MAIZE EAR ROT AND ASSOCIATED MYCOTOXINS IN WESTERN KENYA

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I certify that this work has not been accepted in substance for any degree, and is not concurrently submitted for any degree other than of Doctor of Philosophy (PhD) of the university of Greenwich. I also declare that this work is the result of my own investigations except where otherwise stated.

Dedicated to my family and parents

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Abstract

Maize was established as the most important crop and cob rot as the most important crop protection problem in Western Kenya. The mean percentage rotten maize grain resulting from cob rot was 19% based on the PRA survey in Kapsabet and Tongaren Divisions and on-farm evaluation of the Kenyan hybrids in Tongaren Division in 1998 and 1999 seasons.

The two important factors affecting the incidence of cob rot were the weather conditions at the period of maturation to harvest and stalk borer. Cob rot incidence was found to be strongly correlated with percentage borer damage ($r = 0.87$). *Fusarium moniliforme* was the most frequently isolated fungus in cobs that had stalk borer damage, occurring in 97% of the cobs that had rot and visible borer damage. *F. moniliforme* was found in 80%, *F. graminearum* in 56% and *S. maydis* in 49% of maize samples collected on the farms and markets in Western Kenya.

Deoxynivalenol, zearalenone, fumonisin and aflatoxin were detected in samples collected from the farm and markets in the region. T-2 toxin was absent in all samples collected from the region. The levels of deoxynivalenol and zearalenone detected in the samples ranged from 0-1100 ng/g and 0-550 ng/g respectively and were mainly detected from rotten maize. The presence of zearalenone and deoxynivalenol is reported for the first time in maize in Western Kenya. Fumonisin and aflatoxin levels ranged from 0-2348 ng/g and 0-10 ng/g respectively.

All the rotten maize harvested in the region was utilized as livestock feed and for brewing. Awareness of the potential risks associated with mycotoxins was low among the farmers and the extension workers.

The Kenyan hybrids H627 and H622 were susceptible to *Fusarium graminearum*. The varieties did not show any difference in reaction to *F. moniliforme* and *S. maydis*. This fact was thought to be due to high disease pressure in the field and the genetic background of the hybrids.

Abbreviations

AFB_2	Aflatoxin B_2
AOAC	Association of Official Analytical Chemistry
AR	Analytical reagent
Av	Average
CAN	Calcium ammonium nitrate
CW	Cold and wet
DAP	Di-ammonium phosphate
DON	Deoxynivalenol
FB_1	Fumonisin B_1
ELEM	Equineleucoencephalomalacia
ELISA	Enzyme linked immuno-assay
Ewt	Effective weight
GLS	Gray leaf spot
GPR	General purpose reagent
HH	Hot and humid
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography
IAC	Immuno assay chromatography
IARC	International agency for research on cancer
KARI	Kenya Agricultural Research Institute
LH2	Lower highland zone 2
MAP	Mono-ammonium phosphate
Masl	Metres above sea level
ng	Nanogram

NRI	Natural Resources Institute
OA	Ochratoxin A
PDA	Potato dextrose sugar
PRA	Participatory rural appraisal
ppb	Parts per billion
ppm	Parts per million
SPE	Solid phase extraction column
TLC	Thin layer chromatography
TSP	Tripple super phosphate
UM1	Upper midland zone 1
UM3	Upper midland zone 3
UM4	Upper midland zone 4
UV	Ultra violet
ZEN	Zearalenone
µg	Microgram
µl	Microlitre

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CHAPTER 1: INTRODUCTION

1.1 Maize production and loss to pests and diseases

Maize (*Zea mays L.*) is the most important cereal crop in Kenya that is used primarily for human consumption. Approximately 2.7 million tons of grain is produced annually on about 1.4 million hectares (Anon, 1992). Nationwide loss due to pests and diseases in maize is estimated to be between 20-40% (Anon, 1992). The major maize producing areas of Kenya are located in the Western Highlands of Kenya. In addition to the problem of too much and too little rain and declining soil fertility, production of maize is limited by various diseases and pests (Tables 1.1 and 1.2). Several diseases have been reported affecting maize. They include the leaf spots and other foliar diseases such as leaf blights, rusts, and brown spots; the systemic foliar disease, maize streak; and ear rot and stalk rots (Fajemisin 1985). In the Western Highlands the major diseases of significant importance are blights, ear rots, rust, smut and recently, gray leaf spot (GLS). Ear rot is an important disease problem affecting maize in this region. Yield loss of 13-70% has been reported (Anon, 1986). Ear rot is caused by a number of fungi often infecting the cob as a disease complex. In the Western highlands of Kenya the fungi associated with ear rots include, *Fusarium* species, *Stenocarpella* spp., *Penicillium* spp., *Aspergillus* spp. (Kedera *et al.*, 1992; Kedera, 1998; McDonald and Chapman,1997). Besides causing yield losses, these fungi can produce toxic secondary metabolites called mycotoxins which are associated with human and livestock diseases.

In the Western Highlands, maize is produced under a wide range of ecological conditions. The area basically can be divided into three zones: the highlands which have

high rainfall and altitude ranging from 1500-2300 masl; the mid altitude that has a moderate rainfall, with altitude ranging from 1000-1800 masl and the marginal areas with low rainfall, and altitude ranging from 1000-1800 masl.

Different maize varieties are recommended for each of these ecological zones (Table 1.3). The varieties recommended for the highlands are mainly the hybrid 600 series (Kenyan Agricultural Research Institute and Kenya Seed Company, Kenya), which include H614, H622, H625, H626, and H627 (Appendix 2). These hybrids have a maturity range of 6-8 months, with average yield potential of 7500- 9500 kg/hectare. In the medium altitudes the recommended varieties are H511, H512, and H 513, which have a maturity range of 4-5 months and average potential yield of 3600- 5000 kg/ hectare. In the marginal areas, the recommended varieties are the Katumani composite, DH1 and DH2. The maturity range for these hybrids is 3-4 months and they have average potential yield of 3600 kg/hectare. In the medium altitude and the marginal areas, in addition to these recommended varieties, farmers keep and grow their own local varieties.

1.2 Background to the study

In 1994, surveys were carried out by NRI scientists in Eastern and Southern Africa to ascertain the perceived importance of maize ear rots in these regions. The visits were made to maize researchers and smallholder farmers growing maize in Kenya, Tanzania, Malawi and South Africa. All the national research scientists felt that cob rots were an important constraint to maize production and were concerned about the risks to human and animals resulting from contamination with mycotoxins. With the exception of South

Africa, it was established that there is little information available on quantification of losses due to the ear rot complex or mycotoxins in smallholder farming system in Africa. The present study was developed to address this gap in our knowledge through a social economic and biological investigation of the factors associated with ear rot and mycotoxin contamination. The Western Highlands which form the main maize growing zone of Kenya is characterized by a bi-modal rainfall, with the second rains often coinciding with the maturity of maize crop. The result is often high cob rot incidence.

1.3 Objectives

1. Find out farmers perception and existing practices in relation to maize ear rot complex.
2. Identify the major factors associated with maize ear rot complex in the region
3. Identify major maize ear rot pathogens in Western Kenya.
4. Determine the major mycotoxins associated with maize ear rot in Western Kenya and whether they occur in levels that are a health risk to humans and livestock.
5. Evaluate the current germplasm available to farmers and how agronomic practices might influence maize ear rot incidences

Table 1.1. Most important diseases and the recommended control strategy

Disease	Symptoms	Control
Turcicum leaf blight	Leaves are characterized by spindle shaped gray to tan lesions usually 5-10 cm and 1.3 cm wide. Lesions coalesce to cause all or sections of the leaf to die.	Early planting
Fusarium ear rots	Growth of faint pink-pinkish red mould on silk, kernels and cobs. Cobs may be soft and spongy, tips of ear rotted with reddish brown moulds.	Timely harvest in cases of prolonged rains. Planting varieties with well covered husks (closed tips) and that mature in a downward position
Diplodia ear rots	Light weight discoloured kernels, may observe white mycelium on cob and sheath glued to the cob, husks may dry when rest of the plant is still green.	Proper handling of the grain at harvest
Penicillium rots	Bleached ears Characterized by a powdery brown green or blue green mould on or between kernels	Quick drying of grain to moisture content of 13-15% level before storage
Common leaf rust	Oval to elongate reddish brown pustules on both leaf surfaces, later turn black (Teliospores) and break through epidermis.	Use of resistant and tolerant varieties destruction of alternate hosts
Polysora rust	Cinnamon - brown pustules on the upper surface of leaves, chlorotic and brown leaves (later).	
Head smut	Smutted ears, coarse sori, on eruption reveal dry spores.	Use of certified seeds, rotation (2-3 years), rogue smutted plants and burn before sori erupts, not to feed animals on infected material since spores are re-cycled through manure.
Common smut	White galls on the stalks, leaves, ears, tassels Stunted plants.	
Diplodia stalk rots	Wilting, lodging, stalks are greyish brown in colour when split.	Roguing infected plants
Erwinia rots	Wilting, lodging, stalks are watery (ooze), an ordour when split.	
Gray leaf spot	Pin point lesions surrounded with a halo, later turn gray to tan, rectangular, (2-5 cm long and 0.5 cm wide) running parallel to the veins. Heavy disease, pressure may coalesce and blight entire leaf .	There are no confirmed commercial resistant varieties in Kenya. S. African varieties PAN 6549 & SR 52 are resistant

Source: Based on unpublished recommendations for maize production in western highlands of Kenya National Agricultural Research Centre- Kitale and Regional Research Centre- Kakamega, Kenya.

Table 1.2. Important insect pests and their control strategy

Insect pest	Symptoms or signs or damage	Control	Time of control
Stalk borers	Parallel windowing on the leaves. Dead hearts Tattered leaves and lodging in severe attack	Destruction of alternative hosts i.e. Napier to reduce initial infestation, Early planting, disposal of crop residues-incorporating of stovers the soil by deep ploughing, burning of stover, feeding animals and Chemical control using - Dipterex 4 kg/ha or -Ambush 1G .	-March-April -when slight damage is observed
Chafer grubs	Wilting (dead) heart, presence of adult beetles and larvae in the soil	Use of chemicals-Karate 5% EC, Furadan 5G, Ambush 25%WP. At manufacturers recommended rates, seed dressing with Furadan5G at manufacturers recommended rates	At planting
Maize weevil		Early harvesting, Observe general cleanliness a around the stores (destroy crop residue around the store), Avoid mixing new and old maize stocks in storage, Adequately dry before storage, Chemical treatment of maize kernels with actellic/malathion 2% at manufacturers rates before storage.	At storage

Source: Based on un published recommendations for maize production in western highlands of Kenya National Agricultural Research Centre- Kitale and Regional Research Centre- Kakamega, Kenya.

Table 1.3. Maize growing zones and the recommended varieties

Ecological zones /Districts	Recommended variety	Maturity in months[1]	Yield potential (Bags/Ha)
Highlands with high rainfall	H627	6-8	98
Altitude: 1500-2300 masl	H626	6-8	94
AEZ's: UH, LH, and UM 4, UM I, LH 1-2.	H625	6-8	79
Districts: Uasin Gishu, Trans Nzoia, West Pokot, Keiyo Marakwet, Laikipia, Nakuru, Nandi, Bungoma	H614	6-8	84
Medium altitude with moderate rainfall	H513	4-5	54
Altitude: 1000-1800 masl	H512	4-5	44
AEZ's: UM 1-5, LM 1-2	H511	4-5	40
Districts: West Pokot Keiyo, Marakweti, Nakuru, Kakamega, Bungoma, Nandi,			
Dry land areas, marginal areas with low rainfall	Katumani comp.	3-4	30
Altitude: 1000-1800 masl.	DH1	3-4	40
AEZ'S: LM 3-5	DH2	3-4	40
Districts: West Pokot, Keiyo, Marakwet			

Source: Based on unpublished recommendations for maize production in western highlands of Kenya National Agricultural Research Centre- Kitale and Regional Research Centre- Kakamega, Kenya.

1. Months from planting to physiological maturity

CHAPTER 2: LITERATURE REVIEW

2.1 Distribution and importance of maize ear rots

Ear rots are widely distributed, being found wherever maize is grown. Heavy infestations of the ear rot fungi result in significant yield losses and in addition, these fungi can produce a range of toxins which have been linked with a number of mycotoxicoses and carcinomas of humans and livestock (Julian *et al.*, 1995). *Fusarium moniliforme* occurs worldwide on a great variety of plant hosts and is one of the most prevalent fungi associated with maize (*Zea maydis* L.) in most maize producing areas of the world (Booth, 1971). *Fusarium graminearum* has also a world wide distribution as a soil inhabitant and a serious pathogen that causes root, foot, crown, stem and ear rot and headblight of cereals (Booth, 1997; C.M.I Description of pathogenic Fungi and Bacteria No.22). Various reports exist on the distribution of ear rots in association with specific fungi and the mycotoxins produced. Ear rots caused by *Fusarium moniliforme* and the toxin Fumonisin B_1 have been reported in many countries including the USA, Argentina, Brazil, Egypt, South Africa , China (Coker, 1997) and in Kenya (Kedera, 1992). *Fusarium graminearum* rots and the mycotoxins deoxynivalenol have been reported on maize in Canada, USA, Australia, France, Hungary, Argentina, South Africa, Zambia and Chin. *Stenocarpella maydis* can cause extensive spoilage of maize cobs during wet periods in the late growing periods in South Africa. Toxigenic effects have been identified in S. Africa, USA, and S. America (Coker, 1997)

2.2 Common fungi associated with maize ear rots

Some of the most common fungi that cause ear rot include *Fusarium* spp., such as

Fusarium moniliforme Sheldon, *Fusarium graminearum* Schwabe, and *Stenocarpella maydis* (Berk) Sutton. *Fusarium moniliforme* is associated with hot humid or dry weather, while *Fusarium graminearum* is associated with cool humid environments (Martin and Gilman, 1976). The other fungi causing ear rot of maize are *Nigrospora oryzae* which cause Nigrospora ear rot and *Macrophomina phaseoli* that cause charcoal ear rot. The other secondary organism are *Aspergillus niger* that cause black mould, *Aspergillus flavus* that causes Aspergillus ear rot and *Penicillium* spp. that cause *Penicillium* ear rot. Table 2.1 shows the fungi and type of ear rots caused by the fungi.

Table 2.1. Common fungi associated with maize ear rot

Fungi	Disease	Symptoms	Source
Fusarium moniliforme Sheldon (=*Gibberella fujikuroi* (Sawada)Wollenw)	Fusarium ear rots	Pinkish, red-brown or gray if badly rotten	De Leon, 1984
Fusarium graminearum Schwabe (=*Gibberella zeae* (Schwein.) Perch).	Fusarium ear rots	Reddish-pinkish starting at the tip the cobs	Heseltine, 1977; Koehler, 1942; Sutton, 1982
Stenocarpella maydis (Berk) Sutton (= *Diplodia maydis* (Berk) Sacc).	Diplodia ear rots	Light- weight, discoloured kernels	De Leon, 1984
Stenocarpella macrospora	Diplodia ear rots	light- weight, discoloured kernels	De Leon, 1984
Aspergillus niger	Aspergillus rot	Black mould	Samson and Hoekstra, 1996
Aspergillus flavus	Aspergillus rot	Green mould	Samson and Hoekstra, 1996

2.2.1 *Fusarium moniliforme* Sheldon

Fusarium moniliforme is placed in the section Liseola of the genus *Fusarium*. A section separates species in a large genus into smaller units with similar characteristics (Nelson, 1992). The morphology of the macroconidia is the primary characteristic for defining most species of *Fusarium* (Toussoun and Nelson, 1975).

Species in the section Liseola do not form chlamydospores and both microconidia and macroconidia are endoconidia formed inside the hyphae or conidiophores (Hawksworth, 1983). The conidia form on monophialides or polyphialides. A monophialide is a cell that develops one open end while a polyphialide is a cell that develops two or more open ends from which basipetal succession of conidia develops. *Fusarium moniliforme* consists of at least seven reproductively distinct mating populations designated as A through G (Leslie, 1995). Populations A, D, and E are the most common in maize. Most of the mating populations correspond to particular anomorph species in the Liseola section of *Fusarium*. Both the A and the F mating populations belong to *Fusarium moniliforme* on the basis of morphology (Leslie *et al.*, 1990; Munkvold and Desjardins, 1997). Many of the strains in the A mating population appear frequently on maize and are prolific producers of the mycotoxin, fumonisin. Members of the F mating population produce little or no fumonisin and frequently occur on sorghum (Munkvold and Desjardins, 1997; Leslie *et al.*, 1992). Population D corresponds to *Fusarium proliferatum* strains andt produce copious amounts of fumonisins. Members of population B and E that correspond to *Fusarium subglutinans* strains produce little or no fumonisin (Leslie *et al.*, 1992). Other species of section Liseola that produce fumonisin, include *F. anthphilum, F. napiforme* and *F. nygamai* (Munkvold and Desjardins, 1997). The macroconidia of *Fusarium moniliforme* are sickle-shaped to straight with the dorsal and ventral surfaces almost parallel. The macroconidia are thin-walled with a distinct basal or foot cell and an apical cell that is often elongate. Microconidia are primarily single celled and ovoid to obovoid in shape with a truncate base. Microconidia are borne in long chains and false heads and those borne in chains have

a truncate base. A false head consists of a droplet of moisture at the tip of the microconidiophore (Nelson, 1992). These hold the microcondia in place as they are produced. The microconidiophores are long un-branched and branched monophialides. *Fusarium proliferatum* has the same characteristics as *F. moniliforme* except the microconidia are borne in short chains and false heads on both monophialides and polyphialides (Nelson, 1992). *Fusarium proliferatum* is often misidentified as *F. moniliforme*. It is nearly as common on maize as *F. moniliforme* in temperate regions and can be isolated from symptomatic and asymptomatic tissues, including seed (Munkvold and Desjardins, 1997). *Fusarium subglutinans* differs from *F. moniliforme* in that the microconidia are borne in false heads only on microconidiophores that are both monophialides and polyphialides. The microconidia are fusiform to ovoid to obovoid. Besides this, *Fusarium subglutinans* often produces 0-3 septate elongate, fusiform to spindle shaped microconidia, often referred to as mesoconidia (Pascoe, 1990). *Fusarium anthophilum* differs from *F. moniliforme* in that the microconidia are borne in false heads only and on both monophialides and polyphialides. Microconidia are primarily single celled and are fusiform to ovoid or obovoid in shape or pyriform to napiforme to round with a small basal papilla (Nelson, 1992).

Fusarium moniliforme survives in crop residues but is not usually among the common Fusaria (Leslie *et al.*, 1990). It does not produce chlamydospores but can produce thickened hyphae that apparently prolong its survival (Kommedahl and Windels, 1981). It is seed-borne and seed-transmitted. This factor associates the fungus with seedling blight, however the role of seed transmission in stalk and kernel rot is not established. Strains of this fungus have been traced throughout the plant in

some cases (Kedera *et al.*, 1992; Munkvold, 1992). It has also been reported that seed can be infected with this fungus but with no detrimental effect on the seedling (Kommedahl and Windels, 1981). *F. moniliforme* produces abundant microconidia that are air-borne in maize fields (Kommedahl and Windels, 1981; Nelson, 1992). Rain and wind also serve as a means of spreading inoculum that may be sourced from crop debris and corn in cribs. *Fusarium moniliforme* has been reported to enter more ears than any other fungus, even when ears are covered by husks and free from bird or insect damage (Koehler, 1942). Rot is usually confined to the tip of the cob, or in places damaged by corn borer, or when ears are wet (Warren, 1977). *F. moniliforme* is associated with disease at all stages of development, infecting the root, stalk and kernels (Munkvold and Desjardins, 1997). Symptomless infection of the fungus can also occur throughout the plant. Seed transmitted strains of the fungus can develop systemically to infect the kernels (Munkvold *et al.*, 1997a; Kedera *et al.*, 1992).

2.2.2 *Fusarium graminearum* Schwabe

Fusarium graminearum ear rot is characterized by growth of pinkish mould on the silk, kernels, cobs and husks and the cobs become soft and spongy. Inoculum dispersal is by wind, rain, insect and birds. Entry into the maize ear can occur through wounds or mycelium growing down the silk and cob from germinating spore on the silk (Heseltine and Bothast, 1977; Koehler, 1942; Sutton, 1982). *Fusarium graminearum* produces numerous mycotoxins but the most important economically are deoxynivalenol, nivalenol and zearalenone. *F. graminearum* belongs to the section Discholor of the genus *Fusarium*. Other members in this group include *Fusarium sambicinum*, *F. culmorum*, and *F. sulphureum*. The teleomorph of *Fusarium graminearum* is *Gibberella zeae* (Schwabe) Perch. It is primarily a

pathogen of graminaceous plants. In maize it causes cob rot and stalk rots. Two populations of *F. graminearum* have been described and termed as group 1 and 2. Isolates from group 2 form perithecia readily in nature and in culture on carnation leaf agar, while isolates of group 1 do not form perithecia readily in culture except in compatible crosses. Group 1 isolates cause crown rot of wheat, whereas group 2 isolates are responsible for head scab of wheat and also attack corn cobs. Eugenio *et al.*, 1970 observed the association between production of perithecia and the mycotoxin zearalenone. He suggested that high levels of zearalenone production inhibit perithecia production. The toxin is thought to regulate sexual reproduction in *F. graminearum* (Inaba and Mirocha, 1979). The difference in perithecial production between the two populations of *F. graminearum* is believed to be due to the inherent difference in zearalenone production ability (Marasas *et al.*, 1984). Based on the production of different trichothecenes, *Fusarium graminearum* is divided into two chemotypes: chemotype I, comprises of deoxynivalenol producers while chemotype II are the nivalenol producers (Miller *et al.*, 1991; Mirocha *et al.*, 1989). A distinctive feature of *F. graminearum* is its growth on Potato dextrose agar (PDA), which is usually highly coloured with dense to floccose greyish rose to golden brown mycelium and a dark ruby reverse. Macroconidia are relatively straight and thick-walled, with a foot-shaped basal cell. Members of this species do not produce microconidia.

2.2.3 *Stenocarpella maydis* (Berk) Sutton

Stenocarpella maydis (Berk) Sutton (= *Diplodia maydis* (Berk) Sacc. occurs in nature as an important pathogen of maize. It affects maize kernels resulting in light-weight, discoloured kernels. White mycelia appear on the cob and the sheath may

become glued to the cob. The fungus causes seedling blight, stalk rot and ear rot of maize wherever maize crop is grown intensively. The ear can sometimes be covered completely by mycelia. Bleached ears develop on the husks, until all the husks are dry, even if the rest of the plant is still green (De Leon, 1984).

Stenocarpella maydis ear rot usually progresses upward from the base of the ear and is visible as a conspicuous, coarse, white to greyish-brown mycelial growth over the husks and the kernels. The husks are often glued to the kernels by the white mycelium. Infected kernels have a lusterless appearance and a dull grey to light brown colour. Black fruiting bodies (pycnidia) are normally produced on the invaded husks and kernels as well the rotted stalks late in the season.

Besides *Stenocarpella maydis,* two other species in the genus *Diplodia* cause ear rot of maize. These species are *Diplodia macrospora* Earle and *Diplodia frumenti* Ellis and Everhart. The symptoms of ear rot caused by *D. macrospora* are very similar to those caused by *D. maydis*. The cultural characteristics of the fungi are virtually identical and both are known to produce scolecospores (Hope, 1943). The main distinguishing feature between *Diplodia maydis* and *Diplodia macrospora* is the size of conidia. Conidia from *D. macrospora* are two to three times larger than those of *D. maydis* (Larsh, 1938). On agar media *D. macrospora* grows slower than *D. maydis* and pycnidia are formed less rapidly and abundantly (Johann, 1935a). *Diplodia maydis* also grows readily on synthetic media whereas *D. macrospora* requires abiotin like-growth factor (Wyllie and Morehouse, 1977)

2.2.4 *Aspergillus* spp.

The genus *Aspergillus* is characterized by conidial heads that consists of asexually produced spores (conidia). Conidia are borne in chains from cells (phialides or

sterigmata) developing from an inflated apex or stalk (conidiophore), which arises from a foot cell. In some species the apex of the conidiophore, called the vesicle, is enlarged into a club-like shape. The cells on the vesicle from which the conidia are borne may be arranged in one or two rows commonly referred to as uniseriate and biseriate respectively. Some of the members of this genus include *Aspergillus flavus, A. parasiticus*, *A. niger*, *A. ochraeceous*. *Aspergillus flavus* and *A. parasiticus* are prevalent on crops grown in warmer climates especially groundnut and maize. Although *Aspergillus* ssp. causes post harvest disease, pre-harvest invasion has been reported on cotton seed, groundnut and maize (Wyllie and Morehouse, 1977). Colonies of *A. flavus* are green/yellow to yellow/green in colour and may remain green on Czapek's agar. *Aspergillus parasiticus* colonies on Czapek's agar tend to be dark green and remain green in age. Isolates from these two fungi can produce aflatoxin.

2.3 Mode of infection of ear rot fungi

Colonization of the kernel surfaces by *Fusarium moniliforme* has been shown to occur early in the season with little internal infection until kernel moisture is lower than 34%. *Fusarium moniliforme* is known to infect maize kernels through silk and through kernel damage caused by insects (Headrick and Pataky, 1991; Nelson, 1992). The relative importance of silk infection, insect assisted infection and systemic infection is influenced by the availability of inoculum for each type of infection. Silk infection is the most important pathway for *F. moniliforme* to reach the maize kernels. Other pathways to kernel infection such as seed transmission and systemic infection are less effective (Munkvold *et al.*, 1997a). The movement of *Fusarium moniliforme* systemically from seed to kernel has been demonstrated (*Kedera et al.*,

1992). However, the relative importance of seed transmission to kernel infection is not established. This fungus is known to survive in crop residues but is a minor component of the fungi surviving in crop residue. The spores of this fungus are usually abundant in maize fields during the reproductive growth stages (Leslie *et al.*, 1992; Ooka and Kommedahl, 1977).

Aspergillus flavus and *Fusarium moniliforme* have been described as weak pathogens with similar mode of entry into the corn ears (Marson and Payne, 1984). *Fusarium graminearum* enters the ear via silk and silk channels with infection spreading down the ear from the tip. Insects and birds can be vectors of this pathogen, and wounds created by feeding may pre-dispose the ear to fungal invasion (Hesseltine and Bothast, 1977; Koehler, 1942).

Infection of maize kernels, by *Stenocarpella maydis* (Berk) Sacc also occurs late in the development of the kernels. Entrance of *S. maydis* has been observed to be in the pedicel and penetration through the hilar region. Although the closing layer of the hilum is impermeable to the fungus, the fungus enters the kernel before the closing layer is formed (Johann *et al.*,1935b). It has been observed that lines of maize susceptible to *S. maydis* have delayed or ineffective closure of the hilar orifice (Johann *et al.*,1935b). In Diplodia ear rot, the main pathway of infection of the kernels is through colonization of the fungus from the shank up into the ears (Dorrance *et al.*, 1998). Loss is due to reduced seed weight and seed viability (Dorrance *et al.*, 1998). For the colonization of the shank and stalk tissue, germinating spores penetrate the epidermal cell walls and the host cytoplasm by the formation of an appressorium and enzymatic degradation (Bensch *et al.*, 1992).

2.4 Factors favouring ear rots and mycotoxin contamination

Factors favouring ear rots and mycotoxin contamination are specific to the ear rot pathogen and the type of mycotoxin produced. Environmental factors in the field such as temperature , moisture availability and a susceptible host may influence the level of ear rot in the field. However the conditions that allow for optimum growth of the pathogen particularly temperature may not be the optimum conditions for the production of a particular toxin (Lacey, 1989). Although *F. graminearum* is widely distributed , it is favoured by cool conditions. Similarly the toxins produced by this fungus, such as deoxynivalenol and zearalenone, are more associated with temperate climate or in the highland areas of developing countries which have a cooler climate (IARC, 1993a). *Fusarium moniliforme* is favoured by warm dry conditions. Fumonisin production is also associated by warm dry conditions while zearalenone production is associated with cooler temperatures (Martin and Gilman, 1976). Drought stress with accompanying high temperatures are the factors most frequently reported to increase the likelihood of out breaks of pre-harvest aflatoxin contamination. The influence of temperature, moisture and time on fungal growth and mycotoxin production has been studied under controlled environments. Ochratoxin A can be produced at both low and high temperatures, at low temperatures it is produced by *Penicillium viridicatum* and at high temperatures by *Aspergillus ochraceous*. Changing temperature may change the relative amount or proportions of different mycotoxin produced by a fungus. Under artificial conditions, maximum zearalenone production occurs at 12- 18^{0}C. Canadian isolates of *F. graminearum* have been found to produce mainly zearalenone at 19.5 ^{0}C, and deoxynivalenol at 28^{0}C and both toxins equally at 25^{0}C (Greenhalgh *et al.*, 1983).

The time between harvesting, shelling and drying and the length of storage coupled by environmental factors are important in production of mycotoxin. The environmental factors such as moisture, and temperature will influence fungal growth, sporulation and mycotoxin production. Moisture is a very important parameter in fungal growth and mycotoxin production. Manipulating water availability is a widely used method for the control of fungal colonization and mycotoxin production. In store, regulation of the moisture content of grain is essential to control fungal growth. Also in the growing crop in the field, in addition to its effect on crop growth, water affects susceptibility to fungal invasion and mycotoxin production and the accumulation of leaf litter (Lacey, 1989). Irrigation can be used to combat the increased susceptibility of maize and groundnuts to *Aspergillus flavus* infection and aflatoxin production that results from drought stress. However, it has been found to enhance *Fusarium* spp. especially *Fusarium graminearum* on ears of irrigated fields compared to infection where there was no irrigation (Payne *et al.*, 1986). The infection was associated with irrigation during anthesis, when susceptibility was high, with further colonization exacerbated by lodging and wet conditions close to harvest.

The water availability of stored grain is usually controlled by harvesting as dry as possible, then drying the crop with or without heat before storage. Fungi differ in their tolerance to water availability, the minimum (a_w) for most species colonizing cereal grains is about 0.70. For short term storage, grain should be stored at 0.72 and for longer term at 0.65. The recommended moisture content for short and long term storage is 14.8% and 13.7% respectively (Table 2.3). However on the market seeds are traded at higher a_w (Lacey, 1989; Magan *et al.*, 1984). Table 2.2 shows the

minimum water requirement for the growth and mycotoxin production of various fungi.

The gaseous environment has a great influence on metabolic activity of fungi because toxigenic fungi are aerobic and elevation of CO_2 decreases toxin production (Landers *et al.*, 1967). Reduced levels of O_2 and increased levels of CO_2 have been shown to reduce mould growth and proportionally decrease in toxin production at optimum and sub-optimum temperature (Wilson and Jay, 1979). However excessive aeration of stored grain compared to non-aerated grains has been shown to decrease aflatoxin formation in stored grain (Hesseltine, 1966).

It has been shown that aflatoxin production decreases with increasing pH and degradation of this mycotoxin is higher at pH 5-6.5 than pH 7.0 (Cotty, 1988).

Composition of the substrate will influence the level of mycotoxin production. Substrates with high carbohydrate and lipid composition favour aflatoxin production as in the case of peanuts, wheat, rice and maize (Hesseltine *et al.*, 1966). Foods with high protein content and low carbohydrate like cheese do not favour aflatoxin production (Bullerman, 1981). Some crops such as soy beans have been reported not to favour the production of aflatoxin (Gupta and Venkitasubramian, 1975) or zearalenone production (Eugenio *et al.*, 1970).

Interaction of micro-organisms on the kernels may favour or inhibit one another. Their interaction may be competitive or synergistic on the grain during harvesting, transportation and storage. A notable example of competitive establishment is the inhibition of production of aflatoxin by *A. flavus* due to competition by *Aspergillus niger* (Wyllie and Morehouse, 1977).

Insect damage to developing ears can significantly increase aflatoxin levels in pre-

harvest ears damaged by the corn ear worm (*Helicoverpa zea*), fall army worm (*Spodoptera frugipenda*), European corn borer (*Ostrinia nubilalis*) and maize weevils. The insects increase *Aspergillus flavus* contamination by feeding on and damaging developing kernels and transporting *A. flavus* conidia into the ear. A synergistic relationship has been demonstrated between *A. flavus* and the Southern West corn borer of maize (*Diatraea grandiosella*) in the United States of America. *A. flavus* resistant hybrids were found not to be effective in limiting aflatoxin contamination in the presence of *A. flavus* and the southern west corn borer (Windham *et al.*, 1999). In South Africa, it was shown that *Busseola fusca* or physical damage increased the incidence of kernel infection by *Stenocarpella maydis* (Flett and van Rensburg, 1992). Insect feeding can enhance *A. flavus* infection in maize ears. Insects can invade grain too dry for fungal growth causing heating and releasing water through their metabolic activity. As a result, conditions become more favourable for fungal growth. Insects can disseminate spores on mouth parts and hairs. Beetles may carry *A. flavus* and *Fusarium* spores to maize ears in the field and grain borers may disseminate *A. flavus* and *Penicillium* spores in stored grains (Lacey, 1988).

Table 2.2. Minimum moisture content for fungal growth and mycotoxin production

Species	Mycotoxin	Minimum a_w	
		For growth	For mycotoxin production
Alternaria alternata	Alternariol	0.88	0.9
Fusarium culmorum	Zearalenone	0.90	
Aspergillus flavus	Aflatoxin	0.80	0.83
A. ochraeceus	Ochratoxin A	0.79	0.87
	Penicillic acid		0.87
Penicillium aurantiogriseum	Ochratoxin A	0.80	0.89
	Penicillic acid		0.97
P. viridicatum	Ochratoxin A	0.82	0.86

a_w = water activity

Magan and Lacey, 1984; Northolt and Bullerman, 1982)

Table 2.3. Water content for storage of different seeds without visible moulding for short (3 months) and (long 2-3 year) periods at 15-29 ^{0}C

Grain type	Water content (%) for safe storage	
	short periods ($0.72a_w$)	long periods ($0.65a_w$)
Wheat	15.7	14.6
Maize	14.8	13.7
Barley	14.8	13.6
Oats	14.5	13.4
Soybeans	13.3	11.4

(Lacey, 1988)

2.5 Strategies to minimize ear rots

Ear rots occur in the field, but some ear rot causing fungi continue to cause rotting in the stores depending on the conditions of storage. Some storage fungi such as *Aspergillus flavus* (Wyllie and Morehouse, 1977) may also start in the field depending on the prevailing field conditions. The level of ear rots in the field and store depends on the predisposing factors. Ear rot development in maize fields depends on a number of factors, among them being, physiological resistance of the host, husk protection, declination of the ears, atmospheric moisture, soil fertility conditions, presence of the spores of the pathogen and, in lodged corn, broad contact of ears with the ground (Koehler, 1953). Conditions favourable to the development of one kind of ear rot may not be favourable to the development of others. Strategies to control ear rots, depend on the identification of these factors. Some of these strategies to reduce yield losses due to ear rots may also contribute to reduction of mycotoxin contamination both in the field and storage. Strategies to control ear rots involve those factors contributing to resistance of the host plant and cultural management of the crop both in the field and at the post harvest handling stage.

2.5.1 Cultural methods

Management of the ear rots involves all stages of maize production in the field and post harvest stages. The main factor that determines ear rot infestation in the field is the availability of the ear rot pathogen. The principal source of inoculum for ear rot-causing fungi is host debris such as old corn stocks, ears and stubble left on top of the soil (Trenholm, *et al.*, 1989). Management strategies that involve the removal of the crop debris will lower the inoculum levels. To reduce the inoculum it is important to plough the crop debris into the soil as soon as possible after harvesting (Martin and Johnson, 1982; Teich and Hamilton, 1985). If trash is left on the field after harvesting, mould infection can be carried over to the next year. Surface stubble supports fungal overwintering and production of pycnidia more readily than does buried stubble. Removal of surface stubble by conventional mouldboard ploughing tends to reduce inoculum particularly of *Stenocarpella maydis* (Flett *et al.*, 1998). Byrnes and Carol (1986), indicated that Fusarium ear rot or stalk rot is either decreased or is not affected by conventional tillage. Similar reports by Flett and Wehner (1991), also indicated that Fusarium ear rot was not affected by tillage practice. Survival of *Fusarium moniliforme* has been reported to be poorer on surface corn stubble compared to stubble buried to 30 cm (Nyvall and Kommedahl, 1970). However Skogland and Brown (1988) working on the same fungus, found equal numbers in both buried and surface stubble. They suggested that *Fusarium graminearum* will survive in buried host tissue provided that the tissue integrity is maintained.

Effective land preparation which allows for early planting and effective weed control has been found to assist in control of Fusarium diseases in corn (Seaman, 1982).

Weed control is considered important in the elimination of wild hosts of Fusarium (Martin and Johnson, 1982). In maize, small scale farmers practice several types of land preparation ranging from conventional ploughing and removal of crop residues to different types of minimum tillage or no tillage (Mora and Moreno, 1984). These different practices may have varying effects on the incidence of ear rot. Both incidence and severity of *Diplodia* on the leaves have been found to be influenced by soil management treatments and cropping sequence (Mora and Moreno, 1984). Burial of infected stubble by ploughing has also been found to reduce inoculum and furthermore render it less readily dispersible by wind and rain (Flett *et al.*, 1992). Continuous cropping of maize under zero tillage systems has been found to increase the incidence of ear rots in the field. Zero tillage practice is not common in maize-producing areas of Kenya. Zero tillage is associated with retention of soil moisture, maintenance of constant soil temperature and pH, improved soil structure, and low costs of production. Different maize production and harvesting practices have an effect on the incidence of ear rot. Positive correlation has been observed between cob and stalk rot severity and conservation tillage (Flett and Wehner, 1989; Flett *et al.*, 1992). Saprophytic studies of *Fusarium moniliforme* show that the fungus is not a soil inhabitant (Nyvall and Komedahl, 1968). When host tissue decays the fungus disappears and is only found on tissue resistant to decay (Nyvall, 1970). This work suggests that Fusarium inoculum can be reduced considerably if the crop residue is allowed to decompose before growing the next crop. Investigations have been carried out (Flett *et al.*, 1998) to determine the efficacy of periodic ploughing in reducing maize ear rot caused by *Stenocarpella maydis, Fusarium moniliforme* and *Fusarium graminearum* in reduced-tillage fields. This work was carried out for a

period of over 3 seasons during 1993-96 at Bloekomspruit in South Africa. The studies revealed a positive linear correlation between *Stenocarpella* ear rot incidence and surface stubble mass. Mouldboard ploughed plots had lower stubble mass and *Stenocarpella* ear rot incidence than did reduced tillage practices. A cross-mouldboard plough applied after 1, 2 and 3 seasons of reduced tillage, reduced stubble mass and *Stenocarpella* ear rot incidence in the respective seasons only. *Stenocarpella* incidence increased in the subsequent season in which the original tillage practices were again applied. Alternating practices therefore did not reduce *Stenocarpella* ear rot in the long term. It was therefore concluded that reduced disease incidence can only be achieved by moulboard ploughing during each season. Alternating tillage practices had no effect on ear rots caused by *Fusarium* spp. during all seasons and the conclusion from these findings were that, for long term control of *Stenocarpella* ear rot, mouldboard ploughing seems to be the only viable option (Flett *et al.*, 1998). This work supports the earlier reports by Ullstrup (1964), that infected maize stubble is the major source of *Stenocarpella maydis* inoculum.

Crop sequence and seedbed preparation have been identified as important management practices influencing disease development in maize (Mora and Moreno, 1984). Repeated growing of corn, wheat or other small cereal grains on the same or nearby fields were associated with increases in the inoculum for ear rot causing fungi and population of insects that attack corn plants and spread mould inoculum (Trenholm *et al.*, 1989). To reduce this problem, it was recommended that the cycle be broken periodically to control insect and fungal infestations (Trenholm *et al.*, 1989). Rotations that include crops other than corn or cereals susceptible to *Fusarium* infection have been suggested as a strategy to minimize ear rot and insect

infestations in the field (Martin and Johnson, 1982).

Insects attack has been shown to pre-dispose maize to *A. flavus* and subsequent aflatoxin contamination. In the USA, varieties of maize resistant to *A. flavus* have been shown to become susceptible following damage by the southern west corn borer (Windham *et al.*, 1999). The insects increase *Aspergillus flavus* contamination by feeding on and damaging developing kernels and transport *A. flavus* conidia into the ear. A similar positive correlation has been demonstrated between high populations of western flower thrips (*Frankliniella occidentalis*) and incidence of *Fusarium moniliforme* at the silking stage in USA (Farrar and Davis, 1991). Application of insecticide to control flower thrips resulted in lower incidence of ear rot.

Birds also shred the husks, puncture and ingest the contents of kernels. This damage can facilitate insect attack and mould invasion (Sutton, 1982). In Canada, it has been found that planting infected grain of corn and wheat may give rise to diseased seedlings. High quality and fungicide-treated seed reduced the seed-borne inoculum (Trenholm, *et al.*, 1989). *Fusarium moniliforme* is seed-borne and seed-transmitted and has been found to colonize maize kernels without visible symptoms, although the mechanism for transmission from seed to kernels is not yet clear.

In Kenya, maize is often left in the field well beyond physiological maturity before harvest. When harvest is delayed, maize kernels may be subjected to infection by fungi. (Ochor, *et al., 1987).* Delayed harvest may exacerbate the problem of ear rot (Kedera, *et al.*, 1994). Conversely, harvesting at physiological maturity when moisture content is high increases the risk of mould contamination during post harvest handling (Nagler, 1987).

Lodged stalks with cobs touching the ground provide an opportunity for ear damage

(Martin and Johnson, 1982). This suggests those management strategies that will reduce lodging will lower the incidence of maize ear rots.

Further and additional mould contamination can occur during post harvest and storage. Some field fungi can continue to cause further deterioration in transit or in store when the right conditions occur. This together with storage fungi may inflict serious losses on the kernels. The rate at which maize deteriorates in storage is influenced by a number of factors, of which the most important are moisture content, temperature, the fungi involved, length of storage, rate at which the fungi grow, condition of the grain, location and severity of injuries in the pericarp of the maize kernels (Subhi and Christensen, 1960). Regulation of these factors such as reducing the moisture content quickly to levels below 13%, removal of broken grains and foreign materials and control of insects have been suggested (Lacey, 1989). Moulds have been reported to continue growing on freshly harvested maize unless it is quickly dried to moisture concentrations of 13-15% or below. (Trenholm *et al.*, 1989). In USA, Sauer *et al.*, (1984) reported that the most common *Aspergillus* species on stored maize was *Aspergillus flavus*, occasionally other species of *Aspergillus* were found. *Fusarium moniliforme* was the most common species in corn samples. Other genera identified included *Cephalosporium, Penicillium, Cladosporium, Trichoderma, Nigrospora, Alternaria, Helminthosporium, Mucor, Rhizopus, Chaetomium, Diplodia, Trichothecium,* and *Syncephalastrum.* Although both field and storage fungi are found in the stored grain, freshly harvested grain is relatively low in storage fungi. Field fungi are most abundant in new crop grain (Sauer *et al.*, 1984). A positive relationship has been established between moisture content and percentage of kernels with storage fungi and geographical difference

observed in storage mould levels. Samples higher in storage mould invasion were also higher than average in insect invasion (Sauer *et al.*, 1984). Quick drying of the kernels can therefore arrest the continuation of growth of field fungi and storage fungi and minimize the losses associated with deterioration in transit or store.

2.5.2 Resistance

Development of resistant hybrids is considered to be a viable option to reduce ear rot diseases in corn (Chungu, 1996). The use of these resistant varieties to control the disease is likely to be more effective than the use of cultural measures or chemical treatments, which do not provide adequate control (Barnes and Carroll, 1986). Evaluation of maize germplasm for ear rot resistance has been done in many parts of the world, showing genotypic effects between cultivars. Information on time and method of inoculation, have been developed (Reid, 1992; Chungu, 1996; Shelby *et al.*, 1994; Hart *et al.*, 1981; Campbell, 1995). Selection for resistant genotypes can be made by visual rating of disease severity (Munkvold and Desjardins, 1997). Genetic resistance of maize to *Fusarium graminearum* and *F. moniliforme* besides reducing yield losses due to ear rot, has the greatest potential for minimizing the risk of mycotoxin contamination (Schaafsma *et al.*, 1993). Susceptibility to ear rot and the levels of deoxynivalenol and zearalenone production have been found to be germplasm dependent (Hart, *et al.*, 1982; Cullen, 1983).

Occurrence of *F. graminearum* is sporadic, so relying on natural infection to screen germplasm for resistance is not useful in years when the environment does not favour infection (Reid *et al.*, 1993). The site of resistance to *Fusarium moniliforme* has been suggested to be the pericarp of the kernels by Scott and King (1984). *Fusarium moniliforme* enters the kernels through damage caused by insects (Scott and King,

1984; Farrar and Davis, 1991) and the thickness of the pericarp is an important factor in resistance. Hoenisch and Davis (1994) demonstrated that the pericarp layer on the cap of eight hybrids with high and intermediate level of resistance to ear rot caused by *Fusarium moniliforme* (determined by the percentage of kernels visibly infected by the fungus) was thicker than the pericarp of the susceptible hybrids. In contrast, the aleurone layer of the susceptible hybrids was thicker than the aleurone layer of the resistant hybrids. This suggests that the relatively thin pericarp layer of susceptible hybrids allow easy access of the fungus into the kernels, especially through insect wounds. Scott and King (1984) showed that the genetic contribution to the embryo and endosperm by the pollen has no influence on infection and ear rot caused by *Fusarium moniliforme.* He therefore suggested that resistance to ear rot is under the genetic control of the mother plant.

The severity of ear rot has been found to be positively correlated with intra ear population of ear thrips (*Frankliniella occidentalis* Pergande) and loose husks surrounding the developing ears. Ear husks of resistant hybrids, but not susceptible hybrids, physically excludes the entry of thrips through the silk channel opening during the development when kernels are highly susceptible to infection (Hoenisch and Davis, 1994). Farrar and Davis (1991) found that when insecticide was applied to the ears of susceptible hybrids at green silk stage of development, it completely eliminated *Fusarium* ear rot. It was therefore assumed that, the feeding activity of thrips allowed entrance of the fungus through the pericarp of the kernels. Warfield and Davis (1991) in California USA, showed that there was a positive correlation between population of western flower thrips in the ears and husk looseness and also disease incidence. He concluded that husk tightness is an important trait in breeding

for resistance to ear rots caused by *Fusarium moniliforme.*

Investigation into the growth and ear characteristics of maize, such as ear height, days to 50% silking, prolificacy, number of husks, ear-shank length, and ear declination, in association with ear rot (*Stenocarpella maydis*) in nine commercial maize cultivars was carried out by Flett *et al.*, (1994). The studies revealed that resistance to *S. maydis* was weakly associated with ear declination and husk numbers. Koehler *et al* (1942) also reported a significant correlation between the coverage of the ear by the husks and reduced ear rot development and indicated that the effects of genes governing resistance are additive. Resistance to Stenocarpella ear rot is inherited independently from other ear rot pathogens and to other diseases caused by *Stenocarpella maydis* (Thompson *et al.*, 1971; Hooker, 1956). Although *S. maydis* causes both ear rot and stalk rot in maize, it has been found that hybrids which are resistant to stalk rot are not necessarily resistant to *Stenocarpella* ear rot. Different factors are responsible for resistance to Stenocarpella invasion in the two regions of the plant. Evaluations of corn inbred lines of diverse origins and maturities, including many that were widely used in commercial hybrids production. Hooker (1956) found no significant correlation between basal stalk rot and ear rot. Unlike infection by *Stenocarpella* which is not systemic, infection by *Fusarium moniliforme* can be carried systemically from seed to the kernel.

Asagui *et al.* (1993) investigated the role of phenolic compounds in the resistance of maize kernels to *Fusarium graminearum*. A negative correlation was found between the amount of ear rot observed in the field and the amount of (E)-ferulic acid detected in kernels by high pressure liquid chromatography.

Corn inbred lines high in lysine were found to be highly susceptible to kernel and ear

rots, particularly *Gibberella zeae* and *Stenocarpella maydis* when compared to their normal endosperm, near-isogenic counterparts (Ullstrup, 1970). The hybrids from high lysine corn showed variation in susceptibility depending on the genetic background. Hybrid differences in resistance to storage mould, particularly *Aspergillus* spp. and *Penicillium* spp. as measured by visible mould and the number of propagules have been reported (Friday *et al.*, 1989; Moreno and Chistensen, 1971, Cantone *et al.*, 1983; Tuite *et al.*, 1985).

Maize hybrids genetically engineered with genes from the bacterium *Bacillus thuringiensis* are now commercially available in the USA. These hybrids contain C*ry* genes. These genes produces insecticidal crystalline proteins in the plant tissues. These proteins are toxic to certain insects, particularly the European corn borer (*Ostrinia nubialis*). Maize kernel feeding by *O. nubialis* leads to infection by fungi that contaminate the corn with mycotoxins. Evaluation of these materials revealed that, there was consistently less feeding of the insects on the kernels and less Fusarium ear rot on the transgenic plants than their non-transgenic counterparts. These hybrids exhibited a lower concentration of fumonisin in kernels compared to the non-transgenic hybrids (Munkvold *et al.*, 1999). Evaluation of Bt maize hybrid has also shown that the incidence and severity of Fusarium ear rot and incidence of symptomless kernel infection was low in these hybrids compared to the hybrids lacking the bacterium genes (Munkvold, *et al.*, 1997b)

2.6 Mycotoxins associated with maize ear rots

The major mycotoxins associated with ear rot are the trichothecenes, zearalenone, moniliformin, fumonisins, aflatoxins, and ochratoxin A. The mycotoxins are discussed below and some of the fungi associated with them outlined in Table 2.4.

2.6.1 Trichothecenes

The most important mycotoxins in this group are T-2 toxin, nivalenol, deoxynivalenol (DON) and diacetoxyscirpernol. DON is probably the most widely occurring Fusarium mycotoxin, contaminating a variety of cereals especially maize or wheat. It is reportedly produced by strains of *Fusarium graminearum* and *F. culmorum* (Marasas, *et al.*, 1984) which are common pathogens of cereals particularly wheat and maize. It was first isolated in Japan and later in America as vomitoxin (Vesonder, *et al.*, 1973; Miller *et al.*, 1983). The occurrence of DON has been reported in North America, Europe and Japan but in low concentrations. This toxin is more likely to be produced in the field than in storage (Greenhalgh, 1983; Vesonder, 1981). Its occurrence in cereals in developing nations particularly China (Luo, 1988) and parts of South America and Africa is relatively high in some years (IARC, 1993a). Acute mycotoxicoses affecting large numbers of people, caused by ingestion of deoxynivalenol have been reported in China and India (Miller *et al.*, 1991; Bhat *et al.*, 1989). T-2 toxin is produced on cereals in many parts of the world and is particularly associated with prolonged wet weather at harvest (Coker, 1997).

2.6.2 Zearalenone

Zearalenone is an oestrogen-like metabolite that tends to accumulate in maize ears in storage rather than in the field (Mirocha, 1974). Zearalenone production is associated with cooler temperatures (Martin and Gilman 1976). It is the mostly widely distributed oestrogenic mycotoxin occurring mainly in maize, in low concentrations in North America, Japan, and Europe. However, high concentrations can occur in developing countries, especially when maize is grown under more temperate conditions like the highland regions of the tropics (Coker, 1999).

Exposure to zearalenone contaminated maize causes hyper-oestrogenism in livestock, especially pigs, characterized by vulvar and mammary swelling and infertility. Zearalenone is produced by *Fusarium graminearum*, *F. crookwellense*, *F. culmorum*, and *F. semitectum* and is primarily associated with maize, but is found in low concentration on wheat, barley and sorghum. Concentration in grains in North America, Europe and Japan are generally low, however in some developing countries, exposure may be high, particularly where maize is under temperate conditions (including the highlands) (IARC, 1993a).

2.6.3 Moniliformin

Moniliformin (Sodium or potassium salt of 1-hydroxycyclobut-1-ene 3,4 dione) is a highly toxic compound that was first isolated in 1973 (Cole *et al.*, 1973). This compound causes rapid death and pathological lesions including myocardial degeneration and necrosis in experimental animals (Nelson, 1992). *Fusarium moniliforme* is a weak producer of moniliformin. Marasas *et al.*, (1986) tested several isolates of *Fusarium moniliforme* and found that, only a small percentage of the isolates could produce moniliformin and then only in small amounts. Moniliformin is produced by other *Fusarium* species; *F. subglutinans*, *F. proliferatum*, *F. anthophilum*, *F. avenaceum*, *F. acuminatum*, *F. concolor*, *F. equiseti*, *F. oxysporum*, *F. semitectum*, *F. fusarioides (F. chlamydosporum)*, *F. sporotrichioides*, *F. culmorum* and *F. reticulatum* (Faber *et al.*, 1987).

2.6.4 Fumonisins

Fumonisins form another group of mycotoxin produced by *Fusarium moniliforme*. Six fumonisins (Fumonisin B_1-B_6) have so far been isolated from *Fusarium*

moniliforme and characterized (Thiel *et al.*, 1992). It is also produced by *F. proliferatum* (Ross *et al.*, 1990; Thiel *et al*, 1991), *F. nygamai* (Thiel *et al*, 1991, 1991). Fumonisins are widely distributed in maize products, including those from Europe, although limited numbers of analyses have been published outside South Africa and USA (Thiel *et al.*, 1992). Fumonisin B_1 (1,2,3-propanetricarboxylic acid, 1,1 '-(1-(12-amino-4, 9, 11-trihydroxyl-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanedyl) ester and B_2 (1,2,3-propanetricarboxylic acid, 1,1 '-(1-(12-amino-9,11-trihydroxyl-2-methyltrdecyl)-2-(1-methylpentyl)-1,2-ethanediyl) ester are the major ones produced in nature but the rest are produced in minor quantities. Fumonisin B_1 has been reported in maize or maize products from a variety of agro-climatic regions in the USA, Canada, Brazil, South Africa, Italy and France (Coker, 1999). The toxins occur especially when maize is grown under warm, dry conditions.

2.6.5 Aflatoxins

The aflatoxins are widely considered to be the most important group of mycotoxins and have been associated with mycotoxicoses both in livestock and humans. Aflatoxin-producing moulds occur widely in sub-tropical and tropical climates, throughout the world. Drought stress with accompanying high temperature is one of the most frequent factors reported to increase pre-harvest aflatoxin outbreaks in maize (Payne, 1992). The two most important moulds associated with aflatoxin are *Aspergillus flavus* and *A. parasiticus*. *Aspergillus flavus* produces aflatoxin B_1 and B_2 while *A. parasiticus* produces aflatoxin B_1, B_2, G_1 and G_2 (Dorner *et al.*, 1984). The aflatoxins may be produced, both before and after harvest, on many foods and feeds especially edible nuts, oil seeds and cereals (Coker, 1999).

2.6.6 Ochratoxin A

Ochratoxin A is an important mycotoxin that is produced by *Aspergillus ochraeceus* in warmer climates and *Penicillium verrucosum* in cooler climates. This mycotoxin occurs mainly in wheat and barley growing areas in temperate zones of the northern hemisphere (IARC, 1993b). It also occurs in maize, rice, peas, beans, and cowpeas (Coker, 1999). Developing countries where it has been reported include Brazil, Tunisia, Chile, Senegal, Egypt, India and Indonesia. Pork products are considered to be the most significant dietary source of ochratoxin. Ochratoxin has been detected in pork products in Europe. High levels of ochratoxin have been detected in the blood of individuals in Yugoslavia (IARC, 1993) and have also been detected in milk in Italy (Micco *et al.*, 1991) . Ochratoxin has been linked with the human disease Balkan endemic nephropathy, a fatal chronic renal disease occurring in limited areas of Bulgaria, former republic of Yugoslavia and Romania. Ochratoxin causes renal toxicity, nephropathy and immuno-suppression in several animal species and is carcinogenic in experimental animals (Coker, 1999).

Table 2.4. Some common mycotoxins and fungi that produce them

Toxin	Species responsible
Aflatoxin	*Aspergillus flavus, A. parasiticus, A. wentii*
Diplodiatoxin	*Stenocarpella maydis*
T-2 toxin	*Fusarium sporotrichioides, F. equiseti, F. nivale, F. poae*
Ergotamine	*Claviceps purpurea*
Zearalenone	*Fusarium culmorum, F. graminearum, F .moniliforme, F. equiseti, ,F. tricinctum, Nectria radicicola*
Fumonisin	*Fusarium moniliforme, F. nygamai, F. verticillioides, F. proliferatum.*
Fusarenon- x	*Fusarium nivale*
Moniliformin	*Fusarium subglutinans*
Nivalenol	*Fusarium nivale, Fusarium graminearum*
Ochratoxin	*Aspergillus alliaceus, A. melleus, A. ochraceus, A. ostianus, A. sclerotorium, A. sulphureus, Penicillium verrucosum.*
Patulin	*Aspergillus clavatus, A. giganteus, A. terrus, Byssochlamys niea, Penicillium claviforme, P. expansum, P. urticae*
Penicillic acid	*Aspergillus alliaceus, A. melleus, A. ochraeceus, A. ostianus, A. sclerotorium, A. sulphureus, Penicillium baarnense, P. cyclopium, P. matriti, P martensii, P. Palitans, P. puberulum, P. stoloniferum, P. suaveleolens, P. thomii.*
Verticillin	*Verticillium species*

Source: Martin and Gilman, 1976; Coker, 1997, Samson *et al*,1996

2.7 Diseases associated with mycotoxins in cereal grains

2.7.1 Mycotoxicoses in humans

Diseases caused by mycotoxins are referred to in general as mycotoxicoses. Mycotoxins produced by ear rot fungi have been associated with a range of toxins linked to mycotoxicoses and carcinomas in human and domestic livestock (Gelderblom, 1988).

2.7.1.1 Aflatoxins

In Kenya, there are some records of suspected livestock and human mycotoxicoses. Between 1978 and 1982 widespread mycotoxin contamination in maize and other cereals resulting in a number of deaths, were reported in Kenya (Muraguri *et al.*, 1982, Ngindu *et al.*, 1982 and Manwiller, 1987). These deaths were associated with

aflatoxicoses in cereal grains. In 1981, in Machakos District of Kenya, twenty people were admitted in hospital, and 12 died. Two families where there was acute illness and death, were found to have been eating maize which contained as much as 12000 ppb aflatoxin B_1. Liver tissue necroscopy contained up to 89ppb of this mycotoxin. Mycological cultures of maize grain yielded a mixed growth of *Aspergillus flavus*, *Rhizopus* and *Paecilomyces* spp. on groundnut medium (Ngindu *et al.*, 1982).

High incidences of liver cancer in the population living around Lake Victoria may be associated with consumption of mycotoxin contaminated grain (McDonald, 1996). An epidemiological study was carried out in Murang'a District of Central Province, Kenya by Peers and Linsel (1973). The daily intake of aflatoxin was estimated by analysing samples of food (plate samples) which were principally of cereal origin, but also included honey beers (which also contain grain). The aflatoxin intake was then correlated with the incidence of primary liver cancer. These studies revealed that the mean aflatoxin in ng/kg body wt/day ingested was higher in males than in females (9.8 in and 6.5 respectively) and there were 15 female and 30 male cases of primary liver cancer (age $\geq$ 16, 1967-70). The highest levels of aflatoxin ingestion were found in the populations residing in the lower altitude areas compared to the mid and high altitude areas. Similar studies on the correlation between oral dose of aflatoxin B_1 and the urinary aflatoxin N^7-guanine product revealed that in Murang'a District of Kenya, 12% of urine samples (over1000 samples) tested contained the aflatoxin guanine product. The highest concentration was found in the Western Highlands and the Central Province of Kenya (Autrup *et al.*, 1983, Autrup *et al.*, 1987). Aflatoxin B_1 is a human carcinogen and is one of the most potent hepatocarcinogens known (IARC, 1993d). Association of aflatoxin ingestion and liver cancer has been reported

in Uganda (Korobkin and William's, 1968; Alpert *et al.*, 1968; Alpert and Davidson 1969)), Kenya (Peers and Lindsel, 1973), Thailand (Shank, 1971; Bourgeois *et al.*, 1971), Swaziland (Keen and Martin, 1971a, 1971b), and India (Robinson, 1967).

2.7.1.2 Fumonisins

Two most important areas where mycotoxins have been associated with human oesophageal cancer are the Transkei region of South Africa and Guangxi region of the Peoples Republic of China. Fumonisins produced by *Fusarium moniliforme* have been associated with high incidence of oesophageal cancer in the Transkei region of South Africa (Rheeder *et al.*, 1992; Marasas *et al.*, 1981). Epidemiological studies showed a correlation between the proportion of maize kernels infected with *Fusarium moniliforme*, one of the most prevalent fungi in maize in the Transkei region of South Africa, and oesophageal cancer incidence (Marasas *et al.*, 1981). Follow up studies on kernel infections and fumonisin levels in the high risk and low risk oesophageal cancer areas revealed that significantly higher mean numbers of kernels infected with *F. moniliforme* and correspondingly higher levels of mycotoxins FB_1 and FB_2 were present in maize samples, in high risk oesophageal cancer areas than in low risk areas (Sydenham *et al.*, 1990; Rheeder *et al.*, 1992). Similar studies were carried out in the Peoples Republic of China, comparing the natural occurrence of fumonisins, trichothecenes and zearalenone in corn and wheat from high and low risk areas for human oesophageal cancer. The studies revealed that incidence and mean levels of the toxins were higher in the Lingxian maize, which is an area where the risk of oesophageal is high than Shangqui maize, which is a low risk cancer area (Luo and Katayama, 1990).

2.7.1.3 Trichothecenes

Outbreaks of diseases related to trichothecenes in humans resulting from ingestion of mouldy maize and scabby wheat have been reported in India and China. The symptom involved nausea, vomiting, abdominal pains, diarrhoea, dizziness and headache. Deoxynivalenol and zearalenone were detected in the food samples (Bhat *et al.*, 1990; IARC, 1993a). Ingestion of DON has caused outbreaks of acute human mycotoxicoses in China, India, and rural Japan (IARC,1993a). The symptoms in China included nausea, vomiting, abdominal pains, diarrhoea, dizziness and headache. Nivalenol toxin has been associated with the occurrence of red mould disease in Japan. The symptoms include anorexia, nausea, vomiting, abdominal pains, diarrhoea, dizziness, headache and convulsions (Marasas, *et al.*, 1984). T-2 toxin is associated with 'alimentary toxic aleukia' (ATA), a disease that affected thousands of people in Siberia and led to the elimination of entire villages (IARC,1993e).

2.7.2 Mycotoxicoses in livestock

Mycotoxins can cause both acute and chronic effects in livestock leading to reduced production and possible carry over of mycotoxins to the human food chain. Mycotoxicoses associated with the major ear rot mycotoxins are considered below. Table 2.5 shows a summary of the common mycotoxins and the syndrome or toxic effect on livestock.

2.7.2.1 Diplodiatoxin

Diplodiosis is an endemic neuro-mycotoxicosis of domestic ruminants. In South Africa, it is associated with ruminants grazing on harvested maize in winter (Kellerman *et al.*, 1985). It is caused by the ingestion of maize infected by the

common cob rot fungus *Stenocarpella maydis* (Berk) Sutton. Diplodiosis has been reported in cattle and sheep (Marasas, 1977). Diplodiosis is often the most common bovine mycotoxicosis, the symptom progressing from inco-ordination to paralysis and death (Coker, 1997; Kellerman, 1988). Still birth or death soon after birth has also been reported in calves and lambs in South Africa (Kellerman *et al.*, 1991). Culture material of *S. maydis* isolated from commercial South Africa maize has been reported to cause acute toxicity when administered to rats and ducklings (Rabie *et al.*, 1985).

2.7.2.2 Zearalenone

Zearalenone (ZEN) is an oestrogen that causes swine hyper-oestrogenic syndrome, which is characterized by vulvar and mammary enlargement in immature pigs and induced uterine hypertrophy. It also causes reduced litter size, feed refusal, haemorrhage and male infertility (Marasas *et al.*, 1984).

2.7.2.3 Trichothecenes

Deoxynivalenol (DON) has been reported to induce feed refusal, and decreased weight gain in swine, hens, and rats (Vesonder, 1981; Vesonder *et al.*, 1976; Forsyth *et al.*, 1977). T-2 induces immuno-suppressive activity in animals and causes haemorrhagic disease in animals and is associated with the formation of oral lesions and neuro-toxic effects in livestock. Vomiting is one of the commonest symptom of T-2 toxicosis. It has been found to induce vomiting at doses of 0.1-10 mg/kg body weight in cats, dogs, pigs and duckling (WHO, 1990) as quoted by IARC (1993).

2.7.2.4 Fumonisins

Fumonisins are a group of recently characterized mycotoxins produced by *Fusarium moniliforme*. They occurs throughout the world and are frequently found in maize

(IARC, 1993c). Fumonisin B_1 (FB_1) in maize causes equine leukoencephalomalacia (ELEM) in horses (Ross *et al.*, 1990; Gelderblom *et al.*, 1988), an acute neurological disease of horses and donkeys (Marasas *et al.*, 1988), pulmonary oedema in swine (Ness *et al.*, 1991, Lenn *et al.* 1990, Bill and Lenn 1992, Javed *et al.*, 1993a), mystery disease of swine (David *et al*,. 1992) and mortality in chicks and chick embryos (Javed *et al.*, 1993a; Javed *et al.*, 1993b; Brown *et al.*, 1992). ELEM has been reported in many countries including the USA, Brazil, Egypt, China and South Africa. In swine it induces anorexia, fever, stillbirths, abortions and /or early furrowing, mummified foetus, respiratory diseases, delayed or abnormal oestrous cycle and reduced conception rates (Marasas *et al.*, 1984).

2.7.2.5 Aflatoxins

Aflatoxin poisoning was first reported in England when death occurred to thousands of turkeys, ducklings and other domestic animals fed on groundnut meal contaminated with *Aspergillus flavus* from South America in the 1960's (Blount, 1961). Animals affected by aflatoxicoses show direct damage to the liver in the form of centrilobular necrosis, proliferation of bile ducts and fibrosis and haemorrhage in the intestines. Aflatoxicoses have been reported in England on pigs fed on groundnuts containing aflatoxin (Loosmore and Herding, 1961) and cattle fed on aflatoxin in imported cotton seeds (Loosmore *et al.*, 1964). Aflatoxicoses have also been reported in cattle in the USA (Clegg and Bryson, 1962) and in India (Gopal *et al.*, 1968). Death due to aflatoxin has been reported in goats and pigs in South Africa (Minne *et al.*, 1964), Murrah buffalo in India (Sastry *et al.*, 1965). Aflatoxin induces immuno-suppression in livestock (Coker, 1997).

2.7.2.6 Ochratoxin

Exposure to ochratoxin (OA) seems to occur mainly in wheat and barley growing areas in temperate zones of the northern hemisphere (IARC,1993b). OA toxin has the ability to transfer from animal feeds to animal products. This has been demonstrated by its presence in Europe in pig blood and retail pork (Coker, 1997). Because of these, there exist regulations for the permitted levels of OA in pork products. A provisional tolerable weekly intake of OA of 112 ng/kg body weight per week has been recommended by WHO/FAO joint expert committee on food additives. The minimum acceptable levels of various mycotoxins is outlined in Table 2.6.

Table 2.5. Mycotoxins associated with farm animals and their toxic effects

Mycotoxin	Animal species	Primary syndrome or main toxic effect
Fumonisins	Horses, donkeys and mules	Severe nervous disorder in coordination
Fumonisins	Pigs	Anorexia, fever, stillbirths, abortions and /or early furrowing, mummified foetus, respiratory diseases, delayed or abnormal oestrous cycle, reduced conception rates
	Chicks, ducklings	Death of chicken embryos, mortality of chicks and ducklings
Zearalenone	Pigs	Hyperestrogenic syndrome, characterized by vulvar and mammary enlargement in immature pigs, induced uterine hypetrophy, feed refusal
Zearalenone	Cattle and sheep	Reduction in conception rates and infertility
Deoxynivalenol	Pigs	Feed refusal and emetic syndrome
Aflatoxins	Cattle, swine and dog	Decreased weight gain, rectal prolapse, bile duct proliferation, acute hepatitis.
Aflatoxins	Chicken	Decreased weight gain, egg production and hatchability, hepatotocity, enlarged kidney and carcinogenecity, high mortality, haemorrhagic diseases and death.
Ochratoxin A	Cattle, swine	Nephrotoxicity and teratogenicity
	Chicken	Nephrotoxicity, lecopenia, and high mortality.

Source: Marasas *et al.*, 1984, Panigrahi *et al.*, 1993

Table 2.6. Maximum acceptable levels of some common mycotoxinS in feed and food stuffs

Toxin	Maximum acceptable levels	Effecting countries or organization	Source
aflatoxin B_1	200 ug/kg in raw materials(oil seeds and their derivatives), 5 ug/kg in dairy feeds	European Union	Van Egmond,1989
Aflatoxin B_1	20 ng/g in maize	United states food and drug administration	Gourama and Bullerman, 1995
Ochratoxin A	0-50 ug/kg in food and 100-1000 ug/kg in feed.	UK, Denmark, France, Greece, Hungary, Israel, Netherlands, Brazil, Czechoslovakia.	IARC, 1993b
Zearalenone	1 mg/kg maize	USSR	Van Egmond,1989
Zearalenone	0.2 mg/kg Maize	Brazil	Van Egmond. 1989
Zearalenone	0.03 mg/kg maize	Romania	Van Egmond, 1989
Deoxynivalenol	0.005 mg/kg all feeds	Romania	IARC, 1993a
Deoxynivalenol	2 mg/kg wheat/ wheat products for milling, 0.2 mg/kg finished wheat products	USA	IARC, 1993a
Deoxynivalenol	2 mg/kg uncleaned soft wheat 1 mg/kg infant food	USA	IARC, 1993a
Fumonisin	No regulation		Van Egmond, 1989; IARC, 1993c

2.8 Strategies to reduce mycotoxin in contaminated grains

Prevention of mycotoxin contamination in grain involves the combination of those factors that reduce fungal contamination in the field, as well as prevention of growth of the moulds during harvest and post harvest handling. Mycotoxin may be reduced in contaminated grains by the use of various methods. These methods can be classified as either physical, chemical, or biological. The suitability of the method depends on the type of mycotoxin, type of grain and the desired product.

2.8.1 Physical methods

Many physical methods of decontaminating grain containing mycotoxins have been tried with varying degrees of success. The physical methods include density segregation of contaminated kernels from non-contaminated kernels, in water or saturated sodium chloride or sucrose solution (Huff and Hagler, 1985) and food processing such as milling and baking, cleaning or washing, sieving and dehulling. Although dilution of contaminated grain with clean grain is not a decontamination method *per se* it has been used to reduce the toxicity of contaminated feed stuff. Density segregation, milling, cleaning, and baking does not completely remove DON and ZEN from flour fractions or whole wheat (Trenholm *et al.*, 1991). Some of the physical methods used in decontamination are indicated below.

2.8.1.1 Cooking and processing

Aflatoxin can be reduced by roasting or frying, for a period of up to 30 minutes at 150-200 ^{0}C (Lee *et al.*, 1969). This process degrades aflatoxin by 40-85%. Baking of wheat bread at 90-120 ^{0}C degrades aflatoxin by nearly 80% (Reiss *et al.*, 1978). Other reports indicate that aflatoxins in foods are not readily degradable under

normal cooking conditions (IARC, 1993d). Cooking of the flour-based products does not reduce the level of ZEN appreciably in contaminated flour (Scott, 1990). Baking does not destroy or significantly reduce levels of deoxynivalenol (Bennett and Richard,1996). There are no data on the effects of baking on the level of fumonisins or their transmission into milk and eggs (Norred *et al.*, 1991).

Fusarium mycotoxins survive processing and tend to concentrate in the products generally used for animal feeds such as the bran. Wet milling is the major process used to prepare corn products for human consumption. It has been shown to have a segregating effects on the levels of zearalenone and fumonisin in the chemically diverse products resulting from the process. Distribution of zearalenone in products from wet milling, determined by thin layer chromatography has been found to be in the order of gluten > milling solubles > fibre > germ. Segregation of the toxins in different fractions may have serious implications depending on their use. Some fractions such as the germ may be used for livestock feed and therefore pose a potential health hazard to the animals. Different fractions (grits, germ, floor) produced during dry milling of contaminated corn retain much of the characteristics of the original corn (Bennet and Richard, 1996). Fermentation of maize grains has been found to be ineffective in reducing fumonisin concentration (Bothast *et al.*, 1992). Bennet *et al.* (1978) similarly reported that deoxynivalenol, fumonisin and zearalenone survive most of processing methods and ethanol fermentations, but there is no carry-over of zearalenone, deoxynivalenol or fumonisin in distilled ethanol. The carry over of these mycotoxins in recovered solids from fermentation would increase the potential for animal disorders in the event that they are used as feed. Traditional fermentations for brewing of corn beer found to have as much as 51% carry over of

zearalenone into the finished product (Okoye, 1978). Zearalenone has been detected in Canadian beers (Scott *et al.*, 1993) .

2.8.1.2 Sieving and dehulling

Two physical methods, sieving and dehulling have been reported to reduce the concentration of deoxynivalenol (DON) and zearalenone (ZEN) toxins in contaminated barley, wheat corn and rye (Trenholm *et al.,* 1991). These results have shown that DON and ZEN toxins are not homogeneously distributed throughout the fractions of differing particle size in coarsely ground barley, wheat and maize. Higher concentrations were found in smaller particles, removal of these small particles in maize reduced DON and ZEN by 73 and 79%, respectively. In the same study, hulls of barley and wheat were found to have a higher concentration of the two mycotoxins. Earlier work (Miller *et al.,* 1985) which agrees with these findings, showed that chaff of various cereals contaminated with DON had consistently higher DON concentrations than the inner portions of the kernels. Other studies have suggested that in cases of relatively moderate infestation in wheat, the degree of DON and ZEN toxins contamination was usually greatest at the exterior of the kernels (Lee *et al.*, 1985). A similar distribution is suspected in other grains as well (Trenholm *et al.,* 1991). The highest reduction in the concentration of the two toxins was achieved when both sieving and dehulling were applied.

2.8.1.3 Removal of broken grains and foreign matter

Broken grains and foreign material, such as dust, weed seeds, plant fragments, insect debris and soil, often become concentrated with grain bulks. Such material may differ from the grain bulk and be more susceptible to fungal invasion, providing focus

for hot pockets or invasion of otherwise sound grain by fungi. This may also differ with ventilation and lead to uneven drying, again increasing the risks of moulding and perhaps mycotoxin formation (Lacey, 1989).

2.8.2 Chemical methods

There are only a few chemical methods of decontamination of mycotoxins and these are applicable to aflatoxin decontamination. Some of the patented procedures that exist include the use of compounds like ammonia, calcium hydroxide, hydrogen peroxide, methylamine, and a mixture of calcium and methylamine (Coker, 1999). Ammoniation is the most widely used detoxification process, ammonia is used as anhydrous vapour and aqueous solution (Park *et al.*, 1984; Coker, 1999). Ammoniation has been found to be ineffective for fumonisin B_1 detoxification (Kenneth *et al.*, 1992; Norred *et al.*, 1991).

2.9 Detection of mycotoxins

There are various analytical methods for quantification of mycotoxins in food and feed stuffs, but the particular method used depends on various factors such as number of samples to be analyzed, the cost and availability of equipment. Mycotoxin analyses involve various steps from sample collection and preparation of the samples for analysis, extraction, filtration, clean-up and detection. Various solvents are used depending on the method of extraction. Some of the most common solvents are methanol, acetone and acetonitrile which are often mixed with water in various proportions depending on the method. The weighed sample is mixed with extraction solvent and blended (3 or 5 minutes) or shaken in a flask shaker for 40 - 45 minutes and then filtered. The crude extract is then passed through the cleaned- up stage

which may involve passing the extract through the clean-up columns. The effectiveness of chromatographic methods of analysis is largely determined by the effectiveness of the sample clean-up step (removal of interfering components from the initial crude sample extract). Among the recently developed methods for cleaning up of the extract is the solid phase extraction (SPE), which involves the use of liquid phases bonded on an inert support, contained within a plastic cartridge. A small volume of the crude sample extract is applied to the SPE cartridge which is then washed with water. After drying by passage of air, the toxin is eluted with a suitable solvent. Immuno-affinity cartridges have also been developed for the clean-up of extracts. The crude extract is passed through the cartridge containing monoclonal mycotoxin antibodies adsorbed on to an inert support. The mycotoxin is retained and eluted with a suitable solvent.

The methods used in detection and/or quantification can be put broadly into three groups; the physicochemical, immunoassay methods and the bioassay. The physicochemical methods include the traditional thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), high performance thin-layer chromatography (HPTLC) and gas-liquid chromatography (GLC).

TLC involves the application of concentrated extracted samples on the base line of a silica gel coated plate which separate out by solvent migration. The characteristic fluorescent regions under the UV light are then quantified. The migration data for mycotoxins are only approximate and usually the sample must be compared to a reference standard on the same plate. The TLC technique is usually adopted in the multi-sample analysis to check the presence of mycotoxin before subjecting the samples to methods specifically designed for the quantitative analysis of the specific

mycotoxin. The immuno assay systems include radio-immunoassay (RIA), enzyme linked immuno-assay (ELISA) and immuno-affinity chromatography (IAC). If a large number of samples are to be analyzed, an automated procedure such as high performance liquid chromatography (HPLC), high performance thin-layer chromatography (HPTLC) or enzyme- linked immunosorbent assay (ELISA) are advantageous (Coker, 1999). The automated procedures provide for handling a large number of samples, have high degree of accuracy and precision but have the disadvantage that they bear high capital costs and require skilled analysts. Immuno-chemical technology has been used in the production of tests kits for the rapid analysis of samples of food and feeds. Immuno-affinity clean-up cartridges are used in combination with simple fluorometric devices. IAC is based on competition of binding sites between toxins and specific antibody-antigen molecules on a solid matrix and packed in a column. When a sample is passed through the column, the toxin is bound onto the surface and the rest of the material is eluted using highly polar solvents. The bound toxin is then estimated using a fluorimeter. The RIA procedure is based on the competition between a free antigen which could be in the form of mycotoxin and radioactive labeled antibody to form a soluble reversible antigen-antibody complex. When a mycotoxin is introduced in the form of un-labeled antigen, it will compete with the labeled antigen and bind onto the antibody. The antibody-antigen complex is then used to quantify accurately mycotoxin content in a given sample. RIA can only be used in central laboratories with access to sophisticated apparatus and skilled manpower. It also has the health risk element to operators associated with the exposure to radio-active materials. IAC is highly sensitive and specific, can be used to carry out large number of samples and requires

little or no capital outlay. IAC has the other advantage in that, it can be used to clean up samples for TLC, GLC, or HPLC.

ELISA is based on the use of toxin -enzyme conjugate, linked to an insoluble carrier surface. The resulting complex is used to detect mycotoxins which are quantified spectrophotometrically. The advantages of ELISA are higher sensitivity, low cost of equipment and chemicals, use of non-toxic reagents and no requirement for use of radio-active reagents. Affinity cartridges are commercially available for the analysis of mycotoxins B_1, B_2, G_1, G_2 and M_1, and for the determination of fumonisin B_1, ochratoxin A, deoxynivalenol and zearalenone. Immuno-chemical technology has been applied in development of rapid ELISA methods for a variety of mycotoxins including the aflatoxins, ochratoxin A, fumonisins, zearalenone, T-2 toxin and deoxynivalenol. Card tests have also been developed where the antibody is immobilized within a small indentation on a card similar in size to a credit card. Such tests have been developed for detecting aflatoxin, zearalenone, ochratoxin A and T-2 toxin in maize. Immuno-chemical methods are relatively expensive and the antibody is unstable under high temperatures. They have the advantage that they do not require a highly skilled analyst and are appropriate for the analysis of samples requiring a quick decision.

Bioassay methods are based on the use of a sensitive biologically indicator. Sensitivity of the yeast, *Kluyveromyces marxianus* has been used for detection of a range of mycotoxins by measurement of retardation of growth rate and measuring the optical density of the yeast suspension or measurement of the inhibition of the of galactosidase enzyme by toxins (Dell, 1993; Engler,1996) as quoted by Coker (1997).

CHAPTER 3: SURVEY OF FARMERS PERCEPTIONS AND PRACTICES IN RELATION TO MAIZE EAR ROT

3.1 Background

The survey was carried out in Mbakalo Location in Tongaren Division of Bungoma District and Kapkangani Location of Kapsabet Division of Nandi District. Both Districts fall under high potential maize producing areas of Western Kenya. Tongaren Division has an annual rainfall of 1200-1800 mm. The rainfall is bimodal. It covers a total area of 375 km^2 with a population density of 239 persons per sq. km. There are about 15,000 farm families with an average farm family of eight members. The altitude ranges from 1540-1837 masl. The area is generally flat with average slope of 6%. The main agro-ecological zones are as follows, $UM_1 = 2\%$, UM-3 = 38% and $UM_4 = 60\%$. The UM-3 in the region is described as the marginal coffee and maize zone. The first rains start in March, while the second rains start indistinctly at the beginning of August. The UM-4 is described as the sunflower and maize zone (Jaetzold and Schmidt, 1983). The two zones normally have long to very long cropping seasons or are dividable in two variable cropping seasons. The division is currently divided into four Locations namely Mbakalo, Tongaren, Naitiri and Soysambu. Each Location has an agricultural extension staff. The soils in this area are described as sandy clay loams (Ferralsols and Acrisols). According to the general fertility groups, the soils in this area are classified as low fertility (according to the soil list). However this is subject to local differences. The Division is a settlement area, with people from varied ethnic groups. The majority of the population is from theLuhya community. Initially the area was occupied by the European settlers. After independence the farms were sub-divided

into 15 acre African settlement scheme. Maize is the main enterprise, either grown as a pure stand or intercropped with beans (*Phaseolus vulgaris L*). Other crop enterprises are sunflower, horticulture and sweet potatoes. The current area under (Long rains 1997) maize was 22,083 ha, Beans 9,550 ha, Sunflower 1,700 ha and horticulture 469 ha (Table 3.1). There were 15,000 indigenous cattle and 13,500 improved cattle. Other livestock enterprises included sheep and poultry. Both dairy and oxen were kept for milk and drought power respectively. The Division is geographically isolated because of the poor roads serving the area. There are no tarmac roads within the Division and the seasonal roads serving the area are poor. Kapsabet Division is situated in Nandi District. It has a bimodal rainfall ranging from1400-2000 mm. The population projection for 1997 based on 1989 census was 121,394. The average family size was 7.5 with 15,835 farm families. The Division covers an area of 590 km^2 (59,000 ha). The population density was 205 people/ km^2. The altitude ranges between 1400-2100 masl, with a slope average of 6%. The main Agro-ecological zones are as follows. UM_1-30%, UM_{2-3}-5%, LH_1 - 40%, LH_2 -20%, LH_3 - 5%. The soils in the area of study are generally considered of high fertility (Jaetzold and Schmidt, 1982). The surveyed area included areas in LH-1 and UM-1 (Appendix 3). Previous work at Kapkangani Location in Kapsabet Division (Otieno *et al.,* 1997) on adoption of soil improvement technologies revealed that 100% of the farmers were growing maize. The use of farm yard manure and inorganic fertilizer (DAP) as soil improvement technologies were 29.1% and 24% respectively.

Table 3.1. Area covered by different crops long rains 1997 in Kapsabet and Tongaren

Crop	Crop hecterage in Tongaren	Crop Hecterage in Kapsabet
Maize	22,083	12,149
Beans	9550	544
Horticulture	469	94
Sunflower	1700	-
Millet	-	99
Forest	-	236.5 km^2

Source: Nandi District annual report, 1998
Bungoma District annual report,1998

The two Divisions are net exporters of maize and have a long history of maize production. Relatively, the areas have more farmers that fall under the medium and large scale maize producers. Tongaren Division is the leading Division in terms of maize production in the country.

The purpose of the survey was to obtain base-line data on maize production practices and constraints in relation to cob rots. This information will help improve the understanding of farmers' practices and increase farmers' perceptions of the nature of the causal agents of ear rot complex and potential for mycotoxin hazard to human and livestock consumers. The survey was also to identify agronomic practices that help to minimize grain contamination in pre- and post harvest management. This information is important to the overall project, whose aim is to develop an improved cultivation and storage practices to decrease losses due to cob rots and conduct research to determine if mycotoxins associated with cob rot fungi are present at levels that are high enough to present a risk to consumers of maize flour in the region.

3.2 Methodology

Both individual and group interviews were applied. The enumerators were guided by a prepared checklist to answer specified questions (Appendix 4). During the

initial period the interviewers moved in pairs, one asking questions while the other recorded The extension officer was responsible for organizing the movement within the villages and introducing the group to the farmers. The language used was Luhya, Kiswahili and English. There was no problem with communication as all the farmers interviewed could speak one of the languages. The information collected was recorded in note books. A meeting of interviewers was held to synthesise information gathered during the individual interviews followed the evening after the interviews. Group interviews were done on the last day of the survey in each Location (Fig 3.1). All the farmers interviewed individually were gathered and information collected was discussed and confirmed by the group.

3.3 Results

3.4.1 Crop enterprises

All the (71) farmers in Tongaren and (59) in Kapsabet Divisions ranked maize as their main crop in replies given to individual interviews. The findings were confirmed at group meetings in both areas. The ranking of the top 5 crops grown by the farmers is given in Table 3.2

Table 3.2. Crop enterprises as ranked by farmers in Tongaren and Kapsabet Divisions

	Tongaren		Kapsabet	
Enterprise	Individual rank	Group rank	Individual rank	Group rank
Maize	1	1	1	1
Beans	2	2	2	2
Bananas	4	3	-	-
Cassava	5	4	-	-
Sweetpotato	3	5	-	-
Horticulture	-	-	3	3
Tea	-	-	4	4
Finger millet	-	-	5	5

- = Crop not grown or ranked among the first five)

All the farmers interviewed in the two Divisions grew maize on their farms every year. Maize was grown during the long rains, either as a pure stand or intercropped with beans. Beans (*Phaseolus vulgaris*) was confirmed as the second most important crop to maize in the two study areas. Individual interviews indicated that 60% and 58% of the farmers in Kapsabet and Tongaren Divisions respectively intercropped all or some of their maize with beans. Group interviews in both areas indicated that all the farmers preferred to intercrop their maize with beans as this gave higher returns. Other important crops were identified as sweet potato, cassava, and bananas in Tongaren Division and horticulture, tea, and finger millet in Kapsabet Division. Sometime the group rankings did not always confirm individual ranking. For example, in Tongaren sweet potato was ranked third by individual farmers and fifth by the group. In such cases greater reliance was generally placed on the individual replies which were free from peer pressure, especially from influential farmers or good orators.

Figure 3.1 . Group interview at Sirakalu market in Tongaren Division

3.3.2 Livestock enterprises

The most important livestock enterprise in the two study areas was cattle. Variable cattle breeds were kept in both areas, which include exotic, indigenous and crosses. Some farmers kept local animals mainly oxen for ploughing. Poultry was the second most important livestock enterprise in the Divisions. All the farmers kept indigenous chicken. Less than 1% of the farmers had improved chicken, kept for commercial egg production. The ranking of the livestock enterprises was similar in both areas under study, (Table 3.3) this was also in agreement with group interviews in the two areas.

Table 3.3 Livestock enterprises as ranked by farmers in Tongaren and Kapsabet Divisions

Division	Tongaren		Kapsabet	
Enterprise	Individual rank	Group rank	Individual rank	Group rank
Cattle	1	1	1	1
Sheep	3	3	3	3
Goat	4	4	4	4
Poultry	2	2	2	2
Bee keeping	-	-	5	5

- = Crop not grown or ranked among the first five)

3.3.3 Land use

An estimate of the total land area under maize in Tongaren LR 1997, showed that annual crop land was the most dominant land use type. Maize was the most important crop grown covering over 50% of the farmers cropped land during the long rains season. The other crops grown in the area included beans and sunflower. Perennial crop land was mainly under coffee, bananas and smaller pieces under cassava. Individual farmer interviews provided data on farm size in response to the questions. The data was grouped into 5 land size ranges; < 5, 6 - 11, 11-15, 16-20, and >20 acres (Table 3.4)

Table 3.4. Distribution of land size per household in Tongaren and Kapsabet expressed in percentages

Land area in acres	Tongaren	Kapsabet
< 5	28.6	38.6
6-10	13.2	26.3
11-15	44.4	3.5
16-20	7.8	14.0
>20	5.6	17.5

The average farm size in Tongaren Division from the survey results was found to be 11.8 acres, but holdings varied widely, ranging from 0.5 acres to 64 acres. This estimate of average farm size was consistent with the figure of 10 acres per house hold as given by the group interview. The majority of farms (44.4%) in Tongaren were found to have sizes in the range of 11-15 acres, but the second most common holding (28.6%) was less than 5 acres. Tongaren settlement scheme was originally sold in 15 acre packages, (Section 3.1), survey results strongly indicated a trend towards smaller farms.

In Kapsabet the land holding was variable, 38.6 % had less than 5 acres and 31.5% had over 16 acres of land.

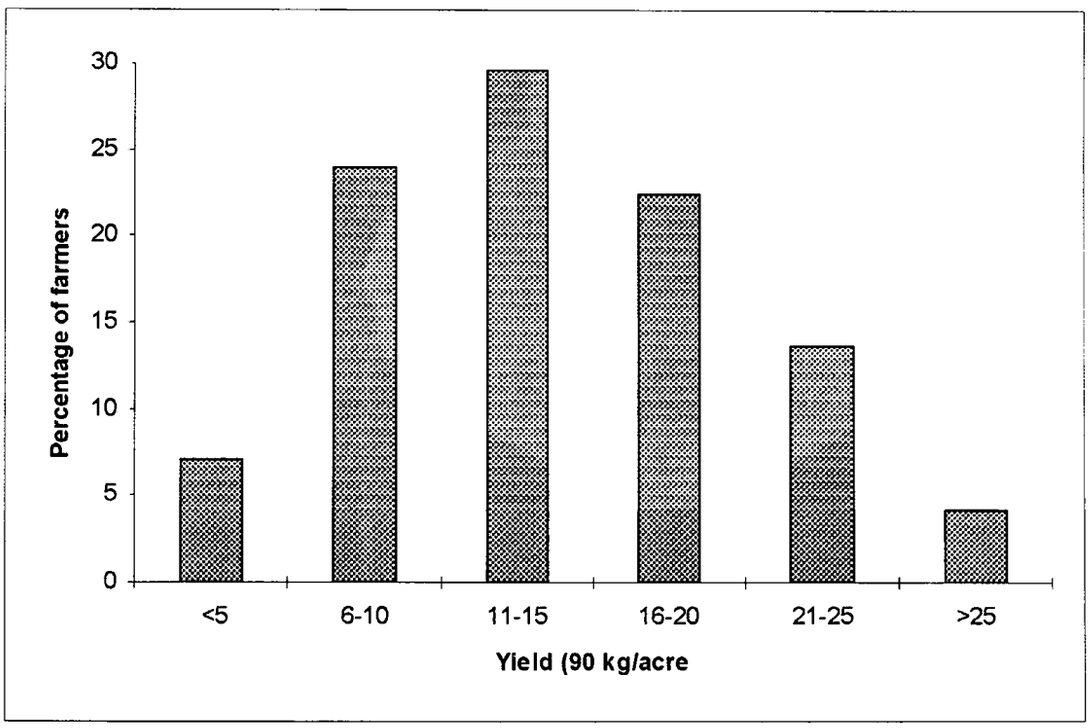
3.3.4 Yield and area under maize

In Tongaren Division the average land size per household under maize for the year 1997 was estimated to be 5 acres. The average yield of shelled maize was calculated based on individual average yield obtained from the farmers interviewed. The yield was given as an estimate in 90 kg bags. The average yield per acre in Tongaren was 15 bags per acre, but the yield on individual farm ranged from 3 to 30 bags per acre. The group meeting confirmed that 30 bags per acre was possible under good

management. The average yield for 71 farmers interviewed in Tongaren is shown in Fig 3.2.

In Kapsabet the average yield of maize per acre was 15 bags. The lowest and the highest yields recorded in Kapsabet Division were 2 and 30 bags per acre respectively. The average area of land under maize cultivation was 3.3 acres. This was equivalent to19.5 % of the total land holding. The smallest size of maize plot was 0.5 acres while the biggest was 30 acres. The average holding was 16.9 acres with the smallest farm size being 1.5 acres and the largest 200 acres. The average yield as given by the group was 15-20 bags per acre, and the lowest and highest yields were 5 bags and 30 bags per acre respectively.

Fig 3.2. Distribution of yield to farm households in Tongaren Division



The results on yield showed that 70% of farmers produced more than 10 bags per acre (900 kg), with 52% falling within the yield range of 10-15 bags per acre. Seven percent produced less than 5 bags per acre (450 kg). Only 4.2 % were able to obtain yields of over 25 bags per acre.

3.4.0 Production practices

3.4.1 Varieties

Most of the varieties grown in Tongaren and Kapsabet Divisions were the recommended Kenyan hybrids. A few farmers grew local varieties on small pieces near the homestead. The common local varieties grown was Namba nane. The hybrids grown were H614, H625, H626, H512, H511 and H622. The farmers preference of varieties was similar in both areas of study. The most preferred hybrid in both areas was H 614. All the farmers interviewed prefer H 614 although for varying reasons. They all indicated that they grow H 614 or at one time have grown the variety. The farmers pointed out that they only bought other varieties when there is no supply of H614. The second and third most preferred varieties were H 625 and H 626 (Table 3.5).

Four farmers in Tongaren Division indicated that the variety H 614 gave better yields even at low fertilizer levels or low rainfall. This contrasted with their experiences of H625 and H626 that were considered to be heavy feeders e.g. require more fertilizers and more rainfall. The group indicated that H 625 had weak and thin stalks that made the variety susceptible to lodging. Varieties H625 and H626 were reported to be very tall, making them prone to lodging during periods strong winds. The farmers reported that H625 produces a single cob but H 614 and H 626 produce double cobs under good management. Variety H 614 was preferred

because it was less susceptible to ear rot and weevil damage, but H 625 was reported to be susceptible to weevil damage. The three varieties H614, H625, H626 were considered to have closed ear tips but H622 has an open ear tip, a factor that was associated with more rotting because of the cobs accumulating water through the open tip. Both groups in the two study areas confirmed that, at physiological maturity, the ears of H614 tend to droop. Ear declination (drooping) was considered as a positive morphological characteristic that tended to reduce ear rot. H614 was also preferred because of its high density grains compared to the other varieties. This was considered as an important attribute in marketing of the grain based on weight. The Kenya Cereals and Produce Broard which is the major marketing outlet pays by weight and not volume.

Table 3.5. Maize cultivars grown in Tongaren and Kapsabet Divisions

Cultivars	Percentage Tongaren	Percentage Kapsabet
H 614	56	51
H 625	28	23
H 626	11	10
H 622	1	1
H 511	1	5
Others	4	10

3.4.2 Farmers' source of seed

Farmers in the two Divisions use certified seed obtained from Kenya farmers association stores or from local stockists. In Tongaren Division seed was obtained from stockists at Tongaren and Naitiri markets. In Kapsabet Division the seed was obtainable from Kapsabet town.

3.4.3 Land preparation

Data derived from individual farmers and confirmed by group interview indicated that ploughing period ranged from November to March, depending on the onset of rainfall. When newly opening the land, ploughing started in November and was ploughed up to three or four times. Most farmers preferred to plough in November to allow for the incorporation of stovers in the soil. Incorporation of the stovers in the soil was considered as a soil improvement strategy rather than a pest or disease management strategy. Up to three ploughings were common among the farmers who owned oxen, while two ploughings was practised by farmers who had no oxen of their own. About 30% of the farmers in Tongaren Division owned oxen for ploughing their land and offered them for hire after ploughing their land. Tractors were available for hire but were considered expensive. A majority of farmers in both areas used oxen for ploughing. The group interview in Tongaren Division pointed out that it was necessary to plough with a tractor every three years because continuous ploughing with oxen caused the soil to become shallow and develop a hard pan resulting in low yields.

3.4.4 Inputs and fertilizer use

The most commonly used fertilizers at planting were Di-ammonium phosphate (DAP), N:P:K-23:23:0, N:P:K- 20:20:0. Fertilizer was either hand drilled in open furrows at the rate of 1-2 bags per acre (50kg bag) or applied in hand-made holes. Farmers who applied fertilizer in holes used less fertilizer -a rate of 1 bag per acre. A rate of 1.5-2 bags per acre was common with farmers who applied the fertilizer in furrows. The proportion of farmers using the various fertilizers and the method of application is shown in Table 3.6. Usually the furrows were opened using oxen at a

spacing of 75 cm. The most preferred fertilizer in Tongaren was N:P:K-23:23:0. (Table 3.7) The fertilizer gave better yields than DAP. 70% of the farmers prepared manure on their farms, but most was used on other crops, mainly vegetables. The farmers had no specific rates of application of manure. Most of them prepared manure from stovers and cow dung. Upon harvesting of maize in November they carried some of the stovers from the field to the cow sheds as feed and bedding for the animals. Animals fed and trampled on them. The manure was normally ready by February for planting. Manure was applied in furrows or holes. Later in the season they applied CAN when maize was knee high, at the rate of 1 bag of 50 kg per acre. Dipterex (Trichlorphon G) was not routinely applied for stalk borer control, it was only applied during dry spells early in the growing season when windowing was observed on the leaves.

All the farmers interviewed used fertilizer on their maize. DAP, manure, compound N:P:K - 23:23:0 and 20: 20:0 were the main fertilizers used at planting maize in the two areas surveyed. None of the farmers used TSP The amount of fertilizer used in planting averaged 1.5 bags per acre (50kg bag) in Tongaren. In Kapsabet it was 1 bag per acre. DAP was most preferred in Kapsabet Division while in Tongaren Division NPK was the most preferred. All the 71 farmers interviewed in Tongaren used CAN, Urea or both on their maize crop. 46/71 applied CAN, 19/71 Urea and 3/71 used both fertilizers on their maize (Table 3.7) Both the individual and group interviews revealed that CAN was the most preferred fertilizer on the maize for top dressing. The amount used averaged 1.5 bags per acre in Tongaren and 1 bag per acre in Kapsabet.

There were 44 responses on the methods of application of fertilizer in Tongaren Division. Three main methods of application, were used, which included application in holes, Hand drilling and spreading on the farm (Table 3.6).

Table 3.6. Fertilizer application in Tongaren and Kapsabet Divisions

Method of application	Percentage in Tongaren	Percentage in Kapsabet
In holes (DAP, NPK)	20.5	32.7
Drilling (DAP, NPK)	29.5	57.7
Spreading(Manure)	20.5	1.9
Drilling (Manure)	15.5	5.8
Drilling (manure & DAP or NPK)	13.7	-
Manure in holes	-	1.9

Table 3.7. Fertilizer use in Tongaren and Kapsabet Division

Type of fertilizer	Percentage in Tongaren	Percentage in Kapsabet
DAP	28.7	75
Manure	4.4	1.4
DAP + Manure	10.4	6.9
NPK-23:23:0	21.8	-
NPK-20:20:0	13.9	-
NPK-20:20:0 + Manure	7.8	-
NPK-23:23:0 + Manure	13.0	-
CAN	62.2	37.5
Urea	35.1	5.6
CAN + Urea	2.7	-
Sulphate of ammonia	-	11.1

3.4.5 Planting

The Planting period was from February and March for the main maize crop depending on the onset of the rains. Some farmers plant small plots of maize in valley/near streams in December. This maize was sold as green maize or boiled or roasted in homes. Very little of this maize was harvested dry. The highest acreage that was observed during the survey for this off season type of maize was 8 acres and all of it was intended for sale as green maize.

3.4.6 Weeding

The farmers carried out two weedings during the growing season. The first weeding was 2-3 weeks after planting. The second weeding was when maize was knee high. Weeding was done by hand but some farmers used oxen during the second weeding.

3.4.7 Harvesting

Harvesting period started at the end of September and went on to November. The main harvesting time was in October. The most common method of harvesting was cutting the maize stalks to the ground followed by hand harvesting within the same week (Fig 3.3). Most farmers preferred to cut and harvest the same day. The main problem with this method was, in cases of heavy and continuous rains which may interfere with the harvesting, more rotting occurs and the maize which is on the ground is also prone to termite attack. Some farmers practised stooking but this was not preferred by many farmers because of too much rotting during heavy rains. Stooked maize looses its white colour and appears yellowish-brown, because of the discolouration arising from the centre of the cob. The maize was left in the field until it dried. Although sun drying was practised by a few farmers, it was considered labour intensive. Only small quantities were sun dried when harvested early in the season for home use. Maize was carried directly to the stores where the drying continued for varying periods of time. The stores were well ventilated to allow further drying.

3.4.8 Storage

Most of the farmers in Tongaren and Kapsabet Divisions had built outside stores for the storage of maize cobs. These stores were mainly made of wood and covered

with grass or iron sheets (Fig 3.4). Traditional stores were also common on some farms. These were either small circular or rectangular (3 m diameter or 3x3 m square) often not raised from the floor (Fig 3.5). Both types of stores were well ventilated to allow for good circulation of air for further drying . The Ministry of Agriculture recommends the width of the stores not to be more than 3 m wide to allow for easy flow of air through the stored grains to facilitate quick drying. Only a few of these stores fall under these recommendations. The stores had varying widths of more than 3 m. Grain storage extension messages are passed to the farmers by the Ministry of Agriculture extension staff as evidenced by the construction of grain storage demonstration structures (Cribs) on the some of the farms in the two study areas. The crib stores (4 x 1.5 m) were raised with rat guards, wooden slat sides and bases with good aeration, metal roof, good for storing equivalent of 25 to 30 x 90kg bags (Fig 3.6). The farmers preferred to harvested their maize when completely dry and transport from the farm directly to the stores. Sun drying was not a common practice in the two divisions. However a majority of farmers tended to pick out the very best cobs for home consumption. In this case the cobs would be sun-dried and stored separately. For the rest of the cobs sun drying was practised when harvested maize cobs were rained on during the harvesting day. The maize was dried for 1-2 days then stored. Before putting the maize in the stores, the store was cleaned and actellic (Pirimophos methyl D) applied on the floor and the walls.

Figure 3.3 : Harvesting of maize by cutting the stalks to the ground followed by hand picking

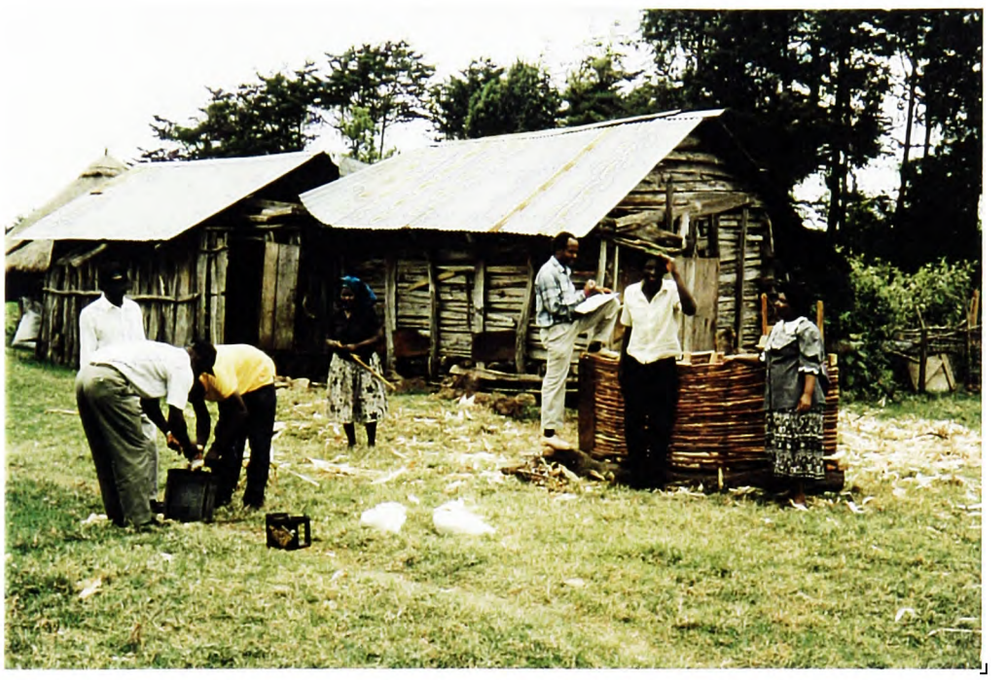


Figure 3.4 : Typical maize storage structure on a large scale farm in Tongaren Division

Figure 3.5 : Traditional storage structure for maize inTongaren Division

Figure 3.6 . Maize crib built as a demonstration on the farm in Tongaren Division.

Figure 3.7 : Stooking method of drying maize in the field

Figure 3.8 · Maize storage structure built out of maize stalks and grass

Figure 3.9 Harvesting on the farm at Tongaren

The maize was stored on cobs in outside stores for a varying period of 1-3 months. The period was dictated by the need to sall for cash. Actellic was not applied on maize grain meant for immediate sale. However shelled maize grain for storage at the farm for longer periods was treated against weevils before storage. Most farmers stored shelled maize inside their houses for future sale or for food. The maize was stored in stacks on the floor in the houses. Shelling was either done by hand, hand driven Sheller, or tractor mounted shellers. The latter two were available for hire.

Store types were coded to determine the types of stores present in the area. Coding for the type of stores is shown below. The most common storage structure was RAUI i.e. rectangular, made up of slats on the sides, raised and roofed with iron sheets (Table 3.8).

Shape: C= Circular, S = Square, R= Rectangular,

Walls: W = Wooven, A = Slats and or wire, M= Mud

Floor: U = Raised up, Raised but covered in mud(filled),G= on ground

Roof: T= Thatch, I = Iron, H = Inside dwelling, C = Crib

Table 3.8. Type of stores in Kapsabet and Tongaren Divisions

Type of store	No. of farmers	Average capacity (90kg bag-shelled)	Percentage of total
RAUI	27	135	75
RAFI	2	100	5.6
RAUIM	2	-	5.6
SAUT	2	80	5.6
RAUT	2	100	5.6
CAUI	1	40	2.8

3.4.9 Drying methods

In Tongaren Division maize was left to dry in the field for 4 weeks after physiological maturity. The maize was left to dry *in situ* or was cut and stooked for further drying (Fig 3.7). The maize was harvested and put directly in the stores where it was left for further drying. However 12% of the farmers in Tongaren sun-dried all or some of their maize cobs, citing conditions of high moisture of the cobs or cobs rained on during harvesting day. There were extreme differences in the two Locations of Tongaren Divisions, in Tongaren Location (80%) of the farmers interviewed were found stooking their maize whereas in Mbakalo Location only 10% were stooking their maize. One farmer indicated that sometimes he may remove the cobs from the store and sun dry for one or two days depending on the moisture levels in the stores. The time for storage of cobs before shelling was variable from 14 days to 5 months. Eighty percent of the farmers stored their maize for 2-3 months. The group interview in Tongaren revealed that early shelling was possible if there were pressing needs to sell and get cash. Storage for 5 months was rare. Kernel drying was common among some farmers, in Tongaren Location 50% of the farmers sun dried their shelled maize for one to two days before bagging. However sun drying was done when there is no ready market, or when the maize was intended for longer periods of storage.

3.5 Uses of maize and stovers

Maize was the main source of dietary carbohydrate. Data obtained from individual interviews and groups showed that maize was ground into floor and made into "Ugali" that was eaten with vegetables, meat or bean stew. This was the main dish for the people and may be eaten twice a day for lunch and dinner. The flour was

also used for making porridge. Maize was mixed with beans and boiled, a dish commonly referred to as "Githeri" or "Maenjera". Green maize was boiled or roasted. These farmers preferred to keep two bags of shelled maize for every member of the household per year. This information was confirmed by the extension officer for the area as the average recommended by the Ministry of Agriculture. Maize stovers had various uses on the farm but most of it was left in the field to rot and incorporated into the soil during ploughing. It was a common practice to leave livestock to graze the stovers in the field after harvest. Only an isolated case of stover burning in the field was reported. Stovers were sometimes removed from the field and stored for feeding animals during the dry season. Cases of stovers being carried from the field as feed and bedding in cattle 'bomas' was common. This later formed manure for use on the farm. Dried stovers were also used as domestic fuel for cooking in some homes and used in building structures on the farms such as chicken houses and maize stores (Fig. 3.8)

Figure 3.10 Seed distribution for on-farm trials in Tongaren

3.6 Production constraints

1. High cost of inputs-the prices of fertilizer and seeds was very high while the price of maize at harvest was low.

2. Timeliness of input availability -farmers reported incidence of shortage of inputs particularly at the time of planting. Sometimes they had to move long distances to fetch them. Variety H 614 that was normally in high demand at the time of planting was often in short supply. The farmers had to move long distances looking for this variety or have to settle for second choice.

3. Poor marketing - maize was marketed by the Kenya Cereal and Produce Board,but this was characterised by late payment and low prices

4. Theft on the farm - theft of maize on the farms and even in the stores was reported to be common.

5. Lack of cash or working capital was generally a problem. When the settlement schemes were established in mid 1960's up to early 1980's credit was made available through Agricultural Finance Corporation. This facility was no longer available, and interest rates were high in commercial banks.

6. Rotting of maize was considered to be a serious problem. The farmers indicated that they have lived with the problem for a very long time. Average rot of 18% were recorded. Most of the farmers were able to quantify the losses in shelled bags and the differences exhibited between varieties.

7. Communication in Tongaren was generally poor. There are many feeder roads but these are poorly maintained and impassable during the rainy season. The farmers do not have their own transport but have to hire public transport which was not readily available or expensive. Farmers cannot move their maize to fetch good

prices. Most of the maize is sold to middlemen who have transport. Kapsabet Division is served by one main tarmac road traversing the Division making the area accessible to the nearby big towns. However the feeder roads are not passable during the wet season.

8. Poor quality seed and fertilizers -the farmers complain that the seed currently supplied is poor. They complain lack of uniformity within the varieties and generally low yields of the varieties.

The farmers listed all the constraints to maize production in both areas of study. The ranking of these constraints were almost similar in both areas. Lack of cash was considered as the most important constraint in both areas. High costs of inputs such as cost of land preparation, fertilizer and seed also ranked highly. Marketing was ranked as an important problem in Tongaren but was not ranked highly in Kapsabet. Similarly communication and delivery of inputs was not a serious constraint in Kapsabet Division but were important in Tongaren Division. Ear rot was ranked seventh overall in both areas (Table 3.9).

Table 3.9. Production constraints as ranked by the farmers

Constraint	Rank	
	Tongaren	Kapsabet
Lack of cash	1	1
Marketing problems	2	5
High costs of inputs	3	2
Communications	4	0
Poor quality of seed	5	4
Delay in delivery of inputs	6	0
Rotting of maize	7	7
Theft on the farm	8	8
Availability of labour	0	6
Lack of farm machinery for hire	0	3
Land scarcity	0	9
Pests	0	10

3.7. Common Pests

Weevils were considered to be the most economically important pest, however most of the farmers used chemicals to control the pest. Actellic (Pirimiphos methyl D) was widely used and the most preferred chemical for control of weevils (Table 3.10). This was applied at the rate of 1kg to 40 bags of 90 kg of shelled maize. The chemical was also applied on cobs during the short term storage before shelling. Stem borers were considered as serious pests, especially during dry spells early in the season. The chemical used for the control of stem borer was Dipterex (Trichlophon G). The recommended rate was 12 kg per hectare.

Table 3.10. Main pests problems as perceived by farmers and control measures adopted

Pests	Farmers' control measures
Stem borers	DDT, Trichlophon, ash, urea
Weevils (in store)	Pirimiphos methyl, Malathion
Locusts	None
Birds (Weavers)	Scaring
Rodents	Trapping
Army worms	None
Termites	None

3.8 Common diseases

The common diseases reported in the study areas were maize ear rot, maize streak, smut and stalk rot (Table 3.11). Maize ear rot was the most important disease problem in the two study areas. In 1995, Tongaren Division experienced a high incidences of maize ear rot. However in Kapsabet Division, there was no specific year that ear rot was considered more or less serious. Thirty percent of the farmers indicated that stalk rot was a problem on their farms, the rest did not recognize it as

problem or were not aware of it. However during the group interview most of the farmers indicated that it was a problem.

Table 3.11. Main disease problems as perceived by farmers and control measures adopted

Disease	Farmer's control measures
Ear rot	Early planting and harvesting, variety
Streak	Roguing
Smut	Roguing
Stalk rot	None

Some of the causes of ear rot cited by the farmers are given below.

1. Heavy rainfall. Rainfall was considered as an important factor influencing ear rot levels in the field especially during crop maturity and harvesting.

2. Lodging was also associated with heavy rotting of cobs. It was caused by strong winds but was also considered to be a varietal factor.

3. Mixing of rotten cobs with healthy cobs during harvesting caused cross contamination. The rotten cobs have high moisture content and if kept together with the other cobs, spread the rotting within the whole lot.

4. Poor storage of maize cobs also result in high incidence of rotting, such factors cited included storing of maize at high moisture content, poorly ventilated of the stores and leaking roofs.

5. Late planting of maize-farmers revealed that late planted maize has a high incidence of ear rotting than early planted maize.

6. There were varietal differences to ear rot resistance within the recommended varieties for both areas. The farmers were aware of these differences and pointed out that, H614 showed less ear rot than H625 and H626, variety H622 was considered to be the most susceptible.

7. Poor timing of harvesting - delayed harvesting was associated with high rotting incidence. Most farmers preferred to stop harvesting and leave the maize in the field rather than harvest when wet. Wet days are normally avoided so that maize with high moisture is not carried to the stores.

8. Methods of harvesting - some methods of harvesting were not preferred because they are associated with high incidence of ear rot, stooking is not preferred particularly in wet seasons because it encourages ear rot.

9. Pests-particularly insect and bird damage were considered to increase rotting of maize due to opening at the tips allowing water to accumulate inside the ears. Stalk borers were also considered to increase ear rot damage.

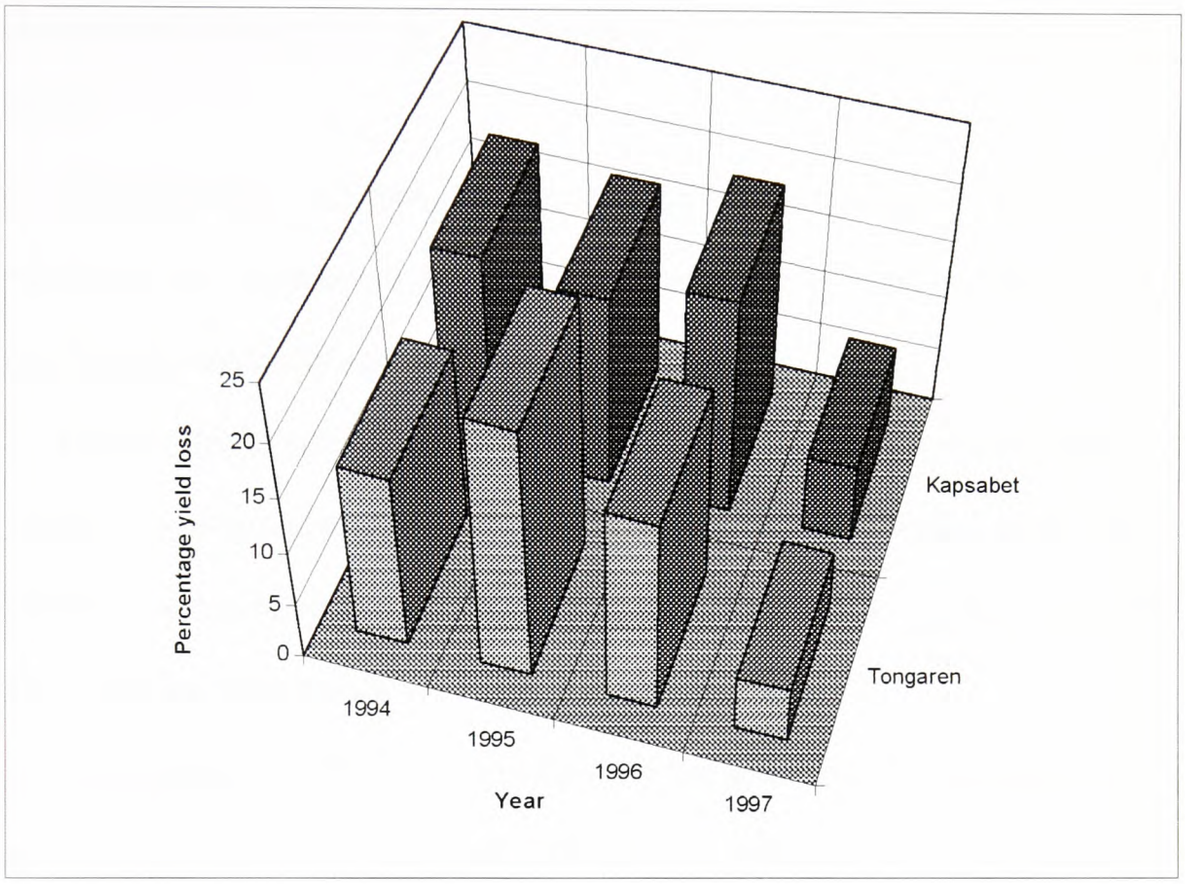
3.9 Yield losses due to ear rot

Farmers were asked to give yield losses associated with maize ear rot for the last three years (1994, 1995, 1996) on their farms. The farmers gave the yield of good maize and rotten maize in bags harvested each year. In Tongaren Division, 83% of the farmers interviewed gave the yield losses for 1996, 71% could remember the yield losses for 1995 and only 37% could remember the yield losses of 1994. Yield losses due to maize ear rot was computed for the three years. The farmers in both areas determine their yields after shelling but could also make quick estimates from the number of unshelled bags harvested or number of ox- carts or trailer loads carried from the field during harvesting. The estimate for yield loss in Tongaren for 1996 averaged 17.4%. The farmers in Tongaren pointed out that they had the highest losses due to maize ear rot in 1995, the average yield loss for this year was 22.7% and in 1994 was 15.7% (Fig 3.11). The mean yield loss estimated for the three years in Tongaren was 18.6%. The 1997 estimates for ear rot were perceived

to be very low, this was at the beginning of the harvesting period. It was generally dry during the harvesting period and the farmers generally expected the rotting to be less.

In Kapsabet the yield losses associated with maize ear rot for the years 1996, 1995, 1994 was estimated to be 19.4%, 17.2% and 18.3% respectively (Fig 3.11). The farmers did not single out a year when the ear rot problem was particularly more serious. The mean yield loss computed for the three year period was 18.3%. In Kapsabet division only 9% of the farmers gave the yield losses due ear rot for the past three years, 57% for the past two and 76% could remember the losses for the previous season.

Figure 3.11. Yield loss due to ear rot in Tongaren and Kapsabet Divisions estimated for 1994, 1995, 1996 and 1997



3.10 Strategies to minimize ear rot

Farmers were asked to give some of the factors or practices that they thought had an impact in reducing ear rot on their farms. The potential impact of these practices in reducing ear rot incidences were discussed in group interviews. These were listed but are not ranked.

1. Early planting of maize. Farmers pointed out that early planted maize had less of an ear rot problem. Although the harvesting of early planted maize will coincide with onset of the short rains, there was less rotting of the cobs. They associated this with less incidence of lodging.

2. Early harvesting tended to minimize losses due to rotting. There was excessive rotting of the cobs in the field if the harvesting was delayed.

3. Sorting out diseased cobs from clean cobs immediately after harvesting to avoid cross contamination of the cobs was considered useful in reducing rotting in the stores.

4. Good storage management. The farmers considered harvesting of cobs when sufficiently dry or drying before storage, and well-ventilated stores with no leaking roofs as important in minimizing rotting in the stores.

5. Variety. The farmers preferred H 614 to the rest of the varieties due to less rotting. It was also considered to be less prone to lodging and has the characteristic of the ears drooping (declining) when drying in the field. H622 was considered to be the most susceptible to ear rot.

6. Crop management. The farmers pointed out that good crop management with good land preparation, weeding, adequate fertilizer, will result in less ear rotting. The reasons cited were, well-managed crop has strong stalks that are less prone to lodging and produces big cobs that bend downwards upon maturing which reduces rotting.

3.12 Uses of rotten cobs

Rotten cobs were used as feed for livestock, especially cattle, in the two study areas. The rotted maize was either ground shelled or unshelled, mixed with mineral salts and fed to cattle. This was particularly given to dairy cows during milking. The farmers who had no animals sold their rotten cobs to other farmers for livestock feed or for brewing purposes. Rotten maize was used by the local people to prepare local Gin (Chang'a) which is sold in some homes. In Tongaren, 6% of the farmers used

the rotten maize for brewing compared to 7% in Kapsabet (Fig 3.12 and 3.13). The proportion of farmers using rotten maize to feed livestock was 65% in Tongaren and 80% in Kapsabet.

Rotted maize was stored for longer periods and fetched better prices during the month of May when maize was scarce on the market. Some farmers preferred to store their rotted maize until May and had separate stores for rotted cobs. The price of rotten maize during May was found to be twice the price of good maize at harvest (October). During the survey period in May we found rotten maize being sold at the local market (Kimilili) by local traders who buy from nearby farms for sale at the market. The traders revealed that, farmers preferred to store the maize until such period to get good prices. This maize sold at the market was mainly bought by Chang'a distillers. The price at this market was Ksh. 34 and Ksh. 45 per 2kg tin for rotten and good maize respectively. The price for the same at the harvesting period was Ksh.6 and Ksh.15 for rotten and good maize respectively. In Tongaren some farmers sold their rotten maize to local feed manufacturing firm for making animal feeds.

Fig. 3.12. Uses of rotten maize in Tongaren Division.

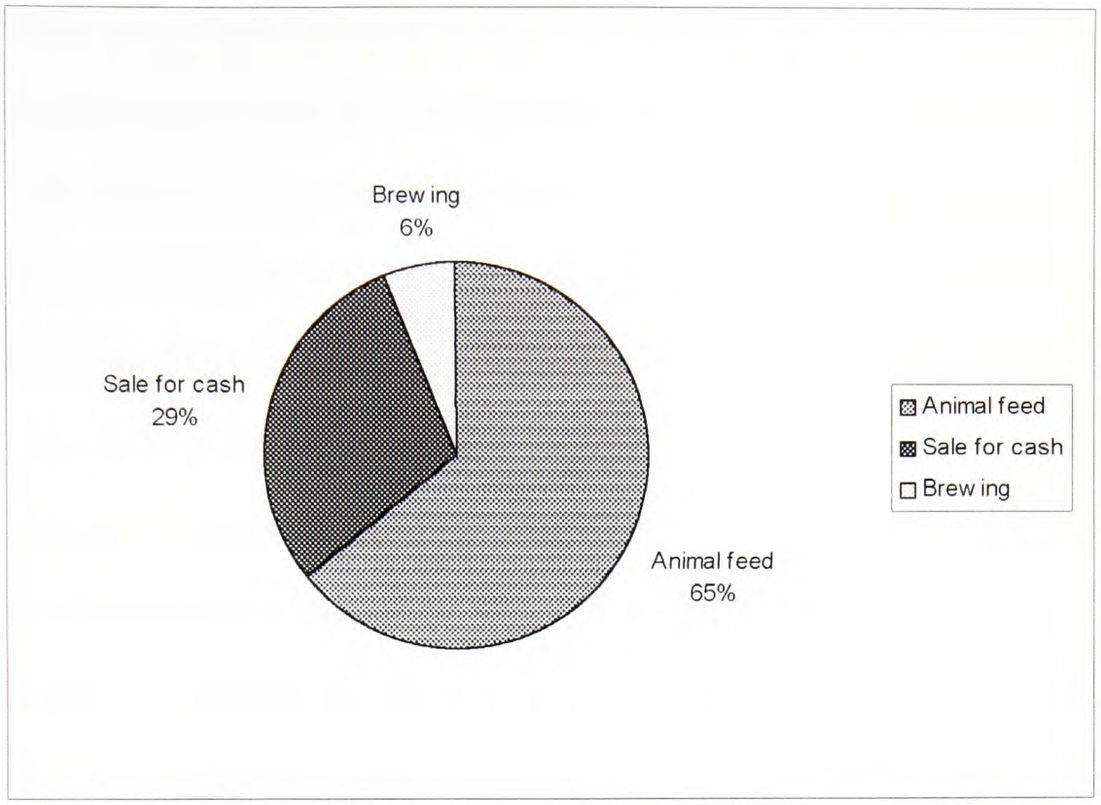
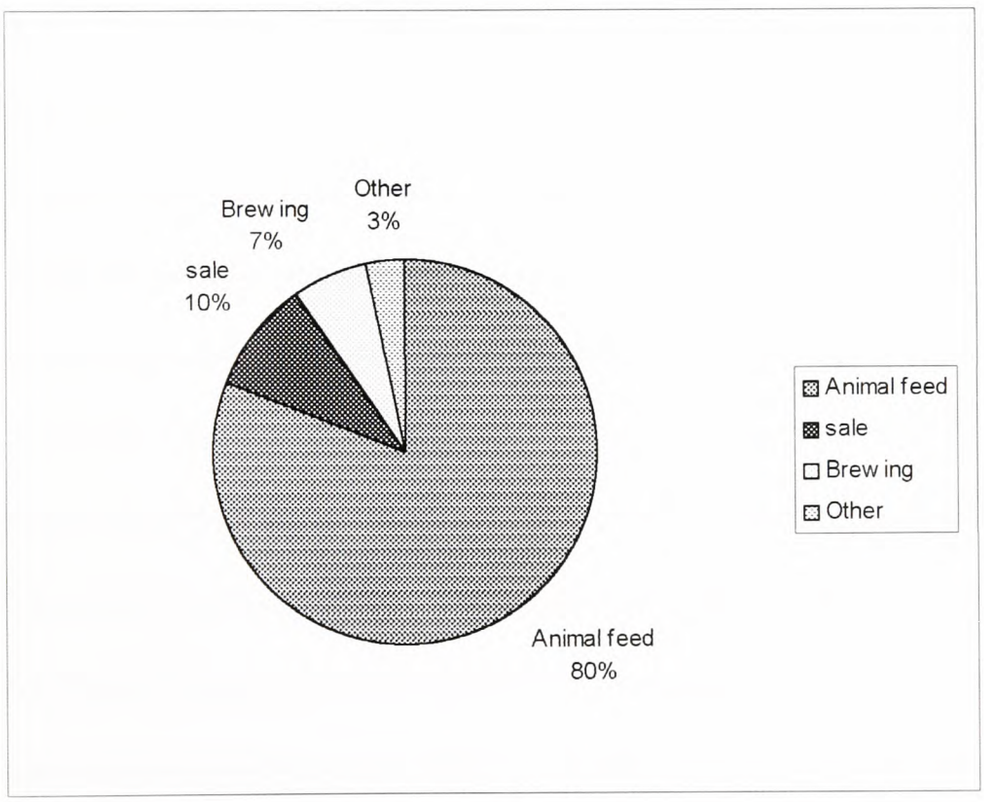


Figure 3.13. Uses of rotten maize in Kapsabet Division



3.12 Discussion

Maize was the most important crop both in Tongaren and Kapsabet Divisions. It is the main source of dietary carbohydrate and main source of income to the communities living in these areas. Bean (*Phaseolus vulgaris*) was the second most important crop, with 60 % of the farmers intercropping maize and beans in the two Divisions. The hybrid 600 series (Kenya seed company-KARI varieties) were the most preferred maize cultivars, with more than 90% of the crop in the two districts surveyed, being planted with the hybrids H614, H625 and H626. The main reasons for favouring H614 appeared to be its relatively high yielding capacity, without having much demand for fertilizer. The fact that it was resistant to rotting and weevils were also regarded as important. Cob rotting was ranked by the farmers as the most important crop protection problem, although it was ranked seventh as a production constraint after a number of issues relating to markets and access to inputs. Stem borers, followed by weevils were ranked as the most important pest problems.

The two most important livestock enterprises were cattle and poultry. Both dairy cattle and oxen for ploughing were kept in both areas. Livestock, particularly dairy cattle were found to consume proportionately large quantities of the rotten maize produced in these areas. In Kapsabet and Tongaren Divisions 80% and 65% of the rotten maize produced in these areas was fed to livestock respectively.

The farmers were not aware of the possible risks associated with mycotoxins. Ear rot fungi are associated with a number of mycotoxicoses that cause human and animal diseases (Julian *et al.*, 1995). In livestock mycotoxicoses associated with grain moulds include equine leukoencephalomalacia (ELEM), an acute neurological

disease of horses and donkeys (Marasas *et al.*, 1988), pulmonary oedema in swine (Ness *et al.*, 1991, Lenn *et al.*, 1990, Bill and Lenn 1991, Javed *et al.*, 1991), Mystery disease of swine (David, *et al.*, 1992) and mortality in chicks and chick embryos (Javed *et al.*, 1993a, Javed *et al.*, 1993b).

Rotten maize was used for brewing in both areas, 6 % and 7 % of rotten maize was used for brewing in Tongaren and Kapsabet respectively. The amount of rotten maize used for brewing could be higher than this as most of the farmers did not want to be associated with brewing because brewing in homes is illegal. Although the local gin made from the brewed maize is less likely to contain mycotoxins, some of the brew was drunk before distillation, posing a danger of mycotoxin exposure. Busaa, a popular local beer is made from maize. It is prepared and sold in homes. It is made from maize of poor quality compared to maize normally used for food in homes. There are risks of mycotoxins exposure to humans who consume beer made from rotten grain or flour from contaminated grain. The mycotoxin zearalenone has been found in beer made from maize and sorghum in Swaziland (Martin, 1974), West African traditional beers made from corn (Okoye, 1978) and in Canadian beers (Scot *et al.*,1993). Based on the studies conducted in Zambia, it was concluded that grain with more than 2% fungal contamination, on the basis of visual inspection, should not be consumed by humans or livestock (Marasas *et al.*,1979).

Although no report on mycotoxicoses in the two areas was available, widespread mycotoxin contamination has been reported in other parts of Kenya ((Muraguri *et al.*, 1982, Ngindu *et al.*, 1982 and Manwiller, 1987). The highest rate of aflatoxin exposure in Kenya has been found to be in the Western highlands and central province (Autrup *et al.*, 1987). Epidemiological studies in South Africa have

indicated the relationship between exposure to fumonisin-contaminated corn and oesophageal cancer in humans (Marasas *et al.*, 1981).

Farmers in both localities identified various factors or practise that they considered important in reducing ear rot. These included varieties, general crop management, harvesting and post harvesting handling. The most important factors were the nature of the cultivar, particularly the morphological characteristics and the weather conditions just before harvest. It was very clear that the farmers were aware of the best varieties in terms of ear rot resistance. Variety H614 was considered to be the best to ear rot resistance and H 622 was the most susceptible. This information was very consistent between farms in the two study areas. Hybrid 614 was released earlier than H625 and H626. The later two have a higher yield potential but H614 still remains the most popular to date. It is clear from the results of this survey that one of the attributes of this variety is resistance to ear rot.

The importance of morphological characteristics of the variety in contributing to ear rot resistance was a frequent factor in both group and individual discussions in both areas of study. The farmers preferred varieties whose ear droops at physiological maturity. This was considered to reduce the amount of water accumulating in ears when it rains and therefore reducing rotting. H614 was considered to have this characteristic. Studies by Fereira *et al*, (1994) revealed some association between ear declination (drooping) and ear rot resistance to *Stenocarpella maydis.*

The coverage of the ear by the husk was considered to be important in minimizing ear rot. Variety H622 was considered the most susceptible to ear rot, and this variety has open ear tips. The farmers preferred varieties with closed ear tips. Short varieties were also preferred to tall varieties because tall varieties are prone to

lodging. H625 was considered to have weak and thin stalks that make it prone to lodging. Lodged stalks with cobs touching the ground provide an opportunity for ear damage. To produce a suitable variety, this information is important to plant breeders to be included in the selection criteria.

The farmers were not aware of the role of crop debris as a source of inoculum for the fungi causing ear rot. The principal source of inoculum for the ear rot fungi is considered to be the host debris such as old corn stocks, ears and stubble left on top of the soil (Trenholm *et al.*, 1989). Debris as a source of inoculum may not be an important factor in the region under study because it has predominately one season of maize crop and little residue may be left in the field by the next planting. The common practice in the area under study was to leave the stovers in the field where they were grazed on and the rest left until the beginning of the new season and ploughed in the soil. Some farmers prefer early ploughing in December which gives time for the decomposition of the stovers in the field before the next planting season which start in the month of March. However, the role of debris in ear rot incidence in this region has not been established. Studies have shown that to reduce the inoculum, it is important to plough the crop debris into the soil soon after harvesting (Martin and Johnson, 1982; Teich and Hamilton, 1985). If trash is left on the field after harvesting, old infection can be carried over to the next year. Farmers gave other alternative uses of stovers such as fuel for cooking, building maize stores and converting into manure. Although this could not be quantified, the amount was relatively small. A few individuals indicated that at one time or the other they burn the stovers in the field but the group disputed this as not a common practice.

Farmers in both locations predominantly use certified seed from the seed companies. The role of seed transmission in the region may not be important as farmers predominantly used certified seed. In Canada, it was found that, planting of infected grain of corn and wheat gave rise to diseased seedlings, but high quality and fungicide-treated seed reduced the seed-borne inoculum (Trenholm *et al.*, 1989). It is unlikely that seed could be an important source of inoculum in Kenya. Weather factors, especially rainfall are considered as an important factor in ear rot incidences. The farmers associated the years of heavy rainfall, especially during the main harvesting periods, to high incidence of ear rot. The two areas have bimodal rainfall, the second rains may coincide with the peak harvest. In both the individual and group interviews, farmers associated the years with most ear rot with heavy rainfall coinciding with crop maturity and harvest.

General agronomic practices were cited as important in control of maize ear rot. Early planting was considered an effective management practice to control ear rot and others include timely weeding. Effective land preparation that allows for early planting and effective weed control has been found to assist in control of *Fusarium* diseases of corn (Seaman, 1982). Weed control is important in the elimination of crop hosts of *Fusarium* (Martin and Johnson, 1982).

Rotation was not considered an important method for reducing ear rot in the field. The areas under study have one maize crop per year, but the second season maize is increasingly becoming important especially in Kapsabet Division. In Tongaren the second maize crop is normally planted in valleys and harvested as green maize for roasting and boiling. Moulds on the trash from this crop and the previous season may be carried over to the next year.

Stalk borer (*Busseola fusca*) was an important pest, from the group discussions the farmers were aware of the control measures of stalk borer. However, the routine for the application of the insecticide to control the pest is not followed, farmers apply when they observe windowing on the leaves which is probably too late to control the pest. Bird damage to cobs was also recorded but no control strategy was available. Stalk borer damage provide avenues for ear rot pathogens. Birds also shred the husks, puncture and ingest the contents of kernels. This damage predisposes the cob to infection by ear rot pathogens.

Post harvest management varied from farmer to farmer. Stooking maize in the field before harvesting during rains was considered to increase the incidence of rotting compared to harvesting when dry and immediate storage. Moisture levels at storage time and method of drying and type of stores were also considered to be important factors influencing ear rot. Poorly aerated, and leaking stores and poor drying were cited as contributing to rotting within the stores. It has been established that mould will continue to grow on freshly harvested maize unless it is quickly dried to moisture levels of 13-15% (Trenholm *et al.*, 1989).

3.14 Conclusions

1. Cob rot was regarded as the most serious biotic constraint to maize production. Losses due to maize ear rot in the areas studied averaged 18%. Previous estimate for loss due to ear rot in this region was broadly put as 30-70 %.

2. Weevils were considered the most important insect pest attacking stored grain.

3. Farmers were aware that cob rot incidence was high and were aware of varietal differences to ear rot problems.

4. The awareness of the potential risks associated with consumption of rotten maize was limited within the two communities. Most of the rotten maize produced in this area is consumed locally either as livestock feed or used for brewing. There is a risk of mycotoxin exposure to both livestock and the people living in these areas.

5. The main factor associated with ear rot incidence in the field was prolonged rainfall during the late growing of the crop and harvesting period.

6. The farmers indicated that good agronomic management, good harvesting and post-harvest handling practices decreased cob rot.

CHAPTER 4: MAIZE EAR ROT INCIDENCE IN MAIZE PLOTS IN WESTERN KENYA

4.1 Background

The maize cob is attacked by a number of fungi which cause rotting of the grain while the cobs are still in the field and the grain may continue to rot during storage. Cob rot in the field is influenced by a number of factors but tends to be significant only in areas where cobs reach maturity during periods of rainfall or high humidity. Cultivation practices and characteristics of the maize cultivar grown, particularly in relation to premature sheath split, also affect the predisposition to cob rot.

Cob rots occur during the growing season, at harvest time based on the harvesting practice or during the post harvest handling. In much of the Western Highlands of Kenya, most farmers harvest their maize in October. This period may coincide with the short rains, making harvesting difficult and resulting in high losses due to ear rot. Harvesting practices and post harvest practices in the region are quite diverse, and range from cutting and 'stooking', cutting leaving the maize to dry on the ground for varied length of time and direct harvesting from standing stalks. This coupled with post harvest handling may influence the levels of ear rot.

The objective of this survey was to provide an estimate of cob rot incidences in one District in Western Kenya and to obtain information on some of the factors associated with high cob rot incidence in order to develop a management strategy for the disease.

4.2 Methodology

The survey was carried out in Tongaren Division during the main harvesting season of October in 1998 and 1999. Tongaren is an upland area where maize is the predominant food crop and rainfall is common at crop maturity.

Farms were selected on the basis that they were growing maize which was ready to be harvested and were accessible to the road. Having contacted the farmer who provided information on size of the field and cultivar grown, thirty cobs were removed from the field by making a diagonal transect walk across the field at a spacing of 2 - 4 metres. A rough estimate was made of percentage lodging. The cobs were then returned to the homestead, the sheath removed and examined for rotting. The number of rotted cobs and severity based on the percentage of rotted grains on each cob was recorded. The number of cobs damaged by stalk borer and birds was noted. The rotted cobs were taken to the laboratory for identification of fungi associated with the rots and healthy cobs were returned to the farmer.

In the laboratory, the cobs were dried to a moisture content of 13% and each individual cob was shelled to constitute a sample. The sample was stored ready for isolation and identification of the fungal pathogen. From each sample a sub-sample of kernels was surface sterilized in 2.5% sodium hypochlorite for two minutes and rinsed twice with sterile distilled water. The kernels were then placed on glycerol agar and PDA containing 5 ml of 5% solution of chloramphenical. Five seeds were placed in each dish, replicated three times. The plates were incubated for 5 days at 25^0C. The colonies growing on the plates were grouped into colony types based on their similarities in appearance. All the colonies that were white to pink in colour were grouped as Type I, and the variations within the group were labeled Ia, Ib, Ic

etc. The pinkish to carmine red were similarly labeled as Type II and the white colonies were labeled as Type III (Section 5.3). One typical colony from each colony type was sub cultured on two plates each of PSA, NSA., and 1.5% malt extract. All the Type I, II and III were plated on PSA and NSA. The type III were put on malt extract. The cultures were placed under NUV light of alternating 12 hr. of light and darkness. The malt extract cultures were first incubated in the dark at 25^0C before being transferred to NUV light for 10 days. For every colony type its appearance on obverse and reverse side on PSA was noted. Those colonies that were put on NSA were observed under a microscope by placing 1 cm^2 agar block of the culture on a slide with a drop of water on a microscope and identified using the identification key for *Fusarium* ssp from laboratory guide for identification of *Fusarium* species by Booth (1977).

4.3 Results:

4.3.1 Survey results.

46 fields were surveyed in 1998 and cob rot incidence ranged from 0-57%. Cob rot occurred in all fields except one. The mean incidence was 22%. Stem borer damage to the cobs ranged from 0-57% with a mean incidence of 12%. At one site where the highest cob rot incidence was recorded, every rotted cob showed signs of borer damage (Fig 4.1). In many cases the borer larvae were still in the cob (Fig 4.2). The various instars of the stalk borer larvae recovered from rotted cobs were buff coloured, some with pinkish tinge and were identified at NRI as *Busseola fusca* Fuller (Fig 4.1). Cobs also showed bird damage but this did not exceed 10% and was absent

Figure 4.1 : Borer damage and borer larva in the cob after harvest

Figure 4.2 : Cobs with typical borer damage and rot at harvest

from more than half the fields. The highest incidence of cob rot damage recorded in the absence of borer damage was 23% (mean 10%).

Based on the mean scores for percentage rotted cobs and the cob rot incidence, an estimate was made of percentage rotted grain in the harvest from these fields. The range was from 0-6% with a mean score of 1.6%.

A correlation analysis was conducted on cob rot incidence against number of days after planting the crop, cob rot incidence against percentage borer damage and borer damage against number of days after planting (Fig 4.3, 4.4 and 4.5). Cob rot incidence was strongly correlated with percentage borer damage (R= 0.87) and to a lesser extent with planting date (R= 0.45). Percentage borer damage however was correlated with planting date (R = 0.60).

Figure 4.3. Association between cob rot incidence and borer damage in Tongaren Division

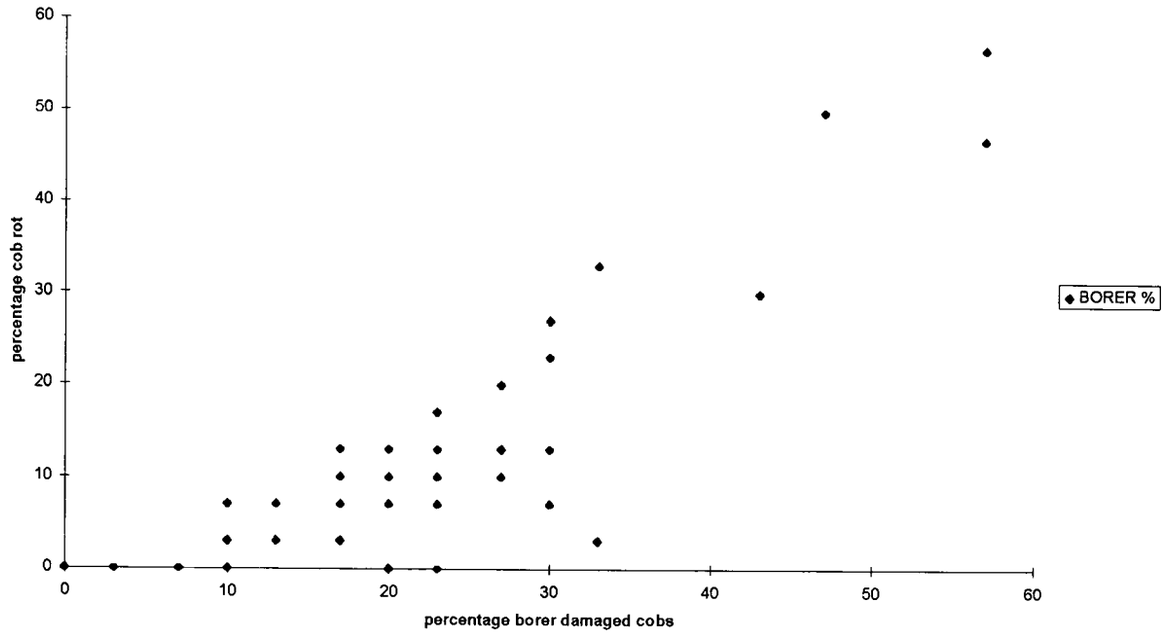


Figure 4.4. Association between cob rot incidence and days after planting in Tongaren Division

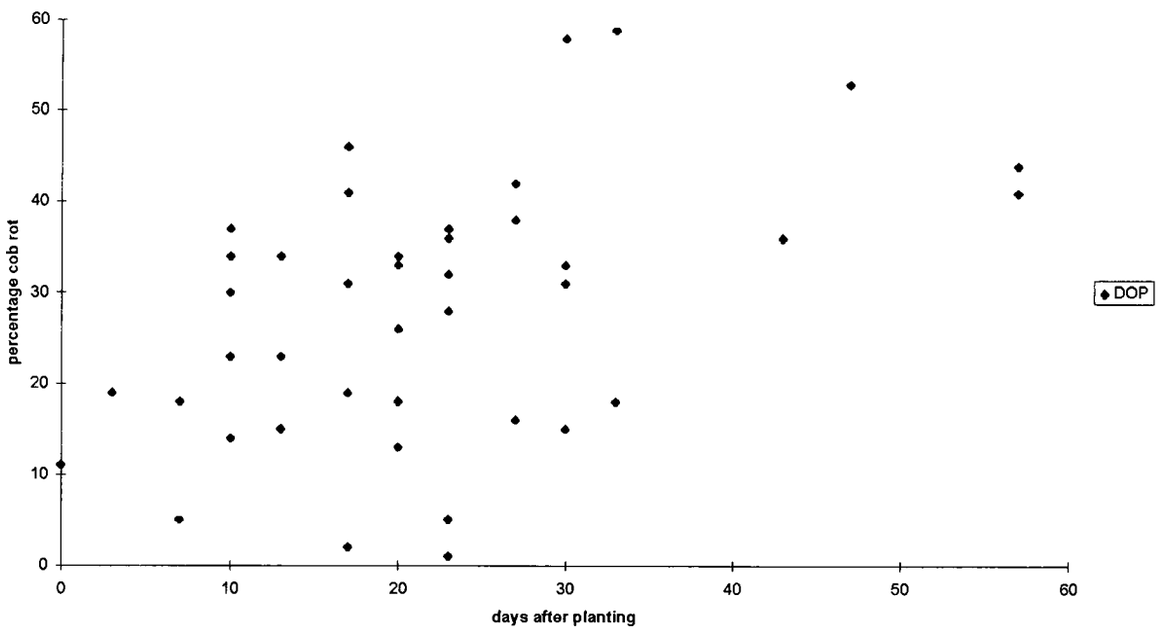


Figure 4.5. Association between borer damage and days after planting in Tongaren Division

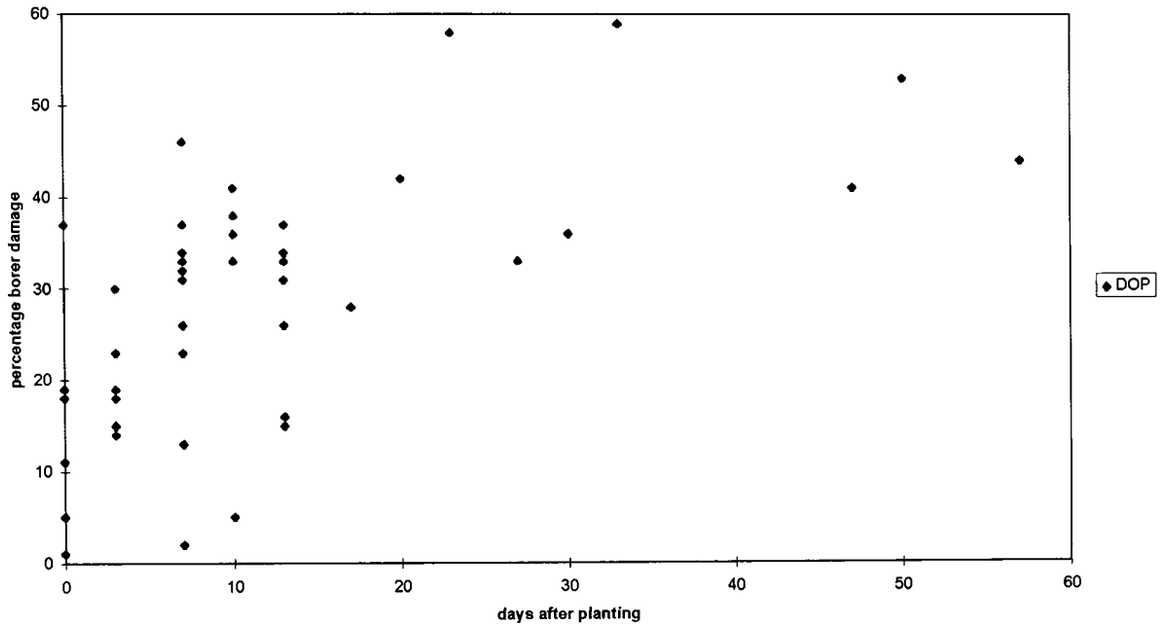


Figure 4.6 Borer damaged cob with *F. moniliforme* infection.

Figure 4.7: Cobs with typical *Stenocarpella maydis* symptoms

The mean incidence of rotten cobs for the crop planted in March, April and May was computed. The result showed that that 34% of the cobs sampled from crop planted in May were rotten compared to 19% and 18% for crops planted in April and March respectively. The incidence of borer damage was highest in the May planted crop. The incidence was 30% compared to 10% and 5% in April and March respectively. Cob rot severity did not show any particular trend, this averaged 25%. Similarly,lodging did not show any particular trend. The average in all the fields sampled was 30%. In 1998 the most predominant variety grown was H614, it was grown by 78% of the farmers, H625 by 13%, and H626 by 7%.

In 1999 cob rot incidence was found to be higher than the previous year. Rotten cobs were found in all farms surveyed. In some farms which were being harvested large heaps of rotten grain were observed being prepared for storage (Fig 4.8).

Figure 4.8 . Rotten maize cobs at the farm ready for storage after harvest

Figure 4.9 : scoring for cob rot and borer incidence during the survey.

The incidence in 1999 ranged from 40-93%. Based on the frequency distribution of the ear rot in the field, 65% of the farms had incidences in the range of 11-30% (Fig 4.10). The mean incidence was 68%. When the three most predominant varieties were compared, it was found that the highest cob rot incidence was in H625. The incidence was 82% compared to 68% and 65% in H 627 and H614 respectively. The mean incidence of borer damage was also found to be higher than the previous year. There was no single field without borer damage to the cobs. The incidence ranged from 10-83% with a mean incidence of 46%. H625 had the highest borer damage incidence of 55% compared to 43% and 46% of H627 and H614 respectively (Table 4.1). Incidence of bird damage ranged from 0-30% and was absent from only 10% of the farm. The mean incidence was 10%.

Based on the mean scores of rot severity and cob rot incidence an estimate of percentage rotted grain in the harvest from these fields was found to be higher than the previous year. This ranged from 1-37% with a mean score of 10%.

Figure 4.10. Frequency distribution of ear rot incidence in farmers' fields in Tongaren Division 1998

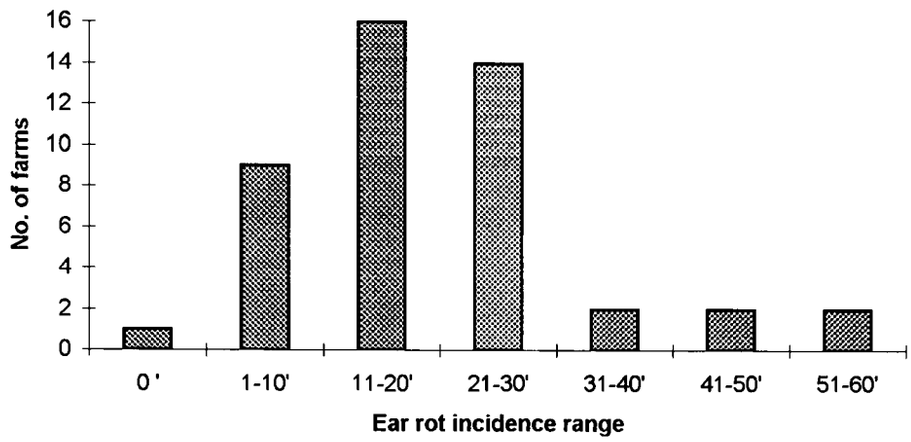


Table 4.1. Comparison of the three main varieties grown in 1999

Variety	Lodging	Rot incidence	Rot Severity	Borer Incidence	Bird damage
H627	17.4	68.2	20.3	43.4	11.9
H614	26.8	65.3	21.9	45.7	6.9
H625	20.0	81.8	18.5	55.0	12.5

Correlation analysis between cob rot incidence and borer damage was found to be positive and significant at 1% (R = 0.62). The number of days after planting was also found to be positively correlated to borer damage. The correlation was significant 1% (R = 0.42). The date of planting was also found to be significantly correlated to cob rot. The result was significant at 1% (R = 0.30). Similar trends were observed in the previous season of 1998.

In 1999 the predominant variety was H 627 which was grown by 60% of the farmers, H614 was grown by 29%, H625 was 5%, H512 and H622 was grown each by 1% of the farmers. The local and unknown varieties were represented by 4% of the overall farms surveyed.

4.3.2 Results of mycological analysis

From 46 farms surveyed in Tongaren Division, 11 farms were sampled in Naitiri, 18 and 17 in Mbakalo and Tongaren Locations respectively. A total of 295 rotted cobs were collected for laboratory mycological analysis. The cobs were visually examined and described . In the laboratory cobs, from 26 farms were analysed (Tongaren-9, Mbakalo-10 and Naitiri-7) constituting 197 cobs. From visual examination, 90 cobs had rot without any signs of visual insect damage, 100 cobs had both rot and borer damage, 14 had rot and both borer and bird damage, only 7 cobs had rot and bird damage. The most predominant fungal pathogens identified were *Fusarium moniliforme*, *Fusarium graminearum* and *Stenocarpella maydis*. From the 197 cobs analyzed, *F. moniliforme* was isolated from 77%,, *F. graminearum* on 34% and *Stenocarpella maydis* on 44% (Table 4.2). *F. moniliforme* was the most predominant pathogen on the cobs that had visible signs of borer damage, being isolated from 97% of the borer damaged cobs. It was also the predominant fungus on cobs that had no visible signs of borer damage, it was isolated on 71% of these cobs. All the cobs that had bird damage were also found to be carrying *F. moniliforme*. *F. graminearum* was found on 36% of all the cobs that had visible symptoms of borer damage, on 27% of the cobs with no visible borer damage and on 33% of cobs that had bird damage. *Stenocarpella maydis* was found on 31% of the cobs that had rot and visible symptoms of borer damage and on 58% of all the cobs that had rot and no visible borer damage. The other fungi identified were *Fusarium moniliforme var. subglutinans*, *Penicillium* spp. and other unidentified ssp. which were found less frequently.

Table 4.2. Results of mycological analysis of cobs sampled from farms in Tongaren

No.	Name of farmer	Location	Sample (Cob)	Appearance of the cob	Fungal pathogen identified
1	Susan Nakhumicha	Naitiri	Cob 1	Big cob, borer damage, rot 5%, Diplodia?	*S. maydis, Penicillium ssp.*
			2	Big cob, borer damage, pinkish rot 5%,	*F graminearum, F. moniliforme*
			3	Big cob, borer damage, rot 15%,	*F. moniliforme, F. graminearum*
			4	Small size cob, borer damage, rot 15%	*F. moniliforme, S. maydis*
			5	Medium size cob, borer damage, rot 10%	*F. moniliforme*
			6	Big cob, borer damage, rot 5%,	*F. moniliforme*
			7	Medium size cob, borer damage, rot 20%	*F. moniliforme, F. graminearum, Penicillium ssp.*
			8	Big cob, borer damage, rot 10%,	*F. moniliforme, S. maydis*
			9	Big cob, borer damage, rot 15%	*F. moniliforme, S. maydis*
			10	Big cob, borer damage, rot 20%	*F. moniliforme, S. maydis*
2	Prisca Masicha	Mbakalo	Cob 2	Big cob, borer damage, rot 5%	*F. moniliforme*
			3	Small cob, rot 90%	*F. moniliforme, F. maydis*
			4	Big cob, borer damage, pinkish rot, scattered kernels 80%	*F. moniliforme, F. graminearum, S. maydis, Penicillium ssp*
			5	Small cob, borer damage, pinkish rot 25%	*F. graminearum, F. moniliforme*
			6	Big cob, borer damage, rot 15%	*F. moniliforme, F. moniliforme var. subglutinans*
			7	Medium cob, borer damage, pinkish rot 25%	*F. graminearum, F. moniliforme*
			8	Small cob, rot 10%	*S. maydis, F. moniliforme*
			9	Medium cob, borer damage, rot 20%	*F. moniliforme*

3	Moses Mwanja	Naitiri	Cob 1	Small cob, 20% rotted	*F. graminearum, F. moniliforme*
			2	Small cob, 10% rotted	*F. graminearum, F. moniliforme*
			4	Medium size cob, rot 5%	*F. moniliforme*
4	Waswa Kulova	Tongaren	Cob 1	Medium size cob, scattered kernels, rot 15%	*F. moniliforme*
			2	Medium size cob, borer damage rot 10%	*F. moniliforme*
			3	Medium size cob, borer damage rot 85%	*F. moniliforme, F. graminearum*
			4	Medium size cob, rot 20%	*F. graminearum, F. moniliforme var. subglutinans*
			5	Medium size cob, borer damage rot 10%	*F. moniliforme*
			6	Medium size cob, rot 35%	*S. maydis*
5	John Barasa	Tongaren	Cob 1	Small cob with 25% pinkish rot	*F. graminearum, F. moniliforme*
			Cob 2	Big cob with borer and bird damage, rot 20%	*F. moniliforme*
			Cob 3	Big cob with borer and bird damage, rot 20%	*F. moniliforme*
			Cob 4	Pinkish rot 10%, borer damage	*F. graminearum, F. moniliforme*
			Cob 5	Borer and bird damage, pinkish rot 15%	*F. graminearum, F. moniliforme*
			Cob 6	Medium sized cob , borer damage, 5% rot	*Fusarium moniliforme, S. maydis*
			Cob 7	Spotted rotten kernels 60%, borer damage	*F. moniliforme*
			Cob 8	Borer damage, rot 20%	*F. moniliforme, S. maydis*
			Cob 9	Borer damage, rot 15%	*F. moniliforme, S. maydis*
6.	Boaz Mukhwana	Tongaren	Cob 1	Rotten kernels spotted on the cob, 40%	*F. moniliforme*
			Cob 2	Small cob, rot 40%, Diplodia?	*S. maydis, F. moniliforme*
			Cob 3	Completely rotten, pinkish rot 100%	*F. graminearum, F. moniliforme var.*

					subglutinans
			Cob 4	60% of kernels rotten spotted on the cob	*F. moniliforme*
			Cob 5	Medium size cobs, pinkish rot 20%	*F. graminearum, F. moniliforme*
			Cob 6	Borer damage and rot 25%	*F. moniliforme, F. graminearum*
7.	Pius Wanyonyi	Tongaren	Cob 1	Small cob, 10% rotted	*F. graminearum*
			2	Small cob, 20% rotted, borer damage	*F. moniliforme*
			3	Small cob, 30% rotted	*S. maydis, F. moniliforme*
			4	Medium size cob, 25% rotted, pinkish rot, borer damage	*F. graminearum, F. moniliforme*
			5	Small cob, 15% rotted, Diplodia?	*S. maydis*
			6	Small cob, 20% rotted, borer damage	*F. moniliforme, S. maydis*
			7	Small cob, 15% rotted, borer damage	*F. moniliforme, S. maydis*
			8	Small cob, 20% rotted	*S. maydis*
			9	Small cob, 50% rotted	*S. maydis*
			10	Small cob, 80% rotted	*F. moniliforme*
			11	Small cob, 15% rotted	*F. moniliforme*
			12	Small cob, 5% rotted	*F. moniliforme, F. graminearum*
			14	Small cob, 40% rotted	*F. moniliforme, F. graminearum*
			15	Big cob, 20% rotted	*S. maydis*
8	Catherine Nelima		Cob 1	Big cob, borer damage, rot 15%	*F. moniliforme, S. maydis*
			2	Big cob, borer damage, rot 10%	*F. moniliforme, S. maydis*
			3	Small cob, borer damage, rot 5%	*F. moniliforme*
			4	Small cob, borer damage, pinkish rot 60%	*F. graminearum*
			5	Small cob, borer damage, pinkish rot 50%	*F. moniliforme, F. graminearum*
			6	Small cob, borer damage, rotted kernels scattered on the cob, rot 40%	*F. moniliforme*
			7	Medium cob, borer damage, pinkish rot 35%	*F. graminearum, F. moniliforme*

9	Wanyama Muganda	Tongaren	Cob 1	Big size cob, rot 5%	*F. moniliforme*
			2	Big size cob, rot 10%	*F. graminearum*
			3	Big size cob, borer damage, bird damage, pinkish rot 60%	*F. graminearum*
			5	Big size cob, rot 5%	*F. graminearum, F. moniliforme*
			6	Big size cob, bird damage, rot 65%	*F. moniliforme*
10	Edward Makhine	Naitiri	Cob 1	Medium cob, rot 5%	*S. maydis*
			2	Small cob, rot 20%	*F. graminearum, F. moniliforme*
			3	Small cob, rot 20%	*F. graminearum, F. moniliforme*
			4	Small cob, rot 5%, Diplodia?	*S. maydis*
			5	Small cob, rot 30%	*S. maydis, F. moniliforme*
11	Robert Masinde 1	Naitiri	Cob 1	Small cob, bird damage, borer damage spotted rotted kernels 25%	*F. graminearum, F. moniliforme*
			2	Small cob, bird damage, borer damage spotted rotted kernels 25%	*F. moniliforme*
			3	Small cob, 10% rot, Diplodia?	*S. maydis*
			4	Small cob, bird damage, borer damage pinkish rot 100%	*F. moniliforme, F. graminearum*
			5	Small cob, borer damage rotted kernels 25%	*F. graminearum, F. moniliforme*
			6	Small cob, borer damage, spotted rotted kernels 70%	*F. moniliforme*
			7	Small cob, spotted rotted kernels 70%	*F. moniliforme*
			8	Small cob, pinkish rot 30%,borer damage	*F. graminearum, F moniliforme var. subglutinans*
			9	Small cob, rotten at the tip 20%	*F. graminearum, F. moniliforme var. subglutinans*
12	Khamala Sitati	Mbakalo	Cob1	Medium cob, rot 5%	*S. maydis, F. moniliforme*
			2	Medium cob, rot 15%	*F. graminearum*
			3	Small cob borer damage, rot 30%	*F. moniliforme, S. maydis*
			4	Small cob, borer damage, rot 15%	*F. moniliforme, S. maydis*
			5	Medium cob, rot 30%	*S. maydis, F. moniliforme*

			6	Small cob, rot 5%. Diplodia?	*S. maydis*
			7	Medium cob, rot 30%	*S. maydis*
13	David Mukaya	Mbakalo	Cob 1	Big cob, rot 10%	*F. graminearum, F. moniliforme*
			2	Big cob borer damage, rot 15%	*F. moniliforme*
			3	Medium cob, borer damage, 25% rot	*F. graminearum, F. moniliforme*
			4	Medium size cob, 40% rot, Diplodia?	*S. maydis, F. moniliforme*
			5	Medium size cob, 40% rot	*F. graminearum, F. moniliforme*
			6	Big cob, 5% rot	*S. maydis*
14	Siangu Khalago	Mbakalo	Cob 1	Medium size cob, 5% rot	*S. maydis*
			2	Small cob, 5% rot, bird damage	*F. moniliforme, S. maydis*
			3	Big cob, 5% rot, bird damage	*F. moniliforme, S. maydis*
			4	Medium size cob, 5% rot	*F. graminearum*
			5	Small cob, 20% rot, Diplodia?	*S. maydis, F. moniliforme*
			6	Small cob, 20% rot, borer damage	*F. moniliforme, F. graminearum*
			7	Big cob 20% rot, bird damage, borer damage.	*F. moniliforme, F. graminearum*
			8	Medium cob, borer damage, 15% rot	*F. moniliforme, F. graminearum*
			9	Medium cob, borer damage, 20% rot	*F. moniliforme, F. graminearum*
15	Philip Olugulu	Mbakalo	Cob 1	Medium cob, borer damage, rot20%	*F. moniliforme, F. graminearum*
			2	Big cob, rot 20%	*S. maydis*
			3	Small cob, borer damage, pinkish rot 30%	*F. moniliforme, F. graminearum*
			4	Big cob, borer damage, rot 5%	*F. moniliforme*
			5	Big cob, borer damage, rot 10%	*F. moniliforme, S. maydis*
			6	Big cob, borer damage, rot 35%	*F. moniliforme, F. graminearum*
			7	Big cob, rot 5%	*F. moniliforme*
			8	Big cob borer damage, rot 5%	*F. moniliforme*
			9	Medium cob, borer damage, rot 30%	*F. moniliforme, F. graminearum*
			10	Big cob borer damage, rot 20%	*F. moniliforme, F. graminearum*
			11	Medium cob, borer damage, rot 10%	*F. moniliforme, S. maydis*
			12	Medium cob, borer damage, rot 5%	*F. moniliforme*
			13	Big cob, borer damage, rot 10%	*F. moniliforme, F. graminearum*

			14	Big cob, borer damage, rot 20%	*F. moniliforme*
			15	Medium cob, borer damage, rot 5%	*F. moniliforme, S. maydis*
			16	Big cob, borer damage, rot 35%	*F. moniliforme, S. maydis*
			17	Medium cob, borer damage, rot 30%	*F. moniliforme, S. maydis*
16	Haroun Wekesa	Mbakalo	Cob 1	Small cob, 60% rotten, Diplodia?	*S. maydis*
			2	Medium size, cob 20% rotted, Diplodia?	*S. maydis*
			3	Medium size cob, 20% rotted, Diplodia?	*S. maydis*
			4	Small cob, 5% rotted	*S. maydis, F. moniliforme*
			5	Small cob, 60% rotted, Diplodia?	*S. maydis*
			6	Medium size cob, 5% rotted, borer damage.	*F. moniliforme*
			7	Small cob, rot 30% rotted, Diplodia?	*S. maydis, F. moniliforme*
-			8	Small cob, 80% rotted	*S. maydis, F. moniliforme*
			9	Small cob, 10% rotted	*S. maydis, F. moniliforme*
			10	Medium size cob, 5% rotted	*F. moniliforme, F. graminearum*
17	Simon Wanjala	Mbakalo			
			Cob 1	Medium sized cob, 30% rotten, no insect or bird damage, likely affected by Diplodia	*S. maydis, F. moniliforme, Penicillium ssp.*
			Cob 2	15% rot with borer damage	*F. moniliforme, F. graminearum*
			Cob 3	50% rotten, rotten kernels spotted on cob, borer damage	*F. moniliforme, F. graminearum*
			Cob 4	10% rotten, rotten kernels spotted on cob, no borer damage	*F. moniliforme, F graminearum*
			Cob 5	Small cob, 5% rotten, bird damage	*F. moniliforme, S. maydis*
			Cob 6	5% spotted rotten kernels, borer damage	*F. moniliforme*
18	Loyce Nakitare	Mbakalo	Cob1	Small cob, 20% rotted (Diplodia)	*S. maydis*
			2	Big cob, 25% rot, borer damage	*F. moniliforme*
			3	Medium cob, rot 15% , borer damage	*F. moniliforme, F. graminearum*

			4	Small cob, rot 10%	*F. moniliforme, S. maydis*
			5	Big cob, borer damage, rot 5%	*F. moniliforme, S. maydis*
			6	Medium cob, borer damage, rot 5%	*F. moniliforme*
			7	Small cob, borer damage, rot 15%	*F. moniliforme, S. maydis*
			8	Small cob, borer damage, rot 75%	*F. moniliforme*
			9	Medium cob, borer damage, rot 40%	*F. moniliforme, F. graminearum*
19	Robert Masinde	Naitiri	Cob 1	Medium size cob, borer damage, rot 15%	*F moniliforme*
			2	Medium size cob, rot 15%, Diplodia?	*S. maydis*
			3	Medium size cob, rot 25%, Diplodia?	*S. maydis, F. moniliforme*
			4	Medium size cob, 25% rot at the tip.	*F. graminearum, F. moniliforme*
			5	Medium size cob, pinkish rot at the tip 25%.	*F. graminearum, F. moniliforme*
			6	Medium size cob, borer damage, pinkish rot 85%	*F. moniliforme, F. graminearum*
20	Simon Wanjala	Simon Wanjala	Cob 1	Medium size cob, rot 30%,Diplodia?	*S. maydis*
			2	Small cob, borer damage, rot 15%	*F. moniliforme*
			3	Small cob, rot 50%, Diplodia?	*S. maydis*
			4	Small cob, rot 10%	*F. moniliforme, S. maydis*
			5	Small cob, rot 1%	*F. moniliforme, F. moniliforme var. subglutinans*
			6	Small cob, rot 5%, Diplodia?	*S. maydis, F. moniliforme var. subglutinans*
21	Moses Situma	Naitiri	Cob 1	Big cob, rot 90%, Diplodia? Fusarium?	*S. maydis, F. moniliforme*
			2	Big cob, rot 30%, Diplodia?	*S. maydis*
			3	Big cob, rot 10%, Diplodia?	*S. maydis*
			4	Small cob 15% rot, Diplodia?	*S. maydis*
			5	Small cob 25% rot, Diplodia?	*S. maydis*
			6	Big cob, rot 15%, Diplodia?	*S. maydis*
22	Maurice Matala	Tongaren	Cob 1	Medium size cob, rot 10%	*S. maydis, F. moniliforme*
			2	Big cob, rot 20%	*F. graminearum, F. moniliforme*

			3	Medium size cob, borer damage rot 20%	*F. graminearum, F. moniliforme*
			4	Medium size cob, rot 5%	*F. , graminearum, F. moniliforme*
23	Nathan Makokha	Tongaren	Cob 1	Small cob, bird damage, pinkish rot 60%	*F. graminearum, F. moniliforme*
			2	Small cob, borer damage, rot 10%	*F. graminearum, F. moniliforme*
			3	Small cob, borer damage, rot 20%	*F. graminearum, F. moniliforme*
			4	Big cob, borer damage, rot 5%	*F. moniliforme, S. maydis*
			5	Medium size cob, borer damage, rot 15%	*F. moniliforme*
24	Alfred Masika	Mbakalo	Cob 1	Small cob, rot 10%	*S. maydis, F. moniliforme*
			2	Medium size cob, borer damage, rot 30%	*F. moniliforme, S. maydis*
			3	Big cob, rot 5%	*F. graminearum, F. moniliforme*
			4	Medium size cob, rot 5%	*S. maydis*
			5	Small cob, rot 25%	*S. maydis*
			6	Small cob, rot 5%	*S. maydis*
			7	Small cob, rot 5%	*S. maydis, F. moniliforme*
			8	Medium size cob, rot 30%	*S. maydis, F. moniliforme*
25	Wycliffe Opose	Mbakalo	Cob 1	Small cob, 20% rot	*F. graminearum, F. moniliforme*
			Cob 2	Medium size cob, rot 20%	*F. graminearum*
			3	Big cob, bird damage, 10% rot	*F. moniliforme, S. maydis*
			4	Medium size cob, 15% rot	*F. moniliforme*
			5	Big cob, rot 30%. Diplodia?	*S. maydis*
			6	Medium size cob, rot 20%. Diplodia?	*S. maydis*
			7	Big cob borer damage, borer damage, rot 10%	*F. moniliforme, S. maydis*
			8	Big cob, borer damage, rot 10%	*F. moniliforme, S. maydis*
			9	Medium size cob, borer damage, rot 25%	*F. moniliforme*
26	Mchanga Cheloti	Mbakalo	Cob 1	Small cob, borer damage, scattered	*F. moniliforme, F. graminearum*

				rotten kernels 40%	
			2	Medium size cob, borer damage, rotten kernels 20%	*F. graminearum, F. moniliforme*
			3	Medium size cob, borer damage, rotten kernels 5%	*F. graminearum, S. maydis*
			4	Small cob, borer damage rotten kernels 10%	*F. moniliforme, S. maydis*
			5	Medium size cob, borer damage, rotten kernels 15%, Diplodia	*F. moniliforme, S. maydis*
			6	Small cob, borer damage rotten kernels 30%	*F. moniliforme*
			7	Small cob, borer damage with rotten kernels 50%	*F. moniliforme, F. graminearum*

4.4 Discussion

Incidence of ear rot differed between the two seasons surveyed. In 1998 cob rot incidence was lower in many of the fields visited with an average of 22% of rotten cobs and 25% rotted grain on the affected cobs. This was equivalent to a mean of 2% rotted grain in the harvest from this area. The incidence of cob rot was very high in 1999, the mean incidence was 68%. There was little difference in the mean severity between the two years. In 1999, the mean severity was 21%. The estimated level of rotten grain in the harvest this year was 10%.

In both years a strong correlation was established between ear rot incidence and borer incidence. From this data it can be concluded that borers are important pre-disposing factor of maize to ear rot pathogens in the region. *Busseola fusca* has been found to increase incidence of kernel infection by *Stenocarpella maydis* (Flett *et al.*, 1992). Control of borers would reduce the incidence appreciably. There was also a close association between damaged cobs and *F. moniliforme* incidence in rotted cob. The main cause of cob damage in the field was due to stalk borer damage. Birds damage was less significant. The other factor found to have a positive correlation with ear rot incidence for the two seasons studied was the number of days after planting. There was a tendency for the late planted crop to have higher ear rot incidence and borer damage. The trend in that direction is probably due to the fact that the incidence of stalk borer damage to the cob was significantly higher in the later planted fields and that, in turn, cob rot incidence was strongly correlated with borer damage. The available recommendation from the Ministry of Agriculture for the control of stalk borers is to apply 4 kg/ha of Trichlorphon G when the crop is knee high. Framers in the area no longer apply this recommendation. The method

provides some control of borers in early stages of the crop but may not control the borers late in the season when ears are formed.

There are health implications of the levels of rotten grain in harvested maize because of the associated mycotoxins. It has been estimated in South Africa that grain which contain 2% rotted grain can contain sufficient mycotoxins to present a health hazard to consumers (Marasas *et al.*, 1979). Although the level of rotted grain in the harvest may be reduced by separation of the rotted cobs from healthy cobs this is not normally sufficient to eliminate all the rotted kernels. This was confirmed by the samples of harvested grain seen during the survey. Much of this was intended for the preparation of flour for human consumption as it contained more than 2% rotted grain. The levels may even be higher in grain meant for sale. As earlier indicated in the mycotoxin survey, the large scale farmers do not normally sort out rotten cobs. Most of this will end in the diet of the maize consumers, increasing the level of exposure to mycotoxins. There is some detoxification of mycotoxins associated with rotted grain when contaminated flour is cooked. However beer is traditionally prepared from grain, particularly during the harvest period as a form of celebration. Heavily rotted grain is used for this purpose and the level of mycotoxins in this beer may be very high. The other use of rotted grain is in livestock feed and animals fed on such grain may be at risk from mycotoxicoses.

Three most frequently occurring pathogens in rotted cobs in the region were *F. moniliforme*, *S. maydis*, and *F. graminearum*. *F. moniliforme* was the most frequent in cob that had stalk borer damage, occurring in 97% of all the cobs that had visible damage. The three fungal pathogens produce mycotoxins. *F. moniliforme* produces fumonisins, *F. graminearum* produces T-2 toxin, deoxynivalenol and zearalenone and

S. maydis produces diplodiatoxin. These toxins are a risk to human and livestock health. The prevalence of these fungal pathogens in the region, posses a potential risk of mycotoxin exposure to maize consumers in the region.

4.5 Conclusion

1. Cob rot incidence was high in Tongaren Division of Western Kenya (22% in 1998 and 68% in 1999).

2. Cob rot incidence was strongly correlated with damage to cobs by stalk borers

3. *Fusarium moniliforme* was the most frequently isolated pathogen from rotted cobs.

The estimate for the mean percentage of rotted grain in the harvest was 1.6% in 1998 and 10% in 1999 (2% is considered to be the threshold level for potential mycotoxin risk).

CHAPTER 5: IDENTIFICATION OF EAR ROT FUNGI FROM FARM AND MARKET SAMPLES

5.1 Background

During the mycotoxins survey representative maize samples from markets in six major towns and four locations in Western Kenya were collected and analysed for ear rot fungi prior to the analysis for mycotoxins in the samples. The samples were collected from markets in the following towns Kakamega, Bungoma, Kitale, Kisumu, Busia, and Siaya. The other lot of samples were collected from farms in Tongaren and Mbakalo Locations in Tongaren Division and Kapsabet and Kapkangani Locations in Kapsabet Division. The objective of the mycological analysis was to provide some information on the prevalence of some of the ear rot fungi associated with cob rot in the region. This information was also to provide an indication of the potential mycotoxins likely to be present in the maize in the region.

5.2 Methodology

In every location 10 kg samples of both 'good' and 'poor' maize were collected on a single visit. The samples were air dried to moisture content of less than 13% and a representative sample of 1/12 portion taken for fungal pathogen analysis using Pascal sample divider. The procedures used for sample collection, handling and preparation for both mycotoxins and mycological analysis are outlined in section 6.4.1.

Isolation of the fungal pathogens was carried out on glycerol agar and Potato dextrose agar (PDA). The fungal colony types were identified on PDA, PSA, SNA and Malt extract.

The kernels from each sample were first surface sterilized in 2.5% (w/v)sodium hypochlorite for 2 minutes and rinsed with sterile distilled water. Five seeds were plated per Petri dish in triplicates on both glycerol agar and PDA.. The Petri dishes were incubated for 5 days at 25^0C and then observed for fungal growth. The various fungi growing on the petri dishes were grouped into colony types based on their similarity in appearance, marked and recorded on the fungal identification record sheet (Appendix 9). Typical colonies from each of the colony types were sub-cultured on PSA and SNA for identification of *Fusarium* species and on 1.5% malt extract for identification of *Stenocarpella* species. The colonies on malt extract were first incubated in the dark for 5 days at 25^0C then transferred to NUV light of alternating cycle of 12 hr. of light and darkness for 10-14 days.

The other colony types were sub-cultured on two plates each of PSA and SNA and also placed NUV light. The cultures on SNA were used for identification based on the morphology of the conidia. SNA medium is transparent and can be viewed directly on the microscope stage. The cultures were viewed by cutting 1 cm^2 of agar with the fungal colony and mounting directly on the slide with a drop of water and cover slip. *Fusarium* pathogens were identified using the taxonomic key to common species of *Fusarium* (Booth, 1977). PSA was mainly used for differentiation of the cultures by pigmentation produced on this media.

5.3 Results

Based on the appearance of the colonies on the PDA and GA agar plates, the colonies were marked and labelled before being sub cultured on SNA PSA and Malt extract. The colonies were grouped into colony types based on their appearances (Table 5.1). Because of variation in the appearances within the groups on the media, the types

were put into sub groups such as Type 1a, Type I b and Type I c, depending on the numbers appearing in each group. All the Type I, Type II and Type III were sub cultured on SNA and PSA because of their frequency of yielding *Fusarium* species. The Type III, were the predominantly white colonies and were sub cultured on PDA and Malt extract for identification of *Stenocarpella* species. Colonies which could not be identified were kept on agar slants. The identification of *Aspergillus* species was based on the appearance of the colonies on PDA. The most common species of *Aspergillus* was *Aspergillus flavus*. The other groups of organisms that could not be identified were also cultured and kept on the agar slants

Table 5.2. Fungi identified from samples collected on market and farms in Western Kenya

Sample	Source (Division/ Location	Description of cobs / Kernels	% rot	Fungi identified
KT97001	Tongaren/Mbakalo	Fair quality kernels, some infected by *Fusarium* spp.	6	*Fusarium moniliforme, F. moniliforme var. subglutinans, Aspergillus flavus, Rhizopus stolonifer*
KT97002	Tongaren/Mbakalo	Very poor quality kernels, shrivelled kernels likely *S. maydis* and *Fusarium* infection	90	*Fusarium moniliforme, Stenocarpella maydis, Rhizopus stolonifer, Fusarium graminearum, Penicillium spp., A. flavus*
KT97003	Tongaren/Tongaren	Good quality white kernels	4	*Fusarium moniliforme, Rhizopus stolonifer, Penicillium spp., Aspergillus spp.*
KT97004	Tongaren/Tongaren	Very poor quality kernels with suspected *Fusarium* spp. and *Stenocarpella spp.* infection.	89	*Fusarium moniliforme, Fusarium graminearum, Penicillium spp., Aspergillus spp., Stenocarpella maydis*
KT97005	Kapsabet/Kapkangani	Good quality white kernels	4	*Fusarium moniliforme, Aspergillus flavus, Fusarium graminearum*
KT97006	Kapsabet/Kapkangani	Poor quality kernels, shrivelled suspected *S. maydis* and *Fusarium* infection	83	*Fusarium graminearum, F. moniliforme var. subglutinans, Stenocarpella maydis*
KT97007	Kapsabet/ Kapsabet	Fair quality white kernels, some browning.	10	*Fusarium moniliforme*
KT97008	Kapsabet/ Kapsabet	Very poor quality kernels suspected *Fusarium* and *S. maydis* infection	68	*Fusarium graminearum, A. flavus, S. maydis, Penicillium Spp., Stenocarpella maydis*
KT97009	Shibuye market (Kisumu)	Fair quality kernels	11	*Fusarium moniliforme, S. maydis, A flavus*
KT97010	Kitale market	Fair quality kernels	13	*F. moniliforme, F. graminearum, A. flavus*
KT97011	Bungoma market	Fair quality kernels	16	*F. moniliforme, F. moniliforme var. subglutinans, F. graminearum, A. niger, A. flavus, Penicillium spp*
KT97012	Busia market	Fair quality kernels	9	*Aspergillus niger, Penicillium spp, F. moniliforme*

KT97013	Siaya market	Fair quality kernels	19	*Fusarium moniliforme, A. niger, Fusarium graminearum, Penicillium spp.*
KT97014	Kakamega market	Fair quality kernels	4	*Fusarium moniliforme, A. niger, Penicillium spp, Rhizopus spp., S. maydis.*
KT97015	Amukura	Good quality white kernels	5	*F moniliforme, A. niger, F graminearum Penicillium spp., A. flavus*
KT97016	Kitale market	Fair quality white kernels	7	*F. moniliforme, F. graminearum, Stenocarpella maydis*
KT97017	Kapsabet/Kapsabet	Fair quality white kernels	86	*F. moniliforme, S. maydis, F. graminearum, F moniliforme var. subglutinans*
KT97018	Kapsabet/Kapsabet	Fair quality white kernels	8	*F graminearum*
KT97019	Kakamega/ market	Fair quality white kernels	7	*F. graminearum, Stenocarpella maydis, F. moniliforme, Penicillium spp.*
KT97020	Siaya market	Fair quality white kernels	13	*F. moniliforme, F. graminearum A. niger, Penicillium spp.*
KT97021	Siaya market	Fair quality kernels	8	*F. moniliforme, A. niger, Rhizopus stolonifer,*
KT97022	Bungoma market	Good quality kernels	19	*F moniliforme, A. niger, A. flavus, Penicillium, spp., F. graminearum*
KT97023	Tongaren/Tongaren	Good quality kernels	2	Clean
KT97024	Tongaren/Mbakalo	Good quality kernels	2	Clean
KT97025	Kisumu market	Good quality kernels	3	Clean
KT97026	Kakamega market	Good quality kernels	3	*F graminearum, S. maydis, A. flavus, F. moniliforme, Penicillium ssp.*
KT97027	Kakamega market	Fair quality kernels	17	*F. graminearum, S. maydis, F. moniliforme,*
KT97028	Kisumu market	Poor quality kernels	73	*S. maydis, A. flavus, F. graminearum, F moniliforme, Penicillium ssp.*
KT97029	Kisumu market	Fair quality kernels	6	*F. moniliforme, Penicillium ssp.*
KT97030	Bungoma market	Fair quality kernels	9	*S. maydis, F. moniliforme*

KT97031	Bungoma market	Poor quality kernels	93	*F. graminearum, S. maydis, F. moniliforme, Penicillium ssp*
KT97032	Busia market	Good quality kernels	7	*S. maydis, F moniliforme, A. niger, Penicillium ssp*
KT97033	Busia market	Fair quality kernels	12	*A. niger, F. moniliforme, Penicillium spp, Rhizopus Spp.*
KT97034	Siaya market	Fair quality kernels	6	*A. niger, F moniliforme, Penicillium spp,*
KT97035	Siaya market	Fair quality kernels	18	*A. niger, F. moniliforme, Penicillium spp, Rhizopus Spp., F. graminearum*
KT97036	Kapsabet/ Kapsabet	Poor quality kernels	76	*A. flavus, F. moniliforme, F graminearum Penicillium spp, S. maydis spp.*
KT97037	Kapsabet/ Kapsabet	Good quality kernels	3	*F graminearum, F. moniliforme var. subglutinans, S. maydis, Penicillium spp., Fusarium moniliforme*
KT97038	Kapsabet/Kapkangani	Poor quality kernels	23	*F. graminearum, F. moniliforme var. subglutinans, S. maydis, Penicillium spp, F. moniliforme*
KT97039	Kapsabet/Kapkangani	Poor quality kernels	92	*F. graminearum, S. maydis, F. moniliforme var. subglutinans, F. moniliforme*
KT97040	Kapsabet/Kapkangani	Poor quality kernels	71	*F. graminearum, S. maydis, F. moniliforme*
KT97041	Kitale market	Good quality kernels	12	*F moniliforme, F. moniliforme var. subglutinans, F. graminearum, A. flavus, Penicillium spp*
KT97042	Kitale market	Poor quality kernels	87	*F. moniliforme, F. graminearum, S. maydis, F. moniliforme var. subglutinans*
KT97043	Tongaren/Tongaren	Poor quality kernels	90	*F. moniliforme, F. moniliforme var. subglutinans, F. graminearum, S. maydis*
KT97044	Tongaren/Mbakalo	Poor quality kernels	79	*Fusarium moniliforme, F. graminearum, A. flavus, S. maydis, Penicillium spp*
KT97045	Tongaren/Mbakalo	Good quality kernels	5	*Fusarium moniliforme*

Of the 45 samples collected from farms and market towns, 26 samples were classified as 'good' by the farmers or traders (all these were either fair good quality kernels) and 19 were poor quality samples. The poor quality maize from the farms were taken from mouldy cobs which had been separated from the good cobs during storage or at harvest. Table 5.3 shows the major pathogens identified in the samples.

Table 5.3. Frequency of occurrence of the main fungi identified in the maize samples

Fungal species	No. of positive samples	Percentage of total
Fusarium moniliforme	36	80
Fusarium graminearum	25	56
Fusarium moniliforme var. subglutinans	10	22
Stenocarpella maydis	22	49
Aspergillus flavus	12	27
Aspergillus niger	8	18

Fusarium moniliforme was the predominant fungal species identified in the samples collected. It was found in 80 % of the samples and occurred both in good quality maize and poor quality maize. *Fusarium graminearum* occurred in 56 % of the sample, *Fusarium moniliforme var. subglutinans* 22%, *Stenocarpella maydis* 49 %, *Aspergillus flavus* 27 % and *Aspergillus niger* in 18 % of the samples. *Aspergillus niger* was only isolated from Busia, Siaya, Amukura and one sample from Bungoma. *Fusarium moniliforme* was found almost in all the locations sampled and markets but *Fusarium graminearum* was absent in samples from Busia, Amukura and Siaya.

5.4 Discussion

Fusarium moniliforme was the most frequently isolated species in maize collected and was found in both mouldy and good kernels. It occurred in 80 % of the samples

collected. *Fusarium graminearum* was isolated in 56 % of the samples, it was found in most of the areas except in Busia, Amukura and Siaya. These areas are considered to be in hot/dry and humid zones. *F. moniliforme* was isolated in the three areas but *F. graminearum* and *Fusarium moniliforme var. subglutinans* were absent in these areas. *Aspergillus niger* was frequent in samples from Siaya, Busia and Amukura. Previous surveys confirms these results that *Fusarium moniliforme* is the most predominant ear rot fungus on maize kernels in the region. Survey of maize grain purchased from market stalls and road side traders in Central and Western Kenya by McDonald and Chapman (1997) reported that 14 % and 9 % of the kernels tested contained for *Fusarium moniliforme* and *Fusarium graminearum* respectively. In another survey in western Kenya, *F. moniliforme* was found to be the most dominant fungus in nine districts surveyed and was found in 60 % of the samples, *F. graminearum* was found in 31 % of the samples and *F. solani* and *F. subglutinans* were in 18 % and 15% of the samples respectively (Kedera, 1999).

Samples of maize ears and stalks collected from farms in Kakamega/Vihiga and Trans-Nzoia district in Western Kenya in 1999, had 40% *F. moniliforme*, 30% *F. graminearum* as the most predominant fungal species (Kedera, 1998). In another previous survey by Kedera 1994 (as quoted by Kedera *et al.*, 1999), *F. moniliforme* to be the most predominant fungal species (82 % of isolates from maize), followed by *F. graminearum* in (9 % of isolates) and *F. subglutinans* (7 % isolates)

5.5 Conclusions

Fusarium moniliforme was the most prevalent fungus identified in the maize samples collected from two divisions (Tongaren and Kapsabet) and the major markets in the region (Kakamega, Busia, Bungoma, Kitale, Kisumu and Siaya).

Only three samples were found to be clean and this constituted 7 % of the samples collected.

The three most frequent ear rot fungi in maize samples in the region were *Fusarium moniliforme*, *Fusarium graminearum* and *Stenocarpella maydis*

CHAPTER 6: SURVEY OF MYCOTOXINS ASSOCIATED WITH MAIZE EAR ROT IN WESTERN KENYA

6.1 Background

Maize ear rot is an important disease problem affecting maize in Western Kenya. Besides causing yield loss, the fungi that cause maize ear rot produce mycotoxins that are a potential risk to both human health and livestock production. In previous surveys in the region (Chapter 3) it was found that both clean and rotten maize harvested in this area was utilized at various levels as either human food or livestock feed. It was therefore important to establish if mycotoxins were present in maize in Western province, at levels which were likely to be detrimental to human and animal health. The survey involved sampling of maize both at market and farm level. The survey was carried out from October 1997, which was the main maize harvesting period in Western highlands of Kenya until August 1998. The vast majority of households in this region grow maize, and at harvest time the majority of maize consumed will be that produced by the farmer and relatively very little will be purchased from the markets. The market sampling was intended to complement the farmer mycotoxin survey. It was to provide information on maize as purchased in town markets, which would have a history of farmers' selection and passage through the traders' system. As the year progresses from harvest in October through to April or May, an increasing proportion of the population of Western province will rely on maize from the markets for the supply of their staple diet.

The survey was therefore designed to determine the presence of mycotoxins in the maize consumed at farm level and that sold at the market. This was intended to generate information on the level of intake of these mycotoxins and provide an

indication of the potential risk of mycotoxin exposure to maize consumers in the region. The objectives of the survey were therefore as follows;

1. To determine the presence and levels of common mycotoxins in the food and feed chain in the region.

2. To determine the levels of common mycotoxins in the pre- harvest and post-harvest system.

3. To find out the correlation between mycotoxins levels and ear rot levels in the harvested maize.

6.2 Mycotoxin survey

6.2.1 Design of mycotoxin surveys

The survey was divided in two major parts. One part of the survey involved the collection of representative maize samples from markets in six major towns in Western Kenya. The towns were Kakamega, Bungoma, Kitale, Kisumu, Busia, and Siaya. In the second part of the survey, samples were collected from farms in identified maize producing areas. These areas comprised of the following locations, Tongaren, Mbakalo, Kapsabet, Kapkangani and Amukura. A mycotoxin sample collection form was developed which included a few background questions on the history of the maize (Appendix 5). In every location 10 kg samples of both good and poor maize were collected on a single visit. The procedures used in sample collection, handling and preparation of samples for both mycotoxin and mycological analysis are outlined below.

6.2.2 Sample Collection

1. Bulk samples of maize weighing at least 10 kg and representing either a Location or a town market were collected. The bulk samples were composed of at least ten incremental samples.

2. Each sample was allocated a number using the coding list (each Location and town was assigned a number and this was recorded on a Sample Collection Form.

3. Incremental samples were combined and put in a labeled cotton bag for transportation to the laboratory.

4. On arrival at the laboratory, each sample spread out on a bench or floor to dry to a moisture level of 13% or less for further storage in the cotton bag, or up to 12% for longer-term storage in a sealed plastic bag. Moisture content was determined by a 'Dickey John' moisture meter.

6.2.3 Sampling at farm level

In the Western highlands, the following climatic maize growing zones were identified and sampling done based on them: cold and wet (CW), average (Av), and hot and humid (HH). Sampling in Kapsabet and Kapkangani locations represented the cold and wet zone, Mbakalo and Tongaren represented the average and Amukura represented the hot humid zone.

1. From each Location two samples were collected, one sample of 'good' maize, from the lot to be consumed by the farmer over the next few days, and the other sample was of 'rotten' maize, using the following procedure.

2. The Extension officer from the selected Location was responsible for identifying two villages in each Location. Five medium-scale (5 to 30 acres) farms in each village were sampled.

3. On each farm 1 kg each of the 'good' and 'rotten' maize was collected.

4. Farmers were asked questions to provide the information required on the Sample Collection Form. The store or stores were then classified according to the coding given on the coding sheet.

5. Moisture content of each sub-sample was determined using a 'Dickey John' moisture meter and recorded on the Sample Collection form (Appendix 5)

6.2.4 Sampling at Markets.

Twenty incremental samples of 0.5 kg were collected from the larger-scale of the available traders, with stocks of 5 x 90 kg bags or more, as follows:

1. Effort was made to try and locate at least two markets in each town, one of which was the main or largest market selling maize. In cases where only one market was available, the market was visited on two different days or all twenty incremental samples were collected on the same day.

2. The method involved first walking through the market sketching the route and marking and sequentially numbering the traders who satisfied the criteria for selection (the criteria was normally 5 bags or more, but in some cases could be reduced if less than 10 traders satisfied it). The criteria used for selection and the number of traders satisfying it was recorded on the sample collection form.

3. Incremental samples of 0.5 kg was bought from each of the 10 traders randomly selected and the quality noted.

4. Questions were asked to complete the 'market' section of the sample collection form.

5. The sub-samples were combined in a labelled cotton bag to constitute a sample and moisture content determined to give the initial moisture and later (average of 3 readings) when the sample was dried in the laboratory.

6.2.5 Transportation of samples

1. Each sample was transported in (55 x 35 cm) cotton bags, to prevent moisture migration and heating which helped to preserve the integrity of the sample during transit. Incremental samples with high moisture content, in excess of 20%, were kept in separate cotton bags for priority drying and possible disposal if heating occurred.
2. The samples were carried inside the vehicle free from rain and direct heating from the sun. This was to keep the samples cool during transportation and minimize microbial changes within the samples.

6.2.6 Sample Drying

1. The samples were spread in a thin layer on cotton bags over benches and floor in the laboratory.
2. Drying continued until moisture levels of $\leq$ 13 % was achieved for subsequent short-term storage in cotton bags.

6.2.7 Sample Storage

1. Samples were stored in cotton bags kept in a large open room on the benches in the laboratory for short-term (not more than 1 month).
2. Long term storage, was used mainly for the samples for mycological analysis ($\leq$ 12 % moisture content). Samples were kept in sealed plastic bags and placed in a deep freezer at - 20 ^{0}C for two weeks to kill insects and their eggs, after which the samples were left to attain room temperature.

3. After removing from the deep freezer, the samples were allowed to dry from the condensation and checked for condensation. The samples were then placed inside labeled plastic bags and closed.

6.2.8 Sample division

1. A rotary cascade divider (Pascal Model 3) was used, with the cone gap set at its widest, to divide the whole kernels (normally the divider is only used for dividing ground materials, but it was important to obtain a representative sample of kernels for mycology).

2. A 1/12 portion of the divided maize sample was taken and kept for mycological analysis. The remaining 11/12 was combined for mycotoxin analysis and returned to the original bag for milling.

3. The same laboratory numbers were assigned to the mycology and mycotoxin samples and recorded on the Sample Record form.

6.2.9 Preparation of mycotoxin laboratory sample

1. The samples were milled in a commercial maize mill. Cross contamination was minimized by running the mill until there was none of the previously milled maize in the system; banging the hoppers to dislodge any maize and discarding the first 500 g of sample and making sure that 'good' maize was milled before 'poor'maize.

2. The milled maize samples were weighed and recorded.

3. The milled mycotoxin samples were divided using the Pascal rotary cascade divider, using a medium cone gap.

4. 1/12 portions as the Laboratory Sample (800 g to 1 kg) ($\leq$ 12 % moisture) were taken and sealed in a plastic bag labeled with the Laboratory Number (KT series).

5. The second 1/12 portion and one of the 1/6 portions, were kept separately, as reference samples. These were labeled and stored in sealed plastic bags, kept in a plastic container with a tight fitting lid.

6.3 Mycotoxin analysis in maize samples

The milled samples were tested for the presence of mycotoxins. The samples were tested at both KARI- Kakamega laboratories and Natural Resources Institute-mycotoxin laboratories. At Kakamega the samples were tested for levels of aflatoxin using the Steiner method (Steiner *et al.*,1992) based on the AOAC 'BF' methods and for the presence of deoxynivalenol (DON) and T-2 toxin by thin layer chromatography (TLC) using Romer #225 solid phase extraction cartridges. At NRI the samples were tested for aflatoxin and Fumonisin B_1 by high performance liquid chromatography (HPLC) methods and for DON, T-2 toxin and zearalenone by high performance thin layer chromatography (HPTLC). The procedures for each analysis are outlined below.

6.3.1 Estimation of aflatoxin in maize (Steiner method based on AOAC, 'BF' method

Analysis of aflatoxin in maize sample was done at KARI-Kakamega laboratory using the Steiner method based on AOAC, 'BF' methods (Steiner *et al*,. 1992; Official methods of analysis of the AOAC., 1990). The AOAC 'BF' method has been officially adopted for the analysis of aflatoxin in groundnuts and groundnut products and has been found applicable to maize.

The method was slightly modified so that toluene:acetonitrile (98:2) was used as the spotting solvent instead of benzene:acetonitrile (98:2). Toluene:acetonitrile spotting

solvent was tested in Natural Resource Institute mycotoxin laboratories in January, 1997 when developing methods for analysis of mycotoxins. It was found to give comparable results to benzene, but being a less toxic compound it was favoured to benzene.

6.3.1.1 Extraction

80 g maize flour was placed in a 1 litre blender jar and 200 ml of methanol and 20 ml of water was added. The mixture was blended for 2 minutes before adding another 60 ml of water and blending for another 1 minute, then filtered though Whatman filter paper No. 2. The filtrate was retained for the clean-up step.

6.3.1.2 Cleaning-up and quantification

To 70 ml of the crude extract in a 500 ml separating funnel, 50 ml of hexane (to remove lipids), 55 ml of water and 2 g of sodium chloride (to prevent emulsion) was added and shaken for 1 minute and the layers allowed to separate. The lower layer was run into a second separating funnel. To the second separating funnel 25 ml of chloroform was added, the mixture shaken and allowed to separate into layers. The lower layer was run into a 250 ml conical flask via a 35 mm funnel two thirds full of anhydrous sodium sulphate (oven-dried). A second 25 ml of chloroform was added and the procedure repeated. The clear extract collected was taken to dryness on a rotary evaporator at 45^0C. The extract was reconstituted in 3 ml chloroform by shaking 1 ml at a time transferring to a 7 ml vial. The extract was taken to dryness at 45^0C under a jet of air.

For semi-quantitative screening the sample was taken up in 400 µl of tolune:acetonitrile (98:2), vortexed for 30 seconds and 2 µl spotted in duplicate on

aluminium-backed TLC plate (Merck 5721). An extra 1200 µl of toluene:acetonitrile (98:2) was added to the extract and vortexed for a another 30 seconds and 2 and 5 µl spots spotted in duplicate on a TLC plate. The plate was developed for 15 minutes in chloroform:acetone:water 88:12:0.2. The plate was dried in a fume hood and observed under long wave length UV light.

For D = 400 µl : If 5 µl spot was not visible, then < 1ppb aflatoxin

If 5 µl was just visible, then > I ppb aflatoxin

For D = 1200 µl : If 5 µl spot was not visible, then < 4ppb aflatoxin

If 5 µl was just visible, then > 4 ppb aflatoxin

If 2 µl spot was not visible, then < 10ppb aflatoxin

If 2 µl was just visible, then > 10 ppb aflatoxin

For quantitative assessment the aflatoxin positive extracts were taken to dryness and then 500 µl of toluene: acetonitrile was added. The extract was re-dissolved by mixing for 30 seconds. An HPTLC plate (Merck, 5547) was spotted with 1, 2, 3, 4, and 5 µl of sample and working standard solutions (Appendix 6.2) for spotting patterns). The plate was developed in chloroform:acetone:water 88:12:0.2 and observed under long wave length UV light. The intensity of the spots were compared and the best equivalence recorded (Appendix 6.1).

Aflatoxin was calculated in µg/kg (ppb) using the equation

$$ppb = \frac{C \times \text{Volume of standard spot (Z)} \times D}{\text{Volume sample spot (Y)} \times Ewt}$$

where C = concentration of standard in ug/ml

D = Dilution in µl (=500), Ewt = Effective weight in final extract = 2 g

6.3.2 Aflatoxin analysis in maize samples using HPLC method

6.3.2.1 Extraction

A 50 g representative sample was placed into a blender jar with 250 ml of acetone :water 80:20 and blended at high speed for 3 minutes (explosion proof blender). The mixture was filtered through Whatman paper No. 1 and retained for clean-up.

6.3.2.2 Cleaning-up and quantification

A phenyl bonded column with a 70 ml reservoir was fitted on a vacuum manifold. 1 g of Hy flo supercel (celite) was added to the reservoir. The column was solvated by adding sequentially 10 ml of methanol and 10 ml of distilled water. The vacuum was set to 15" Hg for a flow rate of 5 ml per minute. Care was taken not run the column dry. To the reservoir 30 ml acetic acid buffer, 3 ml lead acetate, 5 ml sample filtrate (using an absoluter) and 30 ml of acetic acid buffer sequentially. The contents of the reservoir were drawn through the column at a flow rate of 5 ml per minute, care being taken not to run the column dry. The column was washed with 10 ml of distilled water. The column was then dried by drawing in air at maximum vacuum, up to 20" mercury for 5 minutes.

To elute the aflatoxin, the phenyl column with a 25 ml reservoir was fitted onto sodium sulphate column on a dry vacuum manifold containing a 7 ml collection vial. Seven millitres of chloroform was added to the reservoir and the vacuum set at a maximum flow rate of 0.5 ml per minute. The sample collected in the 7ml vial was taken to dryness on a sample concentrator under nitrogen at 45 °C. The aflatoxin levels were then quantified by HPLC by reconstituting the extract in water:acetonitrile:methanol (WAM) and vortexing for 1 minute. The mobile phase was made up of water:acetonitrile:methanol 1200:600:200 plus 238 mg potassium

bromide and 700µl 4 M nitric acid degassed by an ultra sonic bath for 20 minutes. The flow rate of the mobile phase was set at 0.8 ml per minute. Aflatoxin was detected at excitation wave length of 365 nm and emission of 415 nm. Aflatoxin levels were integrated and calculated by a Nelson PCINT (Appendix 6.2)

6.3.3 Detection of T-2 toxin and Deoxynivalenol (DON) by TLC method using solid phase clean-up cartridges

This method was used for screening samples in the Kakamega laboratory for deoxynivalenol and T-2 toxin. The positive samples were taken to NRI for quantitative assessment by HPTLC method. The extraction, filtration and cleaning - up of the sample was the same both for TLC and HPTLC, for the quantification method. This procedure is outlined below

6.3.3.1 Extraction

A 50 g representative sample was placed into a 500 ml quickfit flask and 200 ml acetonitrile- water (84:16 V/V) was added. The flask was shaken for 40 minutes in a flask shaker and the mixture filtered through Whatman filter paper No.1 and retained for clean-up.

6.3.3.2 Quantification by TLC

The extract was reconstituted in 200 µl toluene -acetonitrile (9:1) and 4 µl spotted onto HPTLC plate at 5 mm intervals with the standard positioned on every fifth track separately for T-2 and DON.

6.3.3.3 Quantification of T-2

After spotting the plate was placed into in a TLC tank containing 20 ml of chloroform: acetone (9:1) for 22 minutes. The plate was allowed to dry for 5 minutes. For visualization the plate was dipped briefly in 3% sulphuric acid in methanol then allowed to dry for 5 minutes in the fume cupboard before heating in an oven at 100^0C for 5 minutes. The T-2 spots were checked under (UV) long wave length for positive samples.

6.3.3.4 Quantification of DON

The plate was developed in toluene:acetone (3:1) for 16 minutes and briefly dipped in 3% aluminium chloride in methanol and allowed to dry in the fume cupboard for 5 minutes and heated at 120^0C for 5 minutes. The positive samples were checked under the long wave length in the UV light.

6.3.4 Determination of T-2 toxin and Deoxynivalenol (DON) by HPTLC using solid phase clean-up cartridges (Romer #225)

6.3.4.1 Extraction

A 50 g representative sample was placed into a 500 ml quickfit flask and 200 ml acetonitrile- water (84:16 V/V) was added. The flask was shaken for 40 minutes in a flask shaker and the mixture filtered through Whatman filter paper No.1 and retained for clean-up.

6.3.4.2 Quantification by HPTLC

The extract was reconstituted in 200 µl toluene -acetonitrile (9:1) and 4 µl spotted onto HPTLC plate at 5 mm intervals with the standard positioned on every fifth track separately for T-2 and DON.

6.3.4.3 Quantification of T-2

After spotting the plate was placed into in a TLC tank containing 20 ml of chloroform: acetone (9:1) for 22 minutes. The plate was allowed to dry for 5 minutes. For visualization the plate was dipped briefly in 3% sulphuric acid in methanol then allowed to dry for 5 minutes in the fume cupboard before heating in an oven at 100^0C for 5 minutes. The T-2 spots were checked under (UV) long wave length and quantified by a CAMAG Scanner using CATS3 software at an excitation wave length of 365nm, and emission filter of 400 nm.

6.3.4.4 Quantification of DON

The plate was developed in toluene:acetone (3:1) for 16 minutes and briefly dipped in 3% aluminium chloride in methanol and allowed to dry in the fume cupboard for 5 minutes and heated at 120^0C for 5 minutes. Quantification was done by CAMAG Scanner using CATS software at excitation wavelength of 313 nm and emission filter of 400 nm (Appendix 6.3)

6.3.5 Analysis of Fumonisin B_1 using Fumoni-test affinity chromatography and HPLC

6.3.5.1 Extraction

A 50 g ground sample was placed into a 500 ml blender jar and 5 g of sodium chloride was added. 100 ml of 80% methanol water was added in the blender jar. The mixture was blended at a high speed for 5 minutes, and then filtered through Whatman filter paper No.1.

6.3.5.2 Dilution and filtration

10 ml of the extract was diluted with 40 ml of PBS (Phosphate buffer solution). The diluted extract was filtered through a micro fibre filter.

6.3.5.3 Fumonisin test affinity chromatography

Fumoni-test affinity chromatography column was attached to the outlet of a 10 ml reservoir on a vacuum pump. 10 ml of the dilute extract was pipetted into the reservoir and passed through Fumoni-test column at a stead flow rate of 1 drop per second. The column was then washed with 10 ml of PBS at a steady flow rate of 2 drops per second.

Fumonisin was then eluted with 1ml of HPLC methanol and collected in 7 ml vials. The extract was filtered and 50µl of the extract was mixed with 200µl of OPA (0-Phthaldialdehyde derivatization reagent) and 50µl used for determination of fumonisin level in the sample using system file 5, iso-cratic, 1ml/min, excitation = 335 nm, emission = 440nm (Appendix 6.4).

6.3.6 Determination of zearalenone in maize samples using base clean-up and HPTLC

Quantitative determination of zearalenone in maize was based on high performance liquid chromatographic method (HPLC) adopted by AOAC for the quantitative analysis of zearalenone modified for application to maize, with quantification by high performance thin layer chromatography (HPTLC) by Dawlatana *et al.*, 1998.

6.3.6.1 Extraction

A sample of 25 g of ground maize was mixed with 12.5 g diatomaceous earth (Hyflo super-cel) and 10ml of distilled water in 500ml conical flask. 250ml of chloroform

was added, the flask stoppered, sealed and shaken for 15 min. on a reciprocating shaker before the contents of the flask were filtered through Whatman paper No.1.

6.3.6.2 Cleaning-up

The crude maize extract (25ml) was mixed by swirling with 40 ml saturated sodium chloride solution (45% w/v) in a 250 ml separating funnel. After the addition of 50 ml sodium hydroxide solution (2% w/v), the mixture was shaken for 1 minute before allowing the layers to separate completely. The lower chloroform layer and any extraneous matter at the interface was discarded. The contents of the separating funnel were extracted by a further 50 ml chloroform. After the layers had separated, chloroform layer was removed and discarded. The mixture was acidified by swirling with 50 ml of citric acid solution (10.6% w/v citric acid monohydrate) before being extracted with 50 ml dichloromethane by gently shaking for 1 min. The lower dichloromethane layer and any emulsion at the solvent interface were removed and retained. The aqueous layer was re-extracted with a further 50 ml of dichloromethane. The combined extract were passed through a bed of anhydrous sodium sulphate. The extracts were evaporated to near dryness on a water bath at 40 ^{0}C. The residue was transferred quantitatively to a 7 ml screw neck vial with 1 x 3 ml of dichloromethane and dried under a stream of nitrogen on a sample concentrator. The vials were kept at - 20^{0}C until required for quantification.

6.3.6.3 Quantification

For quantification by HPTLC 200 µl benzene:acetonitrile (98:2) was added using a Finnpipette stepette followed by mixing in a vortex mixer for 1 min. The standards were applied to the HPTLC plates using a semi-automatic plate spotter (Camag,

Nanometer III, Switzerland) set to dispense along a line 12 mm from the bottom edge of the plate. Samples were applied at 10mm intervals with standards positioned on every fifth track. The plates were placed in a 20 x 20 cm vee-bottomed TLC tank containing 20 ml toluene:ethyl acetate:formic (TEF) acid (6:3:1) and developed for 15 min. The plate was dried for 5 min. and zearalenone was quantified by UV fluorescence using an HPTLC scanning monochromatic fluorodensitometer (Camag Scanner II, Switzerland) fitted with a 400 nm emission filter and controlled by CATS 3 software (Appendix 6.5).

6.3.7 Analysis of zearalenone using Zearala-test affinity chromatography and HPTLC

Some of the samples that had high levels of zearalenone were tested using zearala-test affinity columns, which were to act as a confirmatory test for the presence of zearalenone. The recovery of zearalenone using the Dawlatana method in the initial analysis wasfound to be low. The use of Zearala-test chromatography which is more robust was to improve on the clean up and the recovery of zearalenone from the samples. This would also act as a good positive confirmation for zearalenone.

6.3.7.1 Extraction

50 g ground sample was placed into a 500 ml blender jar and 5 g of sodium chloride was added. 100 ml of 80% methanol water was added in the blender jar. The mixture was blended at a high speed for 5 minutes, and then filtered through Whatman filter paper No.1.

6.3.7.2 Dilution and filtration

2 ml of the extract was diluted with 98 ml of water and filtered. 50 ml of the filtrate was passed through the zearala-test affinity column (VICAM). The column was washed twice with 5 ml of water. Zearalenone was eluted from the column with 1.0 ml HPLC grade methanol and collected in 7 ml vials.

6.3.7.3 Quantification

The samples were taken to dryness on a sample concentrator at 45^0C. The dry extracts were taken up in benzene:acetonitrile (98:2) vortexed and 4µl spotted on TLC plates, 12 mm from the bottom edge of the plate at 10 mm intervals with a standard at every fifth track. The plates were developed for 15 min. with 20ml toluene:ethyl acetate:formic acid (6:3:1). The results were read on a CAMAG Scanner II using CATS 3 soft ware (Appendix 6.6)

6.4 Results of mycotoxin analysis

6.4.1 Deoxynivalenol and T-2 toxin levels in maize samples

During the survey 45 samples were collected and analyzed for T-2 toxin and deoxynivalenol. All the samples analyzed for T-2 toxin were found to be negative (below detection limit). The analysis of DON was initially carried out on 17 samples by TLC method using solid phase clean up cartridges at KARI-Kakamega and only two samples appeared to be positive.

Table 6.1. Results of DON and T-2 toxin analysis in maize samples

KARI-Lab ID/No.	NRI-Lab ID/No	Place collected	Quality of maize	% rot	DON ng/g	T-2 ng/g
KT97001	MT99009	Tongaren/Mbakalo	Good quality	6	ND	ND
KT97002	MT99029	Tongaren/Mbakalo	Very poor quality	90	ND	ND
KT97003	MT99030	Tongaren/Tongaren	Good quality	4	ND	ND
KT97004	MT99031	Tongaren/Tongaren	Very poor quality	89	867	ND
KT97005	MT99032	Kapsabet/Kapkangani	Good quality	4	ND	ND
KT97006	MT99033	Kapsabet/Kapkangani	Very poor quality	83	376	ND
KT97007	MT99034	Kapsabet/Kapsabet	Fair quality	10	ND	ND
KT97008	MT99035	Kapsabet/Kapsabet	Very poor quality	68	900	ND
KT97009	MT99036	Kisumu market	Good quality	11	ND	ND
KT97010	MT99037	Kitale market	Fair quality	13	ND	ND
KT97011	MT99038	Bungoma market	Fair quality	16	ND	ND
KT97012	MT99039	Busia market	Fair quality	9	ND	ND
KT97013	MT99040	Siaya market	Fair quality	19	ND	ND
KT97014	MT99041	Kakamega market	Fair quality	4	ND	ND
KT97015	MT99042	Amukura market	Good quality	5	ND	ND
KT97016	MT99043	Kitale market	Fair quality	7	ND	ND
KT97017	MT99044	Kapsabet/ Kapsabet	Poor quality	86	ND	ND
KT97018	MT99045	Kapsabet/Kapsabet	Fair quality	8	ND	ND
KT97019	MT99046	Kakamega market	Fair quality	7	ND	ND
KT97020	MT99047	Siaya market	Fair quality	13	ND	ND
KT97021	MT99048	Busia market	Fair quality	8	ND	ND
KT97022	MT99049	Bungoma market	Fair quality	19	ND	ND
KT97023	MT99050	Tongaren/ Tongaren	Good quality	2	ND	ND
KT97024	MT99051	Tongaren/ Mbakalo	Good quality	2	ND	ND
KT97025	MT99052	Kisumu market	Good quality	3	ND	ND
KT97026	MT99053	Kakamega market	Good quality	3	ND	ND
KT97027	MT99054	Kakamega market	Fair quality	17	ND	ND
KT97028	MT99055	Kisumu market	Poor	73	216	ND
KT97029	MT99056	Kisumu market	Fair quality	6	ND	ND
KT97030	MT99057	Bungoma market	Fair quality	9	ND	ND
KT97031	MT99058	Bungoma market	Poor	93	ND	ND
KT97032	MT99059	Busia market	Good quality	7	ND	ND
KT97033	MT99060	Busia market	Fair quality	12	ND	ND
KT97034	MT99061	Siaya market	Fair	6	ND	ND
KT97035	MT99062	Siaya market	Poor	18	ND	ND
KT97036	MT99063	Kapsabet/Kapsabet	Poor	76	324	ND
KT97037	MT99064	Kapsabet/Kapsabet	Good quality	3	ND	ND
KT97038	MT99065	Kapsabet/ Kapkangani	Poor	23	ND	ND
KT97039	MT99066	Kapsabet /Kapkangani	Poor	92	ND	ND
KT97040	MT99067	Kapsabet/ Kapkangani	Fair quality	71	ND	ND
KT97041	MT99068	Kitale market	Good	12	602	ND
KT97042	MT99069	Kitale market	Very poor quality	87	384	ND
KT97043	MT99070	Tongaren/ Tongaren	Poor	90	1111	ND
KT97044	MT99071	Tongaren/ Mbakalo	Poor	79	323	ND
KT97045	MT99072	Tongaren/ Mbakalo	Good quality	5	ND	ND

ND- Not detected

All the samples were then tested at NRI using a HPTLC method which was more sensitive. The results of the HPTLC analyses (Table 6.1) showed nine positive samples. The DON toxin levels ranged from 200 to 1100 ng/g. The mean levels in the positive samples was 567 ng/g. The overall mean levels of DON in the Kenyan

samples was 113 ng/g. The mean recoveries of DON and T-2 in the spiked samples was over 96% and 94.4 % respectively. All the samples identified as positive were from poor quality maize. DON was detected only in samples collected from the cool wet zone and the moderately cool zone, except for one sample collected at Kisumu market which was classified as hot and humid, but may have originated elsewhere. The positive samples constituted 20 % of all the samples collected and had a high percentage of rotten kernels, ranging from 68-90%. Correlation analysis of the percentage number of rotten kernels and the level of DON showed a positive linear correlation ($r = 0.67$), which was significant at 1% level.

6.4.2 Aflatoxin levels in maize samples

Aflatoxin was analysed in all the samples in Kakamega laboratories using the Steiner method of aflatoxin estimation in maize. Five samples were positive for aflatoxin with levels ranging from 1.8-8.3 ng/g which was consistent with the screening results. At NRI, the samples were subjected to a more accurate method of analysis using HPLC. Twenty nine samples, including the five previously tested, were analyzed. Seventeen samples turned out to be negative and 12 were positive with low levels of aflatoxin. The aflatoxin levels ranged from 0.5 ng/g to 10.3 ng/g. More samples collected from the market were positive for aflatoxin compared to samples collected at farm level suggesting low levels of post-harvest contamination. The levels of aflatoxin were generally low (Table 6.2)

Table 6.2. Results of aflatoxin analysis in maize samples

KARI-Lab ID/No.	NRI-Lab ID/No	Place collected	Quality of maize	% rot	Aflatoxin by TLC	Aflatoxin by HPLC	
						AFB_1	AFB_2
KT97001	MT99009	Tongaren/Mbakalo	Good quality	6	ND	ND	ND
KT97002	MT99029	Tongaren/Mbakalo	Very poor quality	90	1.25ppb	4.0	1.7
KT97003	MT99030	Tongaren/Tongaren	Good quality	4	ND	ND	ND
KT97004	MT99031	Tongaren/Tongaren	Very poor quality	89	ND	1.4	1.0
KT97005	MT99032	Kapsabet/Kapkangani	Good quality	4	ND	1.6	0.8
KT97006	MT99033	Kapsabet/Kapkangani	Very poor quality	83	ND	ND	ND
KT97007	MT99034	Kapsabet/Kapsabet	Fair quality	10	ND	ND	ND
KT97008	MT99035	Kapsabet/Kapsabet	Very poor quality	68	ND	ND	ND
KT97009	MT99036	Kisumu market	Good quality	11	ND	ND	ND
KT97010	MT99037	Kitale market	Fair quality	13	2.5ppb	1.3	ND
KT97011	MT99038	Bungoma market	Fair quality	16	ND	ND	ND
KT97012	MT99039	Busia market	Fair quality	9	ND	0.5	ND
KT97013	MT99040	Siaya market	Fair quality	19	ND	0.3	ND
KT97014	MT99041	Kakamega market	Fair quality	4	ND	1.5	ND
KT97015	MT99042	Amukura market	Good quality	5	3.75ppb	3.2	0.8
KT97016	MT99043	Kitale market	Fair quality	7	8.25ppb	10.2	10.3
KT97017	MT99044	Kapsabet/ Kapsabet	Poor quality	86	ND	ND	ND
KT97018	MT99045	Kapsabet/Kapsabet	Fair quality	8	ND	0.9	0.3
KT97019	MT99046	Kakamega market	Fair quality	7	ND	0.5	0.2
KT97020	MT99047	Siaya market	Fair quality	13	1.25ppb	1.1	1.6
KT97021	MT99048	Busia market	Fair quality	8	1.75ppb	1.1	1.5
KT97022	MT99049	Bungoma market	Fair quality	19	ND	ND	ND
KT97023	MT99050	Tongaren/ Tongaren	Good quality	2	ND	0.5	0.2
KT97024	MT99051	Tongaren/ Mbakalo	Good quality	2	ND	0.6	0.3
KT97025	MT99052	Kisumu market	Good quality	3	ND	ND	ND
KT97026	MT99053	Kakamega market	Good quality	3	ND		
KT97027	MT99054	Kakamega market	Fair quality	17	ND		
KT97028	MT99055	Kisumu market	Poor	73	ND	-	-
KT97029	MT99056	Kisumu market	Fair quality	6	ND		
KT97030	MT99057	Bungoma market	Fair quality	9	ND		
KT97031	MT99058	Bungoma market	Poor	93	ND	-	
KT97032	MT99059	Busia market	Good quality	7	ND		
KT97033	MT99060	Busia market	Fair quality	12	ND		
KT97034	MT99061	Siaya market	Fair	6	ND	-	
KT97035	MT99062	Siaya market	Poor	18	ND	-	-
KT97036	MT99063	Kapsabet/Kapsabet	Poor	76	ND	-	-
KT97037	MT99064	Kapsabet/Kapsabet	Good quality	3	ND		-
KT97038	MT99065	Kapsabet/ Kapkangani	Poor	23	ND	ND	ND
KT97039	MT99066	Kapsabet /Kapkangani	Poor	92	ND	ND	ND
KT97040	MT99067	Kapsabet/ Kapkangani	Fair quality	71	ND	ND	ND
KT97041	MT99068	Kitale market	Good	12	ND	-	
KT97042	MT99069	Kitale market	Very poor quality	87	ND		
KT97043	MT99070	Tongaren/ Tongaren	Poor	90	ND	0.6	0.24
KT97044	MT99071	Tongaren/ Mbakalo	Poor	79	ND	-	-
KT97045	MT99072	Tongaren/ Mbakalo	Good quality	5	ND		-

ND - Not detected

Not analysed by HPLC owing to low levels observed by initial TLC examination

6.4.3 Zearalenone levels in maize samples

Table 6.3. Results of Zearalenone in maize samples using Dawlatana's method of analysis

KARI-Lab ID/No.	NRI-Lab ID/No	Place collected	Quality of maize	% rot	HPTLC method ZEN (ng/g)
KT97001	MT99009	Tongaren/Mbakalo	Good quality	6	ND
KT97002	MT99029	Tongaren/Mbakalo	Very poor quality	90	35.8
KT97003	MT99030	Tongaren/Tongaren	Good quality	4	ND
KT97004	MT99031	Tongaren/Tongaren	Very poor quality	89	20.1.
KT97005	MT99032	Kapsabet/Kapkangani	Good quality	4	ND
KT97006	MT99033	Kapsabet/Kapkangani	Very poor quality	83	42.7
KT97007	MT99034	Kapsabet/Kapsabet	Fair quality	10	12.1
KT97008	MT99035	Kapsabet/Kapsabet	Very poor quality	68	65.4
KT97009	MT99036	Kisumu market	Good quality	11	ND
KT97010	MT99037	Kitale market	Fair quality	13	65.6
KT97011	MT99038	Bungoma market	Fair quality	16	87.1
KT97012	MT99039	Busia market	Fair quality	9	11.4
KT97013	MT99040	Siaya market	Fair quality	9	45.6
KT97014	MT99041	Kakamega market	Fair quality	4	24.2
KT97015	MT99042	Amukura market	Good quality	5	57.3
KT97016	MT99043	Kitale market	Fair quality	7	18.8
KT97017	MT99044	Kapsabet/ Kapsabet	Poor quality	86	159.6
KT97018	MT99045	Kapsabet/Kapsabet	Fair quality	8	30.3
KT97019	MT99046	Kakamega market	Fair quality	7	62.8
KT97020	MT99047	Siaya market	Fair quality	13	48.3
KT97021	MT99048	Busia market	Fair quality	8	ND
KT97022	MT99049	Bungoma market	Fair quality	19	44.3
KT97023	MT99050	Tongaren/ Tongaren	Good quality	2	ND
KT97024	MT99051	Tongaren/ Mbakalo	Good quality	2	ND
KT97025	MT99052	Kisumu market	Good quality	3	24.4
KT97026	MT99053	Kakamega market	Good quality	3	ND
KT97027	MT99054	Kakamega market	Fair quality	17	61.8
KT97028	MT99055	Kisumu market	Poor	73	44.4
KT97029	MT99056	Kisumu market	Fair quality	6	22.4
KT97030	MT99057	Bungoma market	Fair quality	9	15.8
KT97031	MT99058	Bungoma market	Poor	93	252.2
KT97032	MT99059	Busia market	Good quality	7	ND
KT97033	MT99060	Busia market	Fair quality	12	9.8
KT97034	MT99061	Siaya market	Fair	6	13.3
KT97035	MT99062	Siaya market	Poor	18	42.8
KT97036	MT99063	Kapsabet/Kapsabet	Poor	76	46.5
KT97037	MT99064	Kapsabet/Kapsabet	Good quality	3	ND
KT97038	MT99065	Kapsabet/ Kapkangani	Poor	23	68.7
KT97039	MT99066	Kapsabet /Kapkangani	Poor	92	385.6
KT97040	MT99067	Kapsabet/ Kapkangani	Fair quality	71	41.8
KT97041	MT99068	Kitale market	Good	12	58.2
KT97042	MT99069	Kitale market	Very poor quality	87	370.8
KT97043	MT99070	Tongaren/ Tongaren	Poor	90	19.6
KT97044	MT99071	Tongaren/ Mbakalo	Poor	79	74.6
KT97045	MT99072	Tongaren/ Mbakalo	Good quality	5	ND

ND - Not detected

Results of the zearalenone analysis revealed that 73% of the samples were positive for zearalenone using Dawlatana's method, which had a detection limit of 2-3 ng/g (Table 6.3). The levels ranged from 0-386 ng/g but recovery was low, averaging 52%

and revised methodology showed the range to be 0-561ng/g. Zearalenone was detected mainly in poor quality maize. The percentage of rotten kernels in the positive samples ranged from 5-90%. The mean levels of zearalenone in these samples was 70 ng/g. The correlation between percentage number of rotten kernels and the level of zearalenone was significant at 1% (r = 0.6).

Zearalenone was detected in 90% of the area that was classified as cool and wet and included such areas as Kapsabet and Kapkangani, 76% samples in hot and humid areas were positive, the rest of the sample from the intermediate (Average) had 70%. Most of the samples from the hot and humid areas were not representative of the zone as maize sampled on the markets from this area was imported from the other zones. These areas included Siaya, Busia, Kisumu and Amukura.

A zearala-test affinity column was employed to confirm the presence of zearalenone and improve on the recovery of zearalenone in the sample. Three sample extracts produced by Dawlatana's method, two positives and one negative, were passed through the Zearala-test columns. This was done by mixing the dry extracts with 50 ml of 1.6% methanol in water and passed through Zearala-test columns. The resulting Zearalenone levels are given in Table 6.4.

Table 6.4. Zearalenone levels in three samples using dry extracts from Dawlatana method and Zearala-test affinity columns

KARI-Lab ID/No.	NRI-Lab ID/No	Percentage of rotten kernels	Dawlatana method (ng/g)	Dawlatana method + Zearala-test affinity columns (ng/g)
KT97001	MT9909	6	ND	ND
KT97039	MT99066	92	385	329.3
KT97042	MT99069	87	370.8	456.1

ND- Not detected

The TLC spots previously assigned to zearalenone remained approximately the same level, after the highly selective zearalenone clean-up, confirming the presence of zearalenone.

Because of the low recovery of zearalenone using Dawlatana's method, four samples that had high levels of zearalenone were analysed using the complete Zearala-test affinity chromatography and the recovery checked. The results from the complete Zearala-test method had very low levels of zearalenone in samples that originally were known to be high. The results of the two methods are compared in Table 6.5. The recovery of zearalenone through the clean-up step was 100% using Zearala-test compared to 52% using Dawlatana's method. However Dawlatana's method, even with a low mean recovery of 52% during clean-up gave higher levels of zearalenone in the samples than the Zearala-test method. The low results were attributed to the extraction method employed in the Zearala-test method. The results for the two are compared in Table 6.5.

Table 6.5. Zearalenone levels in maize using Dawlatana method and Zearala-test columns

KARI-Lab ID/No.	NRI-Lab ID/No	Percentage of rotten kernels	Dawlatana method (ng/g)	Zearala-test (ng/g)
KT97039	MT99066	92	385.6	48.1
KT97042	MT99069	87	370.8	136.9
KT97031	MT99058	93	252.2	16.1
KT97017	MT99044	26	159.6	39.8

To improve on the Zearala-test affinity method, the extraction method used in Dawlatana's method (chloroform extraction) was used. The extract was first taken to dryness and reconstituted in aqueous methanol before clean up. The result was higher levels of zearalenone were detected in the samples (Table 6.6)

Table 6.6. Zearalenone levels in maize samples tested by Dawlatana method and Zearala-test with chloroform extraction

KARI-Lab ID/No.	NRI-Lab ID/No	Percentage of rotten kernels	Dawlatana method (ng/g)	Zearala-test (ng/g)
KT97017	MT99044	26	159.6	224.8
KT97031	MT99058	93	252.2	294.8
KT97039	MT99066	92	385.6	466.5
KT97042	MT99069	87	370.8	560.9
KT97012	MT99039	9	11.4	61.8
KT97009	MT99036	6	ND	ND
KT97041	MT99068	12	58.2	140.0
KT97010	MT99037	13	65.6	129.0
KT97027	MT99054	17	61.8	175.4
KT97019	MT99046	7	62.8	70.5

ND - Not detected

The use of chloroform:water (84:16) extraction in conjunction with Zearala-test affinity column clean-up gave much higher levels of zearalenone in the samples compared to methanol:water (80:20) extraction as specified in the Zearala-test methodology. The maximum levels found in the samples analyzed was 561 ng/g and the mean recovery was 78%.

6.4.4 Fumonisin B_1 levels in maize samples

Fumonisin B_1 (FB_1) was analysed in 17 samples of both good and poor quality maize. The result showed that the level of FB_1 ranged from 0- 2348 ng/g (Table 6.7). The highest levels of fumonisin were detected in poor quality maize. Correlation analysis between percentage rotten kernels and level of FB_1 showed positive correlation between the two. The correlation was significant at 1% ($r = 0.72$). These results were obtained by eluting the FB_1 with 1 ml of HPLC methanol and using this for quantification by HPLC, without evaporating to dryness. The mean recovery obtained was 87%. When FB_1 was eluted with 1.5ml HPLC methanol and taken to dryness on a sample concentrator at 45^0C over nitrogen gas, the recovery was very low. The loss was assumed to occur when the sample was taken to dryness and

could be due to binding unto the glass vial, despite careful acid washing of the glassware.

Table 6.7. Fumonisin levels in maize samples

KARI-Lab ID/No.	NRI-Lab ID/No	Percentage of rotten kernels	Fumonisin B_1 levels (ng/g)
KT97001	MT99009	6	71
KT97004	MT99031	89	1231
KT97005	MT99032	4	177
KT97006	MT99033	83	550
KT97011	MT99038	86	2348
KT97012	MT99039	9	116
KT97017	MT99044	26	90
KT97023	MT99050	2	43
KT97024	MT99051	2	60
KT97026	MT99053	3	7
KT97028	MT99055	73	323
KT97031	MT99058	93	1364
KT97036	MT99063	76	149
KT97038	MT99065	23	380
KT97039	MT99066	92	2272
KT97042	MT99069	87	386
KT97043	MT99070	90	1191
KT97045	MT99072	0	ND

ND -Not detected

6.5 Discussion

Deoxynivalenol was detected in 20% of the samples analysed, butT-2 toxin was not detected. Deoxynivalenol (DON) and T-2 toxin mycotoxins are produced by *F. graminearum* one of the common fungi associated with cob rot in Western Kenya. DON was only detected in poor quality maize and levels ranged from 0-1100 ng/g. The extraction method used for both DON and T-2 was the same and the recovery was 94% and 96% for T-2 and DON respectively. Percentage rotten kernels and the level of DON showed a linear correlation ($r = 0.67$). DON was detected in maize that originated from the cool wet zone and the moderately cool and wet zone but not in the hot and humid zone except in Kisumu market, where most of the maize sold on the market might have originated from the other zones.

The two mycotoxins are associated with mycotoxicoses in both human and livestock. T-2 toxin is associated with 'alimentary toxic aleukia', a disease that affected thousands of people in Siberia and led to elimination of entire villages. T-2 induces immuno-suppressive activity and causes haemorrhage disease in animals and is associated with neurotoxic effects. Fortunately T-2 was totally absent in all the samples. This finding is consistent with the fact that T-2 is usually produced under conditions of very prolonged wet and cool weather at harvest (IARC, 1993e). Ingestion of DON has been associated with severe outbreaks of acute human mycotoxicoses in China, India, and rural Japan (IARC,1993a). The symptoms in humans are nausea, vomiting, abdominal pains, diarrhoea, dizziness and headache. DON induces feed refusal and decreased weight gain in swine, hens and rats (Vesonder, 1981). The other symptoms of DON poisoning in pigs include vomiting and diarrhoea (Vesonder, 1976). Although this toxin was mainly found in poor quality maize, it is still poses a health hazard because rotten maize harvested in Western Kenya is utilized as livestock feed and for brewing in homes. Feeding trials with farm animals have shown no serious adverse effects of DON at dietary concentrations of 2000 ng/g, in swine, 5000 ng/g in poultry and 6000 ng/g in dairy cattle when fed at the rate of 1% body weight per day (Trenholm *et al.*,1984). Deoxynivalenol is not subject to regulation in any country, however, guidelines, advisory levels and official tolerance levels exist in some countries (IARC, 1983a). In Canada a guide line of 2000 ng/g is given for the occurrence of DON in uncleaned wheat and 1000 ng/g in infant food. In Romania, a tolerance level of 5 ng/g in all feeds is given(Van Egmond, 1989). DON was only detected in poor quality samples, and it was absent or very low in 'good' maize. The presence of DON was confirmed

in three of the positive samples by 2 dimensional TLC. The levels of DON found in this study were low (< 2000 ng/g) relative to Canadian guidelines but more data is required as it is possible to have variation between years or seasons.

Aflatoxin is mainly produced by the fungi, *Aspergillus flavus* and *Aspergillus parasiticus*. Pre-harvest aflatoxin production in maize is associated with factors such as drought stress and insect damage accompanied by high temperatures (25-35^0 C). Post-harvest aflatoxin is associated with inadequate drying during post harvest handling under hot and humid conditions. Aflatoxin is widely considered to be the most dangerous of the mycotoxins. Aflatoxin B_1 is the most potent chemical carcinogen known to man. It is classified as a human carcinogen (IARC, 1993d), causes liver cancer in a wide variety of test animals and is also an immuno-suppressant. In cattle, sheep and dogs aflatoxin causes decreased weight gain, rectal prolapse, bile duct proliferation and acute hepatitis (Panigrahi *et al.*, 1993). In Kenya mycotoxin contamination in cereals grains associated with aflatoxin resulting in death have been reported (Ngindu *et al.*, 1982).

The levels of aflatoxin detected in this study were low, with the maximum levels detected being 10 ng/g. Although conditions for both pre and post harvest aflatoxin contamination do exist in Western Kenya, levels of aflatoxin both at farm and markets were low. The maximum level found in the samples was 10 ng/g. The mean level across the positive samples was 1.7 ng/g and the overall mean levels of aflatoxin in the samples was only 0.6 ng/g. The market samples comprised 59% of the positive samples. This value provides an indication of the overall exposure of aflatoxin B_1 in the samples collected in Western Kenya. The maximum legal limit for aflatoxin B_1 (AFB_1) in raw materials (oil seeds and their derivatives) for animal feed is 20 ng/g in

the European union (IARC, 1993d) and 20 ng/g in maize by the USA food and drug administration (Gourama and Bullerman, 1995). Maximum limits of aflatoxins in food (aflatoxin B_1 or the sum of aflatoxins B_1, B_2, G_1 and G_2) vary from zero detectable up to 50 ng/g (Table 6.8). Aflatoxin B_1 is the most important of the aflatoxins, considered from both the view point of toxicology and occurrence. It is unlikely that commodities will contain aflatoxins B_2, G_1 and G_2 and not B_1, whereas the concentration of the sum of aflatoxins B_2, G_1 and G_2 is generally less than the concentration of B_1 alone (Egmond, 1989). In Kenya there is a regulation on the tolerance levels of aflatoxin of 20 ng/g in peanuts and other vegetable oils by the Food, Drugs and Chemical Substances regulation of the Ministry of Health (Van Egmond, 1989). The mean levels of aflatoxin found in Kenyan maize can be considered to be low for this season. However this may vary between years depending on the environmental conditions.

Table 6.8 Medians and ranges in 1987 and 1996 of maximum tolerated levels (ng/g) for some (groups) aflatoxins and numbers of countries that have these regulations.

	1987			1996		
	Median	Range	No. of countries	Median	Range	No. of countries
B_1 in food stuffs	4	0-50	29	4	0-50	33
$B_1+B_2+G_1+G_2$ in food stuff	7	0-50	30	8	0-50	48
B_1 in food stuffs for children	0.2	0-5	4	0.3	0-5	5
M_1 in milk	0.05	0-1	13	0.05	0-1	17
B_1 in feed stuffs	30	5-1000	16	20	5-1000	19
$B_1+B_2+G_1+G_2$ in feed stuff	30	10-1000	8	50	10-1000	21

Source: Van Egmond, 1999

Zearalenone is an oestrogenic mycotoxin produced by the fungus *Fusarium graminearum*. Zearalenone induces feminization in animals. At high levels it can

interfere with conception, implantation, foetal development and viability of new-born animals. Swine are the most sensitive domestic animals, levels as low as 500 ng/g induces pseudo-pregnancy with failure to cycle (Etienne and Jemmali,1982). Visible signs of zearalenone induced hyperoestrogenism in female swine are swollen vulva and mammary glands, enlargement of the uterus, ovarian changes and infertility. The levels of zearalenone detected in the survey was relatively low from 0-550 n/g but the incidence was high at 73%. The incidence did not appear to be correlated with the climatic zones in which it was collected; the incidence in cool wet was 90%, moderately cool and wet was 70% and the hot humid was 76%. Although it appears that zearalenone was fairly evenly distributed in the three zones it is probable that most of the samples did in fact originate from the cool and wet and the moderately wet zones.

Zearalenone was detected in 68% of the samples that had 10% or more of rotten kernels, but 50% of the samples with less than 10% of the kernels rotten were still found to contain zearalenone. The mean incidence of rotten kernels in the negative samples was 5% and for the positive samples it was 38%, with a mean level of 69 ng/g of zearalenone. The overall mean levels of zearalenone in the samples was 52 ng/g. Zearalenone levels in maize samples with less than 10% rotten kernels was 15 ng/g. Maize found on the Kenyan market for human consumption was considered to be between 0-10% rotten kernels. Based on this finding the level of human exposure was estimated to be 15 ng/g. The level of exposure in grain with more than 10% was 86 ng/g.

In Western Kenya, the bulk of rotten maize is used to feed livestock in homes (over 70%). Rotten maize is also sold to local feed manufacturers as indicated by the

farmers in the PRA survey findings. The use of rotten maize in animal feed, such as dairy meal, exposes the very best of the animals in the country to zearalenone. Zearalenone is however not transmitted from feed to milk to any significant extent (Prelusky *et al.*, 1990). Milling of cereals contaminated with zearalenone concentrates the toxin in the bran fractions. Both zearalenone and DON have been found to be higher in the outer fractions (maize bran) compared to whole maize kernels (Siame and Lovelace, 1989).

The use of these fractions in feed manufacturing increases the risks of exposure to this toxins. The use of the poor quality maize in beer making also posses a very high risk to the consumers in the region. There are no previous records of levels of zearalenone in Kenyan maize and maize based animal feed stuffs.

The maximum legal limit for zearalenone in maize is 1000 ng/g, 200 ng/g and 30 ng/g for USSR, Brazil and Romania respectively (Van Egmond, 1989). The number of countries with specific regulations for mycotoxins in food and animal feed stuffs is increasing over the years. This reflects the general concern that governments have about the potential effects of mycotoxins on the health of humans and animals. However differences are seen in the legal limit that countries have laid down in their regulations.

Two methods of zearalenone analysis were compared in this study. The first method, Dawlatana's method was found to give low recovery (52%) of the zearalenone through the clean up step, when in the presence of the matrix. Zearala-test affinity column clean-up was found to give a much higher recovery, 78%. However the extraction of the toxin from the sample matrix was very low, averaging 12% relative to Dawlatana extraction method. Using a modified method, improved recovery of

over 80% was achieved using Zearala-test clean up. Using this modified method, higher levels were found in the samples than the original levels found by Dawlatana's method. There is a need to fully validate the new hybrid method.

Fumonisins are produced by *Fusarium moniliforme* one of the most common fungal contaminants in agricultural products, especially corn (Sydenham *et al.*, 1990). Fumonisin B_1 is the most frequently occurring and has been reported in USA, Canada, Brazil, S. Africa, Italy and France (Coker, 1999). Fumonisin cause adverse effects in a wide range of animal species, with disease symptoms usually observed at FB_1 levels of 5,000-10,000 ng/g feed, although physiological changes may occur at lower concentration (Munkvold and Desjardins, 1997). Fumonisin is associated with the high rate of oesophageal cancer in the Transkei region of S. Africa (Marasas *et al.*, 1981). In livestock fumonisin causes Equine leukoencephalomalacia in horses (Marasas *et al.*, 1988) and pulmonary oedema in pigs (Ness *et al.*, 1991).

Fumonisin B_1 was found in 95% of the samples and ranged between 0-2348 n/g. The mean levels of fumonisin in the positive samples was 633 ng/g and the overall mean in the samples was 598 ng/g. Maize found on the Kenyan markets for human consumption may contain up to 10% rotten kernels. The mean levels found in samples with less than 10% rotten kernels was 63 ng/g and samples with more than10% rotten kernels had a mean fumonisin level of 1019 ng/g. This study indicated that consumers of maize who restrict themselves to eating good quality maize containing less than 10% rotten kernels will have a relatively low exposure to fumonisin B_1. To date there has been no thorough study of human exposure to FB_1 using good analytical criteria (National toxicological program report review, 1999). However ecological studies of the association between human exposure to *Fusarium*

toxins and oesophageal cancer have been carried out in S. Africa. Marasas *et al* (1981) found the proportion of kernels in both mouldy and healthy maize samples infected by *Fusarium moniliforme*, one of the most prevalent fungi in maize in the Transkei was significantly correlated with oesophageal cancer rates. In other studies in the same area of Transkei (Sydenham *et al.*, 1990), found significantly higher mean numbers of kernels infected with *F. moniliforme* and correspondingly higher levels of mycotoxins fumonisin B_1 and B_2 in mouldy maize samples in the high-risk oesophageal cancer area than in the low-risk area. FB_1 and FB_2 were detected in all samples analysed at levels between 450 ng/g and 18900 ng/g and between 150 ng/g and 6750 ng/g respectively (in samples from the low oesophageal cancer prevalence area), and at levels between 3450 ng/g and 46900 ng/g and between 900 ng/g and 16300 ng/g respectively (in samples from the high oesophageal cancer prevalence area). The estimate of human intake of FB_1 in diets in S. Africa where oesophageal cancer is high, is 200 μg/kg/day (National toxicological program report review, 1999). The levels detected in maize in Western Kenya with less than 10% and more than 10% rotten kernels would give a dietary intake of 0.5 μg/kg/day and 8.3 μg/kg/day respectively. This calculation is based on an average intake of 0.5 kg of maize per person weighing 60 kg. The level detected in this Kenyan study is much lower than those detected in Transkei region. The levels are in fact far lower than the levels found in Bizana district of Transkei (the area considered to have low oesophageal cancer incidence). However, recent animal feeding trials have demonstrated clear evidence of carcinogenic activity of FB_1 in male rats and female mice but no evidence of carcinogenic activity in female rats and male mice (National toxicological program report review, 1999). This findings indicates that FB_1 is

carcinogenic and has serious implications to future national and international regulations. There will be pressure to introduce fumonisin limits at very low levels of perhaps 5 to 10 μ/kg, similar to aflatoxin regulations. This could have a catastrophic effect on the maize industry, because very little maize in the world can currently meet such regulations. There are no regulations or guidelines on the minimum levels of fumonisin in food and feedstuffs (Van Egmond, 1999).

A positive correlation was established between fumonisin levels and percentage rotten kernels ($r = 0.72$). The mean recovery of fumonisin using this method was 87%. The levels of fumonisin B_1 in the maize samples collected were found to be similar to those found previously by Mcdonald in October 1994. Naturally contaminated feed with 166,000 n/g of FB_1 and 48,000ng/g of FB_2 fed orally to swine have been shown to produce acute pulmonary oedema. A diet containing 1000 ng/g of FB_1 fed on rats produced hepatoxicity in four weeks (Gelderblom, 1988).

6.6 Conclusions

1. The mycotoxins deoxynivalenol, zearalenone, aflatoxin and fumonisin were detected in the maize samples collected from Western Kenya and were found mainly in poor quality maize samples. This is the first known report of the occurrence of DON and zearalenone in maize in Western Kenya.
2. T-2 toxin was not detected in any of the samples collected in Western Kenya.
3. The levels of zearalenone occasionally exceed 500 μg/kg and are therefore sufficient to induce pseudo- pregnancy and failure to cycle in pigs.
4. Maize found on the Western Kenyan market for human consumption was estimated to have 10% or less of rotten kernels. Based on this assumption, survey

data indicated that the levels of human exposure to aflatoxin, zearalenone, and fumonisin were within the current guidelines in 1997 and 1998, but large seasonal variations are possible.

5. Recent findings that fumonisin B_1 has carcinogenic activity could eventually lead to the introduction of very low international limits, perhaps down to 5 or 10 μg/kg. Little, if any maize produced in Western Kenya, or the rest of the world, could meet these low limits and the world maize industry would be in jeopardy.

6. Zearala-test (VICAM) method for determining zearalenone gave very low recovery of the toxin from naturally contaminated maize, but this was remedied by a change of extraction solvent.

CHAPTER 7: FIELD EVALUATION OF MAIZE VARIETAL SUSCEPTIBLITY TO EAR ROT PATHOGENS

7.1 Background

The Western highlands form the main maize-producing zone of Kenya. The main varieties grown are H614, H622, H625, H626, and H627 which are recommended for the areas with high rainfall and altitude of 1500-2300 masl. The maturity range of these hybrids is 6-8 months. The other varieties grown in the region are H511, H512 and H513, which are recommended for the medium altitudes with moderate rainfall and altitude ranging from 1000-1800 masl. The maturity range of these hybrids is 4-5 months (Table 1.3)

Yield loss due to ear rot in the region is perceived by farmers and extension workers to be high and is recognized as an important disease problem as previously found in the PRA survey (Chapter3). Some studies have shown variation in losses due to ear rot in these varieties. Previous PRA studies in the region showed that farmers considered variety H614 to have less of an ear rot problem compared to the other varieties and H622 was perceived to be most susceptible to ear rot (Chapter 3). The PRA studies also revealed that ear rot resistance was an important criteria influencing the choice of preferred varieties by the farmers in the region. The predominant ear rot fungi in this region are *F. moniliforme*, *F. graminearum* and *S. maydis* (Kedera, 1998; McDonnell and Chapman, 1997). This is supported by the results of the mycological analysis of samples collected from standing maize on farms just before harvest (Chapter 4) and samples collected from farms and markets (Chapter 5) in 1997- 1998. This study was initiated to evaluate the reaction of five recommended varieties to the three predominant ear rot pathogens under controlled conditions by

artificial inoculation and under farmers' management practices. The study was to provide some information on the performance of five released varieties under farmers' management with particular emphasis on cob rots. In addition, information on the losses associated with cob rot was to be generated both under artificial field inoculation and natural infestations.

7.2 Methodology

Five Kenyan commercial varieties were evaluated for their reaction to ear rot pathogens in the field under artificial inoculation (on-station trials) and in farmers' fields under natural infestation. The varieties evaluated were H614, H622, H626, H627 and H512. Evaluation of the varieties under artificial inoculation was carried out at two locations, KARI-Kakamega and KARI-Kitale research stations for two seasons. The on-farm evaluation was carried out in farmers field in Mbakalo Location of Tongaren Division in Bungoma District.

7.2.1 On-station evaluations

On-station trials were carried out for two seasons during the long rains of 1997 and 1998. Five released varieties (H512, H614, H622, H626 and H627) were evaluated in the field under artificial inoculation with the three fungal pathogens (*Fusarium moniliforme, Fusarium graminearum* and *Stenocarpella maydis*). The experiment was a randomized complete block design with four replications. The three fungal pathogens and the varieties were combined factorially to give 15 treatments. Each plot consisted of 4 rows with 12 plants per row. Five plants/row of each of the inner two rows were inoculated to give a total of 10 inoculated plants per plot. The other

10 plants in the same plot were not inoculated and acted as control. The treatments were as follows

Treatment 1 = *F. moniliforme* x H512

Treatment 2 = *F. moniliforme* x H614

Treatment 3 = *F. moniliforme* x H622

Treatment 4 = *F. moniliforme* x H626

Treatment 5 = *F. moniliforme* x H627

Treatment 6 = *F. graminearum* x H512

Treatment 7= *F. graminearum* x H614

Treatment 8 = *F. graminearum* x H622

Treatment 9 = *F. graminearum* x H626

Treatment 10 = *F. graminearum* x H627

Treatment 11 = S. *maydis* x H512

Treatment 12 = S. *maydis* x H614

Treatment 13 = S. *maydis* x H622

Treatment 14 = S. *maydis* x H626

Treatment 15 = S. *maydis* x H627

Treatment 16 = Non inoculated H512

Treatment 17= Non inoculated H614

Treatment 18 = Non inoculated H622

Treatment 19 = Non inoculated H626

Treatment 20 = Non inoculated H627

7.2.1.1 Preparation of inoculumn

Fusarium moniliforme and *Fusarium graminearum* cultures used for inoculation at Kakamega site were prepared on PDA from diseased cobs obtained from field at Kakamega Research Centre. Diseased kernels were surface sterilized in 2% sodium hypochlorite for 2 minutes, placed on PDA and incubated for five days. The colonies were purified and identified on SNA, kept under near UV light for 7-10 days. The pure cultures were multiplied on PDA, kept under near UV light for 7-10 days for

sporulation to occur. *Stenocarpella maydis* was grown on malt extract agar kept in dark for 5 days and later transferred to near UV light. The cultures used at Kitale site were obtained from Kitale Research Centre and multiplied in similar way. The spores were harvested by shaking in sterile distilled water and the spore count adjusted to 2x 10^6 spores /ml using a haemocytometer. Ears were inoculated by injecting 2ml of spore suspension through the silk channel at 50% silking. The ears were covered with pollination bags for 2 days and then removed. The ears were rated individually for disease severity based on the percentage of kernels rotted on each cob. The number and weight of rotted and clean cobs was recorded in each plot.

7.2.2 On farm evaluations.

On farm trials (OFT) were carried out in Mbakalo Location of Tongaren Division. Only those farmers who had at least 10 acres of land and planting at least three acres of maize every year were selected. Spatial separation was also considered for the ease of visiting them as well as the farmers visiting each other. All farmers who participated in the PRA survey, satisfied the above criteria and were willing to participate were invited to participate in the evaluation of the varieties.

The farmers and the extension staff were invited for a meeting before the onset of the rains and activities to be undertaken during the season were discussed. Roles were also defined, the extension officer was to co-ordinate the field activities and with the help of the scientist, assist in data collection and organize farmers group visits. Only seeds for the five varieties were provided (1 kg each for the five varieties). The other inputs (fertilizers, land preparation, weeding) were provided by the farmer. Each farmer was provided with a notebook for records. The farmers were then left with

seed to plant at the onset the long rains seasons 1998. The trials were harvested in October, 1998.

The experiment was repeated during the long rains season of 1999 with some modifications. From previous studies stalk borer was found to an important predisposing factor to ear rot. The modification was aimed at looking at the effect of stalk borer control on the level of ear rot. The number of farmers was increased to 34 and half of the farmers were provided with Dipterex (Trichlorphon G) for the control of stalk borer, to be applied when the crop was at knee high stage. The remaining farmers were not allowed to apply any control measure for stalk borer.

7.3 Results

7.3.1 On-station experiments

The results of the analysis from Kitale-1997 showed that the yield of rotten maize was different among the treatments. Inoculation with *Fusarium moniliforme* showed a difference between varieties in the yield of rotten maize. H626 had the highest yield of rotten maize under this treatment and was different from H614 and H622. Under *Fusarium graminearum* treatment, variety H626 had the highest yield of rotten maize, it was different from all the other varieties except for H627. The increase in yield of rotten maize was very low when inoculated treatment was compared to non-inoculated treatment. H626 had the highest increase in rotten maize yield of 39% under inoculation with *F. graminearum*. The varieties did not show differences in yield of rotten maize under inoculation with *Stenocarpella maydis* (Table 7.1). There was also no difference observed in the yield of rotten maize between the five varieties under no inoculation. Differences were observed in the yield of clean maize between the treatments. The yield of clean maize in the non-inoculated plots were different

from the yield in inoculated plots. No differences were observed in the number of rotten cobs between treatments.

Table 7.1. Mean yield of clean and rotted maize obtained from 1997 harvest at Kitale

Treatment	Yield in kg/acre			
	Clean	Rotten	Total Yield	% rot
Variety H512				
F. moniliforme x H512	745.4	482.8	1228.2	39
F. graminearum x H512	669.7	318.1	987.8	32
S. maydis x H512	774.4	341.1	1115.5	31
Non- inoculated	742.5	548.7	1291.2	42
Variety H614				
F. graminearum	900.5	389.0	1289.5	30
F moniliforme	906.6	195.8	1102.4	18
S. maydis	643.5	505.4	1148.9	44
Non-inoculated	1405.1	374.0	1778.1	21
Variety H622				
F. moniliforme	588.1	141.5	729.6	19
F. graminearum	829.3	395.9	1225.2	32
S. maydis	913.1	347.5	1260.6	28
Non- inoculated	875.9	503.5	1379.1	37
Variety H626				
F. moniliforme	742.0	604.1	1346.1	45
F. graminearum	584.1	756.5	1340.6	56
S. maydis	569.0	554.0	1123.0	49
Non-inoculated	1148.8	545.6	1694.4	32
Variety H627				
F. moniliforme	1028.6	399.6	1428.2	28
F. graminearum	919.3	553.9	1473.2	38
S. maydis	894.8	471.9	1366.7	35
Non - inoculated	933.0	495.4	1428.4	35
Results of analysis	LSD value = 575.9 at alpha = 0.05	LSD value = 281.7 at alpha = 0.05	LSD value = 796.3 at alpha = 0.05	LSD value = 14.4 alpha = 0.05

Inoculation with the three pathogens had no effect on the yield of clean maize. The highest yields of clean maize was recorded in non inoculated H614 and H626 and were different from the other treatments but not from each other. Although the varieties H614 and H626 had the best yield of clean maize in non inoculated plots they had the poorest yields in plots inoculated with *S. maydis*. Overall, H626 performed well without inoculation but had the poorest yields in inoculated plots

(Table 7.1). When the yield of rotten maize was compared to the overall yield, H614 had the lowest percentage rot of 21% and H512 had the highest percentage rot of 42 % (Table 7.1). Under inoculation with *F. graminearum* H626 had the highest percentage rot of 56% compared to 30% in H614 which had the lowest rot percentage. Similarly H626 had the highest percentage rot of 45% maize under inoculation with *Fusarium moniliforme* compared to the lowest percentage rot of 18% in H614 (Table 7.1).

Table 7.2. Incidence of cob rot in four varieties inoculated with three different pathogens at Kitale 1997

	Disease incidence (%)				
Variety	*F. moniliforme*	*F. graminearum*	*S. maydis*	Mean	Control
H512	38	30	26	31	36
H614	27	25	36	29	25
H622	31	35	31	37	35
H626	40	50	36	45	36
H627	36	40	35	37	31
Mean	34	36	33		33

Table 7.3. Percentage rot in five varieties inoculated with three different pathogens at Kitale 1997

	Percentage rotten grain				
Variety	*F. moniliforme*	*F. graminearum*	*S. maydis*	Mean	Control
H512	39	32	31	34	42
H614	18	30	44	31	21
H622	19	32	28	26	37
H626	45	56	49	50	32
H627	28	38	35	34	35
Mean	29	38	37		35

The highest incidence of rotten cobs was in H627 and H626 inoculated with *F. graminearum* and H626 inoculated with *F. moniliforme*. The mean disease incidence was not different between treatments. The mean disease incidence in plots inoculated with *F. moniliforme*, *F. graminearum* and *S. maydis* was 34%, 36% and 33%

respectively. The mean disease incidence in the non inoculated plots for the five varieties was 33% (Table 7.2)

Table 7.4. Mean yield of clean and rotted maize obtained from 1998 harvest at Kitale

Treatment	Yield in kg/acre			
	Clean	Rotten	Total	% rot
Variety H512				
F. moniliforme	1130.0	794.9	1924.9	41
F. graminearum	1239.0	628.8	1867.8	34
S. maydis	705.8	537.6	1243.4	43
Non-inoculated	1043.0	731.5	1774.5	41
Variety 614				
F moniliforme	2793.0	1218.0	4011.0	30
F graminearum	1043.0	731.5	1774.5	41
S. maydis	2346.0	741.3	3087.3	24
Non-inoculated	2970.0	617.3	3587.3	17
Variety H622				
F. moniliforme	1043.0	476.9	1519.9	31
F. graminearum	1738.0	1519.0	3257.0	47
S. maydis	1314.0	386.5	1700.5	23
Non-inoculated	2346.0	741.3	3087.3	24
Variety H626				
F. moniliforme	2970.0	617.3	3587.3	17
F. graminearum	1281.0	1479.0	2760.0	54
S. maydis	1687.0	842.9	2529.9	33
Non-inoculated	2540.0	773.5	3313.5	23
Variety 627				
F. moniliforme	1670.0	538.7	2208.7	24
F. graminearum	843.9	1614.0	2457.9	66
S. maydis	2516.0	1352.0	3868.0	35
Non-inoculated	4049.0	385.2	4434.2	9
Result of analysis	LSD value =1374 at alpha = 0.05	LSD value = 831.6 at alpha = 0.05	LSD value =920.9 at alpha = 0.05	LSD value = 13.7 at alpha = 0.05

The result of the second season at Kitale in 1998 showed differences in both the yield of clean and rotten maize among the treatments. The number of clean ears and rotten ears were also different among the treatments. The highest number of rotted cobs was found in H627 and H626, inoculated with *F. graminearum*. The two were not different from each other but were different from the other varieties inoculated with the same pathogen (Table 7.4). No difference was observed among the varieties in

the number of rotten cobs in plots inoculated with *S. maydis* and *F. moniliforme* during this season at Kitale. Differences were observed within the varieties in the number of rotten cobs under inoculation with *F. graminearum*, H627 had the highest incidence of rotten cobs. It was not different from H626 and H622 but different from H614 and H512. H627, H626 and H622 had also the highest yield of rotten maize under inoculation with *F. graminearum* and were different from the non inoculated treatments. The three varieties under inoculation had the highest increase in the yield of rotten maize of 319%, 91% and 146% for H627, H626 and H622 respectively when compared to yield of rotten cobs in non inoculated plots. There was no difference in the yield of rotten maize grain between the five varieties under natural infection. Under inoculation with *S. maydis*, H627 had the highest yield of rotten maize which was different from H512 and H622 but was not different from H614 and H626. The incidence of rotten maize was, however, not different between the varieties under this treatment. H626 showed the highest percentage increase of 250% in rotten maize yield under inoculation with *S. maydis* when compared with rotten yield under no inoculation. No difference was observed between the varieties under inoculation with *F. moniliforme*.

There was no difference in the yield of clean maize between varieties under inoculation (Table 7.4). H627 had the highest yield in the non-inoculated plots but registered the lowest yield of clean maize in plots inoculated with *F. graminearum* (Table 7.4). H627 performed well under natural infection and had the lowest yield of rotten maize. The highest percentage rot was recorded in plots inoculated with *F. graminearum* and this averaged 48 %, followed by S. *maydis* 31% and 29 % in plots inoculated with *F. moniliforme*. The mean percentage rot in non inoculated plots

was 23% (Table 7.6). During the second season at Kitale, *F. graminearum* had the greatest effect on percentage rot and was found to be highest in H626 and H627 with a percentage rot of 54% and 65% respectively.

Table 7.5. Incidence of cob rot in five varieties inoculated with three different pathogens at Kitale 1998

	Disease incidence (%)				
Variety	*F. moniliforme*	*F. graminearum*	*S. maydis*	Mean	Control
H512	48	35	69	51	21
H614	34	49	28	37	15
H622	42	52	25	40	21
H626	30	66	42	46	18
H627	40	81	41	54	8
Mean	39	56	41		16

The highest cob rot incidence was observed in H627 and H626 inoculated with *F. graminearum*, 81% and 66% respectively. The mean disease incidence in the five varieties was highest in plots inoculated with *F. graminearum*, 56% and the incidence in the non- inoculated plot was 16%. The other plots inoculated with *F. moniliforme* and *S. maydis* were 39% and 41% respectively (Table 7.5).

Table 7.6. Percentage rot in five varieties inoculated with three pathogens during the 1998 at Kitale

	Percentage rotten grain				
Variety	*F. moniliforme*	*F. graminearum*	*S. maydis*	Mean	Control
H512	41	34	40	35	41
H614	30	47	24	34	17
H622	31	41	23	32	24
H626	17	54	33	35	23
H627	24	65	35	42	10
Mean	29	48	31		23

Table 7.7. Mean yield of clean and rotted maize obtained from 1997 harvest at Kakamega

Treatment	Yield in kg/ acre			
	Clean	Rotten	Total yield	% rot
Variety H512				
F. moniliforme	836.2	608.5	1444.7	42
F. graminearum	477.4	772.8	1250.2	62
S. maydis	570.2	422.2	992.4	43
Non- inoculated	961.3	609.4	1570.7	39
Variety 614				
F. moniliforme	765.1	722.2	1487.3	49
F. graminearum	853.4	648.3	1501.7	43
S. maydis	1060.2	439.8	1500.0	29
Non- inoculated	1236.6	430.9	1667.5	26
Variety 622				
F moniliforme	1035.0	640.2	1675.2	38
F graminearum	660.9	441.8	1102.7	40
S. maydis	524.6	446.9	971.5	46
Non- inoculated	927.8	485.4	1413.2	34
Variety H626				
F. moniliforme	930.3	578.1	1508.4	38
F. graminearum	962.8	496.2	1459.0	34
S. maydis	800.3	491.1	1291.4	38
Non- inoculated	1162.7	470.3	1633.0	29
Result of analysis	LSD value = 402.9 at alpha = 0.05	LSD value = 290.6 at alpha = 0.05	LSD value = 343.6 at alpha = 0.05	LSD value = 13.6 at alpha = 0.05

At Kakamega site, during the long rains 1997, results showed that the effect of different pathogens on the yield of clean maize was different among the varieties. No difference was observed in the yield of rotten maize among the treatments (Table 7 7). However H614 showed the highest increase in the yield of rotten maize of 68% and 51% under inoculation with *F. moniliforme* and *F. graminearum* plots respectively during the 1997 season. Non inoculated plots were not superior to the inoculated in yield of clean maize. The highest percentage rot under no inoculation was 39% in H512 and the lowest 26% in H614. The mean percentage rot in the five varieties *F. moniliforme*, *F. graminearum* and *S. maydis* were 42%, 45% and 39% respectively. Percentage rot in the non-inoculated plots was 32% (Table 7.9).

Table 7.8. Incidence of cob rot in four varieties inoculated with three different pathogens at Kakamega 1997

	Disease incidence (%)				
Variety	*F. moniliforme*	*F. graminearum*	*S. maydis*	Mean	Control
H512	30	56	57	67	36
H614	36	41	39	39	26
H622	27	42	45	38	44
H626	35	48	61	48	31
Mean	47	47	51		35

Table 7.9. Percentage rot in five varieties inoculated with three different pathogens in 1997 at Kakamega

	Percentage rotten grain				
Variety	*F. moniliforme*	*F. graminearum*	*S. maydis*	Mean	Control
H512	42	62	43	49	39
H614	49	43	29	40	26
H622	38	40	46	41	34
H626	39	34	38	37	29
Mean	42	45	39		32

The highest mean percentage rot in the five varieties at Kakamega 1997 occurred in plots inoculated with *F. graminearum.* H614 had the lowest yield losses compared to the other varieties in plots inoculated with *S. maydis*, *F. moniliforme* and the non-inoculated plots. H622 had the highest yield loss in plots inoculated with *S. maydis* and *F. moniliforme*.

Table 7.10. The mean yield of clean and rotted maize obtained from 1998 harvest at KARI-Kakamega

Treatment	Yield in kg/ acre			
	Clean	Rotten	Total	% rot
Variety H512				
F. moniliforme x H512	771.1	600.1	1371.2	44
F. graminearum x H512	1329.0	324.3	1671.5	19
S. maydis x H512	813.9	471.2	1285.1	37
H512	1456.0	417.9	1873.9	22
Variety H614				
F. moniliforme x H614	1479.0	836.7	2315.7	36
F. graminearum x H614	1478.0	492.5	1970.5	25
S. maydis x H614	1308.0	1015.0	2359.0	45
H614	2023.0	454.8	2477.8	18
Variety H622				
F. moniliforme x H622	1028.0	471.5	1499.5	31
F. graminearum x H622	986.0	342.9	1328.9	26
S. maydis x H622	643.0	364.8	1007.8	36
H622	1234.0	397.0	1631.0	24
Variety H626				
F. moniliforme x H626	1607.0	707.3	2314.3	31
F. graminearum x H626	1414.0	429.2	1843.2	23
S. maydis x H626	1135.0	814.3	1949.3	42
H626	2072.0	284.2	2356.2	12
Variety H627				
F. moniliforme x H627	1329.0	513.9	1842.9	
F. graminearum x H627	1201.0	857.4	2058.4	42
S. maydis x H627	728.0	771.2	1499.2	51
H627	1801.0	664.1	2465.1	27
Result of analysis	LSD value =684.4 at 0.05	LSD value = 564.2, at 0.05	LSD value =795.1 at 0.05	LSD value = 11.3 at = 0.05

In the 1988 season at Kakamega there was no difference in the number of rotten cobs between the treatments but the number of clean cobs were different among the treatments. There was also no differences observed in the yield of rotten maize among the treatments. H614 had the highest number of clean ears in the non inoculated plots. The ranking of the clean cobs was H614, H626, H512, H622 and H627. However there were no differences between varieties in the number of clean ears harvested in non-inoculated plots. The yield of clean maize was different among the various treatments. The yields of H626, H614, H627and H512 were not different from the yield of the same variety inoculated with *F. graminearum* and *F.*

moniliforme (Table 7.10). In H622, there was no difference in the weight of clean cobs between the inoculated and the non-inoculated treatments. The lowest yield of clean maize was observed in the varieties inoculated with *Stenocarpella maydis*. This was observed in H622, H512, H627 but no difference was observed among these treatment. The highest incidence of cob rot at Kakamega was in H627 and H626 inoculated with *S. maydis*. The incidence were 55% and 47% respectively. The highest mean disease incidence in the five varieties was in plots inoculated with *S. maydis* (42%) and was lowest in the control (not inoculated) at 24% (Table 7.11). The mean percentage rot due to *F. moniliforme* and *F. graminearum* and *S. maydis* was 34, 28% and 42 % respectively. Mean percentage rot in the in the non-inoculated plots was 21% (Table 7.12). Differences were observed in the total weight with the highest total yield from non-inoculated H614 followed by non-inoculated H627 and H626. H622 and H512 inoculated with *S. maydis* recorded the lowest total yield.

Table 7.11 Incidence of cob rot in five varieties inoculated with three different pathogens at Kakamega 1998

	Disease incidence (%)				
Variety	*F. moniliforme*	*F. graminearum*	*S. maydis*	Mean	Control
H512	30	19	32	27	24
H614	38	24	39	33	23
H622	39	22	36	32	23
H626	31	26	47	34	25
H627	26	41	55	40	23
Mean	33	26	42		24

Table 7.12 Percentage rot in five varieties inoculated with three different pathogens during the1998 at Kakamega

	Percentage rotten grain				
Variety	*F. moniliforme*	*F. graminearum*	*S. maydis*	Mean	Control
H512	44	21	37	34	22
H614	36	25	45	35	18
H622	31	26	36	31	24
H626	31	23	42	32	12
H627	28	42	51	40	27
Mean	34	28	42		21

Table 7.13. Summary for the mean total yield of grain at Kitale and Kakamega for two seasons

Treatment	Yield in kg/ acre					
	Kakamega			Kitale		
	1997	1998	Mean	1997	1998	Mean
Variety H512						
F. moniliforme	1444.7	1371.2	1408.0	1228.2	1924.9	1576.6
F. graminearum	1250.2	1671.5	1460.9	987.8	1867.8	1427.8
S. maydis	992.4	1285.1	1138.8	1115.5	1243.4	1179.5
Mean	1229.1	1442.6		1110.5	1678.7	
Variety H614						
F. moniliforme	1487.3	2315.7	1901.5	1289.5	4011.0	2650.3
F graminearum	1501.7	1970.5	1736.1	1102.4	1774.5	1438.5
S. maydis	1500.0	2359.0	1929.5	1148.9	3087.3	2118.1
Mean	1496.3	2215.1		1180.3	2957.6	
Variety H622						
F. moniliforme	1675.2	1499.5	1587.35	729.6	1519.9	1124.8
F. graminearum	1102.7	1328.9	1215.8	1225.2	3257.0	2241.1
S. maydis	971.5	1007.8	989.65	1260.6	1700.5	1480.6
Mean	1249.8	1278.7		1071.8	2159.1	
Variety H626						
F moniliforme	1508.4	2314.3	1911.35	1346.1	3587.3	2466.7
F. graminearum	1459.0	1843.2	1651.1	1340.6	2760.0	2050.3
S. maydis	1291.4	1949.3	1620.35	1123.0	2529.9	1826.5
Mean	1419.6	2035.6		1269.9	2959.1	
Variety H627						
F. moniliforme	*	1842.9	1842.9	1428.2	2208.7	1818.45
F. graminearum	*	2058.4	2058.4	1473.2	2457.9	1965.55
S. maydis	*	1499.2	1499.2	1366.7	3868.0	2617.35
Mean	*	1800.2		1422.7	2844.9	
Non-inoculated						
H512	1570.7	1873.9	1722.3	1291.2	1774.5	1532.9
H614	1667.5	2477.8	2072.65	1778.1	3587.3	2682.7
H622	1413.2	1631.0	1522.1	1379.1	3087.3	2233.2
H626	1633.0	2356.2	1994.6	1694.4	3313.5	2504.0
H627	*	2465.1	2465.1	1428.4	4434.2	2931.3
lsd	343.6	795.5		796.3	920.9	

* H627 had not been released on the market by the time the trial was planted at Kakamega

Table 7.14. Summary for the mean percentage rotten grain for Kitale and Kakamega for two seasons

Treatment	Percentage rotten grain					
	Kakamega			Kitale		
	1997	1998	Mean	1997	1998	Mean
Variety H512						
F. moniliforme	42	44	43	39	41	40
F. graminearum	62	19	41	32	34	33
S. maydis	43	37	40	31	43	37
Mean	49	33		34	39	
Variety H614						
F. moniliforme	49	36	43	30	30	30
F. graminearum	43	25	34	18	41	30
S. maydis	29	45	37	44	24	34
Mean	40	35		31	32	
Variety H622						
F. moniliforme	38	31	35	19	31	25
F. graminearum	40	26	33	32	47	40
S. maydis	46	36	41	28	23	26
Mean	41	31		26	34	
Variety H626						
F. moniliforme	38	31	35	45	17	31
F. graminearum	34	23	29	56	54	55
S. maydis	38	42	40	49	33	41
Mean	37	32		50	35	
Variety H627						
F. moniliforme	*	28	28	28	24	26
F. graminearum	*	42	42	38	66	52
S. maydis	*	51	51	35	35	35
Mean	*	40		34	42	
Non- inoculated						
H512	39	22	31	42	41	42
H614	26	18	22	21	17	19
H622	34	24	29	37	24	31
H626	29	12	21	32	23	28
H627	*	27	27	35	9	22
lsd	13.6	11.3		14.4	13.7	

* H627 had not been released on the market by the time the trial was planted at Kakamega

7.3.2 On-farm experiments

On-farm trials were carried out during the long rains seasons of 1998 and 1999 in Tongaren Division of Bungoma District. In the 1998 season 22 farmers participated in the trials but data was collected only on 18 farms. Each farmer had five varieties and represented a replicate. The dates of planting varied from March to early May. Harvesting was done in October. Data on lodging, broken stems, bare tips, weight and number of clean and rotten cobs, severity of rotting and borer damage was taken. In 1999 the number of farmers was increased to 34 and were divided into two groups. The first group of 17 farmers were supplied with Dipterex to control the stem borers and the other set did not control stem borers on their plots. Thirteen farmers in each set managed to take care of the plots to harvesting and data were taken on this farms.

In the 1998 season data collected showed the number of broken stems were different between the five varieties. The highest incidence of broken stems was recorded in H622; it was significantly different from H626 and H627, but not significantly different from H614 and H625. The highest incidence of bare tips was observed in H627, and was different from the other varieties (Table 7.15). The other varieties were not different from each other in the number of open tips. There were no differences in the average number of plants harvested in each farm and also on the total area harvested per variety between farms and within farms during this season. Observation also showed differences between the varieties in the number of plants that had root lodging. H622 had the highest incidence of root lodging, it was significantly different from H627 and H626 and H625 and not different from H614 (Table 7.20)

Table 7.15. Comparison of performance of five varieties planted on farm in 1998 in Tongaren Division

Parameter	Variety					LSD value	
	H614	H622	H625	H626	H627		
Mean % borer damage	18	19	19	22	20		
Mean % rot	13	20	16	14	15		
Mean % severity	25	25	20	19	24		
Mean % incidence	22	25	23	19	21		
% Borer damage[1]	1.21	1.21	1.21	1.25	1.23	0.25	ns
% rot[1]	1.10	1.33	1.17	1.16	1.19	0.10	**
% Severity[1]	1.40	1.40	1.30	1.28	1.32	0.14	ns
Broken stems	0.38	0.41	0.38	0.36	0.32	0.04	*
Incidence	1.30	1.38	1.31	1.29	1.38	0.10	ns
Bare tips	26.6	30.7	27.3	27.3	102.9	43.8	**
Root lodging/ha	6709	9716	6612	5035	3697	3070	**
Stand count at harvest/ha	28328	33452	35718	37060	33853	13909	ns
Yield of clean maize kg /ha	4632	2642	3784	4729	3601	987.7	**
Yield of rotten maize kg /ha	716	662	698	786	625	227.7	ns
Total yield kg/ha	5347	3304	4481	5515	4226	1116.8	**

ns - not significant
* - significant at 5% level
* * - significant at 1% level
1- Log transformation

The results also showed that the percentage rot was different between the varieties, H622 had the highest percentage rot and was different from the rest of the varieties. There was no difference in percentage rot between the other varieties. The highest percentage rot was 20% in H622 and the lowest was 13% in H614. H626, H627 and H625 had mean percentage rot of 14%, 15% and 16% respectively (Table 7.16). The mean percentage rot of the five varieties during this year was 16%. The varieties were not different in the severity and the incidence of rotten cobs.

Table 7.16. Mean performance of five varieties and percentage rot in Tongaren Division taken from 18 farms in 1998

Variety	Mean yield of clean (kg/ha)	Yield of rotten (kg/ha)	Total yield (kg/ha)	Percentage yield loss
H614	4632	716	5347	13
H622	2642	662	3304	20
H625	3784	698	4481	16
H626	4729	786	5515	14
H627	3601	625	4226	15
Mean	3880	697	4575	16

The result of the on-farm trials in 1999 from thirteen farms where no Dipterex was applied showed that there were no difference between varieties in the incidence of borer damage on the cobs. The varieties were different in the number of rotten cobs harvested. However, the incidence of rotten cobs was not different between the varieties. H626 had the highest number of rotten cobs per hectare and H614 had the lowest. The percentage rot was significantly different between the varieties, H622 had the highest percentage rot (30%) and was different from the rest of the varieties. The lowest percentage rot was in H614 (19%) but was not different from H625, H626 and H627 (Table 7.17). Mean percentage rotten maize for the five varieties in 1999 was 23%. Differences were observed in the number of broken stems per hectare and the number of bare tips per hectare (Table 7.17).

Table 7.17 Comparison of five varieties with no Dipterex applied to control borer on farms in 1999

Parameter	Variety					LSD value	
	H614	H622	H625	H626	H627		
Mean % borer damage	22	19	22	26	19		
Mean % rot	19	30	20	25	23		
Mean % severity	20	22	19	21	21		
Mean % incidence	34	37	32	37	32		
Borer damage[1]	1.32	1.30	1.31	1.39	1.26	0.10	ns
% rot[1]	1.28	1.48	1.31	1.39	1.35	0.09	**
Severity[1]	1.29	1.31	1.26	1.30	1.27	0.08	ns
Incidence[1]	1.41	1.48	1.40	1.50	1.41	0.09	ns
Broken stems/ha	4941	7543	3686	4619	6689	2099	**
Bare tips/ha	1211	1148	557	1122	2663	562	**
No. of good ears/ha	20245	19140	23252	20967	25287	5046	ns
Root lodging/ha	5155	7062	5266	4880	2802	1739	**
No. of rotten cobs/ha	7497	8463	7617	10625	9723	1283	*
Stand count at harvest	41140	41804	38272	38215	43105	6320	ns
Yield of clean maize kg/ha	3957	2425	3564	3843	3968	756	**
Yield of rotten maize kg/ha	943	1029	881	1281	1152	3228.0	**
Total yield kg/ha	4900	3453	4445	5124	5121	858	**

ns - not significant
* - Significant at 5%
* * -Significant at 1% level
1- Log transformation

H622 had the highest number of broken stems, it was different from H625, H614 and H626 but not different from H627. The highest number of bare tips was found in H627, it was different from the rest of the varieties. H625 had the lowest number of open tips, it was different from all the varieties (Table 7.17). Root lodging was different between the varieties, the highest incidence of root lodging was in H622 and was different from the other varieties. The severity of rotten cobs was also not different among the varieties.

In the farms where Dipterex was applied, no difference was observed in the incidence of borer damage in cobs among the varieties. Differences were observed in the number of rotten cobs and the yield of rotten cobs among the varieties. The highest number of rotten cobs was in H627 and was significantly different from the all the varieties except H625. The lowest number of rotten cobs was in H614. Percentage rot and severity of rotten cobs was different between the varieties. The highest percentage rot was recorded in H622, it was different from all the varieties. The other varieties were not different from each other (Table 7.18). The number of clean ears was different between the varieties. H627 had the highest number of clean ears, it was not different from H625 but was different from the rest of the varieties.

Table 7.18. Comparison of five varieties with Dipterex applied to control borer on farms in 1999

Parameter	Variety					LSD value	
	H614	H622	H625	H626	H627		
Mean % borer damage	22	22	19	20	20		
Mean % rot	16	26	18	19	19		
Mean % severity	26	32	25	26	26		
Mean % incidence	28	33	25	26	27		
Borer damage[1]	1.24	1.21	1.20	1.25	1.22	0.24	ns
% rot[1]	1.17	1.41	1.25	1.25	1.26	0.11	**
Severity[1]	1.33	1.46	1.34	1.36	1.43	0.10	*
Incidence[1]	1.35	1.48	1.75	1.38	1.40	0.09	ns
Broken stems/ha	2698	4848	3785	3001	5701	1657	**
Bare tips/ha	1091	1451	729	1049	2144	555.	**
No. of good ears/ha	19142	19963	25351	21149	28397	4004	**
Root lodging/ha	3918	5533	6596	4126	3380	1844	**
No. of rotten cobs/ha	5921	8741	7886	7335	10193	2199	**
Stand count at harvest/ha	32396	37495	44245	35752	46390	7427	*
Yield of clean maize kg/ha	4077	2869	4708	4261	4826	832	**
Yield of rotten maize kg/ha	759	997	1039	1004	1101	270	ns
Total yield kg/ha	4836	3866	5746	5265	5927	2199	**

ns - not significant
* - Significant at 5% level
* - Significant at 1% level
1- Log transformation

The highest number of broken stems was in H622, it was different from H626 and H614 but not different from H627 and H625. The number of bare tips was highest in H627, it was different from the rest of the varieties but was not different from H622 (Table 7.18)

Comparison was made between the 13 farmers who applied dipterex on their farm and the other 13 who did not apply Dipterex for the control of stalk borers. The percentage rot in farms where Dipterex was applied was lower compared to the farmers where Dipterex was not applied in all the five varieties (Table 7.19). The mean yield of clean grain in farms where Dipterex was applied was also superior to farms where no Dipterex was not applied. The mean disease severity was different between varieties in dipterex applied farms but no differences were observed in farms

where no Dipterex was applied. Borer damage was not different between the varieties in the two groups of farms.

Table 7.19 Percentage rot in five varieties obtained from on-farm trials in Tongaren Division 1999

Treatment	% Rot incidence	% mean severity	% borer damage	Clean yield	Rotten yield	Total yield	Mean % loss
No. Dipterex applied							
H614	34	20	22	3957	943	4900	19
H622	37	22	19	2425	1029	3453	30
H625	32	19	22	3564	881	4445	20
H626	37	21	26	3843	1281	5124	25
H627	32	21	19	3968	1152	5121	23
Mean	34	21	22	3551	1057	4609	23
Dipterex applied							
H614	28	26	22	4077	759	4836	16
H622	33	32	22	2869	997	3866	26
H625	25	25	19	4708	1039	5746	18
H626	26	26	20	4261	1004	5265	19
H627	27	26	20	4826	1101	5927	19
Mean	28	27	21	4148	980	5128	20

8.4 Discussion

From the results obtained in 1998, differences were observed in both the yield of rotten maize and the number of rotten cobs at Kitale. During this season the highest incidence of rotten cobs was in H627 and H622 and this was reflected in the high increase of rotten maize when compared to the same varieties without inoculation. The number of rotten cobs and the yield of rotten maize was higher in the same varieties without inoculation. The two varieties seem to be susceptible to *F. graminearum*. This is an indication of variability in the susceptibility of the varieties studied to the different cob rot fungi. Further research should be conducted under controlled conditions with little or no natural infection. There was a difference in number of rotten cobs in Kitale during the second season. This was only observed in plots inoculated with *F. graminearum*. Non-inoculated treatments did not show

difference in the disease incidences for the two seasons. The results obtained from Kitale and Kakamega during the 1997 season showed that there was no difference in the number of rotten cobs and yield of rotten maize in the varieties in the inoculated and the non-inoculated treatments. The results seem to suggest inoculation was not effective in increasing the disease pressure during this season. The percentage increase in the yield of rotten grain when inoculated and non-inoculated treatments were compared was consistently low with *S. maydis* and *F. moniliforme*. In Kakamega during the two seasons, no differences were observed in the yield of rotten maize between inoculated and non-inoculated plots. Analysis of the incidence of cob rot also showed no differences in the number of rotten cobs. The results show that either the pathogen were not effective in increasing the disease pressure or the level of the disease pressure was high and inoculation had no role. To find out more about the relative pathogenicity of the three fungi it is necessary to evaluate varieties under a controlled environment, where control treatments are free from disease pressure from natural infestations by naturally occurring fungi in the field. Interaction between the early colonizers from natural infestation and the inoculum may have a synergistic or suppressive interaction interfering with the colonization of the cob.

Based on the percentage of rotten grain in the harvest, H614 performed consistently better than the other varieties under natural infection and had the lowest mean percentage rot at the two sites for the two seasons. H622 had the highest rotten grain percentage. The highest total yield under no infection was in H627 and the lowest in H622.

In the 1998 season at Kitale, the varieties H622, and H627 were not different from the other varieties in the yield of rotten maize under natural infestations but under

inoculation with *F. graminearum* the yield of rotten maize grain increased by over 100%. This showed that the two varieties could be susceptible to the pathogen. H627 also showed a high increase in the yield of rotten maize under inoculation with *S. maydis* when compared to the non-inoculated treatment.

Generally the highest percentage rot for the two seasons in Kitale were in plots inoculated with *F. graminearum*. The mean percentage rot over the two seasons was 43% in the five varieties. The mean percentage rot for the two seasons was 30% and 34% for *F. moniliforme* and *S. maydis* respectively. H614 had the lowest mean percentage rot due to *F. moniliforme* and *S. maydis* at Kitale site in both seasons but did not show superiority over H622 and H512 when inoculated with *F. graminearum*. However, it was superior to H626 and H627 in plots inoculated with *F. graminearum*. Since there was no difference in the yield of clean maize in the three varieties, the proportion of rotting was high in the latter two varieties. The two varieties appeared to be more susceptible to *F. graminearum*.

From the result obtained from the on-farm trials, the varieties showed differences in the number of rotten cobs per acre, but cob rot incidence was not significant between the varieties. The mean percentage rot in these varieties showed that H614 had the lowest rot compared to the other varieties. The highest percentage rot was in

Table 7.20. Ranking of the varieties based on the results of the on-farm trials in 1998 and 1999 season

Ranked based on the mean of three trials

Variety	% rot	Bare tips	Lodging	Total yield	Total score	Rank
H614	5.0	3.0	3.0	2.7	13.7	1
H622	1.7	2.0	1.0	1.0	5.7	5
H625	3.3	5.0	2.0	3.0	13.3	3
H626	2.7	3.0	3.7	4.0	13.4	3
H627	3.0	1.0	5.0	3.7	12.7	4

Rank 1-5
1 = Lowest score
5 = Highest score (favourable traits)

H622 and was different from the other four varieties for the two seasons tested. The other four varieties were not different from each other in percentage rot. From the result of the PRA, farmers associated H622 with high percentage rot and it was ranked the poorest based on this criteria. H614 was the best based on this ranking. The variety H622 was also associated with bare tips and lodging, factors that was thought to favour cob rot incidence in the field. The farmers in Tongaren associated bare tips and lodging to high cob rot incidence, the variety H622 was associated with these factors. The result from the on-farm experiment confirms that H622 was prone to lodging and had the highest number of broken stems. It was also ranked second to H627 in terms of bare tips (Table 7.20). The variety H627 is newly introduced and most of the farmers were growing it for the first time. H622 had consistently the highest incidence of root lodging and broken stems. Broken stems and lodged stems will expose the cobs to rotting when in contact to the ground. However environmental factors may also have an influence on root lodging and broken stems other than the variety and therefore make this factor seasonally dependant. It was clear from the PRA that farmers in the region preferred varieties with closed ear tips because they are less prone to rotting. H614 was overall the best ranked variety in

terms of total yield, lodging, bare tips and percentage rot. H622 had the poorest score (Table 7.20). The influence of open tips could not be verified in this experiment. Although the incidence did not reflect the yield loss in grain, this could be explained by the difference in the number of clean cobs harvested, size of the cobs and probably differences in the grain density in the varieties.

Frams where Dipterex was applied had less yield loss due to cob rot. Previous work showed (Chapter 4) significant correlation in borer incidence and cob rot in the same area. Stem borers are controlled early in the season when the crop is at knee high by applying Dipterex granules in the whorl of the plant but the effectiveness of the chemical later in the season to control the borers in the cob could not be verified. However higher percentage of rot was observed in all varieties in plots without Dipterex control when compared to dipterex applied plots. These result obtained for one season seem to suggest that dipterex could reduce borer incidence and probably losses due to cob rot. The varieties H622 was considered to be more susceptible and was ranked overall the poorest in terms percentage rot, lodging bare tips and total yield while H614 was considered to be more resistant and was ranked first. This results are in agreement with farmers ranking of the varieties. Varieties H627 and H622 did appear to be particularly susceptible to *F. graminearum*

7.5 Conclusion

1. Disease incidence was not different in the cultivars even after inoculation both at Kitale and Kakamega. This was also true in non-inoculated plots and in the on- farm trials. The result seem to suggest that these Kenyan hybrids are not different in susceptibility to cob rot.

2. The varieties showed differences in weight of rotted cobs, but this could not provide a basis for conclusion as it might be related to other yield parameters.

3. H627 was consistently the highest yielding cultivar and H622 was the lowest yielding variety.

4. The varieties did not show differences in stalk borer damage.

5. Disease incidence was high even in the absence of artificial inoculation. Artificial inoculation did not increase it by much.

6. *F. graminearum* was the most frequently isolated pathogen and there was some evidence that H627 and H622 might be more susceptible to the pathogen than the other varieties.

7. The results of the on-farm support the view of the farmers that H614 is least prone to rot and H622 most prone.

CHAPTER 8: EFFECT OF AGRONOMIC PRACTICES ON COB ROTS

8.1 Effect of time of harvesting on cob rot

8.1.1 Background

During the Participatory rural appraisal exercise in the two Divisions of Tongaren and Kapsabet, the role of maize production practices and their influence on cob rot were discussed. Delayed harvesting was considered as an important factor affecting ear rot incidences in the field. When maize was allowed to stay in the field for a long period after physiological maturity the losses due to rotting were considered to be higher. However, the time period that maize is left in the field is variable. In Tongaren farmers preferred to leave their maize in the field for a period of four weeks after physiological maturity to allow for drying. Farmers will delay harvesting until the weather condition is dry or if heavy rains occur. Losses due to rot were perceived to be higher when harvesting proceeded during the rainy days. Investigations were therefore carried out to determine the effect delayed harvesting on cob rot in the field on four hybrids grown in the region.

8.1.2 Methodology

The experiment was carried out for two seasons at KARI-Kakamega and KARI-Kitale during the 1997 and 1998 seasons. Four commercial varieties were evaluated. The experiment was a randomized complete block design with four replicates. Each plot comprised of 6 rows of 12 plants each. The inner four rows were harvested at each harvesting interval. The harvesting interval was at 8, 12, and 20 weeks post mid silk stage. The rotted ears were counted (incidence) and rated for percentage of the rotted kernel per cob (severity), and percentage rotted grain recorded.

8.1.3 Results

Table 8.1. Mean yield of clean and rotted maize harvested at three different time intervals at Kakamega in 1997

Treatment	Yield in kg/ acre			
	Clean	Rotten	Total	% rot
8 weeks				
H512	2126	307.7	2433.7	13
H614	3907	435.5	4342.5	10
H622	2553	307.7	2860.7	11
H626	3693	393.8	4086.8	10
H627	1479	527.6	2006.6	26
12 weeks				
H512	2419	491.6	3210.6	16
H614	3390	659.9	4049.9	16
H622	2109	522.9	2631.9	19
H626	3400	437.9	3837.9	11
H627	1363	578.0	1941.0	30
20 weeks				
H512	2331	995.6	3326.6	30
H614	2407	921.5	3328.5	27
H622	1231	1674	2905.5	42
H626	2377	1407.0	3484.0	40
H627	967.4	791.4	1758.8	45
	LSD Value = 1031, a = 0.05	LSD Value = 534.5, a = 0.05	LSD Value = 1179, a = 0.05	LSD value = 11.3 at a = 0.05

During the 1997 season, the yield of both clean and rotten maize was different at the three harvesting intervals (Table 8.1). Differences were observed in the yield of rotten maize between the 8th and the 20th week in H512, H622 and H26 but not in H614 and H627. The total yield harvested was also different at the three different harvesting time intervals. Differences were observed in all the varieties between 8th and 20th week, except in H626 which showed a difference in percentage rot between 12th and 20th week harvesting interval. Percentage rot at 8thth, 12th and 20th week was 14%, 18% and 37% respectively (Table 8.4). Disease incidence was significantly different. H622 and H626 had the highest disease incidence and a difference was observed between the 8th and 20th week in the two varieties. Other varieties did not show differences in disease incidence at the different harvesting time interval. The

overall mean disease incidence at 8thth, 12th and 20th week was 20%, 25% and 47% respectively (Table 8.2). Disease severity was not significantly different in all the varieties at different harvesting intervals but there was a trend towards increasing severity at late harvesting date. The overall disease severity at 8thth, 12th and 20th week was 35%, 39% and 49% respectively (Table 8.3).

Table 8.2. Incidence of cob rot in four varieties harvested at three time intervals at Kakamega 1998

	Cob rot incidence (%)		
Variety	8 weeks	12 weeks	20 weeks
H512	21	25	43
H614	15	20	39
H622	18	23	51
H626	19	18	49
H627	29	37	55
Mean	20	25	47

Table 8.3. Severity of cob rot in five varieties harvested at three times intervals at Kakamega 1997

	Cob rot severity (% rotted grain per cob)		
Variety	8 weeks	12 weeks	20 weeks
H512	23	27	35
H614	38	29	40
H622	35	50	53
H626	33	35	55
H627	48	53	60
Mean	35	39	49

Table 8.4. Percentage rotten grain in five varieties harvested at three time intervals at Kakamega 1997

	Percentage rotten grain		
Variety	8 weeks	12 weeks	20 weeks
H512	13	16	30
H614	10	16	27
H622	11	19	42
H626	10	11	40
H627	26	30	45
Mean	14	18	37

Table 8.5. Mean yield of clean and rotted maize harvested at three different time intervals at Kakamega in 1998

Treatment	Yield in kg/ acre			
	Clean	Rotten	Total	% rot
8 weeks				
H512	2000.0	370.0	2370.0	16
H614	3519.0	407.0	3926.0	10
H622	2889.0	333.0	3222.0	10
H626	2852.0	370.0	3222.0	12
H627	3111.0	1185.0	4296.0	28
12 weeks				
H512	1630.0	555.0	2185.0	25
H614	3593.0	778.0	4370.0	18
H622	1963.0	667.0	2630.0	25
H626	2037.0	259.0	2296.0	11
H627	2148.0	1407.0.	3556.0	40
20				
H512	1778.0	778.0	2556.0	30
H614	1092.0	367.0	1459.0	25
H622	1801.0	778.0	2579.0	30
H626	1148.0	444.0	1592.0	28
H627	1565.0	1340.0	2905.0	46
	LSD Value = 1052.8 a = 0.05	LSD Value = 435.9, a = 5	LSD Value = 1072.8 a = 0.05	LSD value = 12.1 at = 0.05

In 1998, differences were observed in the varieties in percentage rotten grain at the three levels of harvesting. In all the varieties, percentage rot was different between the 8th week and 20th week. In H626 differences were also observed between 8th and 20th week harvesting interval. The highest percentage rot was at the last harvesting interval in H627 and was different from the other varieties. The overall mean percentage rot in the five varieties was 15%, 24% and 32% at 8th, 12th and 20th week respectively (Table 8.8). The yield of rotten cobs was significantly different at the three harvesting intervals. H627 had the highest yield of rotten cobs at week 20 harvesting time interval and was different from the other varieties but not difference in the same variety at 8th and 12th week harvesting interval. The yield of rotten maize was not significantly different at the three time intervals in H512, H614 and H627 but in H622 it was significantly different between the 8th and 20th week. Differences were

observed in the incidence of rotten cobs between the varieties at the three harvesting intervals. This difference was observed in H626, H614 and H627 at the 8th and 20th harvesting intervals but was not different at the other time intervals. The highest incidence of cob rot was in H627 at the 20th week harvesting interval (Table 8.6). The other varieties did not show differences in the incidence of cob rot at the three levels of harvesting. Severity of the rotten cobs did not differ at the three levels of harvesting. The highest disease severity was observed in H626 at the 20th week harvesting interval (Table 8.8). The overall mean severity for the five varieties was 47%, 51% and 52% at 8th, 12th and 20th week respectively (Table 8.7).

Table 8.6. Incidence of cob rot in five varieties harvested at three time intervals at Kakamega 1998

	Cob rot incidence (%)		
Variety	8 weeks	12 weeks	20 weeks
H512	18	28	32
H614	19	19	41
H622	22	25	36
H626	16	32	37
H627	30	44	54
Mean	21	30	40

Table 8.7. Severity of cob rot in five varieties harvested at three time intervals at Kakamega 1998

	Cob rot severity (% rotted grain per cob)		
Variety	8 weeks	12 weeks	20 weeks
H512	47	25	33
H614	48	63	56
H622	35	55	45
H626	50	57	78
H627	53	53	51
Mean	47	51	52

Table 8.8. Percentage rot in five varieties harvested at three time intervals at Kakamega 1998

	Percentage rotten grain		
Variety	8 weeks	12 weeks	20 weeks
H512	16	25	30
H614	10	18	25
H622	10	25	30
H626	12	11	28
H627	28	40	46
Mean	15	24	32

9.1.4 Discussion

The varieties were harvested at three intervals of 8, 12, and 20 weeks post-mid-silk stage. These varieties have a maturity range of 6-8 months. The varieties will reach a physiological maturity at Kakamega in a period of about 180 days. The harvesting interval of 8 weeks post silking stage was to coincide with the physiological maturity followed by an interval of 4 and 12 weeks harvesting periods equivalent to 12 and 20 weeks post silk stage. The result of the data for two seasons of 1997 and 1998 showed that there was a difference in rot percentage between the 8^{th} and 20^{th} week harvesting period but no difference was observed between the 8^{th} and 12^{th} week. Disease incidence was different at the three harvesting intervals in the two seasons in H626 and H622 in 1997 and in H614 and H626 and H627 in 1998. The other varieties did not show differences in disease incidence at the three harvesting intervals. The difference in disease incidence was not consistent in the two years in the varieties except in H626. However the trend in the varieties showed an increase in disease incidence with time of harvest. Although no differences were observed in percent rotten grain between 4 and 8 weeks harvesting intervals, the trend was also towards increased rotting with time. Time before harvest may not be the most important factor in determining percentage rot and disease incidence but the

prevailing weather conditions are crucial. Farmers in Western Kenya leave their maize in the field for 4 weeks after physiological maturity to dry before harvesting. During dry spells the farmers may leave the maize to stay in the field for a longer period. This allows for harvesting and direct storage without the need for further drying. Harvesting too early may result in storage of maize with high moisture which may require further sun drying before storage.

8.1.4 Conclusion

1. Disease incidence and percentage rotten maize increased with the delayed harvest.

2. There was no difference between harvesting at 8 weeks post mid silk stage (at physiological maturity) and 12 weeks post mid silk stage (4 weeks after physiological maturity) in disease incidence and percent rot.

3. Disease severity was not different at the three stages of harvest, but the mean disease severity for the five varieties showed an increasing trend with the time of harvest.

4. It should be recommended that farmers should harvest as soon as possible after the 4 week period from physiological maturity.

8.2 Effect of fertility levels on ear rot incidence

8.2.1 Background

The results of the PRA survey indicated that agronomic practices such as timely weeding, use of fertilizers reduced the level of ear rot. The use of fertilizers was considered by farmers to have an effect on the size of the cobs. Well fertilized cropsproduced bigger cobs (heavy cobs) which tended to bend down (droop) at physiological maturity. The effect was thought to reduce the accumulation of water

in the cobs therefore reducing cob rot incidence. This characteristic was also associated with variety. An investigation was therefore carried out to find the effect of three levels of fertilizer on cob rot incidence in four varieties.

8.2.2 Methodology

Three levels of the recommended fertilizers for maize production were applied to four varieties of maize to find out the effect on ear rot incidence, severity and percentage rot in the field. The fertilizer used was di-ammonium phosphate (DAP) which was applied at planting and calcium ammonium nitrate (CAN) applied as a top dressing when the crop was knee high. The fertilizer levels were, full rate (applied at the recommended rate of 250 kg per hectare), half rate applied at 125 kg per hectare and zero level (no fertilizer applied). The varieties assessed were H614, H622, H626 and Namba nane (Local variety). The three fertilizer levels and four varieties were combined factorially to give twelve treatments. Data was recorded on lodging, open ears, damage by birds and insects, total number of rotten ears and good ears and severity of rotting. The experiment was carried out for two seasons at KARI-Kakamega, during the long rains of 1998 and 1999.

8.2.3 Results

Table 8.9. Mean yield of clean and rotted maize at three levels of fertilizer at KARI-Kakamega in 1998

Treatment	Yield in kg/ acre			
	Clean	Rotten	Total	% rot
No. fertilizer applied				
H614	2888.0	426.0	3314.0	13
Namba nane	2318.0	349.0	2667.0	13
H622	2254.0	445.0	2699.0	17
H626	2286.0	413.0	2699.0	15
1/2 recommended rate				
H614	2604.0	984.0	3587.0	27
Namba nane	2318.0	191.0	2508.0	8
H622	1619.0	232.0	1851.0	13
H626	2984.0	540.0	3524.0	15
Recommended rate				
H614	3397.0	413.0	3809.0	10
Namba nane	2699.0	260.0	2959.0	9
H622	2159.0	349.0	2508.0	14
H626	3334.0	540.0	3873.0	14
	LSD Value = 1086.0 a = 0.05	LSD Value = 507.6, a = 5	LSD Value = 1251.2 a = 0.05	LSD value = 7.7 at a = 0.05

The result from the 1998 season showed that the level of fertilizer did not affect the yield of rotten and clean maize harvested (Table 8.9). Disease incidence was also not significantly different at the three levels of fertility. However the mean disease incidence for the four varieties was lowest in plots where fertilizer was applied at the recommended rate and highest where no fertilizer was applied (Table 9.10). Percentage rotten grain was not different between the three fertilizer treatment but showed similar trends with the highest percentage rotten grain in plots with no fertilizer applied and lowest in plots with the highest level of fertilizer applied (Table 8.12). Severity of rotten cobs was not different at the three levels of fertility and no trend was observed in the mean severity (Table 8.11). The total yield was not different between the three treatments

Table 8.10. Incidence of cob rot in four varieties planted with three levels of fertilizer at Kakamega 1998

	Cob rot incidence (%)		
Variety	No. fertilizer	1/2 recommended rate	Recommended rate
H614	14	21	11
Namba nane	15	6	8
H622	18	13	20
H626	13	12	15
Mean	15	13	14

Table 8.11. Severity of cob rot in four varieties planted with three levels of fertilizer at Kakamega 1998

	Cob rot severity (% rotted grain per cob)		
Variety	No. fertilizer	1/2 recommended rate	Recommended rate
H614	51	58	50
Namba nane	54	16	55
H622	34	43	38
H626	78	44	53
Mean	54	40	49

Table 8.12. Percentage rot in four varieties four varieties planted with three levels of fertilizer at Kakamega 1998

	Percentage rotten grain		
Variety	No. fertilizer	1/2 recommended rate	Recommended rate
H614	13	27	10
Namba nane	13	8	9
H622	17	13	14
H626	15	15	14
Mean	15	16	12

Table 8.13. Mean yield of clean and rotted maize at three levels of fertilizer at KARI-Kakamega in 1999

Treatment (Fertilizer level)	Yield in kg/ acre			
	Clean	Rotten	Total	% rot
No. fertilizer applied				
H614	3610.0	503.0	4112.0	12
Namba nane	2556.0	131.0	2687.0	5
H622	3839.0	499.0	4337.0	12
H626	2921.0	533.0	3454.0	15
1/2 recommended rate				
H614	5878.0	686.0	6563.0	11
Namba nane	5403.0	280.0	5682.0	5
H622	4602.0	557.0	5159.0	11
H614	7703.0	262.0	7965.0	4
H626	5024.0	275.0	5299.0	5
Recommended rate				
Namba nane	5519.0	645.0	6164.0	11
H622	4883.0	468.0	5351.0	9
H626	6539.0	471.0	7011.0	7
	LSD Value = 2151.0 a = 0.05	LSD Value = 484.0, a = 5	LSD Value = 2096. a = 0.05	LSD value = 5.3 a = 0.05

In 1999 the result revealed that the varieties showed significant differences in the yield of clean maize but did not show significant difference in the yield of rotten maize (Table 8.13). Disease incidence was not significantly different at the three levels of fertility but the mean disease incidence of the varieties showed a higher disease incidence in plots with zero fertilizer levels and was low in fertilized plots (Table 8.14). Percentage rotten grain did not show a difference at three fertilizer levels but the mean percentage rotten grain in the non fertilized plots was highest and lowest in plots where full rate of fertilizer recommendation was applied (Table 8.16). Disease severity was not different at the three levels of fertilizer and no trend was observed in the mean disease severity rating for the four varieties at the three levels of fertilizer. Differences were observed in the total yield harvested at this site. Data taken on percentage declined ears was not different at the three different fertilizer levels (Table 8.17).

Table 8.14. Incidence of cob rot in four varieties planted with three levels of fertilizer at Kakamega 1999

	Cob rot incidence (%)		
Variety	No. fertilizer	1/2 recommended rate	Recommended rate
H614	12	11	7
Namba nane	5	8	13
H622	17	9	12
H626	16	9	8
Mean	13	9	10

Table 8.15. Severity of cob rot in four varieties planted with three levels of fertilizer at Kakamega 1999

	Cob rot severity (% rotted grain per cob)		
Variety	No. fertilizer	1/2 recommended rate	Recommended rate
H614	22	28	34
Namba nane	28	42	52
H622	36	29	29
H626	28	70	25
Mean	29	42	35

Table 8.16. Percentage rot in four varieties four varieties planted with three levels of fertilizer at Kakamega 1999

	Percentage rotten grain		
Variety	No. fertilizer	1/2 recommended rate	Recommended rate
H614	12	11	4
Namba nane	5	5	11
H622	12	11	9
H626	15	5	7
Mean	10	8	8

Table 8.17. Percentage declined ears at different fertility levels in 1999

	Declined ears (%)				
Fertility levels	H614	H622	H626	Namba nane	Mean
Zero fertilizer	19	41	19	34	28
1/2 recommended rate	27	76	45	37	46
Full recommended rate	44	48	34	44	43

8.2.4 Discussion

Disease incidence and percentage rotten grain showed a similar trend for the two seasons. The mean disease incidence was highest in plots where no fertilizer was applied and lowest in plots where fully recommended fertilizer was applied. The result obtained from this study however indicated that the level of applied fertilizer did not significantly affect disease incidence, severity and percentage rotten grain. Maize grown in plots with zero fertilizer applied had the highest percentage rotten grain and was lowest in plots with fully recommended level of fertilizer. One observation made was that the inherent fertility at the research centre where the experiment was carried out was generally high and was probably not very suitable for this kind of experiment. Farmers observation that cob rots are higher under low fertilization was based on their fields in Tongaren Division where the general fertility is low and probably this would have been the best site for the trial. However the trends observed in this experiment seem to provide an indication that fertilizer does have an effect on the incidence of rotten cobs and percentage rotten grain. Few reports are available on the effects of fertilizer on cob rots, but studies on stem rot of maize showed that application of K fertilizers decreased incidence of stem rot caused by *Fusarium moniliforme* in K- fixing soils (Siebold, 1974). Disease incidence of root and foot rots of wheat caused *F. graminearum* was found to be highest in fields where no fertilizer was applied and low in fields where fertilizer NPK was applied (Mladenov, 1973). Increasing the rate of nitrogen has been shown to decrease stalk rots caused by *Diplodia maydis* (White, 1978), *Fusarium moniliforme* and *Fusarium graminearum* and *Diplodia maydis* (Warren *et al.*, 1975) in corn. No report was found on the effect of nitrogen on cob rots.

Farmers gave various agronomic practices that they thought would alleviate cob rot problems in the field. It would be argued that any measures that will help improve general fertility will help alleviate this problem. Fertilizers are expensive and farmers may not be able to afford them. The treatment effect could have been greater if it was carried in soil with relatively low fertility, more similar to the farmers fields .

8.5 Conclusion

The levels of di-ammonium phosphate (DAP) applied at planting and calcium ammonium nitrate (CAN) as a top dressing fertilizer applied at fully recommended rate showed a decreasing trend in the incidence of cob rot and percentage rotten grain when compared with the same fertilizer applied at half the recommended rate and zero rate.

CHAPTER 9: GENERAL DISCUSSION AND CONCLUSIONS

Farmers' perceptions and production practices in relation to cob rots was considered as an important element in addressing the cob rot problem in Western Kenya. Farmer participatory methods were therefore used to establish the importance of maize in the region and identify the major production constraints. A participatory rural appraisal was carried out in two administrative Divisions in the Western Highlands of Kenya in 1997. It was established that maize was the most important crop in the two Divisions. Beans (*Phaseolus vulgaris*) was the second most important crop. It was a general and preferred practice to intercrop the two crops, 60% of the farmers applied this practice. Maize was the major stable food crop for the people in the region and was traded for cash and thus contributed to the household income. Rotten maize was used for brewing, livestock feed and sold for cash.

Cob rotting was ranked by the farmers as their most important crop protection problem, although it was ranked seventh as a production constraint after a number of issues relating to markets and access to inputs. Stem borers, followed by weevils were ranked as the most important pest problems.

The most important factors affecting the incidence of cob rot in the field were the cultivars and the weather conditions at the period of maturation to harvest. The varieties grown in the region were H614, H625, H626, H622 and H512. The preferred variety was H614. The reasons for the preference of this hybrid in the region was high and stable yields, less rotting and resistance to weevil attack. H622 was less preferred because it was considered to be prone to rotting. Other factors associated with cob rotting were poor storage and mixing of rotten and non-rotten cobs before placing them in store, leading to cross contamination. Many farmers did

not have stores which conformed to the recommendations from the Ministry of Agriculture. Rotten maize constituted 18% of the total grain harvest based on estimates obtained from farmers for 1994, 1995 and 1996. Rotten maize was mainly used as livestock feed and brewing in homes. On average in the two Divisions, 70% and 7% of the rotten maize was used as livestock feed and brewing respectively. The fact that some of the rotten maize finds its way into the human food chain indicated the potential health hazard due to mycotoxin contamination to the maize consumers in the region. The awareness of this potential health hazard was low among the farmers and the agricultural extension staff. There are numerous accounts in the literature of the severe effects to livestock consuming mycotoxin contaminated maize and the association of this contamination to human health (Marasas *et al.*, 1979, Thiel *et al.*, 1992; Ross *et al.*, 1991).

In an effort to get additional information on some of the factors that are associated with high cob rot incidence in the field, studies to determine the incidence of cob rot in the field were carried out in 1998 and 1999 (Chapter 4). In 1998, it was found that cob rot incidence in maize fields in Tongaren Division ranged from 0-57% with a mean of 22% in all the fields visited.

The incidence of stalk borer damage to the cobs was also high ranging from 0-57%. Cob rot incidence was found to be strongly correlated with percentage borer damage ($r = 0.87$, Fig 4.3). There was a tendency for higher cob rot incidence in fields which were planted later than those planted early but the correlation was not strong. The trend in that direction is probably due to the fact that incidence of stalk borer damage to the cobs was higher in the later planted fields. In 1999 the correlation between cob rot incidence and stalk borer damage was also found to be positive and

significant (r = 0.62). The cob rot incidence during this year was 68% and borer incidence was 46%.

It was evident from the findings that in this region, in addition to rainfall that occurs during the period of crop maturity, stalk borer damage was an important predisposing factor to cob rot. In South Africa, *Busseola fusca* incidence has been shown to increase the incidence of *F. moniliforme* infected ears irrespective of artificial or natural *F. moniliforme* infestation (Flett and Van-Rensburg, 1992). In China, damage caused by the Asian stem borer (*Ostrinia furnacalis*) was found to be the main factor predisposing maize cobs to infection by *F. moniliforme,* accounting for 88% of the rot (Xia-ZhiHong, *et al.*, 1995).

The results, obtained from this study suggest that control of stalk borer will reduce cob rot incidence in this area. Various methods for control of stalk borer have been suggested (Harris and Nwanze, 1992) but have limited successes. Cultural methods such as crop residue destruction or use as fodder and silage have been recommended as a method of control (Adesiyun and Ajayi, 1980; Wahl, 1926 as reported by Harris and Nwanze, 1992). Reduction of *Busseola fusca* through crop residue disposal practices such as cutting stumps, partial burning, deep ploughing, and harrowing have been reported by Macharia (1884) in Kenya. No information was available of a suitable trap crop for maize (maize has been used as a trap crop in sorghum) nor the effects of different rotations on *Busseola fusca* incidence in maize (Harris and Nwanze, 1992). Swaine (1957) found that later sowings of maize in Tanzania were less affected by *B. fusca* than earlier sowings, but in Nigeria early sowing date of sorghum reduced infestation. Results obtained in this study indicated that percentage borer damage was correlated with planting date (r = 0.60). Farmers who plant early

might probably have less incidence of borer damage and less ear rot incidence. In the Western H ighlands of Kenya 60% of the maize was intercropped with beans and the rest of the maize was grown as a monocrop. The other types of intercropping were insignificant. No report was available on the effect of maize/beans intercrop on *Busseola fusca*. But *B. fusca* infestations were found to be higher in sorghum or maize monocrops than in maize/sorghum intercrop (Omolo and Reddy, 1985). In Kenya several, maize and sorghum genotypes with low to medium levels of resistance to *B. fusca* have been reported (Harris and Nwanze, 1992) but there is no indication whether this material has been incorporated into the commercial varieties. Mating disruptions using pheromones are considered potentially effective if applied on maize and other crops grown under plantation conditions (Campion and Nesbitt, 1983). This could be potentially effective in Western Kenya where maize fields are relatively large. However the application of this method as a component of an integrated pest management strategy would be preferable. This approach was tried in Western Kenya on a pilot scale (Saxena *et al*, 1989) using combined effects of several components such as intercropping, adjustment of sowing date, crop residue disposal, and host plant resistance and were reported to reduce stem borer damage to sorghum.

From the PRA survey, farmers were aware of the recommendations given by the Ministry of Agriculture for stalk borer control but none of the farmers followed these recommendations. The recommendation given for control of stalk borer is application of Dipterex granules in the whorls when the crop is knee high. Because of the high cost of inputs for maize production, farmers tended to cut down on some

of the inputs such as Dipterex for the control of stalk borers and tended to buy only critical inputs such as fertilizers and seed.

In the absence of stalk borer control by most of the farmers over a period of years, there is a possibility that the population of stalk borer has built up in the region. This has led to high incidences of stalk borer damage and high incidence of cob rot. Farmers were aware of the high cob rot incidences but tended to blame it on varieties or what they described as the poor quality seed from the seed companies.

For stalk borer control to have a meaningful impact, the control measures should be taken up by all farmers. Through several years of control, the population of this pest might eventually go down and have an impact on cob rot incidences.

Mycological analysis of the cobs collected during the survey for cob rot incidence showed that the most frequently occurring fungal pathogens in rotted cobs in this region were *F. moniliforme*, *F. graminearum* and *S. maydis*. *F. moniliforme* was isolated in 77% of the cobs harvested, *F. graminearum* in 34% and *S. maydis* in 44% of the cobs. *F. moniliforme* was the most frequently isolated fungus in cobs that had stalk borer damage, occurring in 97% of the cobs that had rot and visible borer damage. This finding supports previous reports from South Africa (Flett and Van-Rensburg, 1992) and China (Xia-ZhiHong *et al.*, 1995) that borer damage creates the entry site for infection of the cobs by *F. moniliforme*.

The three main fungal pathogens isolated in Western Kenya are associated with mycotoxins that are a potential risk to human health and livestock production. *F. moniliforme* produces fumonisin, a mycotoxin associated with a number of mycotoxicoses in both livestock and human. Fumonisin B_1 (FB_1) in maize causes Equine leukoencephalomalacia (ELEM) in horses, an acute neurological disease of

horses and donkeys (Ross *et al.*, 1990; Gelderblom *et al.*, 1988) and pulmonary oedema in swine (Ness *et al.*, 1991). Fumonisins are associated with high incidence of oesophageal cancer in Transkei region of South Africa (Rheeder *et al.*, 1992; Marasas *et al.*, 1981).

F. graminearum produces T-2 toxin, deoxynivalenol and zearalenone. T-2 toxin is associated with 'alimentary toxic aleukia' (ATA), a disease that affected thousands of people in Siberia and led to the elimination of entire villages (IARC, 1993e). T-2 induces immuno-suppressive activity, causes haemorrhagic disease and is also associated with the formation of oral lesions and neuro-toxic effects in animals. Ingestion of deoxynivalenol by humans, another toxin produced by *F. graminearum* in human induces symptoms which include nausea, vomiting, abdominal pains, diarrhoea, dizziness and headache (IARC, 1993a). Deoxynivalenol also induces feed refusal, and decreased weight gain in swine, hens, and rats (Vesonder, 1981).

Zearalenone is an oestrogenic compound produced also by *F. graminearum*. It induces hyper-oestrogenic syndrome in pigs characterized by vulvar and mammary enlargement in immature pigs and induced uterine hypertrophy (Marasas *et al.*, 1984).

S. maydis produces diplodiatoxin which is associated with ruminants grazing on harvested maize fields in winter in South Africa. Symptoms which progressing from inco-ordination to paralysis and death have been reported in cattle and sheep (Kellerman, 1988).

An estimate of percentage rotted grain in the harvest in Tongaren Division in1998 and 1999 was 2% and 10% respectively. These levels could be higher in the event that the rotted cobs are not sorted out at harvest or rotting continues under poor post

harvest handling. In South Africa maize with over 2% rotted grain is considered to be unsafe for human consumption because it can contain sufficient mycotoxins to present a health hazard (Marasas *et al.*, 1978).

Both clean and rotten maize in Western Kenya is utilized at various levels as either human food or livestock feed. Investigation was carried out to establish if mycotoxins are present in maize produced in Western province, at levels which are likely to be detrimental to human and animal health. The study involved (Chapter 5) analysis of samples collected from farms and markets. This study revealed that T-2 toxin was absent in all maize samples collected. However, some samples revealed the presence of aflatoxin, zearalenone, deoxynivalenol and fumonisin at varying levels. Deoxynivalenol was found in 20 % of all the samples collected at levels ranging from 200 to 1100 ng/g. It was found predominantly in samples with high percentage of rotten kernels ranging between 68-90%. All the samples identified as positive were from poor quality maize. The poor quality maize forms the bulk of maize that is fed to livestock and used for brewing in homes. Percentage of rotten kernels and the level of DON showed a positive linear correlation ($r = 0.67$).

Available information on guidelines on tolerance levels for this toxin are varied between countries. No tolerance levels were available for maize or maize based product for DON. Only a few countries have legislation on the tolerance levels on wheat and wheat products (Table 9.1)

Table 9.1. Guidelines on advisory levels and official tolerance levels for DON from various countries

Country	Guideline/Tolerance level	Commodity	Source
Canada	2000 ng/g	Uncleaned wheat	Van Egmond, 1989
Canada	1000 ng/g	Infant food	Van Egmond, 1989
USA	1000 ng/g	wheat	Van Egmond, 1989
Romania	5 ng/g	All feeds	Van Egmond, 1989
China	1000 ng/g	wheat	Luo,1998
USA	4000 ng/g	Wheat and wheat products for feed	Van Egmond, 1989
USSR	1000 ng/g	Duram wheat	Van Egmond, 1989

Available information on feeding trials with farm animals have shown no serious adverse effects of DON at dietary concentrations of 2000 ng/g, in swine, 5000 ng/g in poultry and 6000 ng/g in dairy cattle when fed at the rate of 1% body weight per day (Trenholm *et al.*,1984).

From the available information on tolerance levels it appears that the levels of deoxynivalenol found in Kenyan maize samples were low. However this is not conclusive as the data collected is based on analysis of maize grown in one season (1997). The occurrence of this toxin in parts of South America and Africa is reported to be relatively high in some years (IARC, 1993a). The production of this toxin is dependent on environmental conditions and is therefore likely to vary from one year to the next. No legislation was available on levels on DON on maize and maize-based products. The information on the tolerance levels is available only in a few countries on wheat and wheat-based products and it is important to establish a uniform basis for the regulations.

Sporadic aflatoxicoses with high levels of aflatoxin detected in cereals and cereal based products have been reported in Kenya (Muraguri *et al.*, 1982; Ngindu *et al.*, 1982 and Manwiller *et al.*, 1987). Aflatoxin levels in the samples analysed was low

and ranged from 0.5 ng/g to 10.3 ng/g. Most countries have set the maximum permitted levels in foodstuff in the range of 0-50 ng/g and feedstuff of 5-1000ng/g (Table 7.3). In Kenya the regulation on the tolerance levels for aflatoxin is 20 ng/g in peanuts and other vegetable oils. This regulation was set by the Food, Drugs and Chemical Substances Regulation of the Ministry of Health (Van Egmond, 1989). Aflatoxin is predominantly produced in storage under poor storage conditions but there is evidence of the production of this toxin under field conditions (Coker, 1999; Lillehoj *et al.*, 1994). The fact that the level of aflatoxin found in Kenyan maize was low could be due to the fact that the samples analysed were obtained at harvest and probably not subjected to different storage conditions. In Kenya mycotoxin contamination in cereal grains associated with aflatoxin resulting in death have been reported (Ngindu, *et al.*, 1982).

Zearalenone was detected in maize samples and this is the first report on the occurrence of this toxin in Kenyan maize. The highest level detected was 550 ng/g. Levels of 500 ng/g in feed have been reported to induce pseudopregnancy and failure to cycle in pigs (Etienne and Jammali, 1982). The maximum legal limit for zearalenone in maize varies between countries. The legal limit of tolerance for zearalenone in Kenya food and feedstuffs was not available. Only a few countries have a legal limit or advisory level for zearalenone (Table 10.2). The concentration of zearalenone in food in North America, Japan and Europe is considered low, but in developing countries exposure can be high particularly where maize is grown under temperate conditions (including the highlands) (IARC, 1993e). The conditions in theWestern Highlands of Kenya may provide a suitable environment for the production of this toxin.

Table 9.2. Maximum legal limit for zearalenone in food and feedstuffs

Country	Maximum legal limit	Commodity	Source
USSR	1000 ng/g	Maize	Van Egmond, 1989
Brazil	200 ng/g	Maize	Van Egmond, 1989
Romania	30 ng/g	Maize	Van Egmond, 1989
USA	1000 ng/g	Grains, fat and oils	Van Egmond, 1989

Fumonisin B_1 was detected in the maize samples `and ranged from 0- 2348 ng/g. The highest levels of fumonisin were detected in poor quality maize. Correlation analysis between percentage rotten kernels and level of FB_1 showed a positive correlation between the two. The correlation was significant at 1% ($r = 0.72$).

The levels of fumonisin detected in maize samples were considered low when compared to levels found in South African maize (Table 10.3).

The levels of fumonisin found in the samples are similar to levels previously found by Kedera, 1999, McDonald 1996, Doko *et al.*, 1994.

Fumonisin is an important toxin to human health and livestock production, there is need to continually monitor the levels of this toxin in Kenyan maize because of the prevalence of *F. moniliforme* in the maize growing areas. There is also need to establish the minimum tolerance levels in feed and feed stuff.

Table 9.3. Comparison of natural occurrence of fumonisin in samples analysed from different regions

Country	Year *	Level of fumonisin FB_1 (ng/g)	FB_2 (ng/g)	Product	Source
Western Kenya	1997	0- 2348		Maize kernels	Current study
Western Kenya	1996	110-3500		Maize kernels	Kedera *et al.*, 1999
Kenyan markets	1994	252-1544		Maize kernels	McDonald, 1996
S. Africa (Bizana)**	1985	450-18900	150-6750	Maize kernels	Sydenham *et al.*,1990
S. Africa (Kentani)***	1985	3450-46900	900-16300	Maize kernels	Sydenham *et al.*, 1990
Botswana	1994	35-350	35-255	Maize meal	Doko *et al.*, 1996
Mozambique	1994	240-295	75-110	Maize kernels	Doko *et al.*, 1996
Kenya	1994	780	275	Maize kernels	Doko *et al.*, 1996
Zimbabwe	1994	55-1910	0-620	Maize meal	Doko *et al.*, 1996
Malawi	1994	0-115	0-30	Maize kernels	Doko *et al.*, 1996
Zambia	1994	740	380	Maize meal	Doko *et al.*, 1996
Uganda	1994	605	155	Maize kernels	Doko *et al.*, 1996

* Year samples were collected
** Bizana -low oesophageal cancer region
*** Kentani -High oesophageal cancer region

There are no regulations or guidelines on the minimum levels of fumonisin in food and feedstuffs (Van Egmond, 1999). Naturally contaminated feed with 166,000 n/g of FB_1 and 48,000 ng/g of FB_2 fed orally to swine have been shown to produce acute pulmonary oedema. A diet containing 1000 ng/g of FB_1 fed on rats produced hepatoxicity in four weeks (Gelderblom, 1988).

The results obtained in this study show that the levels of DON, zearalenone and fumonisin were within the current regulations, however, there is need to carry out further study for a longer period as these levels may vary from one year to another. Fumonisin is currently considered carcinogenic. Future regulations on the maximum levels of this mycotoxin may be very stringent to affect import and export of maize. All rotten maize produced by the farmer is utilized in one way or another. The fact that this finds its way in the human food chain is a concern to human health. Rotten maize is utilized in brewing, livestock feed and sometimes sold for cash but its value is lower than that of clean maize it. Maize used for brewing is often the poor quality

grain therefore mycotoxin contamination is a high risk, especially in the non distilled beers. Processing and ethanol fermentation does not reduce concentration of fumonisin ,deoxynivalenol and zearalenone (Bothast *et al.*,1992; Bennet *et al.*, 1978). Occurrence of zearalenone has be reported in Canadian beers (Scott *et al.*, 1993), traditional local beers in West Africa (Okoye, 1978) and beer made from sorghum and maize in Swaziland (Martin,1974). Occurrence of fumonisin has also been reported in Spanish beers (Torres *et al.*, 1998). There is need to test mycotoxins in beer locally brewed in homes in this region as it may be contaminated with some of the toxin identified. There is also need to test animal feed. Local feed manufacturers have been reported to buy sunflower seed and rotten maize in this area for feed processing.(Chapter 5). Fusarium mycotoxins survive processing and tend to concentrate in products generally used for animal feed. During wet milling, these toxins tend to accumulate in germ and bran (Bennet *et al.*, 1978). Animal feeds are prepared from this product and therefore are likely to be more contaminated.

The Ministry of Agriculture has been providing extension messages and demonstration storage structures on the farms. The extension package on pre-harvest losses and the risk associated with mycotoxin contamination in grain is lacking. This can be developed into a single extension package for the farmers in Western Kenya.

Besides the need to increase the awareness of the risk due to mycotoxin contamination of maize to consumers in the area, there is need to set up a mycotoxin laboratory for monitoring the levels of these toxins in food and feed stuffs. The laboratory set up at Kakamega during this study with some further improvements in the safety standards and training of technical staff, may be able to provide qualitative

analysis of T-2, deoxynivalenol and aflatoxin. The main limitations to setting up the laboratory would be the requirement for equipment and trained staff to carry out the work.

Fusarium moniliforme was the most predominant fungal species identified in the samples that were analysed for mycotoxins (Chapter 6). It was found in 80 % of the samples and occurred both in good quality maize and poor quality maize. The incidence of *F. moniliforme* was high but many samples that had visibly high levels of rotten kernels yielded low levels of fumonisin B_1. The reason for these low levels in the samples tested could be due to multiple species of *Fusarium* which cause similar ear and kernel symptoms but are not fumonisin producers or are poor producers of fumonisin.

Some of the mating populations of *F. moniliforme* are known to produce little or no fumonisin. *Fusarium moniliforme* can be divided into six distinct mating populations that probably represent different biological species (Munkvold and Desjardins, 1997). Evaluation of the ability of strains of the members of the mating populations A, D, E, and F which are commonly found on asymptomatic and diseased maize and sorghum plants to produce fumonisin B_1 found a large variability in fumonisin B_1 production in the A and D populations and relatively low variability in the strains of the E and F populations (Leslie *et al.*, 1992). Members of population A are prolific producers of fumonisin while members of population F produce little or no fumonisin (Munkvold and Desjardins, 1997; Leslie, 1995). Desjardins *et al.*, (1998) also identified different strains of *F. moniliforme* (Population A) that differed in the level of fumonisin production under *invitro* conditions. The strains of *Fusarium moniliforme* in Kenya may be poor producers of fumonisin. It is important to determine the ability of

Kenyan strains to produce fumonisin. This will be important because in the event that *Fusarium moniliforme* strains in Kenya are poor producers of fumonisin then steps would be taken to avoid the introduction of prolific fumonisin producing strains from elsewhere especially through the liberalized seed market.

Kedera *et al.*, (1999) found high incidences of *Fusarium moniliforme* and low levels of fumonisin in maize samples collected from Kenya. He attributed this to the inability of *F. moniliforme* isolates present in Kenyan maize to produce fumonisins, to the presence of other ear rot fungi, and / or to environmental conditions unfavourable for fumonisin production.

In vitro interaction of *F. moniliforme* with *Penicillium implicatum* has been shown to decrease fumonisin production while interaction with *F. proliferatum* enhance production of fumonisin (Marin *et al.*, 1998). This also suggested that the interaction of *F .moniliforme* with the other microflora may possibly affect the production of fumonisin.

Limited surveys of good quality maize from hybrids grown in Benin and Zambia and various countries in Eastern and Southern Africa also found a high incidence of *Fusarium moniliforme* and low levels of fumonisins in samples analysed (Doko *et al.*, 1996). Environmental conditions in the area of cultivation has been suggested to play an important role in production of fumonisin in maize (Munkvold and Desjardins, 1997; Pascale *et al.*, 1997). There is need to investigate under controlled environment the ability of strains of *F. moniliforme* to produce fumonisin. This will ascertain whether it is due to the inability of the Kenyan strains to produce large amounts of fumonisin or the effect of the environment.

The result of mycological analysis of the samples collected for mycotoxin analysis showed, beside *F. moniliforme* two other predominant cob rot fungal pathogens were *Fusarium graminearum* and *S. maydis*. *F. moniliforme* was isolated in 80% of the samples, *Fusarium graminearum* in 56% and *S. maydis* in 49%. The results are similar to results obtained from other surveys made in the area (McDonald and Chapman, 1997 ; Kedera *et al.*, 1999; Kedera, 1994).

Evaluation of Kenyan hybrid maize (Chapter 7) showed that varieties H627 and H622 were more susceptible than the other hybrid to *F. graminearum* . The varieties had a high incidence of cob rot associated with this pathogen. Inoculation of the varieties with *F. moniliforme* and *S. maydis* did not show difference in disease incidence.

The fact that this was not so may have been due to inability of the isolate used to cause high levels of disease above the natural infestation in the field. Cob rot incidence in the field was generally high under natural infestation. Under natural infection no differences could be detected between the varieties in their susceptibility to cob rot. In South Africa, evaluation of corn varieties for resistance to ear rot caused by *Stenocarpella maydis* showed that screening varieties at high or very low disease potential did not distinguish the moderately resistant hybrids from resistant and susceptible hybrids. It was shown that beyond these points, disease reaction converged, suggesting that at a very low or high disease potentials, the use of cultivars in disease control is limited (Flett and McLaren, 1994). Screening of maize genotypes for resistance to *F. graminearum* infection found significant genotype x isolate interaction for lines with intermediate resistance but not with lines with either high or low resistance (Reid *et al.*, 1993).

The disease pressure during the evaluation of the Kenyan hybrids was considered high during the two seasons of evaluation and the materials being evaluated were considered to be from a similar genetic base. This could be a possible reason for no differences observed in the disease incidence. Another possible reason could be due to the naturally occurring strains being more aggressive than the test isolates inoculated. The three isolates of *F. graminearum*, *F. moniliforme* and *S. maydis* were previously isolated from the two sites of study the previous season. The five hybrids, H614, H622, H626, H625 and H627 are the predominant varieties grown in this area and are considered to have the same genetic base (Kedera, 1999). This possibility could account for the minimal difference in the varieties. However the varieties H614 was found to be less prone to rot and H622 more prone. Under natural infestation variety H614 had the lowest mean percentage rotten grain at the two sites and H622 had the highest. Although some farmers grow H512 it was found to be grown by less than 1% of the farmers. It is recommended for lower ecological zone. H627 had the highest total yield of grain at both sites. The fact that this variety appeared to be susceptible to *F. graminearum* may give it a disadvantage in being accepted by the farmers.

On-farm evaluation which gave a broader assessment of the varieties in terms of parameters and the actual practice under which these varieties are grown was carried out in Tongaren Division (Chapter 7). Cob rot incidence in the field under natural infestation during the two seasons was high and therefore provided a valid assessment for the varieties for cob rots. The incidence was 20-25 % in 1998 and 30-40 % in 1999. The varieties assessed were H614, H622, H625, H626 and H627. These varieties did not differ in the severity and disease incidence, similar observation

was made in the non inoculated plots in on station experiment. A fact that could be associated with the similarity in genetic background and the level of disease pressure which was high.

The fact that the varieties were not different in borer damage could be another explanation for no differences in disease incidence in the field. Stalk borer was previously identified as an important predisposing factor to cob rot (Chapter 4). The effect of stalk borer may have minimized varietal difference.

Percentage rotten grain was different between the varieties. In 1998, H622 had the highest percentage rotten grain of 20% and H614 had lowest percentage rotten grain of 13%. The trend was the same in 1999, but the overall percentage rot was higher. H627 gave the highest total yield. The mean percentage of rotten grain obtained from three on-farm trials in 1998 and 1999 was 19.5%. The mean estimate given by the farmers in Tongaren Division 1994, 1995 and 1996 was 18 %. The two estimates appear to be in agreement and may therefore be considered a reliable estimates for percentage rotten grain in Western Kenya.

Farmers preferred H614 because it was considered to suffer less ear rot and was said to have other attributes such as resistance to weevil damage, ability to give better yields with less fertilizer. Based on percentage rot, bare tips, lodging and total yield in three on farm trials, H614 ranked as the best variety and H622 as the poorest variety. Variety H627 was found to have the highest number of open tips. One of the reasons for farmers not liking H622 was because it has open tips, a fact that they associated with high incidence of rotting. Although H627 may perform well in terms of yields and lodging, farmers may find it difficult accepting this new variety. H627 is a new variety and farmers were trying for the first time in 1998.

Results from agronomic trials (Chapter 8) revealed a trend of increasing disease incidence and percentage rotten grain with increasing time from physiological maturity to harvest. Harvesting four weeks after physiological maturity would be ideal as there was no difference in disease incidence and percentage rotten cobs with the harvest at physiological maturity. The disadvantage of harvesting at physiological maturity would be high moisture content of the cobs which would necessitate further sun drying which is labour intensive and not reliable because of the rains which might interfere with the process. Farmers in the region practice various methods of quick drying in the field such as stooking and cutting the stalks for maize to dry on the ground.

Trials on fertilizers revealed a trend of increasing disease incidence and percentage rot in plots with reduced fertilizer application. Although specific information on the effect of fertilizer on cob rot was not available, information on effect of fertilizer on maize stalk rot showed that incidence of stem rot of maize caused by *Fusarium moniliforme* is decreased by application of potassium fertilizers. Increasing the rate of nitrogen has been shown to decrease *Diplodia maydis*, *F. moniliforme and F. graminearum* stalk rots in maize (White, 1978; Warren, 1975). This was an observation made by the farmers, who stated that well fertilized maize had less cob rot incidence and probably should have been carried out in farmers plots where soil fertility is low. These experiments were carried out in at KARI Kakamega where the general soil fertility is high. Although there were no difference at the three fertilizer levels, the trend was consistent for the two year period of study. Investigation under farmers plots where soil fertility is low would confirm this observation.

General conclusions

1. Cob rot is the most important crop protection problem on maize in the region studied.

2. Based on farmers estimates and estimates made in three on-farm trials in 1998 and 1999, percentage rotten grain resulting from cob rots in the region is 19%.

3. The most important factors affecting incidence of cob rot in Western highlands are considered to be the nature of the cultivars, rainfall during maturation to harvest and stalk borers.

4. The current varieties grown in the region are considered to be susceptible to cob rots. Varieties H627 and H622 are considered particularly susceptible to *F. graminearum.*

5. H614 is considered to be superior to the other varieties based on farmers ranking (Chapter 3) and the ranking based on results obtained from on- farm trials (Chapter 7) on percentage rotten grain, lodging, total yield of grain and bare tips. H622 was the poorest variety.

6. Damage to the cobs caused mainly by stalk borer (*Busseola fusca*) was the main factor predisposing cob rot to infection by *F. moniliforme*. When considering resistant cultivars to cob rots for Western Kenya, it is important to consider the resistance to stalk borers. Even the most resistant cultivar is likely to succumb to cob rot in the presence of stalk borers.

7. The potential for exposure of mycotoxins in Western Kenya is very high as most of the rotten maize is utilized directly in brewing or as livestock feed.

8. For the first time, mycotoxins deoxynivalenol and zearalenone have been reported in the region. Though the levels appeared to be low, the results are based on samples

obtained from maize grown in 1997. There is need for further surveys on these mycotoxins as their levels may vary between seasons. Recent findings indicate that fumonisin is carcinogenic, this may lead to more stringent regulations of this toxin in maize to very low levels.

9. Although there is currently no legislation on the tolerance levels of fumonisin, the levels of fumonisin obtained in this study can be considered as low when compared to the levels reported in South Africa. The current levels are in agreement with the levels obtained by other workers in previous studies in the region.

10. Due to the prevalence of *Fusarium moniliforme* in Western Kenya and low levels of fumonisin, there is need to screen *F. moniliforme* strains to determine their ability to produce fumonisin.

11. *Fusarium moniliforme*, *Fusarium graminearum* and *Stenocarpella maydis* were identified as the most predominant cob rot fungal pathogens.

12. Farmers should harvest their maize four weeks after physiological maturity.

13. The effect of fertilizer on the incidence of cob rots should be investigated further, probably in farmers fields where fertility is low.

14. There is a need to carry out studies on mycotoxin contamination in local beer and maize-based animal feed.

Recommendations for cob rot control:

1. Plant early (Late February/ early March);

2. Apply adequate fertilizer;

3. Plant H614 and avoid H622;

4. Harvesting at 4 weeks after physiological maturity, longer periods should be avoided;

5. Apply the existing recommendations for control of stalk borer;

6. Post harvesting handling such as separating good cobs from rotten cobs, removal of cobs affected by stalk borer before placing in the stores;

7. Apply insecticide to control insects in the stores.

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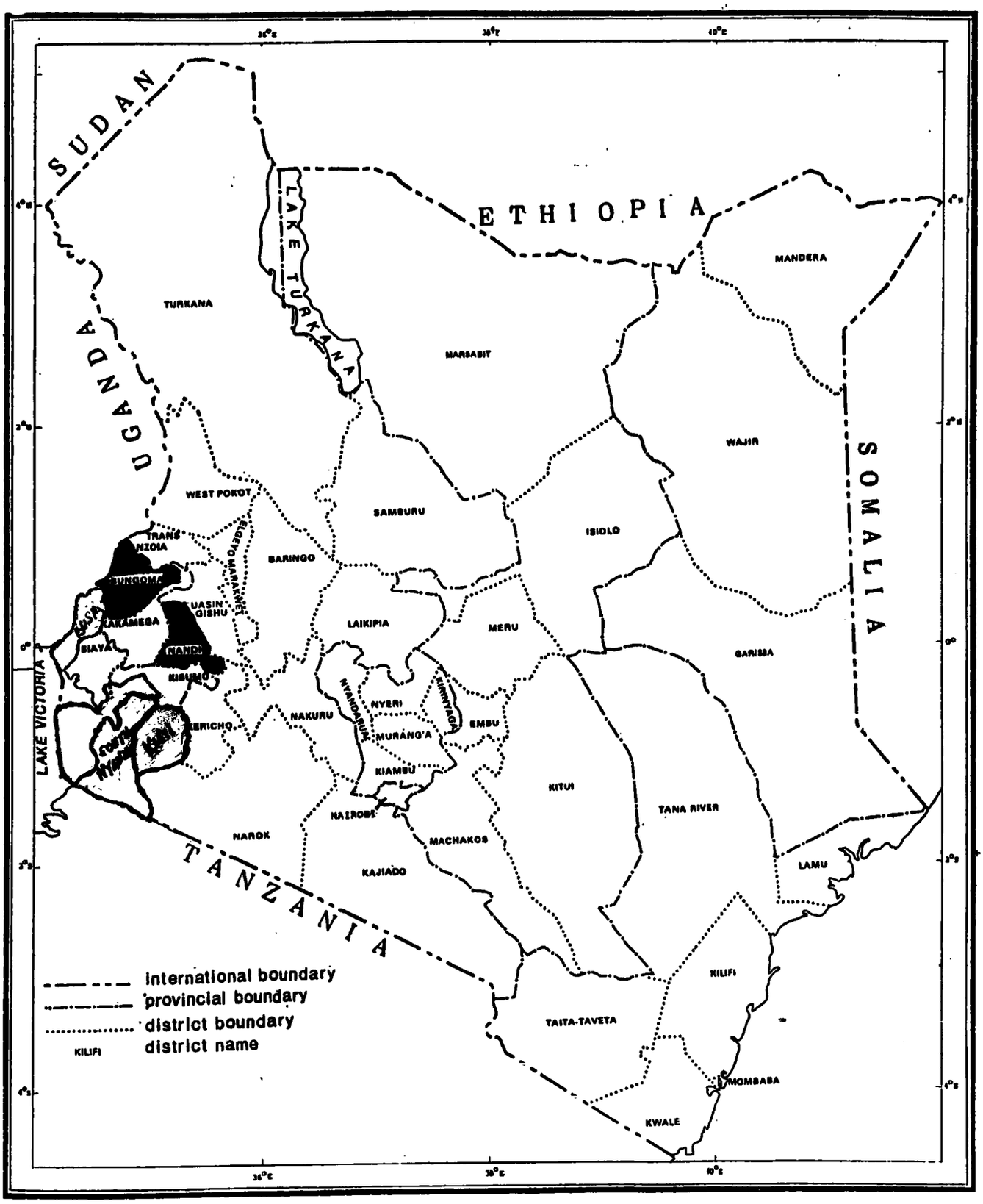
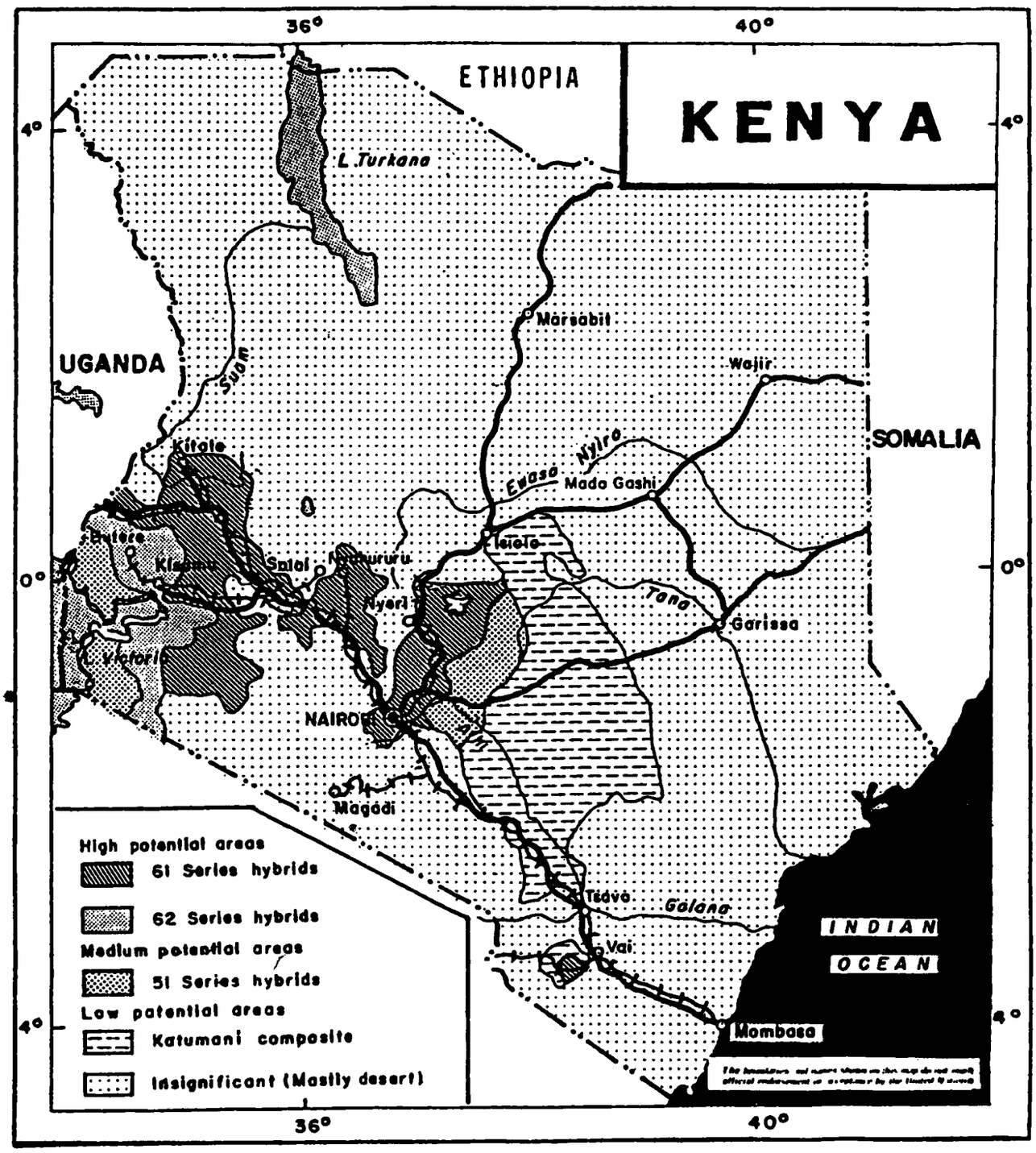
Appendix 1: Administrative map of Kenya showing the Districts surveyed (Bungoma and Nandi)



Appendix 2: Maize seed distribution in Kenya



Source: Ndambuki, 1987

Appendix 3: Descriptions of farming systems zones

Agro-ecological zones	Altitude m.a.s.l	Annual mean temp ^{0}C	Annual mean rainfall (mm)
TA 1 Tropical alpine -Sheep	3000-3500	10.0-7.0	1000-1300
TA 2-Tropical- Alpine zone Forest	Not defined		
UH 1-Upper Highland zone Forest/sheep and Dairy	Not defined		
LH 1- Lower Highland zone Tea/Dairy zone	1950-2400	18.0-15.2	1650-1800
LH 2- Lower Highland zone Wheat/maize/ pyrethrum zone	1800-2200	18.8-16.4	1300-1700
UM 1 Upper middle zone Coffee/ Tea zone	1500-1950	20.6-18.8	1620-1800
UM 2- Upper middle zone Main coffee zone	1500-1800	20.6-18.8	1350-1700
UM 3 Upper middle zone Marginal coffee zone	1500-1800	20.6-18.8	1200-1600
UM 4 Upper middle zone Sunflower-maize zone	1500-1800	20.6-18.8	1200-1500
LM 1- Lower midland zone Sugarcane zone	1350-1500	21.5-20.6	1600-1800
LM 2- Lower midland zone Marginal sugarcane zone	1350-1500	21.5-20.6	1400-1650
LM 3- Lower midland zone Cotton zone	1200-1400	22.4-21.2	1200-1500

Appendix 4 Checklist for PRA survey

How much land do you put under maize?

What is your total land area?

What yields do you get? (90kg bags/acre)

What do you do with your maize?

What do you use for ploughing?

What varieties of maize do you plant?

Are there any particular varieties you prefer and why?

When do you normally harvest maize on your farm?

How do you harvest your maize?

How do you dry your maize?

How do you store your maize?

What do you do with the rotten cobs?

What do you do with maize stovers after harvesting?

Is ear rot an important problem on your farm?

What do you think causes ear rot of maize?

How much of you produce ends up rotten?

Year	Good(90kg bags) shelled	Rotten(90kg bags) shelled
1996		
1995		
1994		

What do use rotten cobs for?

How are you trying to solve the problem of maize ear rot on the farm?

Are there any particular practices you think when applied can alleviate ear rot problem?

Appendix 5: Mycotoxin Sample Collection record Form

Sample Number:		Market / Round / Year Code
	Market	M / 1 / 97 /
		Farm /Round / Year /Location/ G*& P* quality
	Farmer	F / 1 / 97 / /

Date:

District:	
Division:	
Location:	
Town(s):	
Village: 1	
Village: 2	

Commodity (If NOT maize)

MARKET SURVEY

Market Name 1:

Trader No:	1	2	3	4	5	6	7	8	9	10	Mean
Stock (90 kg bags)											
Quality (Good*/FAQ*/Poor*)											

Select FAQ or Good if possible

Market Name 2:

Trader No:	11	12	13	14	15	16	17	18	19	20	Mean
Stock (90 kg bags)											
Quality (Good*/FAQ*/Poor*)											

No. of increments:
Increment weight kg):
Cost/increment (K
Cost/sample (KS):

FARMER SURVEY	*Village 1*					*Village 2*					*Mean*
Farmer No:											
Moisture content (%): Good											
Poor											
Variety:											

Time field drying: (Round 1 in October only)

a). Standing on plant (weeks)										
b). In stooks (weeks)										

Type of store: Good									
(From which sample is taken) Poor									
Time in store: Good									
(From which sample is taken) Poor									

Comments:

Appendix 6.1. Flow diagram for Steiner method of aflatoxin analysis in maize samples

EXTRACTION

Weigh out 80 g ground maize into a 1 litre blender jar

Add 200 ml methanol + 20 ml water

Blend at high speed for 2 minutes

Add 60 ml water

Blend for further 1 minute

Filter through Whatman No. 1 filter paper

↓

CLEAN-UP

Measure 70 ml filtrate into a 500 ml separating funnel

Add 50 ml hexane (to remove lipids)

Add 55 ml water

Add 2 g sodium chloride (to prevent emulsions),
ensuring that none becomes attached to the socket
for the stopper, otherwise it will leak

SHAKE funnel for 1 minute, releasing pressure
frequently by removing the stopper
Allow layers to separate

Run off lower layer into second separating funnel (250 ml)

Add 25 ml chloroform

SHAKE funnel for 30 seconds., releasing pressure

Allow layers to separate

Run off lower layer into 250 ml round bottomed flask

via a filter paper containing 2 g anhydrous sodium sulphate.

Repeat steps 16-19 with second 25 ml chloroform.

Run lower layer through same bed of sodium sulphate
into above round bottomed flask.

Take to dryness on a rotary evaporator at 45°C

Transfer to 7 ml vial using 3 x 1 ml chloroform

Take to dryness at 45 °C under jet of air
(Note: nitrogen gas preferred to air)

|

|

SEMI-QUANTITATIVE TLC

Assumes that minimum detectable quantity of aflatoxin on a TLC plate is 0.1 ng
Add 1 ml toluene:acetonitrile 98:2 to the dry extract

Vortex mix, 30 seconds

Spot 5 µl spots in duplicate on TLC plate
[the 5 µl spot is visible, then > 1 ppb]

for 1 ppb Screening Plate

For semi-quantitative range >1<4>4<10>10
Add extra 3 ml benzene:acetonitrile to the extract

Vortex mix 30 seconds

Spot 2 and 5 µl spots in duplicate on TLC plate

Develop plate for 15 minutes in chloroform:acetone:
water 88:12:0.2

Dry plate for 5 minutes in a fume hood and observe
under long-wave UV light
D=1 ml: If 5 µl spot not visible, then < 1 ppb aflatoxin
If 5 µl spot just visible, then > 1 ppb aflatoxin

D=4 ml: If 5 µl spot not visible, then < 4 ppb

If 5 µl spot visible, then > 4 ppb
If 2 µl spot not visible, then < 10 ppb
If 2 µl spot visible, then > 10 ppb aflatoxin

If >1 ppb aflatoxin

QUANTITATIVE TLC

Take extract to dryness under stream of air (nitrogen preferred) in water bath at 45°C.

Add 500 µl toluene:acetonitrile and vortex mix 30 sec.

Spot HPTLC plate with 1, 2, 3, 4, and 5 µl of sample and working standard solutions.
Develop plate for 15 minutes in chloroform:acetone: water 88:12:0.2
Dry plate and observe under long-wave UV

Compare intensity of spots under long-wave UV light and record best equivalence.

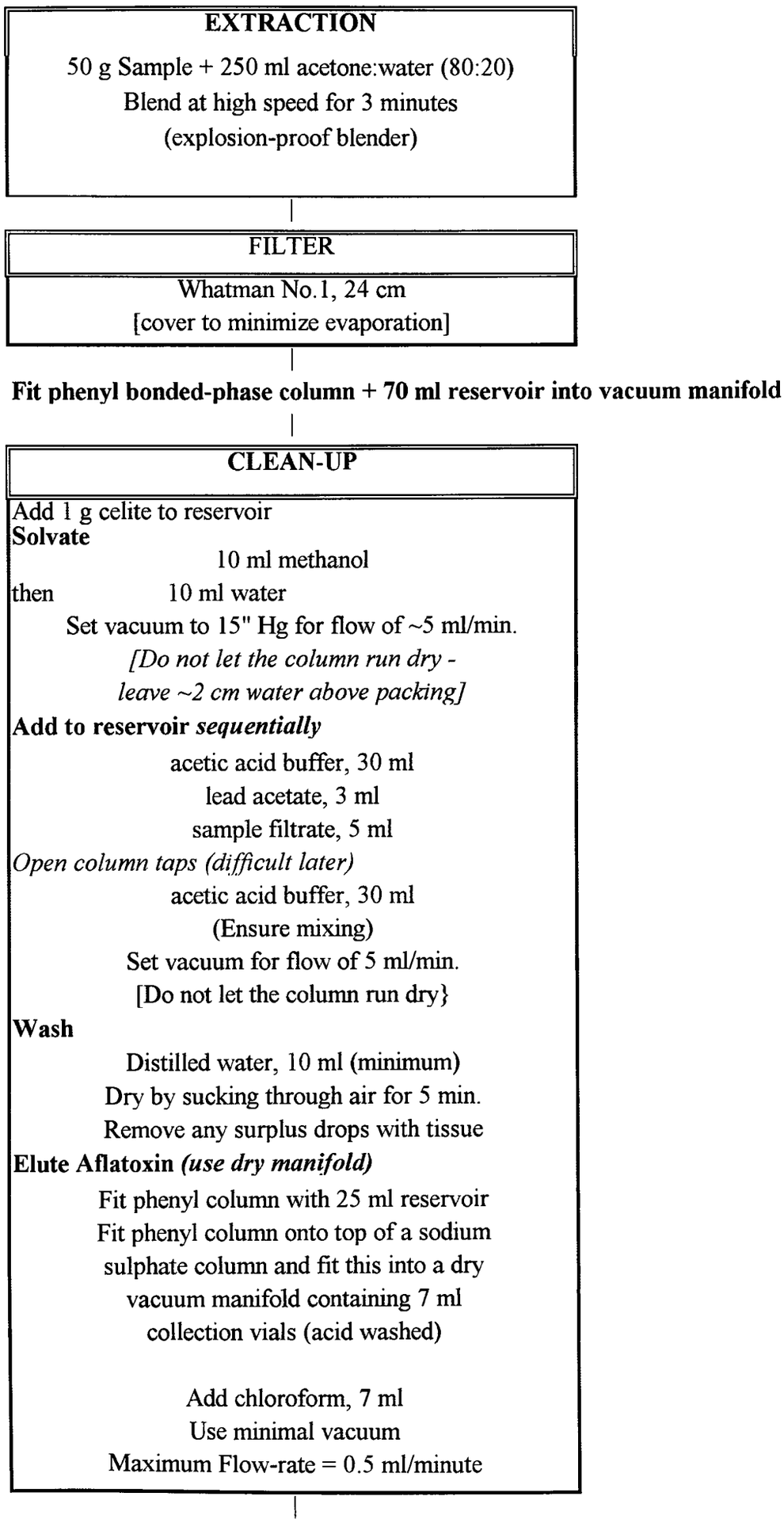
Y µl sample - Z µl standard

Calculate result in µg/kg (ppb) using equation: ppb =

$$\frac{C \times \text{Volume of standard spot (Z)} \times D}{\text{Volume sample spot (Y)} \times EWt}$$

Where C = concentration of standard in µg/ml,
D = Dilution in µl (=500)
EWt = Effective weight in final extract (=20 g)

Appendix 6.2. Flow diagram for analysis of aflatoxin using HPLC



SAMPLE CONCENTRATION

Take to dryness on sample concentrator
under N_2 at 45 °C

HPLC

Add 5 ml WAM to extract and vortex 1 min.

System File 4, iso-cratic

Column:

ODS1 (250x4.6 mm Spherisorb)

Mobile Phase:

Water: acetonitrile: methanol
1200:600:200

Plus 238 mg potassium bromide

Plus 700 µl 4M nitric acid

(De-gassed on sonic bath for 20 minutes)

Flow: 0.8 ml/minute

Post-Column Derivatisation:

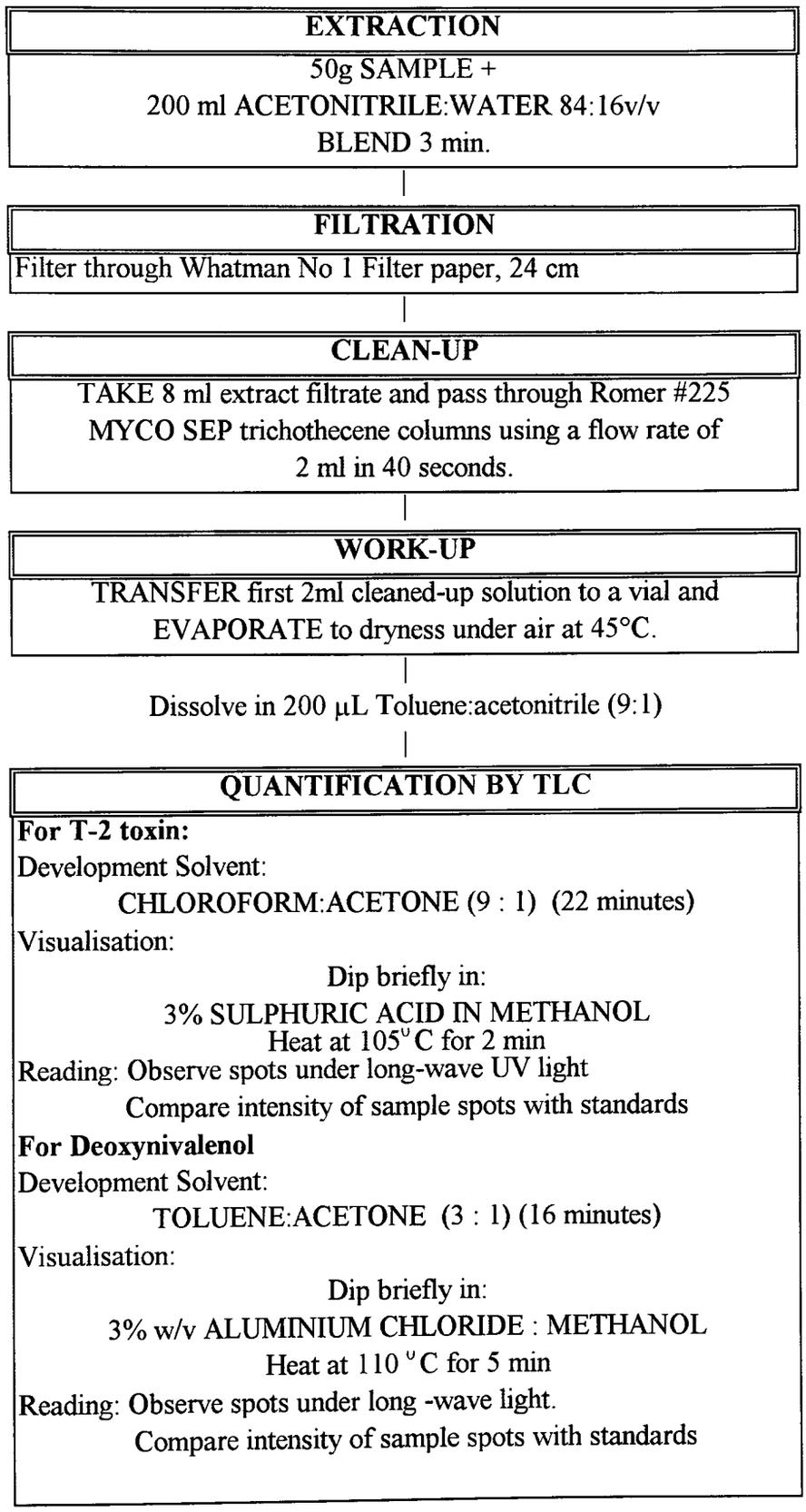
KOBRA CELL, 100 mV

Fluorescence Detector:

Excitation: 365 nm
Emission: 440 nm

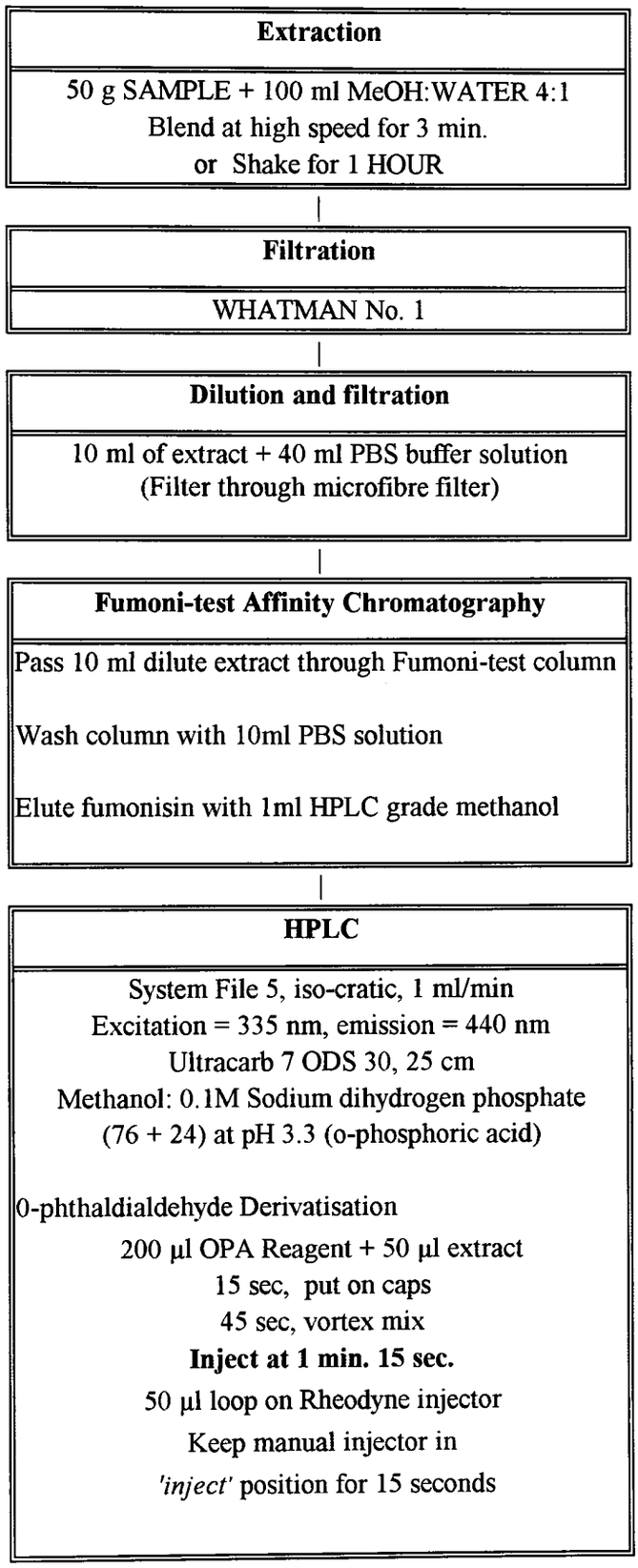
Data Acquisition: Nelson PCINT

Appendix 6.3. Flow diagram for Analysis of T-2 toxin and Deoxynivalenol (DON) using solid phase clean-up cartridges



Source. Gibbs, J. *et al.*,1996

Appendix 6.4. Flow diagram for fumonisin analysis by Fumoni-test affinity chromatography and HPLC.



Appendix 6.5. Flow diagram of the procedure for quantitative determination of zearalenone in maize using base clean-up and HPTLC

EXTRACTION

25 g SAMPLE + 12.5 g diatomaceous earth
+ 10 ml water + 250 ml chloroform

Shake in conical flask, 15 min, reciprocating shaker

|

FILTRATION

WHATMAN No. 1, 24 cm

|

BASE CLEAN-UP

25 ml ext+40 ml NaCl+50 ml NaOH solution

Mix by swirling , shake 1 min, allow layers to separate

Discard lower chloroform layer

Add 50 ml chloroform , repeat previous step, discard

Add 50 ml citric acid solution, swirl, add 50 ml Dichloromethane to extract zearalenone
Pass through a bed of 40 g anhydrous sodium sulphate

Repeat previous step with another 50 ml dichloromethane

Dry extract on water bath at 40^{0}C to near dryness

Transfer residue to 7 ml vial with 1x3 ml Dichloromethane

Dry under Nitrogen on concentrator
Kept at -20^{0}C until required

|

HPTLC

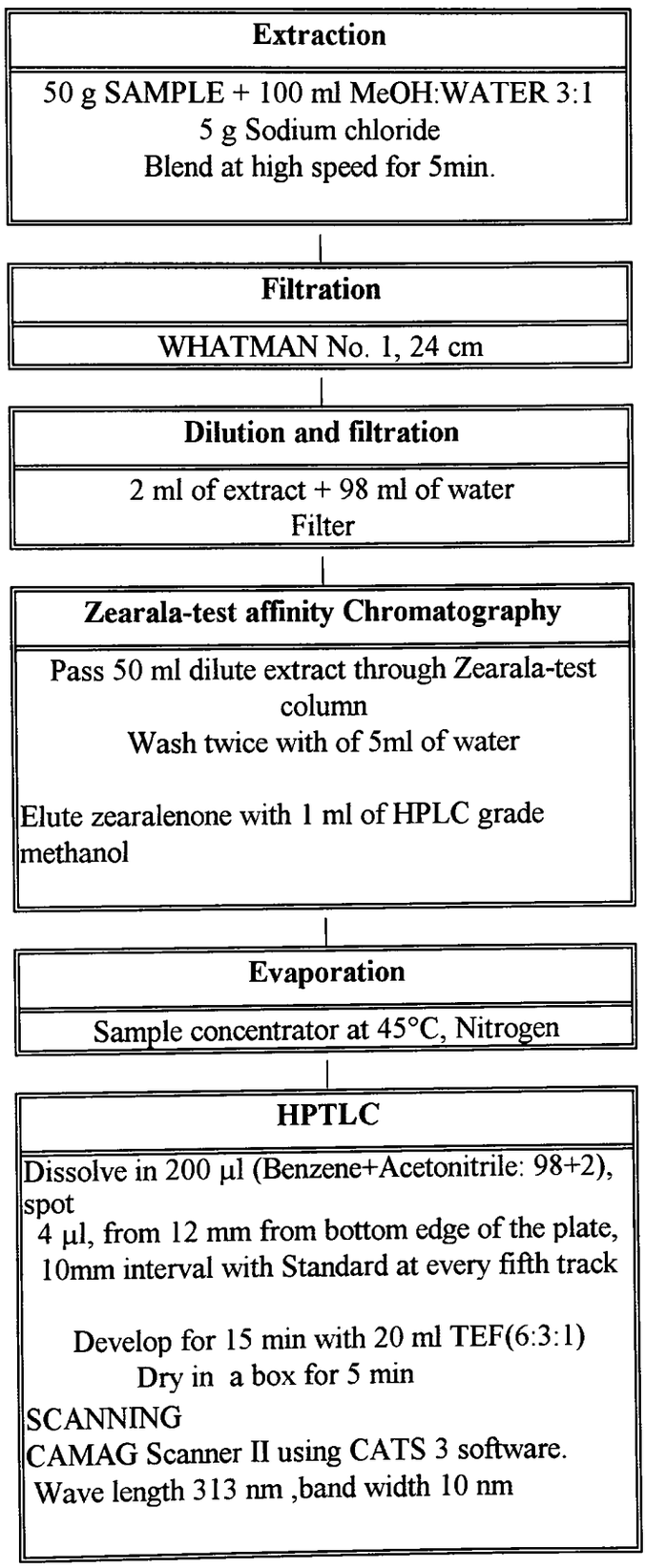
Dissolve in 200 µl (Benzene+Acetonitrile: 98+2), spot 4 µl, from 12 mm from bottom edge of the plate, 10mm interval with Standard at every fifth track

Develop for 15 min with 20 ml TEF(6:3:1)
Dry in a box for 5 min

SCANNING

CAMAG Scanner II using CATS 3 software.
Wave length 313 nm ,band width 10 nm

Appendix 6.6. Flow diagram for analysis of zearalenone using Zearala-test affinity chromatography and HPTLC



Appendix 7: Location and Samples collected for mycotoxin analysis.

Sample	Location/ market	Date collected	Qua. of Kernels	Final % MC	Initial %MC
KT 97001	Tongaren/Mbakalo	8/10/97	Good quality	12.3	16.9
KT 97002	Tongaren/Mbakalo	8/10/97	Very poor	12.5	20.4
KT 97003	Tongaren/Tongaren	9/10/97	Good quality	12.5	18.5
KT 97004	Tongaren/Tongaren	9/10/97	Very poor	12.6	17.9
KT 97005	Kapsabet/Kapkangani	15/10/97	Good quality	12.3	16.2
KT 97006	Kapsabet/Kapkangani	15/10/97	Poor quality	12.3	15.7
KT 97007	Kapsabet/ Kapsabet	17/10/97	Fair quality	12.4	19.0
KT 97008	Kapsabet/ Kapsabet	17/10/97	Very poor	12.7	22.8
KT 97009	Kisumu town	16/10/97	Good quality	12.3	
KT 97010	Kitale market	16/12/97	Fair quality	123	15.8
KT97011	Bungoma market	17/12/97	Fair quality	12.4	15.7
KT 97012	Busia municipal market	19/12/98	Fair quality	12.5	17.0
KT 97013	Siaya market	19/12/98	Fair quality	12.6	15.7
KT 97014	Kakamega l market	13/5/98	Fair quality	12.3	12.2
KT 97015	Amukura	18/12/98	Good quality	12.9	19.0
KT 97016	Kitale market	13/5/98	Fair quality	12.4	14.3
KT 97017	Kapsabet/Kapsabet	28/5/98	Poor quality	2.3	14.7
KT 97018	Kapsabet/ Kapsabet	28/5/ 98	Fair quality	12.3	13.9
KT97019	Kakamega market	15/5/ 98	Fair quality	12.3	14.0
KT 97020	Siaya market	15/5/ 98	Fair quality	12.1	13.7
KT 97021	Busia market	18/5/98	Fair quality	12.3	13.7
KT 97022	Bungoma market	18/5/98	Fair quality	12.2	13.0
KT 97023	Tongaren/ Tongaren	23/5/98	Good quality	2.1	13.2
KT 97024	Tongaren/ Mbakalo	23/5/98	Good quality	12.3	13.7
KT97025	Kisumu market	26/5/98	Good quality	12.5	13.7
KT97026	Kakamega market	2/6/98	Good quality	12.3	14.8
KT97027	Kakamega market	2/6/98	Fair quality	12.4	13.5
KT97028	Kisumu market	3/6/98	Poor	12.7	14.4
KT97029	Kisumu market	3/6/98	Fair quality	12.6	13.9
KT97030	Bungoma market	4/6/98	Fair	12.3	15.0
KT97031	Bungoma market	4/6/98	Poor	12.4	14.6
KT97032	Busia market	4/6/98	Good	12.5	14.1
KT97033	Busia market	4/6/98	Fair	12.6	14.8
KT97034	Siaya market	5/6/98	Fair	12.5	14.9
KT97035	Siaya market	5/6/98	Poor	12.5	13.8
KT97036	Kapsabet/Kapsabet	8/6/98	Poor	12.4	13.0
KT97037	Kapsabet/Kapsabet	8/6/98	good	12.3	13.9
KT97038	Kapsabet/ Kapkangani	9/6/98	Poor	12.6	13.3
KT97039	Kapsabet /Kapkangani	9/6/98	Poor	12.5	13.5
KT97040	Kapsabet/ Kapkangani	15/6/98	Fair	12.3	13.1
KT970041	Kitale market	15/6/98	Good	12.2	14.6
KT97042	Kitale market	15/6/98	Poor	12.3	14.9
KT97043	Tongaren/ Tongaren	16/6/98	Poor	12.5	14.7
KT97044	Tongaren/ Mbakalo	16/6/98	Poor	12.4	15.0
KT97045	Tongaren/ Mbakalo	17/7/98	Good	12.2	14.9

Appendix 8: Framers who participated in on-farm trials in1998 and 1999 in Tongaren division

Bramwell Khisa

Emily Khisa

Edward Makila

Hudson Makheti

Christopher Makokha

David Mokaya

Benjamin Opuche

Paul Opicho

Bernard Luva

Isaiah Kakai

Barnabas Wambeo

Zainabu Wanyama

John Mwanzi

Huroun Wekesa

Makokha Wetekhela

Ronald Wanyama

Joshua Wanyama

Dunston Mwaturo

Jacob Osore

Jackson Nabibya

Colletta Musamali

Stephen Nyongesa

Mary Obose

William Wambaya

Patrick Achero

Appendix 9.1: Fungal identification record sheet

Sample	Source (Division/Location	Media	Colony types	Fungi identified
KT97001	Tongaren/Mbakalo	GA	Type I a, Type I b, Type III a Type IV	*Fusarium moniliforme, Fusarium moniliforme var. subglutinans, Aspergillus flavus, Stenocarpella maydis, Rhizopus stolonifer*
KT97002	Tongaren/Mbakalo	GA	Type I a, Type II b, Type III a, Type VI	*Fusarium moniliforme, Stenocarpella maydis, Rhizopus stolonifer, Fusarium graminearum, Penicillium spp.*
KT97003	Tongaren/Tongaren		Type I a, Type II b, Type V, Others	*Fusarium moniliforme, , Rhizopus stolonifer, Fusarium graminearum, Penicillium spp., Aspergillus spp.*
KT97004	Tongaren/Tongaren		Type I a, Type II b, Type V, Others	*Fusarium moniliforme, , Fusarium graminearum, Penicillium spp., Aspergillus spp.*

Appendix 9.2 Media for isolation and identification

SNA medium (Snyder and Nash agar medium)

SNA media was prepared by mixing then following chemicals in 1 litre of distilled water and autoclaving at 121^{0}C for 20 minutes.

1.0 g KH_2PO_4
1.0 g KNO_3
0.5 g $MgSO_4 .7H_2O$
0.5 g KCl
0.2 g Glucose
0.2 g Sucrose
20 g Agar

Potato Sucrose agar (Potato Sucrose Agar)

500 ml Potato extract
20 g Sucrose
20 g Agar
500 ml distilled water

Glycerol agar

Glycerol agar was prepared by suspending 50 g of agar in 1 litre of distilled water. The suspension was gently heated and stirred until it dissolved completely and 2 ml of chloramphenicol 5% solution was added followed by 5 ml of glycerol .The mixture was then autoclaved at 121°C for 20 minutes.

Malt extract

Malt extract was prepared by suspending 15 g of malt extract in 1 litre of distilled water and 2 ml of chloramphenicol 5% solution was added. The mixture was autoclaved at 121°C for 20 minutes.

Appendix: 10

Results of on-farm trials at Tongaren 1998

FWTG- field weight of good cobs
FWTR- field weight of rotten cobs
YldHaG- Yield of good maize (Kg/Ha)
YldHaR- Yield of rotten maize (Kg/Ha)
Yld total - Total yield
M CG- moisture content of good maize
M CR- moisture content of rotten maize

Bstem- No. broken stems
Bstem/ha- No of broken stems /Ha
Rootlog- Root lodging
Rootlog/ha- Root lodging per Ha
StandH- Stand count at harvest
Btips- No. of bare tips at harvest

%bstem- Percent broken stems
GoodNo- No of good cobs
GoodNo/ha- No of good cobs/Ha
RotNo-No. of rotten cobs
RotNo/ha- No. of rotten cobs /ha
% BorerD - % Borer damage
Btips/ha- No. of bare tips at harvest/Ha

Dip - Dipterex applied farm
Nodip- no dipterex applied farm

Year	Farm	Variety	FWTG	FWTR	MCG	MCR	Net area	YldHaG (K	YldHaR(K	Yldtotal	% rots	Bstem	Bstem/ha	Rootlog	Rootlog/ha	StandH	StandH/ha	Btips	Btips/ha	%bstem	%rootlog	GoodNo.	GoodNo/h	RotNo.	RotNo/ha
1998	1	614	242	47	20	30	683	2752	445	3197	14	64	936	374	5473	1194	17471	1	9	5	31	709	10375	121	1771
1998	1	622	32	22	21	30	266	928	540	1468	37	158	5940	103	3872	500	18797			32	21	790	29699	183	6880
1998	1	625	202	66	20	24	417	3799	1109	4908	23	179	4293	625	14988	1225	29376	1	12	15	51	349	8369	265	6355
1998	1	626	286	46	20	31	404	5337	726	6063	12	140	3465	350	8663	1155	28589	1	20	12	30	356	8812	333	8243
1998	1	627	85	18	22	32	326	1993	351	2344	15	24	736	75	2298	528	16181	5	156	5	14	307	9409	350	10726
1998	2	614	336	40	20	33	464	5671	540	6212	9	306	6593	245	5279	1283	27645	2	52	24	19	211	4546	306	6593
1998	2	622	112	22	22	38	684	1251	179	1431	13	654	9561	800	11696	1555	22734	1	13	42	51	401	5863	266	3889
1998	2	625	239	93	19	24	565	3330	1147	4477	26	588	10416	582	10310	1491	26413	1	18	39	39	1028	18211	106	1878
1998	2	626	361	50	17	25	561	5201	614	5815	11	145	2585	125	2228	1139	20303	2	34	13	11	1112	19822	207	3690
1998	2	627	241	38	19	20	727	2620	387	3007	13	84	1156	1056	14533	1320	18167	6	84	6	80	922	12689	202	2780
1998	3	614	154	86	23	31	235	4946	2318	7264	32	418	17787	245	10426	1426	60681	0	17	29	17	706	30043	89	3787
1998	3	622	39	58	24	26	286	1007	1396	2402	58	872	30511	750	26242	2208	77257			39	34	1010	35339	187	6543
1998	3	625	124	83	22	30	362	2607	1464	4071	36	346	9558	247	6823	1509	41685			23	16	571	15773	86	2376
1998	3	626	141	56	22	29	273	3947	1327	5274	25	350	12816	184	6737	1185	43391			30	16	820	30026	248	9081
1998	3	627	201	52	24	30	622	2405	543	2948	18	402	6460	46	739	1457	23413			28	3	778	12502	239	3841
1998	4	614	174	19	27	34	436	2833	265	3097	9	377	8653	186	4269	1425	32706	1	14	26	13	491	11269	230	5279
1998	4	622	104	60	27	39	546	1360	611	1971	31	454	8317	364	6668	1480	27111	0	2	31	25	837	15332	195	3572
1998	4	625	205	38	26	30	450	3291	540	3831	14	69	1532	201	4464	548	12170	3	60	13	37	483	10726	262	5818
1998	4	626	175	44	27	32	381	3289	711	4000	18	14	367	84	2205	672	17638	1	16	2	13	356	9344	295	7743
1998	4	627	135	50	29	39	727	1282	383	1665	23	5	69	16	220	563	7748	1	7	1	3	579	7969	138	1899
1998	5	614	201	33	17	21	193	8408	1251	9658	13											417	21584	310	16046
1998	5	622	101	41	20	26	627	1263	441	1705	26											378	6029	355	5662
1998	5	625	132	28	16	26	316	3426	609	4035	15											582	18406	184	5819
1998	5	626	89	22	18	26	117	6102	1272	7374	17											333	28462	292	24957
1998	5	627	75	17	23	27	144	3913	795	4708	17											378	26250	221	15347
1998	6	614	261	35	17	29	817	2578	281	2859	10	770	9421	124	1517	1178	14413	0	2	65	11	308	3769	143	1750
1998	6	622	178	44	17	21	603	2376	524	2899	18	494	8188	180	2984	844	13990	1	8	59	21	464	7691	166	2752
1998	6	625	346	33	20	22	525	5178	444	5622	8	606	11534	84	1599	1006	19147	0	4	60	8	680	12943	48	914
1998	6	626	390	24	20	28	506	5990	320	6310	5	540	10672	52	1028	1164	23004	0	2	46	4	1014	20040	224	4427
1998	6	627	330	31	21	21	584	4341	388	4729	8	662	11339	70	1199	866	14834			76	8	1043	17866	243	4162
1998	7	614	212	17	21	23	292	5626	400	6027	7	128	4387	697	23886	1218	41741	1	41	11	57	598	20493	42	1439
1998	7	622	79	14	24	27	197	2992	458	3450	13	674	34283	356	18108	1450	73754	1	41	46	25	801	40743	161	8189
1998	7	625	80	13	21	24	245	2527	379	2906	13	528	21525	140	5707	1660	67672	0	8	32	8	268	10925	146	5952
1998	7	626	191	18	25	25	207	6751	609	7360	8	675	32656	140	6773	1660	80310	2	97	41	8	923	44654	272	13159
1998	7	627	99	13	22	26	227	3311	379	3690	10	548	24109	55	2420	1404	61769	10	418	39	4	912	40123	250	10999
1998	8	614	227	32	23	37	511	3319	357	3676	10	469	9171	53	1036	1036	20258	1	18	45	5	428	8369	102	1995
1998	8	622	201	67	29	31	656	2120	647	2767	23	900	13720	98	1494	1182	18018	0	5	76	8	580	8841	177	2698
1998	8	625	290	29	23	40	477	4571	331	4902	7	427	8946	9	189	1228	25728			35	1	518	10853	171	3583
1998	8	626	366	43	23	31	552	4970	493	5463	9	505	9152	93	1685	1240	22472			41	8	454	8228	338	6125
1998	8	627	209	26	26	29	484	3136	346	3483	10	236	4874	17	351	1176	24287	2	31	20	1	673	13899	413	8530
1998	9	614	190	55	16	34	278	5637	1189	6825	17						0					633	22762	99	3560
1998	9	622	114	88	15	23	412	2281	1504	3785	40	582	14126	429	10413	1308	31748	2	44	44	33	659	15995	269	6529
1998	9	625	190	42	17	20	397	3884	768	4651	17	134	3379	34	857	728	18356			18	5	921	23222	207	5219
1998	9	626	167	56	20	23	423	3082	944	4026	23	85	2011	20	473	728	17223	0	7	12	3	953	22546	141	3336
1998	9	627	149	35	18	21	429	2790	593	3383	18						0					785	18320	154	3594
1998	10	614	340	64	20	24	408	6523	1098	7620	14	514	12607	139	3409	1354	33211			38	10	416	10204	245	6009

Appendix 10 (continued)

1998	10	622	231	48	21	30	947	7748	326	8074	4	592	6254	264	2789	1059	11187			56	25	617	6518	420	4437
1998	10	625	246	36	28	23	352	4881	718	5599	13	295	8381	249	7074	1216	34545			24	20	727	20653	254	7216
1998	10	626	219	58	22	31	504	3321	728	4049	18	176	3492	239	4742	1100	21825			16	22	625	12401	407	8075
1998	10	627	220	55	20	27	175	9835	2096	11931	18	157	8951	82	4675	1632	93044	3	182	10	5	613	34949	408	23261
1998	11	614	314	6	21	30	378	6432	110	6542	2	360	9524	238	6296	1626	43016	0	3	22	15	117	3095	85	2249
1998	11	622	229	13	22	28	454	3831	190	4021	5	660	14550	396	8730	1980	43651	0	9	33	20	419	9237	113	2491
1998	11	625	287	8	20	26	324	6901	176	7077	2	705	21779	290	8959	2599	80290			27	11	190	5870	118	3645
1998	11	626	254	12	19	26	312	6393	271	6664	4	526	16881	371	11906	2473	79365	0	6	21	15	292	9371	148	4750
1998	11	627	271	29	22	27	312	4601	614	5215	12	394	12644	156	5006	2056	65982	1	35	19	8	514	16496	174	5584
1998	12	614	126	50	16	26	374	2753	906	3659	25					186	4968	4	101			632	16880	109	2911
1998	12	622	82	70	19	25	374	1725	1286	3011	43					1174	31357	6	147			962	25694	104	2778
1998	12	625	159	101	19	24	374	3344	1877	5221	36					1224	32692	1	21			876	23397	141	3766
1998	12	626	120	91	17	21	374	2528	1758	4286	41					1052	28098	4	107			459	12260	119	3178
1998	12	627	156	59	18	23	374	3322	1114	4436	25					1611	43029	2	59			987	26362	235	6277
1998	13	614										149		103		1134		1				616		91	
1998	13	622	305	39	18	27	229	10731	1132	11863	10	172	7524	136	5949	1277	55862	1	48	13	11	702	30709	187	8180
1998	13	625	223	17	21	29	255	6755	446	7201	6	137	5379	35	1374	1659	65135	2	82	8	2	919	36082	95	3730
1998	13	626	230	26	22	23	261	6728	696	7423	9	214	8193	93	3560	1398	53522	0	4	15	7	694	26570	95	3637
1998	13	627										157		102		1537		1				718		104	
1998	14	614		17	20	21						125		221		876		0				586		276	
1998	14	622	153	38	24	23	400	2829	671	3500	19	297	7434	516	12916	1054	26383	0	5	28	49	685	17146	290	7259
1998	14	625	157	47	23	31	389	3034	765	3798	20	228	5860	92	2364	671	17245			34	14	664	17065	198	5089
1998	14	626	161	31	20	26	343	3661	615	4276	14	787	22951	226	6591	1822	53135	0	6	43	12	518	15106	258	7524
1998	14	627	156	30	22	27	346	3428	582	4010	15	246	7116	160	4628	1094	31646	7	197	22	15	1023	29592	166	4802
1998	15	614	222	34	18	21	430	4175	574	4750	12	272	6324	85	1976	1225	28482			22	7	575	13369	157	3650
1998	15	622	118	79	20	31	809	1140	625	1766	35	378	4672	479	5921	1686	20841			22	28	640	7911	401	4957
1998	15	625	224	64	18	26	658	2740	659	3399	19	404	6137	687	10436	1779	27024			23	39	694	10542	176	2674
1998	15	626	273	48	18	20	498	4410	711	5120	14	183	3675	76	1526	1335	26807	0	2	14	6	717	14398	413	8293
1998	15	627	234	33	18	21	447	4186	539	4725	11	226	5053	357	7981	1901	42499	1	18	12	19	737	16477	406	9077
1998	16	614	135	33	19	24	452	2361	515	2876	18	452	10007	345	7638	1038	22980	1	31	44	33	621	13748	351	7771
1998	16	622	152	66	18	31	502	2407	840	3247	26	415	8269	688	13708	1460	29089	1	16	28	47	803	15999	403	8029
1998	16	625	78	22	19	21	423	1461	381	1842	21	847	20024	545	12884	1620	38298	1	12	52	34	158	3735	168	3972
1998	16	626	134	47	20	23	430	2432	772	3204	24	954	22176	337	7834	1731	40237			55	19	737	17132	75	1743
1998	16	627	126	42	19	21	294	3391	1033	4424	23	416	14150	198	6735	1338	45510	1	34	31	15	706	24014	89	3027
1998	17	614	212	50	19	22	324	5158	1107	6265	18											448	13827	168	5185
1998	17	622	154	57	19	25	1012	1211	391	1602	24											916	9053	266	2629
1998	17	625	233	36	21	25	396	4545	623	5168	12											583	14722	131	3308
1998	17	626	267	46	22	23	288	7101	1126	8227	14											703	24410	235	8160
1998	17	627	141	32	22	27	720	1492	297	1789	17											928	12889	219	3042
1998	18	614	64	7	23	31	464	1035	90	1124	8	1036	22352	724	15620	2268	48932	0	4	46	32	597	12880	133	2869
1998	18	622	78	6	25	35																962		418	
1998	18	625	55	4	27	32	211	1833	119	1951	6											581	27509	196	9280
1998	18	626	127	17	24	30	244	3878	453	4332	10											506	20755	290	11895
1998	18	627	107	22	27	34	913	831	143	973	15	769	8423	361	3954	1798	19693	1	14	43	20	518	5674	171	1873

Appendix 10 (continued)

Results of on-farm trials in 1999 at Tongaren

Year	Farm	Variety	Treatt	FWTG	FWTR	MCG	MCR	Net area	YldHaG (K	YldHaR(	Yldtotal	%Loss	Bstem	Bstem/ha	Rootlog	Rootlog	StandH	StandH/	Btips	Btips/ha	%bstem	%rootlog	GoodNo.	GoodNo/h	RotNo.	RotNo/ha	Severity
1999	1	614	Dip	208	27	23	30	300	4928	575	5503	10	83	2771	248	8280	960	32053	36	1202	9	26	710	23706	164	5476	19
1999	1	622	Dip	138	64	28	29	315	2913	1340	4254	32	106	3370	480	15262	1336	42480	29	922	8	36	700	22258	375	11924	35
1999	1	625	Dip	202	30	22	23	235	6169	914	7083	13	77	3281	142	6050	860	36643	40	1704	9	17	566	24116	128	5454	18
1999	1	626	Dip	162	37	27	29	232	4673	1046	5719	18	178	7663	110	4735	887	38183	18	775	20	12	403	17348	206	8868	34
1999	1	627	Dip	229	35	25	29	263	5982	866	6848	13	144	5471	71	2698	1133	43047	87	3305	13	6	931	35372	194	7371	78
1999	2	614	Dip	218	18	22	24	261	6000	483	6483	7	73	2801	160	6140	946	36301	34	1305	8	17	474	18189	86	3300	15
1999	2	622	Dip	208	78	23	30	255	5815	1970	7785	25					1542	60494					962	37740	418	16399	30
1999	2	625	Dip	196	17	20	25	231	6231	509	6740	8	84	3630	265	11452	910	39326	32	1383	9	29	737	31850	75	3241	9
1999	2	626	Dip	229	40	20	22	286	5913	1011	6924	15	75	2626	182	6373	934	32703	28	980	8	19	709	24825	121	4237	15
1999	2	627	Dip	195	31	20	23	222	6434	970	7404	13	152	6835	169	7599	1179	53013	43	1933	13	14	790	35522	183	8228	19
1999	3	614	Nodip	109	19	19	23	211	3825	641	4466	14	36	1705	19	900	1223	57907	44	2083	3	2	349	16525	265	12547	15
1999	3	622	Nodip	61	37	21	17	352	1265	787	2052	38	93	2639	46	1305	1159	32889	51	1447	8	4	356	10102	333	9449	25
1999	3	625	Nodip	53	41	23	20	253	1471	1175	2646	44	31	1227	31	1227	977	38662	32	1266	3	3	307	12149	350	13850	20
1999	3	626	Nodip	32	33	20	26	181	1281	1224	2505	49	58	3206	17	940	767	42399	33	1824	8	2	211	11664	306	16915	20
1999	3	627	Nodip	44	24	25	25	274	1116	603	1720	35	97	3545	15	548	890	32529	28	1023	11	2	401	14656	266	9722	20
1999	4	614	Dip	259	18	22	25	353	5272	343	5614	6	29	822	67	1899	1365	38690	46	1304	2	5	1028	29138	106	3005	10
1999	4	622	Dip	272	35	23	19	390	4931	669	5600	12	54	1385	81	2077	1333	34179	101	2590	4	6	1112	28513	207	5308	16
1999	4	625	Dip	173	26	23	28	337	3629	505	4134	12	22	654	70	2080	1372	40761	11	327	2	5	922	27392	202	6001	18
1999	4	626	Dip	151	12	24	27	343	3090	227	3316	7	18	525	56	1632	821	23929	31	904	2	7	706	20577	89	2594	11
1999	4	627	Dip	296	46	23	24	277	7535	1150	8684	13	23	831	21	758	1262	45576	18	650	2	2	1010	36475	187	6753	16
1999	5	614	Nodip	113	19	23	24	263	3021	503	3525	14	32	1216	36	1368	985	37424	21	798	3	4	571	21695	86	3267	25
1999	5	622	Nodip	139	38	24	25	434	2223	604	2827	21	144	3318	177	4078	1152	26544	50	1152	13	15	820	18894	248	5714	25
1999	5	625	Nodip	155	37	24	30	400	2711	590	3301	18	127	3172	87	2173	1165	29096	7	175	11	7	778	19431	239	5969	25
1999	5	626	Nodip	82	30	26	29	169	3277	1178	4455	26	58	3424	30	1771	642	37898	23	1358	9	5	491	28985	230	13577	25
1999	5	627	Nodip	137	24	25	30	251	3752	618	4370	14	139	5529	30	1193	1213	48250	27	1074	11	2	837	33294	195	7757	25
1999	6	614	Nodip	125	54	23	24	212	4206	1772	5978	30	99	4674	231	10907	1206	56941	10	472	8	19	483	22805	262	12370	20
1999	6	622	Nodip	45	36	22	26	191	1681	1294	2975	44	122	6401	376	19727	1053	55247	8	420	12	36	356	18678	295	15477	20
1999	6	625	Nodip	73	17	21	27	102	5228	1124	6351	18	30	2950	135	13274	447	43953	3	295	7	30	579	56932	138	13569	20
1999	6	626	Nodip	64	37	24	29	187	2387	1294	3682	35	76	4062	252	13469	1095	58525	6	321	7	23	417	22288	310	16569	20
1999	6	627	Nodip	52	36	24	30	147	2485	1578	4062	39	92	6246	119	8079	794	53904	5	339	12	15	378	25662	355	24100	20
1999	7	614	Nodip	133	41	18	21	190	5264	1563	6827	23	90	4742	227	11960	958	50474	61	3214	9	24	582	30664	184	9694	20
1999	7	622	Nodip	64	44	18	19	248	1921	1323	3243	41	236	9501	488	19646	1144	46055	29	1167	21	43	333	13406	292	11755	20
1999	7	625	Nodip	84	37	19	25	210	2970	1211	4180	29	117	5574	279	13292	1315	62649	5	238	9	21	378	18009	221	10529	20
1999	7	626	Nodip	97	34	20	24	124	5767	1943	7710	25	123	9943	126	10186	582	47049	12	970	21	22	308	24899	143	11560	20
1999	7	627	Nodip	152	45	22	25	149	7308	2091	9399	22	172	11559	70	4704	814	54704	70	4704	21	9	464	31183	166	11156	20
1999	8	614	Dip	224	14	21	27	223	7296	416	7713	5	21	943	58	2603	603	27065	90	4039	3	10	680	30521	48	2154	7
1999	8	622	Dip	263	48	24	23	292	6308	1180	7488	16	109	3739	129	4425	822	28199	134	4597	13	16	1014	34786	224	7684	18
1999	8	625	Dip	322	33	24	28	230	9787	934	10721	9	67	2909	257	11159	1099	47720	53	2301	6	23	1043	45289	243	10551	12
1999	8	626	Dip	215	14	21	25	206	7568	461	8029	6	9	436	51	2472	558	27048	68	3296	2	9	598	28987	42	2036	7
1999	8	627	Dip	260	46	23	24	292	6338	1112	7451	15	62	2123	22	753	848	29041	227	7774	7	3	801	27432	161	5514	17
1999	9	614	Dip	130	29	20	28	273	3518	691	4209	16	99	3633	77	2826	724	26569	22	807	14	11	268	9835	146	5358	35
1999	9	622	Dip	201	43	17	24	534	2874	567	3441	16	284	5321	138	2586	1282	24021	43	806	22	11	923	17294	272	5096	23
1999	9	625	Dip	275	44	20	25	326	6219	916	7135	13	163	5005	150	4605	2436	74793	7	215	7	6	912	28001	250	7676	22
1999	9	626	Dip	135	22	23	24	152	6241	986	7227	14	21	1382	71	4671	544	35789	14	921	4	13	428	28158	102	6711	19
1999	9	627	Dip	143	28	27	25	238	3996	807	4803	17	130	5453	81	3398	1010	42366	25	1049	13	8	580	24329	177	7424	23
1999	10	614	Nodip	180	38	22	23	274	4728	995	5724	17	379	13822	127	4632	1498	54632	26	948	25	8	554	20204	165	6018	20
1999	10	622	Nodip	150	51	21	28	390	2814	860	3674	23	351	9002	246	6309	1363	34958	44	1128	26	18	753	19313	333	8541	20
1999	10	625	Nodip	283	41	20	26	428	4876	653	5529	12	169	3953	258	6035	1437	33614	18	421	12	18	1046	24468	229	5357	20
1999	10	626	Nodip	179	62	18	24	242	5566	1792	7358	24	75	3106	102	4224	755	31263	36	1491	10	14	448	18551	168	6957	20
1999	10	627	Nodip	239	60	21	24	370	4695	1131	5826	19	225	6084	130	3515	1470	39751	103	2785	15	9	916	24770	266	7193	20
1999	11	614	Dip	122	17	20	29	225	4021	494	4515	11					917	40828					583	25957	131	5833	18
1999	11	622	Dip	131	29	20	26	305	3128	635	3763	17					1356	44401					703	23019	235	7695	25
1999	11	625	Dip	188	38	22	23	295	4574	907	5480	17					1295	43928					928	31479	219	7429	19
1999	11	626	Dip	149	27	24	28	235	4418	746	5164	14					891	37883					597	25383	133	5655	18
1999	11	627	Dip	169	31	23	21	239	5024	939	5963	16					1286	53875					972	40721	226	9468	19

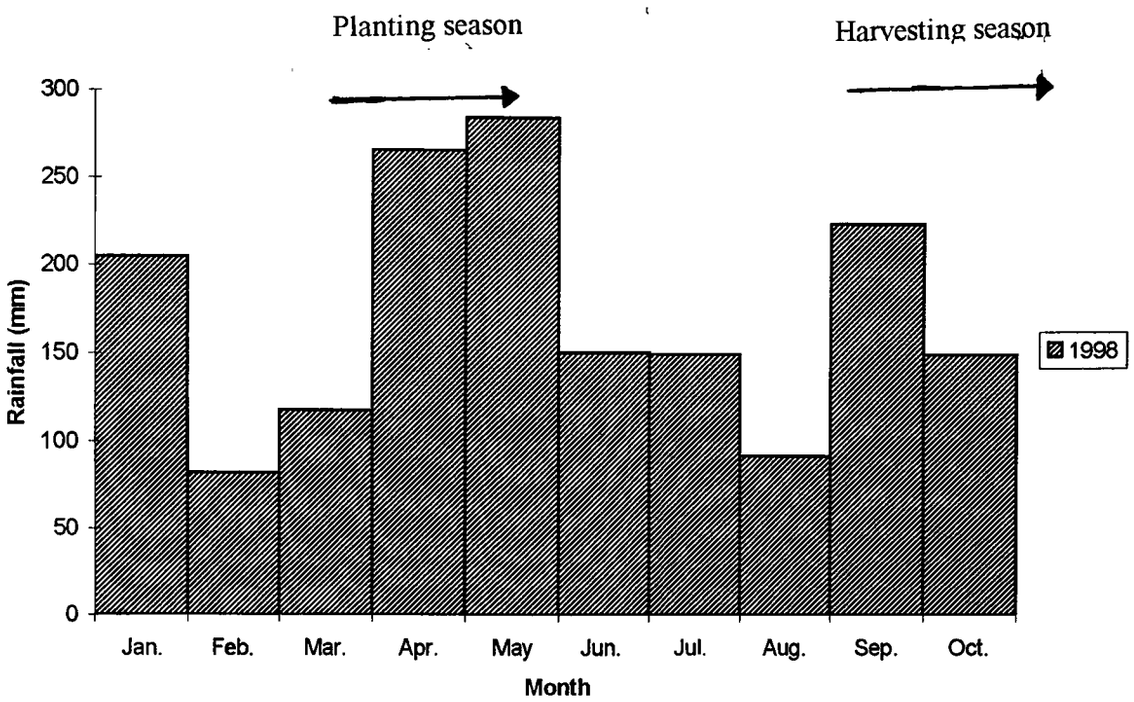
Appendix 10 (continued)

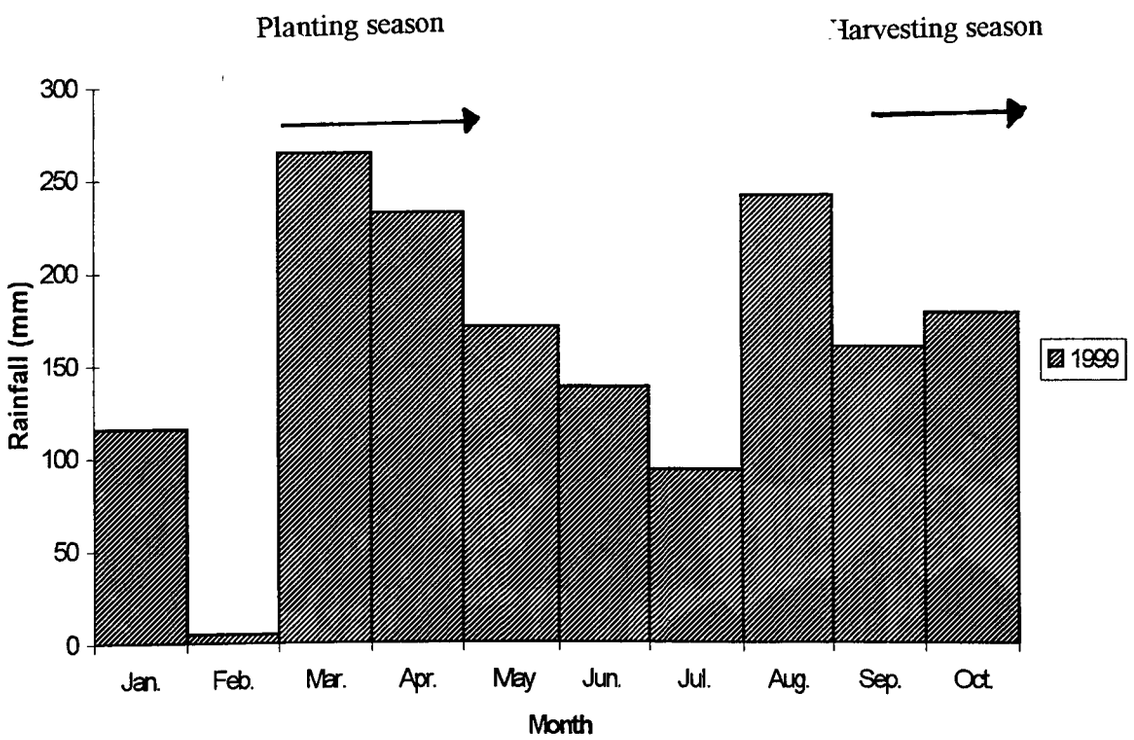
1999	12	614	Nodip	150	41	19	30	228	4895	1160	6055	19	43	1890	69	3033	826	36308	20	879	5	8	581	25538	196	8615	20
1999	12	622	Nodip	106	50	22	24	277	2734	1270	4004	32	145	5238	142	5130	1304	47110	36	1301	11	11	506	18280	290	10477	20
1999	12	625	Nodip	112	31	32	24	284	2461	765	3226	24	34	1196	46	1619	1258	44265	9	317	3	4	518	18227	171	6017	20
1999	12	626	Nodip	101	61	22	30	239	3039	1643	4682	35	41	1718	13	545	853	35735	25	1047	5	2	454	19020	338	14160	20
1999	12	627	Nodip	100	44	22	31	254	2805	1105	3911	28	100	3931	14	550	1336	52516	77	3027	7	1	673	26454	413	16234	20
1999	13	614	Nodip	151	16	23	21	262	4055	442	4497	10	186	7091	80	3050	805	30690	11	419	23	10	633	24133	99	3774	20
1999	13	622	Nodip	149	42	18	25	322	3486	895	4381	20	204	6330	120	3723	1100	34130	31	962	19	11	659	20447	269	8346	20
1999	13	625	Nodip	228	38	17	28	370	4698	672	5370	13	152	4113	220	5952	1275	34497	23	622	12	17	921	24919	207	5601	20
1999	13	626	Nodip	229	29	23	24	398	4100	508	4608	11	153	3845	366	9198	1192	29957	27	679	13	31	953	23951	141	3544	20
1999	13	627	Nodip	204	35	23	27	296	4882	783	5665	14	301	10172	64	2163	1154	39000	114	3853	26	6	785	26529	154	5204	20
1999	14	614	Dip	91	44	24	33	253	2501	1069	3570	30	72	2847	32	1265	730	28865	8	316	10	4	416	16449	245	9688	37
1999	14	622	Dip	115	62	31	34	581	1265	650	1915	34	189	3254	103	1773	1333	22951	52	895	14	8	617	10623	420	7231	41
1999	14	625	Dip	196	55	22	28	319	4434	1137	5571	20	54	1692	30	940	1074	33657	12	376	5	3	727	22783	254	7960	26
1999	14	626	Dip	159	84	23	33	316	3547	1628	5175	31	50	1580	67	2118	973	30752	26	822	5	7	625	19753	407	12863	39
1999	14	627	Dip	165	43	25	30	283	4038	963	5002	19	95	3359	29	1025	1118	39533	35	1238	8	3	613	21676	408	14427	40
1999	15	614	Dip	10	9	22	26	274	262	224	486	46	144	5248	187	6815	860	31341	25	911	17	22	117	4264	85	3098	42
1999	15	622	Dip	68	17	24	23	270	1751	433	2184	20	172	6363	213	7880	1225	45320	17	629	14	17	419	15501	113	4181	21
1999	15	625	Dip	20	10	20	28	187	793	357	1150	31	88	4716	205	10986	931	49893	12	643	9	22	190	10182	118	6324	38
1999	15	626	Dip	35	16	23	30	176	1398	563	1962	29	65	3687	186	10550	795	45094	27	1531	8	23	292	16563	148	8395	34
1999	15	627	Dip	81	22	23	31	212	2704	662	3366	20	99	4679	168	7940	1076	50851	20	945	9	16	514	24291	174	8223	25
1999	16	614	Nodip	230	35	23	27	307	5289	769	6058	13	102	3326	39	1272	846	27584	25	815	12	5	632	20606	109	3554	20
1999	16	622	Nodip	210	48	24	36	287	5108	971	6080	16	327	11382	68	2367	1128	39262	36	1253	29	6	962	33484	104	3620	20
1999	16	625	Nodip	225	47	22	22	260	6221	1288	7509	17	82	3149	53	2035	1234	47389	11	422	7	4	876	33641	141	5415	20
1999	16	626	Nodip	160	41	24	25	223	5003	1274	6277	20	92	4122	43	1927	913	40905	17	762	10	5	459	20565	119	5332	20
1999	16	627	Nodip	281	72	36	27	251	6595	1935	8530	23	181	7208	34	1354	1045	41617	87	3465	17	3	987	39307	235	9359	20
1999	17	614	Dip	270	13	19	23	258	7764	356	8120	4	11	427	58	2249	846	32803	19	737	1	7	616	23885	91	3528	13
1999	17	622	Dip	124	25	21	24	357	2526	479	3005	16	31	868	93	2605	1128	31597	50	1401	3	8	702	19664	187	5238	21
1999	17	625	Dip	283	23	25	22	242	8091	691	8782	8	11	454	294	12129	1234	50908	6	248	1	24	919	37913	95	3919	9
1999	17	626	Dip	206	22	21	27	253	5938	583	6521	9	5	197	53	2092	913	36030	12	474	1	6	694	27388	95	3749	12
1999	17	627	Dip	218	19	21	24	224	7069	579	7648	8	18	804	81	3619	1045	46693	56	2502	2	8	718	32082	104	4647	13
1999	18	614	Nodip	147	46	22	22	251	4224	1314	5538	24	53	2114	115	4587	1168	46590	51	2034	5	10	586	23375	276	11009	9
1999	18	622	Nodip	111	38	21	22	292	2781	937	3718	25	210	7184	111	3797	1352	46254	64	2190	16	8	685	23435	290	9921	8
1999	18	625	Nodip	136	27	18	25	209	4913	877	5789	15	144	6890	135	6459	1104	52823	16	766	13	12	664	31770	198	9474	6
1999	18	626	Nodip	129	25	19	22	197	4870	907	5777	16	38	1925	53	2685	751	38045	25	1266	5	7	518	26241	258	13070	10
1999	18	627	Nodip	213	35	18	21	285	5612	900	6511	14	110	3862	97	3406	1474	51756	137	4810	7	7	1023	35920	166	5829	3
1999	19	614	Dip	127	50	19	24	310	3039	1121	4160	27	66	2128	90	2902	953	30732	13	419	7	9	575	18542	157	5063	22
1999	19	622	Dip	101	53	22	35	455	1593	686	2280	30	207	4547	154	3383	1453	31920	25	549	14	11	631	13862	224	4921	26
1999	19	625	Dip	130	42	22	24	343	2718	842	3561	24	258	7522	108	3149	1357	39563	9	262	19	8	720	20991	221	6443	24
1999	19	626	Dip	151	50	21	31	262	4171	1194	5365	22	80	3050	97	3698	908	34617	23	877	9	11	549	20930	175	6672	24
1999	19	627	Dip	137	27	24	28	244	3911	738	4649	16	146	5986	72	2952	1121	45961	18	738	13	6	750	30750	195	7995	21
1999	20	614	Nodip	42	39	31	34	277	958	851	1809	47	256	9249	131	4733	1153	41655	16	578	22	11	235	8490	321	11597	20
1999	20	622	Nodip	42	30	24	36	225	1317	786	2103	37	142	6311	109	4844	1014	45067	21	933	14	11	356	15822	128	5689	20
1999	20	625	Nodip	55	52	28	33	702	512	457	970	47	201	2862	82	1168	1104	15720	20	285	18	7	370	5268	197	2805	20
1999	20	626	Nodip	40	37	26	27	156	1745	1598	3343	48	182	11667	104	6667	626	40128	24	1538	29	17	241	15449	234	15000	20
1999	20	627	Nodip	85	46	27	33	220	2601	1288	3889	33	316	14364	70	3182	1078	49000	73	3318	29	6	460	20909	253	11500	25
1999	21	614	Nodip	212	29	17	22	277	5843	752	6595	11	88	3181	182	6580	992	35864	39	1410	9	18	552	19957	127	4591	25
1999	21	622	Nodip	104	37	22	26	235	3180	1075	4254	25	281	11947	146	6207	1181	50213	34	1446	24	12	692	29422	110	4677	53
1999	21	625	Nodip	294	35	17	23	510	4388	488	4876	10	138	2705	106	2078	1106	21682	33	647	12	10	1083	21231	134	2627	11
1999	21	626	Nodip	246	33	17	21	301	6243	801	7044	11	80	2657	43	1428	996	33079	36	1196	8	4	779	25872	103	3421	31
1999	21	627	Nodip	303	33	18	23	395	5759	594	6353	9	75	1898	39	987	1161	29378	93	2353	6	3	887	22444	77	1948	25
1999	22	614	Dip	24	81	24	21	256	651	2306	2957	78	183	7162	160	6262	999	39100	10	391	18	16	177	6928	467	18278	73
1999	22	622	Dip	16	40	26	25	235	447	1162	1609	72	150	6372	173	7349	1166	49533	9	382	13	15	137	5820	363	15421	73
1999	22	625	Dip	32	99	23	28	250	890	2598	3488	74	182	7292	92	3686	1164	46635	8	321	16	8	171	6851	369	14784	68
1999	22	626	Dip	50	65	22	25	292	1228	1540	2768	56	232	7934	71	2428	1653	56532	19	650	14	4	252	8618	320	10944	56
1999	22	627	Dip	42	60	29	34	202	1355	1801	3156	57	364	18065	81	4020	1468	72854	21	1042	25	6	302	14988	544	26998	64
1999	23	614	Nodip	124	19	21	20	226	3979	601	4580	13	193	8525	285	12588	1033	45627	22	972	19	28	517	22836	83	3666	25
1999	23	622	Nodip	104	53	21	24	284	2657	1306	3963	33	384	13502	347	12201	1249	43917	26	914	31	28	579	20359	182	6399	14
1999	23	625	Nodip	137	26	21	17	262	3803	741	4544	16	158	6037	297	11349	1247	47650	15	573	13	24	607	23194	139	5311	25

Appendix 10 (continued)

1999	23	626	Nodip	130	34	20	26	207	4633	1117	5750	19	89	4304	147	7108	834	40329	24	1161	11	18	501	24226	153	7398	23
1999	23	627	Nodip	87	34	23	28	279	2212	822	3034	27	130	4668	140	5027	1246	44740	36	1293	10	11	444	15943	135	4847	29
1999	24	614	Dip	166	41	18	25	280	4436	992	5428	18	58	2069	115	4103	867	30931	24	856	7	13	583	20799	195	6957	25
1999	24	622	Dip	50	71	18	21	270	1400	1910	3310	58	341	12630	171	6333	1194	44222	52	1926	29	14	340	12593	331	12259	49
1999	24	625	Dip	172	70	17	25	303	4356	1572	5928	27	112	3694	157	5178	1253	41326	15	495	9	13	708	23351	366	12071	34
1999	24	626	Dip	92	52	22	27	235	2815	1489	4304	35	92	3908	84	3568	867	36831	25	1062	11	10	415	17630	283	12022	41
1999	24	627	Dip	133	80	20	25	306	3223	1811	5033	36	203	6645	104	3404	1324	43339	66	2160	15	8	640	20949	401	13126	39
1999	25	614	Dip	148	39	18	25	336	3314	795	4109	19	51	1517	56	1665	870	25870	27	803	6	6	694	20636	176	5233	20
1999	25	622	Dip	130	76	21	26	402	2344	1278	3622	35	213	5300	153	3807	1130	28116	45	1120	19	14	717	17840	413	10276	37
1999	25	625	Dip	170	90	19	26	381	3310	1620	4930	33	174	4572	295	7751	1143	30032	18	473	15	26	737	19364	406	10667	36
1999	25	626	Dip	208	81	24	30	331	4394	1573	5967	26	100	3023	171	5169	972	29383	10	302	10	18	621	18773	351	10611	36
1999	25	627	Dip	220	91	17	25	327	5128	1921	7049	27	267	8173	78	2388	1206	36915	78	2388	22	6	803	24579	403	12335	34
1999	26	614	Nodip	41	33	24	27	248	1157	891	2047	44	67	2697	35	1409	326	13124	28	1127	21	11	158	6361	168	6763	20
1999	26	622	Nodip					215					114	5307	53	2467			13	605							
1999	26	625	Nodip	60	45	23	29	206	2077	1413	3490	40	84	4086	37	1800	525	25535	25	1216	16	7	268	13035	257	12500	20
1999	26	626	Nodip	59	44	22	30	206	2050	1374	3423	40	125	6062	68	3298	443	21484	20	970	28	15	224	10863	219	10621	20
1999	26	627	Nodip	68	63	24	29	268	1766	1532	3298	46	211	7888	46	1720	621	23215	69	2579	34	7	312	11664	309	11551	19

Appendix 11: Rainfall data for Tongaren in 1998 1nd 1999





Appendix 12: Analysis of variance of evaluation of varieties

Appendix 12.1: Analysis of variance table: Yield of clean maize at Kitale (1997)

Source	D.f.	Sum of Squares	Mean Squares	F - value	Probability
Replication	3	362648.850	120882.950	0.8652	
Variety (V)	4	725074.732	181268.683	1.2974	0.3254
Error	12	1676628.935	139719.078		
Pathogen (P)	3	886428.427	295476.142	2.9649	0.0420
VXP	12	1530076.750	127506.396	1.2794	0.2636
Error	45	4484663.114	99659.180		
Total	79	9665520.808			

Appendix 12.2: Analysis of variance table: Yield of rotten maize at Kitale (1997)

Source	D.f.	Sum of Squares	Mean Squares	F - value	Probability
Replication	3	578326.718	192775.573	3.8184	0.0393
Variety (V)	4	743526.071	185881.518	3.6819	0.0353
Error	12	605826.596	50485.550		
Pathogen (P)	3	204046.652	68015.551	1.7388	0.1726
VXP	12	580222.461	48351.872	1.2361	0.2895
Error	45	1760191.726	39115.372		
Total	79				

Appendix 12.3: Analysis of variance table: Yield of clean maize at Kakamega (1997)

Source	D.f.	Sum of Squares	Mean Squares	F - value	Probability
Replication	3	21059.273	7019.758	0.0466	
Variety (V)	3	701128.099	233709.366	1.5499	0.2680
Error	9	1357139.177	150793.242		
Pathogen (P)	3	1428682.749	476227.583	6.0338	0.0019
VXP	9	774101.116	86011.235	1.0898	0.3941
Error	36	2841337.715	78926.048		
Total	63	7123448.129			

Appendix 12.4: Analysis of variance table: Yield of rotten maize at Kakamega (1997)

Source	D.f.	Sum of Squares	Mean Squares	F - value	Probability
Replication	3	13925.427	4641.809	0.0525	
Variety	3	100022.113	33340.704	0.3770	
Error	9	795838.513	88426.501		
Pathogen	3	331527.246	110542.415	2.6920	0.0606
Variety x Pathogen	9	298880.911	33208.990	0.8087	
Error	36	1478290.342	41063.621		
Total	63	3018584.554			

Appendix 12.5: Analysis of variance table: Number of clean maize cobs at Kitale (1998)

Source	D.f.	Sum of Squares	Mean Squares	F- value	Probability
REP	3	22.55	7.517	1.32	0.2773
TRT	19	346.45	18.234	3.20	0.0004
Error	57	324.95	5.701		
Non-additivity	1	1.30	1.305	0.23	
Residual	56	2323.65	5.779		
Total	79	693.95			

Appendix 12.6: Analysis of variance table: Incidence of rotten maize cobs at Kitale (1998)

Source	D.f.	Sum of Squares	Mean Squares	F- value	Probability
REP	3	41.64	13.879	4.22	0.0092
TRT	19	154.64	8.139	2.47	0.0044
Error	57	187.61	3.291		
Non-additivity	1	6.13	6.134	1.89	0.1744
Residual	56	181.48	3.341		
Total	79	383.89			

Appendix 12.7: Analysis of variance table: Yield of clean maize for Kitale(1998)

Source	D.f.	Sum of Squares	Mean Squares	F- value	Probability
REP	3	5861132.45	1953710.817	2.08	0.1135
TRT	19	85823393.11	4517020.690	4.80	0.0000
Error	57	53642203.14	941091.283		
Non-additivity	1	2969206.06	2969206.064	3.28	0.0754
Residual	56	50672997.08	904874.948		
Total	79	154326728.70			

Appendix 12.8: Analysis of variance table: Yield of rotten maize at Kitale season (998)

Source	D.f.	Sum of Squares	Mean Squares	F- value	Probability
REP	3	3051971.91	1017323.972	2.95	0.0403
TRT	19	11833104.11	622794.953	1.81	0.0446
Error	57	19658838.50	344891.904		
Non-additivity	1	1901662.65	1901662.655	6.00	0.0175
Residual	56	17757175.85	317092.426		
Total	79	34543914.52			

Appendix 12.9: Analysis of variance table: Total yield at Kitale season 2 (1998)

Source	D.f.	Sum of Squares	Mean Squares	F- value	Probability
REP	3	5165282.19	1721760.730	4.07	0.0109
TRT	19	81714203.84	4300747.570	10.17	0.0001
Error	57	24112349.54	423023.676		
Non-additivity	1	48600.77	48600.768	0.11	
Residual	56	24063748.57	427909.800		
Total	79	110991835.57			

Appendix 12.10: Analysis of variance table: Number of clean cobs at Kakamega (1998)

Source	D.f.	Sum of Squares	Mean Squares	F- value	Probability
REP	3	9.05	3.017	0.93	0.4324
TRT	19	161.55	8.503	2.62	0.0026
Error	57	184.95	3.245		
Non-additivity	1	0.12	0.125	0.04	
Residual	56	184.83	3.300		
Total	79	355.55			

Appendix 12.11: Analysis of variance table: Incidence of rotten cobs at Kakamega season 2 (1998)

Source	D.f.	Sum of Squares	Mean Squares	F- value	Probability
REP	3	6.00	2.000	0.85	0.4703
TRT	19	34.70	1.826	0.78	0.7198
Error	57	133.50	2.342		
Non-additivity	1	0.25	0.246	0.10	
Residual	56	133.25	3.380		
Total	79	174.20			

Appendix 12.12: Analysis of variance table: Yield of clean maize grain at Kakamega season 2 (1998)

Source	D.f.	Sum of Squares	Mean Squares	F- value	Probability
REP	3	812554.16	270851.388	1.16	0.3332
TRT	19	12216885.41	642993.969	2.75	0.0017
Error	57	13314946.50	233595.553		
Non-additivity	1	271577.55	271577.545	1.17	0.2849
Residual	56	13043368.95	232917.303		
Total	79	26344386.07			

Appendix 12.13: Analysis of variance table: Yield of rotten maize at Kakamega (1998)

Source	D.f.	Sum of Squares	Mean Squares	F- value	Probability
REP	3	360300.10	120100.013	1.06	0.3733
TRT	19	3419831.75	179991.145	1.59	0.0912
Error	57	6458590.04	113308.597		
Non-additivity	1	425974.47	425974.469	3.95	0.0516
Residual	56	6032615.58	107725.278		
Total	79	10238721.90			

Appendix 12.14: Analysis of variance table: Total yield of maize grain at Kakamega (1998)

Source	D.f.	Sum of Squares	Mean Squares	F- value	Probability
REP	3	1569891.85	523297.285	1.66	0.1859
TRT	19	14131734.71	743775.511	2.36	0.0066
Error	57	17972126.09	315300.458		
Non-additivity	1	343619.35	343619.351	1.09	0.3006
Residual	56	17628506.74	314794.763		
Total	79	33673752.65			

Appendix 12.15: Analysis of variance table: Number of broken stems in On-farm trials Tongaren 1998

Source	D.f	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	17	0.216311	0.015451	4.91	
REP*Units*Statum					
Variety	4	0.080053	0.020013	6.36	< 0.001
Residual	48	0.151095	0.003148		
Total	66	0.383657			

Appendix 12.16: Analysis of variance table: Percentage yield loss in on-farm trials in Tongaren 1998

Source	D.f	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	17	3.51856	0.20697	9.60	
REP*Units*Statum					
Variety	4	0.52118	0.13013	6.04	< 0.001
Residual	48		0.02156		
Total	66	5.23105			

Appendix 12.17: Analysis of variance table: Number of bare tips stems in five varieties in on-farm trials in Tongaren 1998

Source	D.f	Sum of Squares	Mean Squares	F - value	Probability
REP Stratum	17	130131	8675	2.24	
REP*Units*Statum					
Variety	4	89466	22367	5.78	0.001
Residual	48	127609	3867		
Total	66	257188			

Appendix 12.18: Analysis of variance table: Incidence of cob rot in five varieties in on-farm trials in Tongaren 1999 (no dipterex)

Source	D.f	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	12	2.05583	0.17132	13.52	
REP*Units*Statum					
Variety	4	0.10712	0.02678	2.11	0.094
Residual	48	0.59546	0.01267		
Total	64	2.65516			

Appendix 12.19: Analysis of variance table: Incidence of cob rot in five varieties in on-farm trials in Tongaren 1999 (dipterex)

Source	D.f	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	12	2.46952	0.20579	16.03	
REP*Units*Statum					
Variety	4	0.12900	0.03225	2.51	0.054
Residual	48	0.61623	0.01284		
Total	64	3.21475			

Appendix 12.20: Analysis of variance table: Severity of rotten cobs five varieties in on-farm trials in Tongaren 1999

Source	D.f	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	12	2.05583	0.17132	13.52	
REP*Units*Statum					
Variety	4	0.10712	0.02678	2.11	0.094
Residual	48	0.59546	0.01267		
Total	64	2.65516	2.65516		

Appendix 13: Analysis of variance: Effect of time of harvesting on cob rot incidence

Appendix 13.1 Analysis of variance table: Percentage rotten grain at different at different harvesting interval (1998)

Source	D.f.	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	3	237.66	79.	0.88	
REP*Units* Stratum					
TRT	14	6434.47	459.60	5.08	<.001
Residual	42	3439.93	90.52		
Total	59	9042.50			

Appendix 13.2: Analysis of variance table: Incidence of rotten cobs at different at different harvesting interval (1998)

Source	D.f.	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	3	0.08689	0.02896	0.53	
REP*Units* Stratum					
TRT	14	1.89767	0.13555	2.48	0.013
Residual	42	2.08082	0.05476		
Total	59	3.89014			

Appendix 13.3 Analysis of variance table: Yield of clean maize grain at different at different harvesting interval (1998)

Source	D.f.	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	3	3153175	1051058		
REP*Units* Stratum					
TRT	14	35727948	2551996	4.72	<.001
Residual	42	20556130	540951		
Total	59	57089194			

Appendix 13.4 Analysis of variance table: Yield of rotten grain at different at different harvesting interval (1998)

Source	D.f.	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	3	129991	43330	0.47	
REP*Units* Stratum					
TRT	14	7920311	565737	6.10	<.001
Residual	42	3524191	92742		
Total	59	10561594			

Appendix 14: Analysis of variance of effect of fertilizer on cob rot incidence

Appendix 14.1. Analysis of variance table: Incidence of rotten cobs at different fertilizer levels (1998)

Source	D.f.	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	3	0.14018	0.04673	0.83	
REP*Units* Stratum					
TRT	11	1.96753	0.17887	3.17	0.05
Residual	33	1.86010	0.05637		
Total	47	3.96780			

Appendix 14.2: Analysis of variance table: Incidence of rotten cobs at different fertilizer levels (1999)

Source	D.f.	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	3	0.4797	0.1599		
REP*Units* Stratum					
TRT	11	1.7191	0.1563	1.36	0.237
Residual	33	3.7880	0.1148		
Total	47	5.9867			

Appendix 14.3: Analysis of variance table: Percentage rotten grain at different fertilizer levels (1998)

Source	D.f.	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	3	0.4860	0.1620	1.35	
REP*Units* Stratum					
TRT	11	1.5452	0.1405	1.17	0.342
Residual	33	3.9527	0.1198		
Total	47	5.9840			

Appendix 14.4: Analysis of variance table: Percentage rotten grain at different fertilizer levels (1999)

Source	D.f.	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	3	0.7863	0.2621	1.98	
REP*Units* Stratum					
TRT	11	1.5815	0.1438	1.09	0.400
Residual	33	4.3578	0.1321		
Total	47				

Appendix 14.5: Analysis of variance table: Severity of rotten grain at different fertilizer levels (1999)

Source	D.f.	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	3	1.1827	0.3942	1.74	
REP*Units* Stratum					
TRT	11	3.1439	0.2858	1.26	0.288
Residual	33	7.4693	0.2263		
Total	47	11.7958			

Appendix 14.6: Analysis of variance table: Severity of rotten grain at different fertilizer levels (1998)

Source	D.f.	Sum of Squares	Mean Squares	v.r.	Probability
REP Stratum	3	0.2986	0.0995	0.92	
REP*Units* Stratum					
TRT	11	3.8846	0.3531	3.25	0.062
Residual	33	3.5814	0.1085		
Total	47	7.7646			