CHAPTER ONE: INTRODUCTION

IMPORTANCE OF MAIZE IN BENIN

Maize (*Zea mays* L.) is a cereal crop grown throughout the word. It was introduced to Africa from South America during the 16th century (FAO 1996). Maize plays an important role in the diet of millions of African people due to its high yields per hectare, its ease of cultivation and adaptability to different agro-ecological zones, versatile food uses and storage characteristics (Asiedu 1989). The total production of Africa in 2001 was estimated to be about 42 millions tons (FAO 2002).

In Benin, maize is the most cultivated cereal, grown all over the country and the bulk of the production is by small-scale farmers, mainly for home consumption. In 2001, it occupied about 600 000 ha with a total production of 662 958 tons (FAO 2002). Today, maize has become the second cash crop after cotton and its production is increasing yearly. It is a dietary staple food for more than 70 % of the population (CIMMYT 1990, Hounhouigan 1994). It is estimated that more than 246 g are consumed per person per day in Benin (Hounhouigan *et al.* 1999). But generally, the consumption is higher in the Southern and Central parts of the country (274 – 373 g per person per day) than in the North where it is just entering into the food habits of people (Hounhouigan 1994). Maize is consumed in various and numerous traditional fermented or unfermented products including porridges, pastes, dumplings, cakes, fritters and beverages (Nago 1997). About 40 different ways of maize processing have been recorded in Benin (Nago 1997).

IMPORTANCE OF INSECTS AND FUNGI IN MAIZE

In the field as well as in the store, many pests and parasites attack maize and during the storage period, insects are most often considered as the principal cause of grain losses (Gwinner *et al.* 1996). However, fungi are also important and rank second as the cause of deterioration and loss of maize (Ominski *et al.* 1994). Kossou and Aho (1993) reported that fungi could cause about 50 – 80 % of damage on farmers' maize during the storage period if conditions are favourable for their development. The major genera commonly encountered in maize in tropical regions are *Fusarium*, *Aspergillus* and *Penicillium* (Samson 1991, Orsi *et al.* 2000).

FUSARIUM SPECIES AND THEIR IMPORTANCE IN MAIZE

Fusarium species are ubiquitous in soils. They are commonly considered as field fungi invading more than 50 % of maize grains before harvest (Robledo-Robledo 1991). The genus includes many phytopathogenic species that cause serious diseases in maize, consequently affect growth and yield of the crop, and lead to losses of billions dollars each year to farmers throughout the world (Doyle 1998). Several *Fusarium* species are found associated with maize including *F. verticillioides* (Sacc.) Nirenberg (previously known as *F. moniliforme* Sheldon), *F. proliferatum* (Matsushina) Nirenberg, *F. graminearum* Schwabe and *F. anthophilum* (A. Braun) Wollenweber, but *F. verticillioides* is likely to be the most common species isolated worldwide from diseased maize (Lawrence *et al.* 1981, Scott 1993, Munkvold and Desjardins 1997). Doko *et al.* (1996) reported *F. verticillioides* as the most frequently isolated fungus from maize and maize-based commodities in France, Spain and Italy. Likewise, Orsi *et al.* (2000) found in Brazil that *F. verticillioides* was the predominant *Fusarium* species on maize. In general in Africa, very little information is available on *F. verticillioides* occurrence on maize. Reports of surveys conducted in some African countries however showed it as the most prevalent fungus on maize (Marasas *et al.* 1988, Allah Fadl 1998, Baba-Moussa 1998, Kedera *et al.* 1999).

F. verticillioides is an endophyte of maize establishing long-term associations with the plant (Baba-Moussa 1998, Pitt and Hocking 1999). Symptomless infection can exist throughout the plant in leaves, stems, roots, grains, and the presence of the fungus is in many cases ignored because it does not cause visible damageμ to the plant (Munkvold and Desjardins 1997). This suggests that some strains of *F. verticillioides* produce disease in maize and others do not (Bacon and Williamson 1992).

F. verticillioides infects maize at all stages of plant development, either via infected seeds, the silk channel or wounds, causing grain rot during both the pre- and postharvest periods (Munkvold and Desjardins 1997). Figure 1 shows *Fusarium* spp. damage on maize cob. A diagrammatic illustration of the disease cycle of *F. verticillioides* in maize is proposed on Figure 2 showing the following possible infection pathways:

- Infection from seed to cob and further to grain through systemic movement in stalk,
- Infection from root to grain through stalk and cob,
- Infection from airborne or water-splashed conidia to silk and further to grain,

- Infection through wounds caused by insects that can also act as vectors of inoculum (Munkvold and Desjardins 1997).

Fig. 1: Apparently healthy maize cob (left) and *Fusarium* – infected maize cob (right)

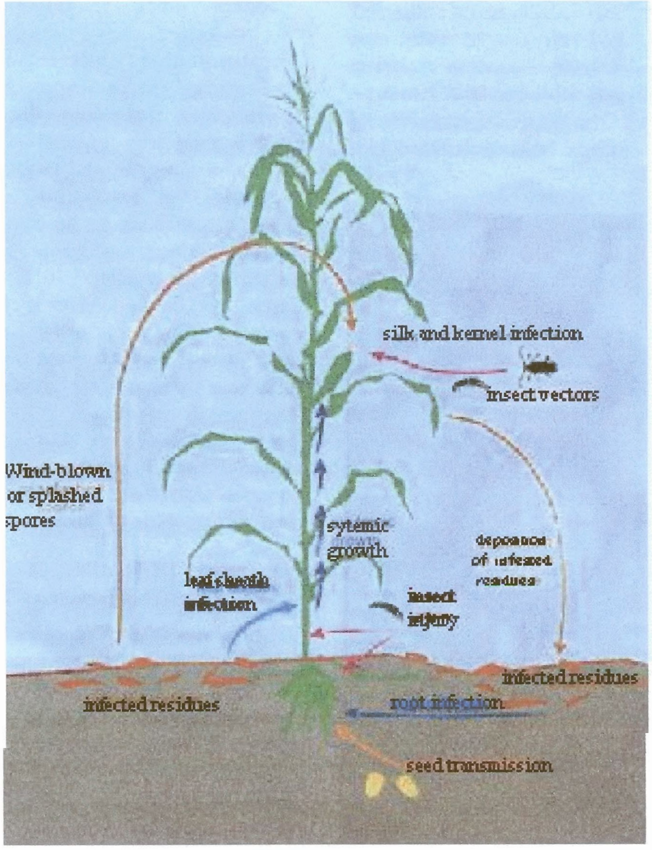

Fig. 2: Disease cycle of *F. verticillioides* on maize showing various infection pathways

<u>Source</u>: Munkvold and Desjardins (1997)

FUMONISINS AND THEIR TOXICOLOGICAL EFFECTS

Maize contamination by fungi not only renders grains unfit for human consumption by discoloration and reduction of nutritional value, but can also lead to mycotoxin production. Mycotoxins are poisonous secondary metabolites produced by some fungi in staple foods and foodstuffs. Many of them are considered to be important worldwide, but the five most often reported and well documented are deoxynivalenol/nivalenol, zearalenone, ochratoxin, aflatoxins and fumonisins (Pittet 1998, Pitt 2000). There is ample evidence that mycotoxin problems affect the agricultural economies of many countries in the world, mainly the African countries. The FAO estimated that each year, between 25 % and 50 % of the world's food crops are contaminated by mycotoxins (Mannon and Johnson 1985). The direct impact of mycotoxins on the staple product quality constitutes an important danger for human health and among them fumonisins produced by some toxigenic *Fusarium* species on maize and maize-based foods and feeds increase the risk.

Fumonisins are recently discovered mycotoxins. In 1988, their chemical structure and biological activity were elucidated in South Africa (Gelderblom *et al.* 1988, Marasas 2001). Since the discovery of these toxins, numerous studies have been undertaken to investigate them further. The interesting results found so far have been thoroughly reviewed (Norred 1993, Riley *et al.* 1993, Cardwell and Miller 1996, Gelderblom *et al.* 1996, Shephard *et al.* 1996, Marasas 1996, IPCS 2000, Bolger *et al.* 2001, Marasas 2001, WHO 2002). These reviews mainly highlighted:

- Events leading to the discovery of the fumonisins,
- The toxicological effects of these toxins,
- Their worldwide occurrence in maize and maize-based foods and feeds,
- Their association with animal and human diseases,
- Their impact on animal and human health.

Fumonisins have been found as very common contaminants of maize-based foods and feeds in the United States of America, China, Europe, South America and Africa (Sydenham *et al.* 1991, Thiel *et al.* 1992, Visconti and Doko 1994, Shephard *et al.* 1996). To date, a total of 28 fumonisin analogs have been identified and characterised (Rheeder *et al.* 2002). The most abundant found in naturally contaminated foods and feeds are FB_1, FB_2 and FB_3 (Shephard *et al.* 1996, Rheeder *et al.* 2002).

Fumonisins are produced by several *Fusarium* species (Marasas 2001) including:

- *F. verticillioides* (Sacc.) Nirenberg,

- *F. proliferatum* (Matsushima) Nirenberg,
- *F. nygamai* Burgess & Trimboli,
- *F. anthophilum* (A. Braun) Wollenweber,
- *F. dlamini* Marasas, Nelson & Toussoun,
- *F. napiforme* Marasas, Nelson & Rabie,
- *F. thapsinum* Klittich, Leslie, Nelson & Marasas,
- *F. globosum* Rheeder, Marasas & Nelson.

Amongst these, *F. verticillioides* and *F. proliferatum* are by far the most prolific fumonisin producers (Shephard *et al.* 1996). They produce the highest amounts of toxins: up to 17900 mg kg^{-1} of FB$_1$ have been recorded in cultures for the former and 31000 mg kg^{-1} FB$_1$ for the latter (Rheeder *et al.* 2002).

Maize is the product in which fumonisins are most abundant (Shephard *et al.* 1996). Fumonisins have been also detected but at lower levels in sorghum (Shetty and Bhat 1997, Leslie and Marasas 2001), rice (Abbas *et al.* 1998) and spices (Pittet 1998). Fumonisins can contaminate maize foods and feeds as a result of the *Fusarium* invasion before and after harvest (Doko *et al.* 1995).

Fumonisins have emerged as a highly visible animal and human health safety concern since they have been associated with many animal diseases such as leukoencephalomalacia (LEM) in horses (Marasas 1996), pulmonary oedema syndrome (PES) in pigs (Harrison *et al.* 1990, Colvin and Harrison 1992), and hepatocarcinogenesis in rats (Gelderblom *et al.* 2001). With respect to humans, studies on the prevalence of oesophageal cancer in regions of South Africa, China, Italy and Iran, revealed an association between this disease and the consumption of maize contaminated by *Fusarium* spp (Franceschi *et al.* 1990, Rheeder *et al.* 1992, Chu and Li 1994, Marasas 1996, Shephard *et al.* 2000, Wang *et al.* 2000). The International Agency for Research on Cancer (IARC) evaluated in 1992 the toxins derived from *F. verticillioides* as possibly carcinogenic to humans, belonging to the group 2B carcinogens (IARC 1993). More recently, based on the research results obtained so far, FB$_1$ has been evaluated as possibly carcinogenic to humans (group 2B) (IARC 2002).

Although the effects of fumonisins on humans are not yet well understood, legislation is being put in place to regulate commercial exchanges of fumonisin-contaminated maize and maize-based foods. The US Food and Drug Administration (FDA) recommended that the fumonisin levels are not higher than 4 mg kg^{-1} in human foods, and that controlling fumonisins to that level can reduce exposure to the toxin (FDA 2000a, FDA 2000b). In Switzerland, tolerance levels for fumonisins of 1 mg kg^{-1} in dry maize products intended for human consumption were proposed (Marasas *et al.* 2001). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) allocated a group provisional maximum tolerable daily intake (PMTDI) of 0.002 mg kg^{-1} body weight for FB_1, FB_2 and FB_3, alone or in combination (WHO 2002). With respect to animals, the total fumonisin maximal tolerable levels recommended by FDA in maize-based feeds are 5 mg kg^{-1} for horse and rabbit, 60 mg kg^{-1} for ruminants (cattle, sheep, goat) and 100 mg kg^{-1} for poultry (chicken, turkey, duckling) (FDA 2000c).

The mechanism of action of fumonisins in animal diseases is quite complex, but it appears that the toxins mainly cause disruption of lipid metabolism in cells, an event that can lead to cellular deregulation or toxic cell injury and finally to cell death (Wang *et al.* 1991, Riley 1998, Marasas *et al.* 2001).

Fumonisins are found phytotoxic. FB_1 can indeed damage a wide variety of plants including maize (Scott 1993, Lamprecht *et al.* 1994). Doehlert *et al.* (1994) showed that the presence of high levels of fumonisins in maize seeds might have deleterious effects on seedling emergence. Elongation of maize radicles was inhibited by about 75 % after 48 hours of imbibition in 100 mg kg^{-1} of fumonisins and amylase activities in seeds significantly decreased as well.

Fumonisins are also found to be relatively stable molecules, heat stable (Alberts *et al.* 1990, Howard *et al.* 1998), light stable (IARC 1993), and stable in stored products when these are kept in airtight at very low temperatures.(Gelderblom *et al.* PROMEC Unit, Medical Research Council, Tygerberg, South Africa, 2002, unpublished data). or γ-irradiated (Visconti *et al.* 1996). However, instability of fumonisins in contaminated products over time has been shown (Scott *et al.* 1999, Kim *et al.* 2002). Fumonisins are also water soluble (IPCS 2000).

FACTORS INFLUENCING INFECTION OF MAIZE WITH *FUSARIUM* SPECIES AND FUMONISIN DEVELOPMENT

Infection of maize with *Fusarium* species and its contamination by fumonisins are generally influenced by many factors including environmental conditions (climate, temperature and humidity), insect infestation and pre- and postharvest handling. These factors do not influence infection independently but most often there are complex interactions.

Influence of abiotic factors

Environmental factors

Worldwide surveys showed high levels of fumonisins associated with warmer and drier climates (Shephard *et al.* 1996) and when weather conditions are favourable for *Fusarium* infection (Marasas *et al.* 2001). At the same location, fumonisin contamination is not necessarily the same from one year to another. Hennigen *et al.* (2000) found in Argentina a marked difference in terms of fumonisin contamination for the same maize varieties during two consecutive growing seasons, due to the fact that environmental conditions may differ from one growing season to another.

Studying the effect of climatic conditions on fumonisin occurrence in freshly harvested maize in different regions of the State of Parana in Brazil, Ono *et al.* (1999) detected higher fumonisins levels in maize samples from the Northern and Central-Western regions compared to that from the South. The authors suggested that it could be due to the differences in rainfall levels during the month preceding harvest (92.8 mm in South, 202 mm in North).

Physiological stress during the period just preceding maize harvest, due to drastic oscillations in rainfall and relative humidity, is likely to create favourable conditions for fumonisin production (Visconti 1996). Shelby *et al.* (1994) suggested that dry weather at or just prior to pollination of maize might be an important factor for fumonisin production in maize. On the other hand, Doyle (1998) reported that late season rainfall increases infection of maize with *F. verticillioides*, the main fumonisin producer. All this leads to the conclusion that some climatic events such as changes in rainfall patterns or stress during the last stages of maize plant development in the field are likely to have a great influence on fumonisin production in maize before harvest.

Furthermore, temperature and moisture conditions during the growing season as well as during storage are often pointed out to affect maize infection by *Fusarium* spp. and fumonisin synthesis. In this connection, water activity (a_w), the water available for fungal growth, depending on the relative humidity of air around maize grain, grain moisture content and temperature, plays a key role. Velluti *et al.* (2000) working *in vitro* on fungal competition on maize found that the growth rate of *F. verticillioides* was higher at a temperature of 25 °C, whereas at 15 °C, growth was much lower. These researchers also found that at a constant temperature, the growth rate of *F. verticillioides* increased with water activity.

Some authors suggest that the best temperature for production of fumonisin B_1 in maize is 20 °C (Scott 1993). Marin *et al.* (1999) rather found in a study on fumonisin production under different environmental conditions that the toxin was optimally produced at 30 °C and 0.98 a_w. However, Alberts *et al.* (1990) showed that the mean FB_1 yield obtained at 25 °C (9500 mg kg^{-1}) was significantly higher than that at 20 °C (8700 mg kg^{-1}) and 30 °C (600 mg kg^{-1}). Munkvold and Desjardins (1997) reported that *F. verticillioides* generally grows in grain when moisture content is more than 18 – 20 %.

Agricultural practices

It has been reported that late planting of maize with harvesting in wet conditions favours disease caused by *F. verticillioides* (Bilgrami and Choudhary 1998), and the prevalence of this fungus is considerably increased with wet weather later in the season (Al-Heeti 1987). Moreover, repeated planting of maize and other cereal crops in the same or in nearby fields favours fungal infection by increasing the fungal inoculum and insect population that attack maize plants (Bilgrami and Choudhary 1998, Doyle 1998). Lipps and Deep (1991) found that the rotation maize/nonhost crop of *Fusarium* was better than maize/maize, as the former was less favourable to *Fusarium* disease outbreaks than the latter. Weed control also affects fungal infection in maize fields because it helps to eliminate nonhost weeds on which *Fusarium* can also be found (Bilgrami and Choudhary 1998).

Maize characteristics

The type of maize cultivar and grain characteristics such as colour, endosperm type, chemical composition and stage of development may also influence fungal infection and subsequent fumonisin production. Late-maturing maize cultivars in which grain moisture content decreases slowly below 30 % are most susceptible to *Fusarium* disease (Manninger 1979). It is thought maize cultivars with upright cobs, tight husks (Emerson and Hunter 1980), thin grain pericarp (Riley and Norred 1999), and an increased propensity for grain splitting (Odvody *et al.* 1990) are likely to be more susceptible to *Fusarium* infection. Tight-husked varieties favour *Fusarium* problems because of slow drying (Dowd 1998).

Fumonisins are found more concentrated in the pericarp and germ of the grain than in the endosperm, so that removal of those outer parts by mechanical processes such as dehulling can significantly reduce the toxin in maize (Charmley and Prelusky 1994, Sydenham *et al.*

1995, FDA 2000b). However, influence of maize grain colour on fumonisin contamination does not seem to be clear. Shephard *et al.* (1996) reported that in some years, fumonisin levels were significantly lower in yellow than in white maize, but the reverse situation was observed in other years.

Regarding the effect of grain endosperm characteristics (dent, flint, or semi-dent) on contamination by fumonisins, Hennigen *et al.* (2000) compared contamination of maize varieties of flint endosperm to that of dent type and did not find significant differences. Shelby *et al.* (1994) tested fifteen maize hybrids and found no significant correlation between starch, lipid, fibre, and protein contents and fumonisin production in maize.

Grain age may also influence fumonisin production in maize. Warfield and Gilchrist (1999) found higher levels of fumonisins in maize grains at the dent stage and significantly lower levels in grains at the immature stage, suggesting that production of the toxin may begin early in cob development and increase as the grains reach physiological maturity. Likewise, Chulze *et al.* (1996) reported that contamination of maize by fumonisins was greater after physiological maturity.

Postharvest operations

Pre- and postharvest handling and processing (sorting, washing, dehulling, milling, fermentation, cooking) favourably or unfavourably affect fungal infection and fumonisin production in maize. Mechanical damage during and after harvest may offer entry to the fungal spores either in maize cobs or grains. Dharmaputra *et al.* (1994) found that motorised shellers can cause mechanical damage on grains providing entry points to fungal spores. Substantial amounts of fumonisins (up to 74 %) can be removed by simply washing maize grains, immersing them in water and by removing the upper floating fraction, as contaminated grains generally have a low density (Shetty and Bhat 1999). These authors also found that removal of the toxin is more significant (about 86 %) if salt is added to the water during that process. Likewise, sorting and removal of small, broken and visibly contaminated grains during processing can significantly reduce toxin levels (Charmley and Prelusky 1994, Doyle 1998). Steeping maize grains in water has also been found effective in reducing fumonisin content (Canela *et al.* 1996). In contrast, fermentation of maize does not seem to reduce fumonisin levels (Shephard *et al.* 1996, Desjardins *et al.* 2000).

As for milling, Bennett *et al.* (1996) found that by wet-milling fumonisin-contaminated maize, the toxin distribution in the different fractions is as follows: very little or no fumonisin in the starch fraction, but detectable fumonisins in fibre, germ and steep water fractions. This indicates that maize-based foods derived from the starch fraction are likely to contain less fumonisins than that derived from the other fractions. After dry-milling contaminated maize, fumonisins levels were found lower in grits and higher in germ, bran and fines (Bolger *et al.* 2001). It has also been shown that fumonisin levels decrease as the level of refinement of maize meal during milling increases (Shephard *et al.* 1996).

Regarding cooking, it has been observed that fumonisins are fairly heat-stable and that ordinary cooking does not substantially reduce the toxin (Alberts *et al.* 1990, Scott 1993). Significant removal of fumonisins is more likely to occur only when temperature during cooking is more than 150 °C (Bolger *et al.* 2001).

Although some processing methods potentially can be selected as favourable ways to reduce fumonisin levels in maize-based products, it is important to keep in mind that their success would depend on many factors including the moisture content of the product, the degree of contamination, distribution of the toxin in the product, and the presence of additives (Charmley and Prelusky 1994, Bolger *et al.* 2001).

Influence of biotic factors

Storage insects

Insects also play an important role in infection of maize by *Fusarium* spp. They can act as wounding agents or as vectors spreading the fungus from origin of inoculum to plants (Dowd 1998). Wounding by insects may provide an opportunity for the fungus to circumvent the natural protection of the integument and establish infection sites in the vulnerable interior (Bilgrami and Choudhary 1998). Borers and insects of the family Nitidulidae are most often cited as favouring maize infection by *Fusarium* spp. They include among others the lepidopteran stem and cob borers (*Ostrinia nubilalis*, *Sesamia calamistis*, *Eldana saccharina*, *Mussidia nigrivenella* and *Busseola fusca*), thrips and sap beetles (family Nitidulidae) (Flett and Van Rensburg 1992, Munkvold and Desjardins 1997, Cardwell *et al.* 2000, Ako *et al.* 2003). Sobek and Munkvold (1995) found in the USA that damage caused by the European maize borer *Ostrinia nubilalis* increased infection by *F. verticillioides* by three- to ninefold over those with simple mechanical damage. Moreover, larvae of *O. nubilalis* can also act as vectors of *F. verticillioides* by carrying inoculum from plant surfaces into maize cobs (Munkvold *et al.* 1997). In South Africa, Flett and Van Rensburg (1992) showed that

Busseola fusca infestation significantly increased the incidence of *F. verticillioides*- infected maize cobs, irrespective of whether the cobs are artificially inoculated with the fungus or not. In a recent study in Benin, it has been observed that cob/stem infection by *F. verticillioides* positively correlated with infestation of *Eldana saccharina*, *Cryptophlebia leucotreta*, *Mussidia nigrivenella* and *Sesamia calamistis* (Schulthess *et al.* 2002). Regarding the beetles, it has been shown that not only nitidulid beetles are strongly implicated in *F. verticillioides* infection, but also cucurlionid and silvanid species positively correlated with fungal infection (Cardwell *et al.* 2000).

All these findings pose the problem of cause and effect relationships between fungal infection and insect infestation on maize plants. It is likely that the presence of *F. verticillioides* promotes insect attacks (Schulthess *et al.* 2002) and insect infestation favours fungal infection (Dowd 1998). *F. verticillioides* may be introduced into the stem and cob via insects (Munkvold and Carlton 1997). Likewise, incidence of infection by *F. verticillioides* in maize stems is a source for cob infection by the fungus, not only through movement of the fungus, but also through increased activity of stem borers (Baba-Moussa 1998). On the other hand, *F. verticillioides* produced volatiles that are quite attractive to nitidulid beetles (Bartelt and Wicklow 1999). It has been shown that fecundity, laying of eggs and survival of larvae of *Eldana saccharina* were significantly higher on inoculated maize plants (Ako *et al.* 2003). The authors also found that development time of *Carpophilus dimidiatus* was lower and its fecundity higher on infected grain than on non-infected grain. Schulthess *et al.* (2002) suggested that keeping the plant free of the fungus could be an effective way to reduce insect damage to both stem and grain. On the other hand, any action also to avoid insect infestation is useful for reducing infection of maize by *F. verticillioides* (Riley and Norred 1999).

Fungal interactions

Interactions among fungi in maize also constitute an important factor influencing fungal infection and subsequent mycotoxin production. Harvested maize grains in the tropical zones contain mycelium and spores of several fungal species including mainly *Fusarium*, *Aspergillus* and *Penicillium* that can come into contact, grow and compete for food if environmental conditions are favourable. As far as *Fusarium* species are concerned, many research reports highlighted their interaction with other fungi. Velluti *et al.* (2000) showed that populations of *F. verticillioides* and *F. proliferatum*, the most important fumonisin producers, are markedly reduced by the presence of *F. graminearum*, and that fumonisin B_1

(FB_1) production by them can be significantly inhibited as well in the presence of *F. graminearum*. On the other hand, Marin *et al.* (1998) found that *F. verticillioides* and *F. proliferatum* are generally very competitive and dominant against *Aspergillus flavus* and *Penicillium* spp., especially at a_w more than 0.96. This inhibition can lead to significantly reduced aflatoxin contamination in infected grains (Zummo and Scott 1992).

ATTEMPTS TO CONTROL *F. VERTICILLIOIDES* AND TO DETOXIFY OR REDUCE FUMONISIN LEVELS IN MAIZE

There is strong evidence that due to its endophytic habit, control of *F. verticillioides* in the field is very difficult. Novel control strategies are being investigated and some reported technologies include:

- The use of an endophytic bacterium (e.g. *Bacillus mojavensis*) as a biological control agent on maize seed (Bacon and Hinton 2000).
- The use of an iodine-based product called Plantpro 45™ as a biocompatible control of the fungus. The active ingredient of that product has been used as a disinfectant in human and animal health care products (Yates *et al.* 2000).
- The use of non-producing strains of *F. verticillioides* aiming to minimise fumonisin levels in maize (Plattner *et al.* 2000).
- The use of genetic engineering approach such as engineering plants (*Bt*-maize) and *in planta* detoxification of fumonisins in maize (Munkvold and Desjardins 1997, Munkvold *et al.* 1997, Munkvold *et al.* 1998).

Additional investigations are however needed to render some of those technologies more applicable.

Decontamination of fumonisins in maize and maize-based products by means of chemical reactions is the object of many research studies. Fumonisins are quite stable molecules and their destruction is likely to be also quite difficult. Ammonisation, initially used for detoxify products from aflatoxins has been investigated for fumonisin reduction but does not always give satisfactory results. Scott (1993) reported that treatment of *F. verticillioides* culture material with 2 % of ammonium hydroxide at 50 °C decreased by 89 % fumonisin concentration, but only 32 % of toxin reduction was later measured after four days air-drying. In contrast, nixtamalisation, the alkaline cooking of maize for tortilla production in Central America, significantly reduces fumonisin concentration in maize (Dombrink-Kurtzman *et al.* 2000). However, Voss *et al.* (1996) found that nixtamalised *F. verticillioides* culture remained toxic. This indicates that reduction in detectable fumonisins does not necessarily result in reduced toxicity.

It is therefore clear that detoxification of mycotoxins in foods is not so easy. Sinha (1998) suggested that it must be economical, simple, easy to be applied by unskilled person, not too time-consuming, capable of removing all traces of the active toxin without hazardous chemical residues in the decontaminated food, and does not impair the nutritional quality of the food.

Considering the above-mentioned review of existing findings on fumonisin contamination, several points arise and need to be emphasised:

1. Contamination of food commodities by fumonisins has become a serious food safety problem throughout the world. People are more and more aware that the fumonisins, in addition to aflatoxins, constitute a real threat to human and animal health. However, in contrast to aflatoxins, fumonisins are less documented. Indeed, they are recently discovered mycotoxins and more research studies are urgently needed in order to understand more about them.

2. Some information is available on factors contributing to fumonisin production and on those able to reduce fumonisin levels in foods. However, research results on some factors remain uncertain, or are not applicable to a developing country situation. The need for more information about environmental and agroecological influences, fumonisin toxicology in respect to human and animal health, prevention methods against fungal infection and fumonisin contamination, methods to use for reducing the toxin in foods and other aspects of fumonisins, is great enough to challenge scientists to undertake further research on these topics.

3. To date in Africa, apart from South Africa, very little information is available on the natural occurrence of both *Fusarium* and fumonisins, although this part of the world is most often suspected of having potentially higher levels of fumonisins due to its position in tropical and subtropical zones. Work undertaken so far in a few African countries basically consisted of sporadic surveys of farmers' stores and retail markets, mostly basing data measurements on a relatively small number of samples (Shephard *et al.* 1996). It is a matter of great concern that in Africa, millions of people are consuming contaminated maize and maize-based foods daily without being aware of the danger. Efforts are, however, to be saluted in investigating fumonisins contamination in maize and maize-based foods in some African

countries other than South Africa such as Benin, Cameroon, Ghana, Kenya, Zambia and Zimbabwe (Shephard *et al.* 1996, Doko *et al.* 1995, Hell *et al.* 1995, Kedera *et al.* 1999, Kpodo *et al.* 2000, Gamanya and Sibanda 2001, Ngoko *et al.* 2001). Consequently, there is great need for more investigations on the continent, mainly in the maize production and consumption zones. The present study enters this framework.

OBJECTIVES

By targeting Benin, a West-African country, this study aims indeed to contribute to efforts of African countries to guarantee foods of good quality by specifically focusing investigations on:

- Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in the different agroecological zones of Benin,
- Some factors including insect infestation, indigenous storage systems, mechanical shelling and dehulling methods influencing the occurrence of fumonisins in maize in Benin,
- Impacts of some traditional processing techniques in use in Benin on maize contamination by fumonisins.

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