL) of the Republic of Cameroon to assess the biological and physical constraints on maize grain quality. Questionnaires were administered to 108 farmers in 1997. A backward regression analysis revealed that several agricultural practices were associated with the infection of maize grains by *Fusarium* species and subsequently contamination with fumonisin. Harvesting maize in June (11.1%), sorting right from the field (16.7%), drying maize over the fireplace with husk (19.4) or without husk (33.3%) or in the cribs, were factors that significantly reduced the infection by *F. moniliforme* and the risk of contamination of fumonisin. Yellow maize was less contaminated with fumonisin compared to white maize. The storage weevil, *Sitophilus zeamais*, significantly increased the risk of contamination by fumonisins. Other factors such as harvesting in August, storing in bags, maize as previous crop, and the education level of the farmers were non-significant factors retained by the regression analysis.

Keywords: Cameroon, cultural practices, fumonisin, maize, *Sitophilus*

Maize (*Zea mays* L.), one the most important commodities produced in Cameroon, is cultivated on about 600,000 ha for a total annual grain production of 800,000 metric tons (Ayuk-Takem, 1996). Since the devaluation of the CFA (Communauté Financiere Africaine) currency, maize production has increased markedly but is still not keeping pace with the demand for maize. Production could be higher if factors such as soil infertility, poor managerial skills of farmers, high cost of inputs, environmental stresses, pests and diseases in the field and in storage were not disrupting the actual yield potential of the available local and improved varieties (Ayuk-Takem et al., 1982). Leaf, stem and ear diseases occur in all the production areas (Cardwell et al., 1997; Ngoko et al., Chapter 2). Leaf and stem diseases cause a decrease in the production by lowering photosynthetic activities, interrupting nutrient transport and causing lodging. Ear rots in the field and/or in the store affect grain quantity and quality especially in areas where maize is harvested under high rainfall. Although 40 % of the farmers sort out damaged grain before selling and /or consuming, bad grains in most cases end up in local breweries, feeds and, during hardship conditions, in food (McHugh, 1994).

The infection of grain by mycotoxin producing fungi is well established. Marasas et al. (1988) reported *Fusarium moniliforme* Sheldon as the predominant fungus contaminant of maize in South Africa. Booth (1971) noted that *F. moniliforme* was a common contaminant fungus of maize throughout the world. Logrieco et al. (1995) reported that *F. proliferatum* (Matsushima) Nirenberg (34 %) and *F. moniliforme* (54 %), were the predominant species in infected maize ears in Italy. *Fusarium moniliforme* is known to produce fumonisins, which are carcinogenic mycotoxins (Gelderblom et al., 1988; Marasas, 1996; Miller, 1996). The contamination of maize and maize products by mycotoxins has been associated with several human and animal diseases. It is well documented in the medical and veterinary literature that mycotoxin related diseases occur in many parts of the world, particularly in Africa (Adhikari et al., 1994; Allen et al., 1992; Cova et al., 1990; Gelderblom et al., 1988; Marasas, 1995, 1996, 1997; Marasas et al., 1988; Rheeder et al., 1992; Ross et al., 1993; Shephard et al., 1992, 1995; Sydenham et al., 1990; Thiel et al., 1991; Wild, 1993; Yang, 1980; Zarba et al., 1992) Investigations have been conducted to single out factors that enhance the infection of maize grain by *Apergillus flavus* Link: Fr. and production of aflatoxin in Nigeria (Udoh, 1997) and in Benin (Hell, 1998). Given the farming practices and the weather conditions under which maize is produced, harvested, dried and stored, it is likely that mycotoxin contamination occurs also in Cameroon.

The objective of this work was to identify traditional farming, harvesting, drying and storage practices related to fungal invasion and mycotoxin contamination of stored maize in the Western Highlands (WHL) of Cameroon.

MATERIALS AND METHODS

Sample collection

A questionnaire (Appendix 1) was administered to 108 farmers randomly selected in the WHL in 1997. This interview was conducted with the same farmers, simultaneously when maize samples were collected for mycological and chemical analyses (Chapter 4). "Pidgin English", a popular communication language of the area was used. The questionnaire was conducted in collaboration with an extension worker who usually served as facilitator at each location. The questions were related to maize production practices such as harvest techniques and time, drying, and storage methods. The survey was carried out only in the WHL because during a survey conducted in 1996, we noticed that storing maize was not a common practice in the Humid Forest because maize is usually consumed fresh.

The altitude in the WHL ranges between 800 m and 2500 m above sea level. Bamunka is located at 1100 m a. s. l. in the Ndop valley surrounded by mountains with peaks at 2600 m. The rainfall ranges from 1000 mm to 1500 mm per annum with temperatures from 18^0 C to 35^0 C. Bali, with two cropping seasons receives 1500 mm to 2000 mm rainfall a year and is located in the Guemba plateau with altitude approximately 1000 m and the temperature similar to that of the Ndop valley. Njinikom, one of the most important maize production and consumption areas in the province, is situated at 1500 m a. s. l. The rainfall is also bimodal and the temperature varies between 18^0C and 30^0C. The peak rainy season is in August-September which corresponds to the main harvesting period in the region.

Data analysis

The analysis of the questionnaire was done by computing the frequency of each variable using SAS for windows version 6.02. Correlation coefficients were computed to determine the relationships between the significant variables for samples collected in 1997 in the WHL. A stepwise backward linear regression was used to identify constraints that were significantly associated with grain maize quality deterioration and fumonisin contamination. Fumonisin concentration (Chapter 4) was used as the dependent variable, and the crop management practices as binomial independent variables.

RESULTS

Farming practices

Several agricultural practices associated with harvesting and post-harvest storage were recorded. Considering the harvesting practices in the WHL, 11.1 % of farmers declared that they harvested in June, 23.6 % in July, 63.9 % in August and a few harvested late planted maize in September (Table 1). Farmers did not take into consideration the weather conditions of the day of harvest. Some claimed (31.8%) that they harvested and transported their maize from the field to their houses on a wet day. About 3 % of the farmers reported that they stacked the cobs in the field, covered them with banana (*Musa accuminata* Colla) leaves or tarpaulin for a night pending transportation. Different harvesting procedures (with husk, stacked in the field and harvested on a wet day), were identified (Table 1). Most farmers (77.8 %) reported that they harvested with husk. In general, harvesting was done by hand as soon as maize got into the hard dough stage. Most farmers harvested some green ears for family consumption, especially from fields not very far from the house.

Sorting started in the field for 16.7 % of the farmers who said that they removed rotten cobs and ears with bad tip cover from the lot before drying. The rest did not sort at all either out of ignorance or because they thought it would reduce their crop. Bad maize was fed directly to animals (33.3%), mostly chickens and pigs, or sold in the local market (20%), some of which may be mixed with some good maize and used to make meals or snacks (10%); other farmers (36.6%) either threw it away or did not give a clear answer.

Four drying methods were identified in most locations (Table 1), i. e. cobs dried with husks in a "banda" (a sort of platform built with bamboo) or under the ceiling of the house (19.4%); cobs without husk over the fireplace (16.7%); 33.3 % used air- or sun drying either hung under the ceiling or in a "banda", with or without husk, and 18.1% used a crib. The drying period varied between one month (66%) and two months (23%). Concerning the cleaning of the drying and storing structure, 88.9 % reported that they usually clean the drying and storing structure every year before the new maize is brought in. Almost one half (48.6%) of the farmers said that they disinfected these structures using actellic powder (perimiphos-methyl), a common commercial insecticide sold in the region or with fresh leaves of a common local tree (*Cupressus* sp.). These fresh leaves were burnt about an hour before cobs were brought in for drying and/or storing.

Seven kinds of storage were identified in the WHL (Table 1). Most farmers (70.6%) stored their maize where it was dried with or without husk. Maize cobs harvested without husks are kept in a well constructed crib with bamboos or wire mesh and covered with a corrugated iron roof by 18.0% of the farmers. Less than a quarter (20%) of farmers reported that they use platforms constructed in around their houses for storing and 31.0% said that they stored in the banda in their kitchen. Different types of bags (9.7%) and boxes or drums (9.7%) were preferred by some farmers. Few farmers reported that they stored cobs on the bare ground for a short period. In the WHL, 79.2% said that they poured all the cobs on the bare ground before sorting, while 12.5 % had a concrete floor for the manipulation of cobs before drying and hand shelling. Compared to 16.7% of farmers who stored maize in houses with grass roofs, 80.6% had corrugated iron roofs that they claimed enhanced the drying of grains. About 50 % had white, 8 % yellow, and 41 % mixed color maize (data not shown).

Four categories of farmers were identified in the WHL (data not shown). Those who did not attend school (27.4 %), those who attended primary school (44.4%), those who attended secondary and high school (13.9%) and 11.1% university degree holders who either actually farmed or were landlords hiring laborers for their farming activities.

Analysis of factors affecting fumonisin contamination in WHL

The relationships between the potential biological and physical constraints and the fumonisin concentration in the maize samples are summarized in Table 2. Drying maize over the fireplace (p=0.001) or in a crib (p=0.006) significantly reduced fumonisin contamination. Insect attack, especially *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), significantly (p=0.0012) increased the contamination of maize grain. Sorting rotten cobs prior to leaving the field also reduced fumonisin contamination significantly (p=0.033). Harvesting in June was significantly (p=0.041) related to lower fumonisin contamination. Yellow maize was less contaminated with fumonisin than white maize, but only marginally significantly

($p=0.052$). Fumonisin levels were numerically reduced in maize handled by women, but not statistically significantly ($p>0.05$). There was no significant difference between storing in boxes and storing in bags. Maize as previous crop had no significant effects on the fumonisin contamination of the samples analyzed. Maize handled by non-educated persons was not significantly different from maize collected from educated farmers. When field production factors (soil texture, soil fertility, weeds) were regressed against the maize grain quality, no significant relationship was found between them.

In summary, several groups of potential factors affecting the contamination of maize by fumonisin were identified in the WHL in 1997 and some recommendations are made about these in Table 3.

DISCUSSION

The results of the present study have clearly shown that it is very important for maize farmers in Cameroon to harvest maize on sunny days and to dry the maize immediately after harvest in order to reduce fumonisin contamination. Drying with or without husk over the fireplace or in cribs was associated with reduced fumonisin contamination. Hell (1998) noted that aflatoxin levels increased throughout storage in poorly dried maize stacks. Harvesting of maize during wet days increased the labor, the grain moisture content and its susceptibility to infection by fungi and subsequent mycotoxin contamination. The use of cribs should be improved in areas with altitudes of 1600m and higher as drying of grain is linked to the size of the crib and the circulation of air between the cobs.

This work also demonstrated that the storage weevil *S. zeamais* significantly increased fumonisin contamination of maize. The role of insects in the dispersal of fungi is well established. Setamou et al. (1998) found that damage by the cob-borer *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) was positively related to aflatoxin contamination. Flett & Van Rensburg (1992) found that *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and physical damage increased the incidence of *F. moniliforme*. Consequently, the control of *S. zeamais* and *B. fusca* should reduce fumonisin contamination and lead to grain quality improvement.

Sorting bad cobs before drying reduced fumonisin contamination. Borgemeister et al. (1994), Udoh (1997) and Hell (1998) found that sorting bad grain reduced losses due to insects and aflatoxin contamination after harvest. The contamination of a maize stack by molds is related to the dispersal capacity of the fungi within the lot. So once the moldy cobs are removed the chances of preserving good grain quality improve. Sorting should therefore be considered as a continuous process in grain management from harvest to final utilization. Given the fact that moldy grain is an important source of mycotoxin contamination, the extension services should emphasize the sorting of bad cobs prior to leaving the field and continuous sorting before consumption.

In this study *A. flavus* was isolated only rarely and aflatoxin B_1 was detected in very few samples. Other fluorescing compounds detected may have been breakdown products of aflatoxin. Although aflatoxins (B_1 & G_1) were detected in only about 8% of the samples (Chapter 4), it should not be considered as a guarantee against *A. flavus* and *A. parasiticus* Speare invasion. Weather conditions prevailing in the WHL in 1997 may not have favored the development of *Aspergillus spp. a*nd subsequent aflatoxin contamination.

The infection of grain by fungi and subsequent mycotoxin contamination depends on weather conditions as well as pre- and post-harvest agricultural practices. Harvesting in June and July resulted in a decrease of the fumonisin concentration in maize in 1997 compared to harvesting in August. The temperature in the WHL in June and July varied between 20 and 25^0C and the relative humidity was approximately 70%; but harvesting at this period meant planting early in March which depended on rainfall and farmers' priorities. Harvesting in August was detrimental to maize grain quality in 1997. This corresponded to the peak of the rainy season with rains falling almost every day. This pattern needs to be followed up in on-station research to determine the time favorable for the development of *Fusarium* species. This knowledge will serve as baseline information to design a new cropping calendar for farmers.

Fumonisin contamination was lower in yellow maize compared to white maize. These data are similar to those found by Rheeder et al. (1992). Given the fact that white maize is preferred by 51% of the population of the WHL (McHugh, 1994), emphasis should be placed on breeding white maize for resistance to *F. moniliforme* ear rot.

No significant relationship was found between storage methods and fumonisin contamination. The negative relationship identified between storing in boxes and fumonisin contamination was not significant. Conversely storing in bags was positively related to fumonisin contamination, but not significantly. Boxes are made up of wood and the chances of damage from rodents, insects and household tools are reduced. This may explain why better quality maize grain is found in this type of structures. Hell (1998) and Udoh (1997) investigated maize storage structures and aflatoxin contamination in Benin and Nigeria and found that storing in bags reduced the infestation of maize grain by *Aspergillus flavus* and subsequent aflatoxin contamination. The conditions conducive to the development of *F. moniliforme* and *A. flavus* could explain this apparent contradiction. Although the control of maize grain by chemical or local insecticides was not retained by the regression analysis in this study, Awuah (1996) and Cardwell & Dongo (1994) suggested some natural products to control insects and storage fungi which could improve the quality of the maize grain stored in bags or boxes. Care should, however, be taken in using natural insecticides. Hell (1998) reported that the use of *Khaya senegalensis* as a stored grain protectant against insects, increased the risk of aflatoxin contamination. This plant may have stimulated the breeding of insects, which enhanced the dispersal of aflatoxin-producing fungi in the stores. Therefore, the knowledge of the insecticidal and fungicidal properties of local plants should be taken into consideration during investigations.

A negative relationship between gender and fumonisin contamination of maize grain revealed that maize handled by women had lower fumonisin levels compared to maize handled by men. This may be explained by the fact that most women usually sort bad cobs and grain before drying, storing or cooking. More investigations need to be carried out on the role of women in determining maize grain quality.

Although emphasis was placed on the post-harvest practices, it must be understood that inappropriate agronomic techniques such as repeated maize cropping in the same field may lead to infection by mycotoxin producing fungi in the field. Maize as previous crop was retained by the regression analysis as a factor that contributes to fumonisin contamination. Therefore, continuous maize cropping in the same field should be discouraged and rotation with legumes should be encouraged..

The analysis of factors contributing to fumonisin contamination showed that the level of education was not significantly related to fumonisin contamination. Similarly Jaminson & Lau (1982) and Mook (1981) found that levels of education were not necessarily an improvement over zero education in predicting technology adoption by small scale farmers in the developing world. In contrast, Carlson & Mueller (1987) reported that there is a strong positive effect of formal education of the farmer on his or her perception of the cost-benefit. Although this study did not reveal any role of education of farmers in reducing fumonisin contamination, these results may be misleading because some university graduates hired other people to farm for them. Consequently, their skills and knowledge may not be used in actual farming.

Post-harvest factors such as period of harvest, methods of drying and storing techniques had significant effects on the contamination of maize grain by fumonisin. While on-station research should be carried out to identify the combination of factors that will reduce the con+tamination of maize by mycotoxins, education campaigns should be initiated to create awareness about the relative importance of the post-harvest practices of handling maize grains in order to minimize the risk of fungal infection and subsequent mycotoxin contamination in rural areas.

RECOMMENDATIONS

- **To the Institute of Agricultural Research for Development (IRAD)**

  Efforts should be aimed at reinforcing the maize improvement programmes which need to breed for resistance to the major maize diseases and pests in Cameroon in collaboration with other national and international research and teaching institutes.

  An illustrated manual of maize diseases and insects should be prepared to assist farmers in their identification.

- **To the extension services and farmers**

  Use improved maize seeds tolerant to the major diseases of your region
  Plant maize in a well balanced fertilized soil under reduced stress
  Harvest on sunny days at physiological maturity in June or July
  Avoid stacking maize overnight in the fields after harvest
  Avoid spreading maize cobs without husks on the bare ground
  Sort bad cobs before drying and continue to sort during drying
  Dry maize as soon as possible after harvest
  Shell cobs as soon as the grain is dry
  Treat maize grain with insecticides against insect pests
  Store maize in a dry environment with good air circulation
  Always sort bad grain before consumption

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Table 1. Farmers' harvesting, drying and storing practices in the Western Highlands of Cameroon

Practices	Respondents (%) n=108
Period of harvest	
June	11.1
July	23.6
August	63.9
September	1.4
Cleaning of the drying/storing place	88.9
Harvesting	
With husk[a]	77.8
Stack[b]	2.8
Wet[c]	31.8
Sorting	
Yes	16.7
No or uncertain	83.5
Drying	
Banda with fire	19.4
Hanging with fire	16.7
Sun/air drying	33.3
Crib	18.1
Storing	
Drying places	70.6
Crib	18.0
Platforms	20.0
Bandas	31.0
Bags (assorted types)	9.7
Boxes or drums	9.7
Bare ground for (short time)	1.5
Other	8.5
1 month	66
2 months	23
Use of bad maize	
Animal feeds	33.3
Sales	20.0
Consumption	10.0
Other	36.6

[a]With husk = maize harvested with husk
[b]Stack = cobs were stacked in the fields for at least a day pending transportation
[c]Wet = maize was harvested and/or transported on a wet day

Table 2. Significant crop management factors affecting fumonisin contamination of maize in the Western Highlands of Cameroon in 1997 as calculated by a stepwise backward regression analysis

Variable	Parameter estimates	t	P>t	R^2	F
				0.75	0.005
Intercept	145.0	1.25	0.001		
Drying (over fire)	-170.5	-3.54	0.001		
Drying (crib)	-264.5	2.31	0.006		
Sitophilus zeamais	81.9	0.66	0.012		
Sorting (rotten cobs)	-672.5	-0.81	0.033		
Harvest (June)	-130.4	-0.44	0.041		
Yellow maize	-81.9	-0.45	0.052		
Harvest (August)	249.8	0.31	0.149		
Storing (boxes)	-470.1	-1.33	0.192		
Storing (bags)	938.7	-1.14	0.259		
Gender (women)	-329.5	0.10	0.359		
Previous crop (maize)	56.2	0.61	0.530		
Education level	23.2	0.80	0.701		

Word(s) in parentheses refers to the attribute that has an effect on reducing or increasing the risk of contamination.

Table 3. Summary of potential factors affecting fumonisin contamination of maize from rural areas of the Western Highlands of Cameroon in 1997

Factors	Effects on contamination by fumonisins	Recommendation
Harvest time	Reduces	Harvest at physiological maturity, preferably in June
Harvesting period	Reduces	Harvest on sunny days. Discourage harvest and transportation on wet days
Drying methods	Reduce	Dry immediately after harvest over the fireplace or any other source of heat
Sorting time	Reduces	Sorting immediately after harvest, before drying and storing. Continuous sorting before consuming
Storing techniques	Reduce	Shell, treat and store grain in bags/boxes in appropriate rooms or treat cobs and store in cribs
Weevil outbreaks	Increase	Treat maize cobs or grain with appropriate insects protectants to reduce fumonisin contamination
Previous crop	May Increase	Fumonisin levels tended to increase where maize was produced on a continuous basis. Rotation with legumes should be encouraged
Yellow maize	Reduces	Encourage the production of yellow maize
Gender	May Reduce	Women tended to handle maize better than men (sorting)

SUMMARY

Maize (*Zea mays* L.), the staple food crop of the majority of the population of Cameroon, is damaged by insects and diseases from the fields to the stores. As a result, the quantity and the quality of harvested grain is reduced. This study was undertaken to identify constraints associated with the production and post-harvest losses of this commodity in two ecological zones of Cameroon from 1995 to 1997.

Farmers' perceptions of diseases and pests play an important role in their acceptance of new pest management technologies. From the survey conducted to assess their perceptions, farmers reported that borers (*Busseola fusca*) were the main constraint to maize production in the Humid Forest and Western Highlands. Locusts (*Zonecerus variegatus*) and rodents were the second most important limiting factor in the Humid Forest. The storage weevil (*Sitophilus zeamais*) was the most damaging storage insect. Diseases were not generally known by farmers who could only recognize smuts and ear rots by the visible damage caused by them. While the period of the outbreaks of insect infestation was not reported with precision, most farmers reported that diseases occurred at the mid-season. Control practices were not well established.

Disease surveys conducted from 1995 to 1997, revealed that lowland blight (*Bipolaris maydis*, Diplodia leaf spot (*Stenocarpella macrospora*) and sheath blight (*Rhizoctonia solani*) were the most important maize diseases in the Humid Forest, while highland blight (*Exserohilum turcicum*) and grey leaf spot (*Cercospora zeae-maydis*) prevailed in the Western Highlands. Phaeosphearia leaf spot (*Phaeosphaeria maydis*) was specific to the Western Highlands with a negative relationship with grey leaf spot. *Busseola fusca* infested maize plants at all stages of growth with high prevalence in the Humid Forest.

The identification of factors affecting maize yield demonstrated that diseases, insects and their interactions with soil infertility, soil texture, weeds, and maize varieties were responsible for the reduction of maize production. Yield reductions were 30% and 33.6% respectively, in the Humid Forest in 1995 and 1996 due to *Stenocarpella macrospora*, *Puccinia polysora* and *Rhizoctonia solani*. In the Western Highlands, *Cercospora zeae-maydis*, *Busseola fusca*, stem diseases, and physiological spot caused yield reductions of 51.2%, and 37.9% in 1996 and 1997, respectively.

Mycological and chemical analyses of maize grain collected from 72 farmers' stores showed that several pathogens were associated with grain quality deterioration. *Nigrospora* spp. were the most frequently isolated fungi on kernels, followed by *Fusarium moniliforme* and *Fusarium graminearum*. *Aspergillus* spp. were rare in both zones. Fumonisin B_1, deoxynivalenol and zearalenone were detected in maize samples at levels ranging from 300 to 26,000ng/g, 100 to 1300 ng/g, and 50 to 110 ng/g, respectively. This is the first report on the natural occurrence of these *Fusarium* mycotoxins in maize in Cameroon.

Surveys conducted to identify the biological and physical factors that enhanced the infection of maize kernels by fungi and the contamination with fumonisin , identified several agricultural techniques related to grain quality in the Western Highlands. Harvesting in June (11.1%) or July (23.6%), sorting right from the field (16.7%), drying over the fireplace with husk (19.4) or without husk (33.3%) and storing shelled grain in bags (19.4%) or boxes (9.7%) reduced fumonisin contamination. Continuous production of maize on the same field, harvesting in August, and the infestation by the weevil *Sitophilus zeamais* were factors that increased fumonisin contamination. Crop rotation, sorting maize during all the post-harvest processes and the treatment of maize grain with appropriate insecticides should decrease the risk of contamination by fumonisin.

Continuing collaborative research should aim at understanding farmers' needs and priorities, investigating the epidemiology of maize diseases, screening for resistance to the most important maize diseases and improving harvesting, sorting, drying and storing methods in Cameroon.

OPSOMMING

Mielies (*Zea mays* L.), die stapelvoedsel van die meerderheid van die bevolking van Kameroen, word beskadig deur insekte en siektes, vanaf die land tot in opberging. Gevolglik word die hoeveelheid en die gehalte van die ge-oeste graan verlaag. Hierdie studie is onderneem om die beperkings op die produksie van mielies en na-oes verliese in twee ekologiese gebiede van Kameroen gedurende 1995 tot 1997, te identifiseer.

Boere se persepsies omtrent siektes en plae speel 'n belangrike rol in hulle aanvaarding van nuwe plaagbeheer metodes. In die opname om hulle persepsies te evalueer, het boere aangemeld dat boorders (*Busseola fusca*) die belangrikste beperking op die produksie van mielies in die "Humid Forest" en die "Western Highlands" was. Sprinkane (*Zonecerus variegatus*) en knaagdiere was die tweede belangrikste beperkende faktor in die "Humid Forest". Die kalander (*Sitophilus zeamais*) was die insek wat die meeste skade in opberging veroorsaak het. Siektes was nie algemeen bekend aan boere nie en hulle kon slegs brande en kopvrotte herken aan die sigbare skade wat hierdie siektes veroorsaak. Alhoewel die tydperk wanneer insekplae uitbreek nie noukeurig gerapporteer kon word nie, het die meeste boere aangedui dat siektes in die middel van die seisoen voorkom. Beheermaatre4ls was nie goed gevestig nie.

Siekte opnames wat vanaf 1995 tot 1997 uitgevoer is, het aangedui dat laeveld skroei (*Bipolaris maydis*), Diplodia blaarvlek (*Stenocarpella macrospora*) en blaarskede skroei (*Rhizoctonia solani*), die belangrikste mieliesiektes in die "Humid Forest" was, terwyl ho4veld skroei (*Exserohilum turcicum*) en grys blaarvlek (*Cercospora zeae-maydis*) oorheersend was in die "Western Highlands". Phaeosphaeria blaarvlek (*Phaeosphaeria maydis*) was beperk tot die "Western Highlands" en het 'n negatiewe verwantskap met grys blaarvlek vertoon. *Busseola fusca* het mielieplante in alle groeistadia aangeval met 'n ho4 voorkoms in die "Humid Forest".

Die identifikasie van faktore wat die opbrengs van mielies be■nvloed, het aangedui dat siektes, insekte en hulle interaksies met grondvrugbaarheid, grondtekstuur, onkruide en mielie vari4teite, verantwoordelik was vir die verlaging in opbrengs. Oesverliese in die "Humid Forest" was 30% en 33.6%, respektiewelik, in 1995 en 1996 as gevolg van

Stenocarpella macrospora, *Puccinia polysora* and *Rhizoctonia solani*. In die "Western Highlands" het *Cercospora zeae-maydis*, *Busseola fusca*, stamsiektes en fisiologiese vlek oesverliese van 51.2% en 37.9% in 1996 en 1997, respektiewelik, veroorsaak.

Mikologiese en chemiese analises van mielies wat versamel is van 72 boere se opbergingsplekke het aangetoon dat verskeie patogene betrokke was by die verlaging van die gehalte van die graan. *Nigrospora* spp. Was die mees dikwels geïsoleerde fungi vanaf mieliepitte gevolg deur *Fusarium moniliforme* en *Fusarium graminearum*. *Aspergillus* spp. was skaars in beide gebiede. Fumonisin B_1 , deoxynivalenol en zearalenone is vasgestel in mieliemonsters teen vlakke vanaf 300 tot 26000 ng/g, 100 tot 1300 ng/g, en 50 tot 110 ng/g, respektiewelik. Hierdie is die eerste aanmelding van die natuurlike voorkoms van hierdie *Fusarium* toksiene in mielies in Kameroen.

Opnames wat gedoen is om die biologiese en fisiese faktore te identifisser wat die infeksie van mieliepitte deur fungi en die kontaminasie met fumonisin bevorder, het verskeie landboukundige tegnieke geïdentifiseer wat die gehalte van graan in die "Western Highlands" beïnvloed. Oes in Junie (11.1%) of Julie (23.6%), sortering op die land (16.7%), droogmaak oor die vuurherd met die kopblare aan (19.4%) of sonder die blare (33.3%) en die opberging van gedorste graan in sakke (19.4%) of in kiste (9.7%), het fumonisin kontaminasie verlaag. Aanhoudende produksie van mielies op dieselfde land, oes in Augustus en besmetting met die kalander, *Sitophilus zeamais*, was faktore wat fumonisin kontaminasie verhoog het. Wisselbou, sortering van mielies gedurende al die na-oes prosedures en die behandeling van mielies met geskikte insekdoders behoort die risiko van fumonisin kontaminasie te verminder.

Voortgesette kollaboratiewe navorsing moet gerig wees daarop om boere se behoeftes en prioriteite te verstaan, om die epidemiologie van mieliesiektes te ondersoek, om te toets vir weerstandbiedendheid teen die belangrikste mieliesiektes, en om die metodes van oes, sortering en opberging van mielies in Kameroen te verbeter.

Appendix

Appendix 1. Questionnaire

Appendix 2. List of maize diseases, fungal pathogens and insect pests

Appendix 3. List of plants

Appendix 1

Questionnaire

QUESTIONNAIRE ON FARMERS' MAIZE POST HARVEST PRACTICES

Ecological zone...
village..
Farmer..
Gender..
Period of production...
Education No school( ) Primschool( ) Secschool( ) High school/Univ( )
Seeds used( ) local( ) improved( )
Were the seeds treated with a pesticide? yes ( ) no( )
If yes which one (s)..
Do you have a sample or a used container? yes( ) no( )
Did you harvest on a rainy day? yes( ) no( )
did you harvest with husks yes( ) no( )
Did carry your maize to the house the same that you harvested? yes( ) no ( )
Did stack maize in the field pending transportation? yes ( ) no( )
How long did maize stay in the fieldsa day two days( ) more than 2 days( )
Did you sort rot cobs from the field yes( ) no( )
Which kind of problems did you have in the field? ear rots ( )
insect damage ( )
rodents( )
birds( )
thieves( )
What was the color of your maize? White( ) Yellow( ) mixed color( )
Where did you dry your maize Banda( )hang( ) under the ceiling of the roof( ) in the field( ) crib( )
What is your source of heat Firewood( ) sun( ) air-dry( )
How do you store your maize where it was dried crib( ) bags( ) boxes( ) drums( )
Do you store your maize shelled or on the cobs? shelled( ) cobs( )
Do you sort again before storing? yes( ) no( )
What do you do with bad maize? Animal nutrition( ) sell( ) consume( ) thrown away( )
Do you treat your maize before storing? What products do you used
Actellic powder( ) Diesel( )vetivar grass( )Cypress( ) methylparaphen( )
What are your major problems in storage Insects( ) molds( ) lack of knowledge( ) Rodents( )

Appendix 2

List of maize diseases, fungal pathogens and insect pests

Common names	Fungal pathogen	Authorities
Diseases		
Brown spot	*Physoderma maydis*	Miyabe
Common smut	*Ustilago maydis*	DC
Diplodia leaf spot	*Stenocarpella macrospora*	(Earle) Sutton
	=Diplodia macrospora	Earle
Ear rots	*Fusarium* spp.	
	Fusarium moniliforme	Sheldon
	Fusarium graminearum	Schwabe
	Fusarium proliferatum	(Matsushima) Nirenberg
	Fusarium subglutinans	(Wollenw. & Reink.) Nelson, Toussoun & Marasas
	Stenocarpella spp.	
	Stenocarpella maydis	(Berk.) Sutton
	=Diplodia maydis	(Berk.) Sacc.
Storage molds	*Aspergillus flavus*	Link:Fr
	Aspergillus parasiticus	Speare
Grey leaf spot	*Cercospora zeae-maydis*	Tehon & Daniels
Head smut	*Sporisorium reilianum*	(Kühn) Langdon & Fullerton
	=Sphacelotheca reiliana	(Kühn) Clint
Helminthosprium blight	*Helminthosporium carbonum*	Ullstrupp
Highland blight	*Exserohilum turcicum*	(Passerini) Leonard & Suggs
Highland rust	*Puccinia sorghi*	Schw.
Lowland blight	*Bipolaris maydis*	(Nisikado & Miyake) Shoemaker
Lowland rust	*Puccinia polysora*	Underw.
Phaeosphearia leaf spot	*Phaeosphaeria maydis*	(P. Henn) Rane, Payak & Renfro
Sheath blight	*Rhizoctonia solani*	Kühn

Insects	**Scientific names**	**Authorities (Family)**
Grass hoppers	*Zonecerus variegatus*	Linne
Leaf hopper	*Cicadulina mbila*	Naude
Stem borer	*Busseola fusca*	Fuller (Lepidoptera:Noctuidae)
Stem borer	*Eldana saccharina*	Walker (Lepidoptera:Pyralidae)
Cob borer	*Mussidia nigrivenella*	Ragonot (Lepidoptera:Pyralidae)
Weevils	*Sitophilus zeamais*	Mostschulsky (Coleoptera:Curculionidae)

Appendix 3

List of plants

Common names	Scientific names	Authorities
Maize	*Zea mays*	L.
Soybean	*Glycine max*	(L). Merr.
Cowpea	*Vigna unguiculata*	(L).Walters
Beans	*Phaseolus vulgaris*	L.
Kezem	?	?
Cypress	*Cupressus* spp.	
Cassava	*Manihot esculenta*	Crantz
Groundnuts	*Arachis hypogaea*	L.
Melon	*Cucumis melo*	L.
Cocoyams	*Xanthosoma mafafa*	L.
Okra	*Hibiscus esculentus*	L.
Banana	*Musa acuminata*	Colla
Plantain	*Musa balbisiana*	Colla
Potatoes	*Solanum tuberosum*	L.
Cocoa	*Theobroma cacao*	L.
Coffee	*Coffea arabica*	L.
Spear grass	*Imperata cylindrica*	(L.)Beauv.
Bokassa grass	*Chromolina odorata*	(L.)Beauv.