Mycotoxin levels in subsistence farming systems in South Africa

by

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that it has not, in its entirety or part, been previously submitted for a degree to any other university.

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ABSTRACT

Fusarium spp. and *Aspergillus* spp. are toxin-producing fungi associated with maize and groundnut. *Fusarium verticillioides* produces fumonisins in maize, and *Aspergillus flavus* produces aflatoxins in maize and groundnut kernels. Both toxins are responsible for carcinogenesis in humans and animals. Contamination of maize and groundnut with mycotoxins is often most severe in rural areas where subsistence farmers are unaware of their existence and follow agricultural practices that might contribute to their production. A questionnaire was, therefore, compiled to investigate agricultural decisions in rural areas that may influence mycotoxin contamination of crops. During 2006 and 2007, maize and groundnut samples were collected in the Eastern Cape, KwaZulu-Natal (KZN), Limpopo, and Mpumalanga provinces. Mycotoxin levels were quantified using the ELISA technique, and the incidence of *Fusarium* spp. in maize grain was determined by plating maize kernels out on *Fusarium* selective medium. Fumonisin-producing *Fusarium* spp. were also quantified using real-time PCR (TaqMan). The incidence of *A. flavus* and *A. parasiticus* in groundnut was determined by plating out kernels on potato dextrose agar. Fumonisin contamination levels in maize samples ranged from 0-21.8 parts per million (ppm) and aflatoxin levels ranged from 0-49 parts per billion (ppb), depending on the region where samples were collected. Aflatoxin levels in groundnut ranged from 0-160.1 ppb. *Fusarium verticillioides* was the most common *Fusarium* sp. in maize followed by *F. subglutinans* and *F. proliferatum*, respectively. Regression analyses showed a positive correlation between fumonisin-producing *Fusarium* species when determined by real-time PCR and fumonisin concentration (r^2=0.866). Regression analyses further showed a highly significant positive correlation between *A. flavus* and aflatoxin contamination (r^2=0.10235). Samples from northern KZN contained levels of mycotoxins that were far in excess of the maximum levels set by the Food and Drug Administration in the USA. In South Africa there are currently no regulations with regard to the maximum allowable levels of fumonisin in human food. The high incidence of mycotoxin contamination of human food in subsistence farming systems indicates the need for awareness programmes and further research.

OPSOMMING

Fusarium spp. en *Aspergillus* spp. is toksien-produserende fungi wat met mielies en grondbone geassosieer word. *Fusarium verticillioides* produseer fumonisiene in mielies, terwyl, *A. flavus* aflatoksiene in mielies en grondbone produseer. Beide toksiene is karsinogenies vir mens en dier. Die vlakke van toksien-kontaminasie is meestal die ergste in landelike gebiede waar bestaansboere onbewus is daarvan. Landboupraktyke wat deur die boere toegepas word vererger dikwels die probleem. ‘n Vraelys is saamgestel om vas te stel watter landboupraktyke in landelike gebiede toegepas word, en hoe dit toksien-kontaminasie in mielies en grondbone beinvloed. In die 2006 en 2007 seisoene is mielie- en grondboonmonsters in Kwa-Zulu-Natal (KZN), die Oos Kaap, Limpopo en die Mpumalanga provinsie versamel. Toksien-vlakke is gekwantifiseer deur gebruik te maak van die ELISA tegniek. Die insidensie van *Fusarium* spp. in mielies was bepaal deur pitte op *Fusarium*-selektiewe agar uit te plaat. Fumonisien-produserende *Fusarium* spp. was ook gekwantifiseer deur van kwantitatiewe PCR (TaqMan) gebruik te maak. Die voorkoms van *A. flavus* en *A. parasiticus* is bepaal deurdat mielie- en grondboonpitte op aartappel dekstrose agar uit te plaat. Fumonisien-vlakke in die mieliemonsters het gewissel van 0-21.8 dele per miljoen (dpm), terwyl aflatoksienvlakke gewissel het van 0-49 dele per biljoen (dpb), afhangende van die omgewing waar monsters versamel is. Aflatoksien vlakke in die grondboonmonsters het gewissel van 0-160.1 dpb. *Fusarium verticillioides* is die meeste vanuit mielies geisoleer, gevolg deur *F. subglutinans* en *F. proliferatum*. Regressie analises het ‘n positiewe korrelasie tussen fumonisien konsentrasie en fumonisien-produserende spp. aangedui waar daar gebruik gemaak is van die kwantitatiewe PCR (r^2= 0.866). Regressie analises het ‘n hoogs betekenisvolle positiewe korrelasie getoon tussen *A. flavus* en aflatoksien kontaminasie (r^2=0.10). Monsters van noordelike KZN het toksienvlakke bevat ver bokant die maksimum toelaatbare vlakke is soos bepaal deur die Food en Drug Administrasie in die Verenigde State van Amerika. Daar is tans geen regulasies in Suid Afrika wat die maksimum toelaatbare vlakke van fumonisiene in voedsel vir menslike gebruik bepaal nie. Die hoë voorkoms van mikotoksien-kontaminasie in bestaansboer-sisteme, dui die belangrikheid van verdere navorsing en bewusmakings-progamme aan.

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PREFACE

Fusarium verticillioides (syn = *F. moniliforme*) (Sacc.) Nirenberg and *Fusarium proliferatum* (Matsushima) Nirenberg according to Nelson *et al*. (1983) and Leslie and Summerell (2006), are fungal pathogens that cause Fusarium ear rot and produce fumonisins in maize, while *Aspergillus flavus* Link ex Fr, and *Aspergillus parasiticus* Speare produce aflatoxins in maize, groundnut and cottonseed.

Contamination of crops by mycotoxins is a problem in subsistence farming systems in South Africa, as it results in mycotoxicoses in humans and animals. Subsistence farmers often follow agricultural practices that enhance mycotoxin production, and then consume contaminated food, which is sometimes the only source of food available. Control of mycotoxin production, while desirable, is not always possible to achieve since their production is as a result of the interactions between weather, plant genotype and pathogen. Good agricultural practices and hazard analysis critical control points, however, can be applied to reduce mycotoxin levels in crops.

Chapter 1 provides a general overview of mycotoxin production in subsistence farming systems. This chapter also places an emphasis on maize and groundnut production in South Africa. Aflatoxin and fumonisin-producing organisms, factors influencing the occurrence of aflatoxins and fumonisins in maize and groundnut, implication of aflatoxin and fumonisin to human health, and the control of aflatoxins and fumonisin production, are discussed.

In **Chapter 2**, agricultural practices in subsistence farming systems in the Eastern Cape, KwaZulu-Natal, Limpopo, and Mpumalanga provinces of South Africa were investigated. The knowledge gained will contribute to the understanding of the farming practices in rural areas and their impact on mycotoxin production and how mycotoxin contamination can be reduced.

The level of fumonisins in the Eastern Cape, KwaZulu-Natal, Limpopo, and Mpumalanga provinces were determined in **Chapter 3**. The distribution of *F. verticillioides*, *F. proliferatum* and *Fusarium subglutinans* (Wollenw. & Reinking) P. E. Nelson, Toussoun & Marasas were also determined. The fumonisin-producing

Fusarium spp. were quantified by both plate counts and quantitative PCR. The incidence and distribution of *Fusarium* spp., fumonisin and aflatoxin in maize was further correlated with temperature and rainfall data.

Chapter 4 depicts the level of aflatoxins in groundnut and the distribution of aflatoxin-producing fungi in groundnut samples collected in KwaZulu-Natal, Limpopo, and Mpumalanga provinces of South Africa. These provinces represent the major regions where subsistence farmers plant groundnuts. Knowledge related to the occurrence and levels of aflatoxin in groundnuts is important when considering strategies for their reduction and the reduction of human and animal diseases associated with consumption of aflatoxin-contaminated food.

Chapter 1

Mycotoxin production in subsistence farming systems in South Africa

INTRODUCTION

Mycotoxins are secondary metabolites of fungal origin that are harmful to both animals and humans (Nelson *et al*., 1993). Important mycotoxins that significantly impact on human and animal productivity include aflatoxins, fumonisins, T-2 toxin, deoxynivalenol or nivalenol and zearalenone, and ochratoxin A (Table 1). It was only in the 1970's, however, that technology allowed researchers to isolate these toxins and study their effects on crops, livestock and humans. Approximately 25% of all agricultural crops produced in the world are contaminated with mycotoxins (Fink-Gremmels, 1999). Exposure to human food or animal feed contaminated with mycotoxins through ingestion, inhalation or absorption through the skin are known to result in mycotoxicoses, i.e. reduced performance, depressed immunity, and sickness or death in humans and animals (Sargeant *et al*., 1961; Chao *et al*., 1991; Marasas, 1995; Lanyasunya *et al*., 2005; Nyamogo and Okioma, 2005).

Mycotoxins have been a problem since the Middle Ages. Numerous human ergotism outbreaks occurred in Europe as a result of consumption of ergot-contaminated rye (*Secale cereale* L.) caused by *Claviceps* spp. (Nicholson, 2007). In 1960, 100 000 turkeys (*Meleagris gallopava* L.) died in England due to aflatoxins, which were subsequently linked to contaminated groundnut (*Arachis hypogaea* L.) meal imported from Brazil (Sargeant *et al*., 1961). During 1990, 13 children died from aflatoxicoses in Malaysia (Chao *et al*., 1991) and over 100 human deaths were attributed to aflatoxins in Kenya during the 2004/2005 cropping season (Lanyasunya *et al*., 2005; Nyamogo and Okioma, 2005). A correlation between fumonisin B_1 (FB_1) contamination of maize and the occurrence of human oesophageal cancer was found in the Transkei region of the Eastern Cape Province, South Africa (Rheeder *et al*., 1992). Rheeder *et al*. (1992) found extremely high levels of fumonisin B_1 of more than 117 µg/g in maize from the Transkei. In the Lixian County, Henan Province, China, Chu and Li (1994) found levels of FB_1 of up to 155 µg/g, while Yoshizawa *et al*. (1994) reported levels of up to 11 300 µg/g in the same region. FB_1 and its analogues have also been found in the United States (Missmer *et al*., 2006), Italy (Ritieni *et al*., 1997) and Iran (Yazdanpanah *et al*., 2006).

The "Green Revolution" that saved hundreds of millions of lives, mainly in Asia and South America, between the middle 1960s and the 1980s largely bypassed subsistence farmers in Sub-Saharan Africa (SSA) (Southgate and Graham, 2006). As a result, subsistence farmers in Africa did not benefit from technologies such as fertilizers, pesticides or hybrid seeds. Diets in SSA are deficient in energy intake levels compared to those in every other part of the world resulting in the occurrence of widespread malnutrition and poor health (Southgate and Graham, 2006) emanating from poor nutrition and possibly consumption of mycotoxin-contaminated food which is sometimes the only food available.

The sustainability of subsistence farming in South Africa is hampered by land tenure policies because almost all subsistence land is held by the state under communal tenure. A substantial population of subsistence farmers, therefore, have access to only small pieces of arable land (Adey, 2007). These policies negatively affect the farmer's ability to seek loans from financial institutions for the improvement of their farming systems, purchasing of the necessary agricultural inputs, storage, transport and marketing of the produce since the farmers can not used their lands as collateral.

The Provincial Departments of Agriculture provide extension services to subsistence farmers in South Africa. The focus of these services is geared towards stimulating the adoption of modern farming practices such as provision of seed, fertilizer and pesticides where available, crop protection and planning. However, food safety issues such as the mycotoxin awareness and mycotoxin control are not addressed in these farming systems due to a paucity of available information on mycotoxins. The Agricultural Research Council and the Medical Research Council and Universities are currently involved in mycotoxin research in subsistence farming systems.

This review will summarise information on the two most important mycotoxins produced in grain crops in South Africa namely, aflatoxins and fumonisins. Aflatoxins are most commonly associated with groundnuts (Deiner *et al*., 1987) and fumonisins are the most important mycotoxins in maize (*Zea mays* L.) (Thiel *et al*., 1992; Shephard *et al*., 1996).

MAIZE AND GROUNDNUT PRODUCTION IN SOUTH AFRICA

Maize is an annual summer crop and is the most widely cultivated crop in South Africa. As a staple food, its average production amounts to approximately 9 million tons per year, of which 7.4 million tons is consumed locally as food and feed (www.southafrica.co.za). Commercial farmers produce the bulk of the maize crop (over 8 million tons) under irrigation and dry land conditions (www.fao.org). Subsistence farmers produced 317 056 tons during the 2006 season under dry land conditions (www.fao.org). Average human maize consumption in typical subsistence farming systems varies according to age group. Shephard *et al*. (2007) found that the actual maize consumption per person per day in the 1-9 years and 10-17 years age groups was 246 and 368 g, respectively, in the Bizana and Centane districts of the Eastern Cape province of South Africa. They also found that adult (18-65 years) consumption of maize was 379-456 g per person per day in the Bizana and Centane districts, respectively (Shephard *et al*., 2007).

South Africa produces between 60 000 and 70 000 tons of groundnuts annually of which only 16.5% is grown under irrigation. Groundnut kernels contain about 14% linoleic acid (Inchbald, 2000), an essential fatty acid in the human diet. Groundnuts are an important proteins source, as they contain between 17 and 25.2% protein (Prathiba and Reddy, 1994) making the crop an important food source for rural people.

AFLATOXINS

Aflatoxins are a group of structurally related polyketide mycotoxins that contaminate field crops such as groundnut, maize, cottonseed (*Gossypium* spp. L.), and rice (*Oryza sativa* L.) (Mirocha and Christensen, 1974). In addition, milk and milk products can be contaminated as the result of cows being fed on aflatoxin-contaminated feed (Wiseman and Marth, 1983; Wild *et al*., 1987). The four primary aflatoxins are aflatoxin B_1, B_2, G_1 and G_2.

Aflatoxin-producing organisms:

Aflatoxins are produced by four *Aspergillus* spp. These include *Aspergillus flavus* Link ex Fr, *Aspergillus nomius* Kurtzman, Horn & Hesseltine, *Aspergillus parasiticus* Speare, (Gourama and Bullerman, 1995) and *Aspergillus tamarii* (Goto *et al*., 1996). The agronomically and economically most important are the closely related *A. flavus* and *A. parasiticus*, which are soil-borne fungi that grow on living and decaying plant matter. *Aspergillus flavus* can be distinguished from *A. parasiticus* by its smooth spores and yellow-green colonies on potato dextrose agar (PDA) medium (Klich and Pitt, 1988). *Aspergillus parasiticus* produces dark yellow-green conidia with nearly spherical vesicles that produce roughened conidia. It can be readily distinguished from *A. flavus* by its rough-walled conidia (Klich and Pitt, 1988). Of the two fungus species, *A. parasiticus* is more common in groundnuts probably due to its adaptation to soil environments, while *A. flavus* is more common in maize and cottonseed due to its adaptation to above-ground environments (Deiner *et al*., 1987).

Life cycle of *Aspergillus* spp.:

Aspergillus flavus is a saprophytic fungus that survives on dead plant tissue and sometimes behaves as a weak and opportunistic pathogen (Yu *et al*., 2005). The sources of inoculum for *A. flavus* and *A. parasiticus* are sporogenic sclerotia (Wicklow and Donahue, 1984; Deiner *et al*., 1987; Calvo *et al*, 1999), conidia and mycelia that over-winter in plant debris (Fig. 1) (Scheidegger and Payne, 2003). In fields repeatedly cropped to groundnut or rotated between groundnut, maize and cotton, conidia from sporogenic sclerotia are the primary source of *A. flavus* inoculum (Wicklow and Donahue, 1984; Deiner *et al*., 1987). Conidia adjacent to the developing groundnut fruit germinate in the soil following the release of carbon and nitrogen substrates by injured groundnut pegs and result in colonisation of the fruit (Griffin, 1972; Cole *et al*., 1984; Sanders *et al*., 1985). Hot humid conditions favour the release of spores on plant residues, and these spores are dispersed by wind through the field (Scheidegger and Payne, 2003). Conidia that adhere to insect bodies are physically moved to plant parts and flowers in groundnut or silks in maize (Deiner *et al*., 1987). Smaller, generally immature kernels are more easily infected in a shorter period of time than kernels in more mature pods (Cole *et al*., 1982). Infection of groundnut kernels at other maturity stages are relative to the survival of the fungus and not necessarily to a new infection at a later stage of maturity (Sanders *et al*., 1981,

Deiner *et al*., 1987). *Aspergillus flavus* does not always establish a successful systemic infection in groundnut plants (Griffin and Garren, 1976).

Factors influencing the occurrence of aflatoxin in maize and groundnut:

The relative amount and proportions of various aflatoxins in food crops and products is a consequence of interactions among the *Aspergillus* spp. present, the host crop and the environment. Stressful environments in maize and groundnut crops, in particular, favour aflatoxin production. Drought and high temperatures, insect and physical plant damage and soil type and tillage practices, provide these stresses (Deiner *et al*., 1987).

Drought stress and temperature:

Drought stress and elevated temperatures predispose groundnut and maize kernels to *A. flavus* infection and concomitant aflatoxin production (Jones *et al*., 1980; Cole *et al*., 1984; Lisker and Lillehoj, 1991). Under these conditions, *A. flavus* is more competitive than antagonistic bacteria and fungi such as *Aspergillus niger* Tiegh in the soil (Griffin and Garren, 1973; Cole *et al*., 1982; Hill *et al*., 1983), and influence the germination and growth of *A. flavus* spores in groundnut flowers and maize silks (Widstrom, 1979; Sanders *et al*., 1984). In warmer soils, the groundnut plant blooms slightly earlier than usual in the morning. This exposes the flower to more severe infections due to early morning dew and a longer period of high humidity required for spore germination and infection. Higher air, soil, plant and pod temperatures and drought reduce the metabolic activity of groundnut plants that results in a breakdown of inherent resistance mechanisms such as the induction of β-1,3-glucanase and phytoalexins (Cole *et al*., 1985; Manda *et al*., 2004). In maize, elevated temperatures increase the parasitic ability of *A. flavus* and the associated infection processes in developing maize kernels (Jones *et al*., 1980). Aflatoxin levels are high in plants grown at temperatures between 26 and 30^{o}C. Water stress increases the levels of aflatoxin by ten times in maize kernels, and maize plants exposed to drought stress are more susceptible to *A. flavus* infection than unstressed plants. The reduced leaf area in drought-stressed plants increases infection levels in maize by increasing the silks' accessibility to fungal conidia (Jones *et al*., 1981).

In cooler soils, a relatively low level of pod infection and concomitant aflatoxin production in groundnuts will occur (Sanders *et al*., 1984). Cole *et al*. (1984) found

that at 24.8°C or lower, kernels from undamaged pods grown under drought conditions 5 cm below the soil surface were not contaminated with aflatoxin. During the later stages of groundnut growth, the minimum mean temperature in the portion of soil influenced by the developing groundnut fruit (geocarposphere) required for aflatoxin formation is approximately 25.7°C, the optimum is between 28 and 30.5°C, and the maximum is 31.3°C (Cole *et al*., 1984; 1985; Sanders *et al*., 1985). Preharvest aflatoxin contamination in maize is higher on sandy coastal plain soils than on finer-textured soils because the sandy soils have less water-holding capacity and drought interferes with nutrient uptake by maize plants (Jones *et al*., 1981).

Aspergillus flavus grows at a minimum water activity (a_w) of 0.78 while *A. niger* grows at a minimum a_w of 0.88. At a_w above 0.88, *A. niger* grows more vigorously than *A. flavus* and inhibits aflatoxin formation (Wicklow *et al*., 1980). It also degrades any aflatoxin that is produced by *A. flavus* (Tsubouchi *et al*., 1980). Irrigation has been shown to be very effective in reducing *Aspergillus* spp. infection and aflatoxin development in maize and groundnut crops (Jones *et al*., 1981; Cole *et al*., 1982) because it reduces soil temperatures. Manda *et al*. (2004) found higher aflatoxin levels under natural conditions when compared to continuous irrigated conditions.

Insect and physical damage:

Drought and increased temperatures alone does not result in aflatoxin production by *A. flavus* in sound mature maize and groundnut kernels (Hill *et al*., 1983; Wilson and Stansell, 1983; Cole *et al*., 1985; Deiner *et al*., 1987). Aflatoxin contamination increases with increased kernel damage (Hill *et al*., 1983; Cole *et al*., 1984). Insect damage to kernels increases the risk of kernel invasion by wound pathogens such as *A. flavus* in maize and groundnut (Stephenson and Russell, 1974; Lisker and Lillehoj, 1991). During storage, insect infestation increases production of aflatoxin in maize and groundnuts by increasing grain moisture levels, through respiration, to levels that can support fungal growth (Williams *et al*., 2004).

Soil type and tillage practices:

Minimum and no tillage increase the risk of *A. flavus* infection, as sclerotia from the previous crop remain on or near the soil surface where they produce conidia and result in high populations of *A. flavus* (Wicklow and Donahue, 1984; Zablotowicz *et al*.,

2007). Kernel infection and aflatoxin B_1 contamination were lower in clay-rich soils such as vertisols than in moderately leached forest soils such as alfisols (Mehan, *et al*., 1991). This may be due to the low moisture availability and favourable soil aeration in alfisols (Mehan, *et al*., 1991). Variation in soil mycoflora may result in suppression of *A. flavus*. In vertisols, *A. niger* and *Macrophomina phaseolina* (Tassi) Goid dominate and may compete with or be antagonistic to *A. flavus* (Mehan *et al*., 1991).

Other factors:

In vitro studies have shown that the substrate available to *A. flavus* and *A. parasiticus* influences aflatoxin production in maize and groundnut (Davis and Deiner, 1968; Fink-Gremmels, 1999; Manda *et al*., 2004). Glucose, ribose, glycerol and maltose support fungal growth and high levels of aflatoxin production, while carbon sources such as peptone, lactose and xylose support fungal growth but do not produce high levels of aflatoxin (Davis and Deiner, 1968). Other simple carbohydrates that are readily oxidised through both the hexose monophosphate shunt and glycolytic pathways support fungal growth and aflatoxin production, while the Krebs cycle intermediates are poor substrates (Davis and Deiner, 1968). Woloshuk *et al*. (1997) indicated that low levels of carbon activate a regulatory mechanism for aflatoxin biosynthesis. Increasing the carbon concentration intensifies the induction process and these carbon sources are metabolised by alcoholic fermentation. Woloshuk *et al*. (1997) also hypothesised that, after colonising the embryo, *A. flavus* produces an extra-cellular amylase that rapidly increases the supply of fermentable sugars, and that these sugars are responsible for the induction of aflatoxin biosynthesis.

Linoleic acid induces increased asexual spore development and sclerotium production in *A. flavus* and *A. parasiticus* (Calvo *et al*., 1999). The sporogenic activity of linoleic acid is subject to light regulation (Calvo *et al*., 1999), while sclerotia are produced in the dark (Calvo *et al*., 1999). The production of sclerotia in the dark (underground) is probably the source of inoculum for above-ground or storage infection and concomitant aflatoxin production in groundnut kernels (Gourama and Bullerman, 1995).

Implications of aflatoxins for human and animal health:

Aflatoxins suppress the immune system of susceptible populations in humans such as young children and HIV/AIDS patients (Williams *et al*., 2004). In a study in Gambia it was found that secretory immunoglobulin A in saliva may be reduced by dietary levels of aflatoxin in children resulting in reduced levels of antibodies (Williams *et al*., 2004). In Ghana, changes in the percentages of immune cell subsets following aflatoxin B_1 exposure reduced cell immunity, decreased human resistance to infections and reduced immune responses to vaccinations (Williams *et al*., 2004). Aflatoxin is a carcinogen and acute aflatoxicosis results in human deaths (Lanyasunya *et al*., 2005; Nyamogo and Okioma, 2005). Gong *et al*. (2002) demonstrated that in Benin and Togo, children exposed to the highest level of aflatoxin had a 2 cm lower height gain than those exposed to the lowest level. Aflatoxins also cause oxidative stress, liver necrosis, haemorrhage and death in broiler chickens, pigs and cattle (Eraslan *et al*., 2005; Osweiler, 2005).

Management and control of aflatoxins:

Aflatoxin occurrence and severity in field crops is largely a matter of uncontrollable natural events. The complete elimination of aflatoxin from human food, while desirable, is almost impossible to achieve, as aflatoxin is distributed in a wide range of agricultural products where it is an unavoidable natural contaminant (Gourama and Bullerman, 1995). However certain practices such as the "farm to fork" policy of the European Union (EU) can be put in place to reduce aflatoxin levels. Hazard analysis critical control points (HACCP) are employed to reduce unacceptable aflatoxin levels from insect damage to the developing crop in the field or when the mature crop is exposed to moisture prior to harvest or during storage, handling, and transportation. Small-scale industries, subsistence production and food insecurity makes the economics and enforcement of aflatoxin regulations impractical (Vincelli and Parker, 2002).

Cultural practices used in controlling aflatoxins:

Early harvesting and the rapid drying of maize and groundnut kernels to moisture levels below 15% effectively stop aflatoxin accumulation (Payne *et al*., 1986; Gourama and Bullerman, 1995). Storage conditions at low temperatures and reduced humidity are important components for reduced *A. flavus* growth and aflatoxin production. Storage facilities must be regularly monitored to ensure early detection and control of insect infestations. Old grain residue must not be mixed with new grain and storage areas must be sanitised before new grain is stored (http://ec.europa.eu).

Resistant plants:

Planting *A. flavus*-resistant genotypes is the best approach to aflatoxin management and control. Mehan *et al*. (1991) found that maize genotypes with resistance traits such as husk tightness, kernel pericarp barrier, or wounded kernel resistance showed lower levels of seed infection by *A. flavus* and other fungi than did susceptible genotypes, irrespective of soil types. Maize hybrids that possess resistance through the production of kernel proteins that inhibit either fungal growth or aflatoxin production are available, but their level of resistance is inadequate to prevent unacceptable aflatoxin concentration (Munkvold, 2003). Transgenic maize hybrids that express the *Bacillus thuringiensis* (*Bt*) gene CryIAb that is toxic to certain insect pests are effective in reducing aflatoxin. Reduction of aflatoxin contamination occurs where transgenic hybrids are planted in areas with high infestations of the Southwestern corn borer (*Diatraea grandiosella* Dyar) (Munkvold *et al*., 1999), *Busseola fusca* Fuller (Lepidoptera: Noctuidae), and *Chilo partellus* (Swinhoe) (Van Rensburg, personal communication).

Resistance to aflatoxin contamination in groundnut is probably due to resistance to pod infection, resistance to seed invasion and colonisation by the fungus, and resistance to aflatoxin production (Mehan, 1989). However, Cantonwine *et al*. (2003) found that seed colonisation by *A. flavus* does not appear to be a significant plant trait that affects the field resistance of groundnut to aflatoxin contamination.

Drought-tolerant groundnut genotypes have reduced aflatoxin levels and higher yields than drought-susceptible genotypes when subjected to late season planting and drought stress (Holbrook, *et al*., 2004). Under drought conditions, the receding

groundnut foliar canopy allows solar radiation to strike the bare soil surface, raising the temperature of the geocarposphere, thus promoting the production of aflatoxin (Sanders *et al*, 1981; Cole e*t al*., 1982; 1985).

Biological control:

Biological control of toxigenic *A. flavus* strains can be achieved by the application of atoxigenic *A. flavus* strains to maize, groundnut and cotton fields (Brown *et al*., 1999; Cotty, 2005). Application of 11 kg/ha of an atoxigenic *A. flavus* strain with a food source such as wheat (*Triticum aestivum* L.) or sorghum (*Sorghum bicolour* L.) once a year resulted in the displacement of over 80% of aflatoxin-producing *Aspergillus* spp. in Arizona and Texas in the United States (Phillips *et al*., 2005).

Physical control:

Sanitation practices such as mechanical or hand sorting can reduce aflatoxin levels by removing low-density mould-infected kernels (Fandohan *et al*., 2005). Dehulling of kernels by removing the outer layers of kernels reduces the aflatoxin content by up to 92% in maize (Siwela *et al*., 2005) because the outer layers favour the accumulation of aflatoxin. Prado *et al*. (2003) found that gamma irradiation at doses of 15, 20, 25, and 30 kiloGray (kGy) resulted in a 55-74% aflatoxin B_1 reduction in groundnut kernels. Ogunsanwo *et al*. (2004) found positive correlations between loss of aflatoxin and dry roasting conditions. Roasting at 140^{o}C for 40 minutes reduced aflatoxin B_1 and G_1 by 58.8% and 64.5%, respectively, while roasting at 150^{o}C for 30 minutes resulted in 70% and 79.8% reduction in aflatoxin B_1 and G_1, respectively. Cooking and steaming for 1 hour under pressure reduces aflatoxin by up to 60% (Fandohan *et al*., 2005).

Chemical control:

Cooking and steeping maize in an alkaline solution, such as calcium hydroxide, significantly reduces levels of aflatoxin in a process called nixtamalisation (Mendez-Albores *et al*., 2004). Ring opening of the aflatoxin chemical structure occurs at 100^{o}C followed by decarboxylation leading to the loss of the methoxy group from the aromatic ring of the aflatoxin molecule. Aflatoxin G_1 and G_2 are more susceptible to chemical hydrolysis than aflatoxin B_1 and B_2 because of the presence of two ether linkages in the G group compared to the B group which possess a single ether linkage

(Ogunsanwo *et al*., 2004). Ammonia at 0.5-7% coupled with long exposure time, ambient temperature and pressure has been successfully used to inactivate aflatoxin in contaminated commodities such as groundnut meal, cottonseed and maize. This process has been approved by safety and regulatory agencies such as Food and Agricultural Organisation (FAO), Food and Drug Administration (FDA), and United States Department of Agriculture (USDA) (Moustafa *et al*., 2001).

Aflatoxin bioavailability was reduced in the gastrointestinal tract of animals by chemisorbents such as activated carbon, bentonite, clays, and aluminosilicates (Machen *et al*., 1988). A NovaSil clay (NS) can act as a selective enterosorbent of aflatoxins when mixed with animal feed at inclusion rates as low as 0.25% (w/w) (Phillips *et al*., 2005). At this inclusion rate, NS sequesters aflatoxin resulting in reduced aflatoxin in the gastrointestinal tract and neutralisation of its toxic effects. It does, however, not interfere with vitamin and micronutrient uptake. Long-term studies at Texas A&M University and in a Phase I Adverse Events trial at Texas Tech University confirmed the relative safety of NS in rodents (Phillips *et al*., 2005). NS will be a promising novel, inexpensive and easily disseminated remedy to aflatoxin management in Africa once Phase II human studies to determine its efficacy are completed (Phillips *et al*., 2005).

FUMONISINS

Fumonisins are regarded as the most important mycotoxins found in maize and maize-based products (Thiel *et al*., 1992; Shephard *et al*., 1996). Fumonisins were first isolated by Gelderblom *et al*. (1988). Since then, a family of 28 fumonisin analogues have been characterised and grouped into fumonisins A, B, C, and P series. The naturally occurring fumonisin B (FB) analogues in maize, comprising of FB_1, FB_2, FB_3 (Sydenham *et al*., 1990), are toxicologically important (Bezuidenhout *et al*., 1988) and posses cancer-promoting activities (Gelderblom *et al*., 1988). FB_1 typically accounts for 70-80% of the total fumonisins produced in maize and is the most toxic (Gelderblom *et al*., 1988; Leslie *et al*., 1992), while FB_2 accounts for 15-25% and FB_3 usually makes up 3-8% (Branham and Plattner, 1993). The other analogues may occur in naturally contaminated maize at levels below 5% of the total fumonisins present (Musser *et al*., 1996).

Fumonisin-producing organisms:

Fumonisins are produced by several *Fusarium* spp. from sections Liseola, Dlaminia, Elegans and Arthrosporiella (Table 2). The most important producers of FB_1 are *Fusarium verticillioides* (Sacc.) Nirenberg (syn = *F. moniliforme* Sheldon) and *Fusarium proliferatum* (Matsushima) Nirenberg according to Nelson *et al*. (1983) and Leslie and Summerell (2006), because of their association with maize and sorghum (Shetty and Bhat, 1997), wide geographic distribution, their association with animal and human diseases, and their overall production of high levels of fumonisins (Rheeder *et al*., 2002). *Fusarium verticillioides* is economically the most important pathogen of maize. Yield and poor grain quality losses attributed to it and other *Fusarium* spp. amount to hundreds of millions of dollars annually (Charmley *et al*., 1995). In addition to the fumonisin-producing *Fusarium* spp., *Fusarium graminearum* Schwabe is known to produce zearalenone and deoxynivalenol in maize, while *Fusarium subglutinans* (Wollenw. & Reinking) P. E. Nelson, Toussoun & Marasas, produces moniliformin (Thiel *et al*., 1982; Faber *et al*., 1988).

Fumonisin contamination and Fusarium ear rot are distinct but related aspects of infection in maize by *F. verticillioides* (Fandohan *et al*., 2003; Starr *et al*., 2006). Apart from Fusarium ear rot, *F. graminearum* causes Gibberella ear rot to maize (Miller, 2001). The symptoms of Fusarium ear rot and Gibberella ear rot range from non-symptomatic infections to severe rotting of all plant parts (Munkvold and Desjardins, 1997). Early infection of maize by *F. verticillioides* can also cause plant malformation, which produces twisted foliage and tillers that are likely to affect maize yield (Pokkah boeng disease), stem etiolation, and multiple-ear phyllody, thereby promoting infestation of the plant by lepidopterous and coleopteran pests (Cardwell *et al*., 2000). *Fusarium verticillioides* also causes seedling blights and stalk and root rots. All these symptoms reduce yield and seed quality of maize (Wilke *et al*., 2007).

Life cycle of fumonisin-producing *Fusarium* spp:

Fusarium verticillioides is a natural endophyte of the maize plant. The endophyte colonises the internal tissue of the plant at some point during its lifecycle, regardless of whether it is beneficial, detrimental or of neutral significance to the plant (Sikora *et al*., 2003). Some data suggest that the relationship between *F. verticillioides* and maize is mutualistic, with the fungus producing metabolites such as fusaric acid and

gibberellins for the benefit of the plant (Van Wyk *et al*., 1988; Wicklow, 1994; Munkvold and Desjardins, 1997), and protecting maize seedlings against *F. graminearum* (Van Wyk *et al*., 1988). Zummo and Scott (1992) indicated that *F. verticillioides* reduces infection of maize by *A. flavus*, thereby reducing aflatoxin production.

Fusarium verticillioides survives in buried maize stalks as thickened hyphae in soil where there is poor aeration, moist but not wet soil, low soil temperature, and little or no competition with other fungi and bacteria (Nyvall and Kommedahl, 1968; 1970). The soil borne hyphae germinate and infect the germinating seed and roots and move up the plant through systemic growth (Munkvold and Desjardins, 1997). The fungus also produces macroconidia and airborne microconidia from sporulation on the tassels or previous crop residue (Munkvold and Desjardins, 1997). The mode of kernel infection by *F. verticillioides* is both through systemic infections from contaminated seeds (Foley, 1962; Munkvold and Carlton, 1997) and through the silk channel by airborne spores (Koehler, 1942; Munkvold and Carlton, 1997; Galperin *et al*., 2003). While entrance through the silk channel is the most important means of infection, only a small percentage of infected kernels become symptomatic (Munkvold *et al*., 1997). Silk colonisation by *F. verticillioides* starts from the tip of the ear downward (Headrick *et al*., 1990). Infection is enhanced by late-season rainfall as well as the physiological state of the silks after pollination (Headrick *et al*., 1990; Bush *et al*., 2004). Direct invasion of kernels can also occur through weak points such as silk scars and stress cracks in the pericarp and through the pedicel (Odvody *et al*., 1997). The fungus is released back to the soil via infected stalks and in infected seed for the new plantings (Munkvold and Desjardins, 1997).

Factors contributing to the occurrence of fumonisins in maize:

Fusarium verticillioides grows well above 26^{o}C (Miller, 2001) and is a mildly virulent pathogen associated with warm, dry weather conditions of the subtropics and dry land maize farming that induces plant stress (Oren *et al*., 2003). Environmental conditions such as high relative humidity, intermittent rainfall at or just prior to pollination and insect damage favour the development of *Fusarium* kernel rot and significant fumonisin accumulation in maize (Bankole and Adebanjo, 2003; Fandohan *et al*., 2003).

Drought, temperature and pH:

Drought stress prior to and during silking favours *Fusarium* rots and fumonisin production (Warfield and Gilchrist, 1999). In dry environments, *F. verticillioides* competitively excludes other fungi and bacteria by consuming nutrients available in the plant (Reid *et al*., 1999). The natural occurrence of fumonisins is more of a result of drought-stress than temperature stress (Warfield and Gilchrist, 1999; Miller, 2001). Plant stresses such as decreasing soil moisture, and high soil and air temperatures during the later stages of maturity and dry down, exacerbates silk cut. This result in the pre-harvest occurrence of one or more lateral splits in the kernel pericarp of plants exposed to rapidly increasing environmental stress. This loss of kernel integrity exposes the kernel tissues and embryo to either pre-harvest or post-harvest attack by insects and fungi, primarily *F. verticillioides* (Odvody *et al*., 1997).

Maize kernels become conducive for fumonisins production as soon as they are infected with *F. verticillioides* (Bush *et al*., 2004). *Fusarium verticillioides* infection is greatest when silks are green-brown and brown. Fumonisin contamination occurs as kernels approach physiological maturity (Warfield and Gilchrist, 1999; Bush *et al*., 2004) and increase during the season until the harvest date. The optimum production of fumonisin occurs under relatively high oxygen tensions and low pH (approximately 2) due to organic acids produced by starch metabolism of well-rotted maize (Miller, 2001).

Insects:

Drought stress increases insect feeding on maize (Munkvold and Desjardins, 1997). The wounds produced by the insects such as Lepidopteran borers provide the sites for infection of maize ears and stalks by airborne or rain-splashed inoculum of *F. verticillioides* (Munkvold and Desjardins, 1997). Some lepidopterous and coleopteran pests are attracted by and survive longer or have lower mortality on plants infected with *F. verticillioides* (Schulthess *et al*., 2002). Kernel damage alone favours Fusarium ear rot and fumonisin accumulation (Munkvold *et al*, 1997; Clements *et al*., 2003). Maize stalk borers, mainly *B. fusca* infestations, physically damage maize kernels, thereby increasing the incidence of *F. verticillioides* infection in maize (Flett and Van Rensburg, 1992), which may lead to production of fumonisin by this fungus. Other insects such as the European corn borer *Ostrinia nubilalis* Hübner

(Lepidoptera: Pyralidae, Pyraustinae) and its larvae act as vectors, spreading the fungus over long distances (Munkvold and Desjardins, 1997). Insect damage increases infection by *F. verticillioides* between three and nine times compared to simple mechanical damage (Sobek and Munkvold, 1995).

Plant genotype:

Yellow maize is more susceptible to *F. verticillioides* infection than white maize, but white maize is a better substrate for fumonisin production (Rheeder *et al*., 1995). Hybrids and breeding lines differ in their susceptibility to *F. verticillioides* infection and concomitant fumonisin concentration. Hybrids grown outside their range of adaptation are more susceptible to fumonisin accumulation (Shelby *et al*., 1994; Doko *et al*., 1995). Also, hybrids with high lysine content are more susceptible to *Fusarium* ear rot than normal varieties (Visconti, 2002). Genetically modified maize hybrids with *Bt* genes encoding for the δ-endotoxin CryIAb and other Cry proteins that are toxic to insects tend to have less severity of Fusarium ear rot, lower fumonisin concentrations in grain than non-*Bt* maize due to reduction in insect herbivory (Dowd, 2000). The morphology of maize varieties may influence their susceptibility to *F. verticillioides.* Koehler (1942) found more than twice as much *F. verticillioides* ear rot in ears with open husks when compared to closed husks, possibly because adequate tight husk cover is considered key to protecting the ear from fungi and insects (Warfield and Davis, 1996).

Agricultural practices:

Maize residue can act as a long-term source of Fusarium spp. inoculum for infection of maize plants because the survival of *F. verticillioides, F. proliferatum* and *F. subglutinans* decreases more slowly in fields with surface residue than in fields with buried residues (Cotton and Munkvold, 1998). Late planting of maize and repeated planting of maize and other cereal crops in the same or in adjacent fields increase fungal inoculum as well as maize pests resulting in increased fungal infection (Dowd *et al*., 2001).

Post harvest handling and storage:

The risk of fumonisin production increases in pre- and post-harvest maize kernels prior to drying. Harvested moist maize is most favourable for toxin production

(Warfield and Gilchrist, 1999) and contamination of maize by fumonisins increases after physiological maturity (Chulze *et al*., 1996). A moisture level above 18-20% favours *F. verticillioides* growth and concomitant fumonisin contamination during storage (Munkvold and Desjardins, 1997). Subsistence farming practices such as the storage of grain on the floor and non-control of weevils and other insects may promote fungal growth and mycotoxin contamination.

Implications of fumonisins for human and animal health:

Fumonisins can be found in visibly mouldy and symptomless infected kernels. A correlation between fumonisin B_1 (FB_1) contamination of maize and the occurrence of human oesophageal cancer was found in the Transkei region of the Eastern Cape Province, South Africa (Rheeder *et al*., 1992). Rheeder *et al*. (1992) found extremely high levels of fumonisin B_1 of more than 117 parts per million (ppm) in maize from the Butterworth and Centane districts in Transkei. In the Lixian region of China, Chu and Li (1994) found levels of FB_1 of up to 155 ppm, while Yoshizawa e*t al*. (1994) reported levels of up to 11 300 ppm in the same region.

Subsistence farmers are also exposed to fumonisins through the consumption opaque traditional beer containing suspensions of maize-based solids that is home-brewed by rural women using homegrown maize as the primary ingredient. Mouldy grain that is usually separated from the visibly non-mouldy grain by hand sorting after harvest is frequently used as a component because it is believed to impart a certain desirable flavour to the beer (Rheeder *et al*., 1992; Shephard *et al*., 2005). Fumonisin exposure also occur through the consumption of dried traditional African vegetables known as *morogo* that grow as weeds in maize growing fields (Van der Walt, *et al*., 2006).

Fumonisin B_1 has also been associated with high incidence of neural tube defects in babies of mothers consuming fumonisin-contaminated maize along the Texas-Mexico border (Missmer *et al*., 2006). However, maternal folic acid protects the foetus against NTD and fortification of all enriched cereal grain products with folic acid result in the reduction in NTD (Green, 2002). Supplementing the daily diet of women that have a history of NTD-affected pregnancies with 4 000 µg of folic acid is recommended, while 400 µg of synthetic folic acid per day is recommended for all women of child bearing age (Green, 2002).

Fumonisins are structurally similar to sphingolipid precursors such as sphinganine, which are responsible for maintaining membrane and lipoprotein structure, interaction between individual cells, regulation of growth factor receptors, mediation of cell growth, differentiation and cell death (Merril *et al*., 1997). This structural similarity enables fumonisins to be potent competitive inhibitors of ceramide synthase, an enzyme that catalyses sphingosine synthesis in rats. It thereby causes interruption of sphingolipid biosynthesis, as well as the disruption of cellular lipids, fatty acid accumulation and cell proliferation, oxidative stress, lipid peroxidation and peroxisome proliferation (Riley *et al*., 2006).

Exposure to FB_1 in feeds shows a remarkable variation in clinical symptoms across animal species. The most dramatic manifestation of maize feed contaminated with fumonisin is equine leukoencephalomalacia (ELEM), a neurotoxin disease of horses (*Equus caballus* L.), and donkeys (*Equus asinus* L.) that is induced by FB_1 (Marasas *et al*., 1988; Kellerman *et al*., 1990). This disease is characterised by liquifactive necrosis of the white matter of the brain. The clinical symptoms of the disease are apathy, somnolent appearance with protruding tongue, reluctance to move backwards, aimless circling, and ataxia (Nelson *et al*., 1993). Horses are the most sensitive animals to fumonisin toxicity, and levels ranging from 0.2-126 ppm FB_1 were found in feed samples associated with outbreaks of ELEM in North America, South America, and South Africa (Wilson *et al*., 1985; Bezuidenhout *et al*., 1988). Contaminated feeds containing FB_1 levels as low as 8 ppm FB_1 exposes ponies to an elevated risk of ELEM development (Wilson *et al*., 1992; Marasas, 1995).

Porcine pulmonary oedema in pigs (*Sus scrofa domestica* L.) is associated with the consumption of fumonisin-contaminated feed (Kriek *et al*., 1981; Harrison *et al*., 1990). This disease is characterised by extremely marked oedema and massive hydrothorax with the thoracic cavities overfilled with golden-yellow liquid (Harrison *et al*., 1990). Pigs consuming naturally contaminated feeds containing approximately 100 ppm FB_1 are at risk of developing porcine pulmonary oedema (Marasas, 1995). FB_1 induces hepatocellular carcinoma, cholangiofibrosis and cholangiocarcinoma in rats (Gelderblom *et al*., 1991). A number of species-specific effects have been experimentally induced by fumonisins on other target organs such as

immunosuppression in chickens (*Gullus domesticus* L.), toxicity to broiler chicks and chicken embryos, and nephrotoxicity in rabbits (Marasas, 1995; Visconti *et al*., 1998).

Management and control of fumonisins:

Procedures to decontaminate maize and maize-products from fumonisins should be easy to use, inexpensive, and should not lead to the formation of other toxic compounds, revert to the toxic state of the fumonisin, or alter the nutritional and palatability properties of the grain and its products (www.mycotoxins.org). Methods for the control of fumonisin levels in food can be physical, chemical or biological.

Physical methods:

The physical removal of broken kernels and other fine material from bulk shipments of maize by means of a wire screen before processing results in the reduction of total fumonisin content by between 26-69% (Sydenham *et al*., 1995), hand sorting mouldy from non-mouldy maize grain before consumption also reduces fumonisin exposure (Desjardins *et al*., 2000; Fandohan *et al*., 2003). Gamma irradiation causes a 20% total fumonisin reduction when maize was sterilised with 15 kGy of γ-irradiation (Visconti *et al*., 1996). Bennett and Richard (1996) found that starch from fumonisin-contaminated maize prepared by wet milling did not contain fumonisins. The gluten and fibre fractions, however, contained considerable amounts of fumonisin and required further decontamination before use in animal feed. The stability of fumonisins is time-temperature dependent (Jackson *et al*., 1996). Although fumonisins are fairly heat stable, a significant reduction of 16-100% in fumonisin contamination occurs when moist or dry maize is heated at temperatures above 160^{o}C for 20-60 minutes (Scott and Lawrence, 1994; Jackson *et al*., 1997; Castelo *et al*., 1998; Katta *et al*., 1999).

Chemical methods:

Specific processes used in food preparation such as nixtamalization of maize dough can reduce the fumonisin content but the hydrolysed fumonisin may be nearly as toxic as unaltered FB_1 (Murphy *et al*., 1996). Lu *et al*. (1997) found that non-enzymatic browning that occurred in the presence of a primary amine, a reducing sugar, and water at pH above 7, resulted in the removal of the primary amine group from the fumonisin molecule and concomitant reduction in detectable FB_1 levels. Treatment of

contaminated maize grain with a combination of hydrogen peroxide and sodium bicarbonate greatly reduced the toxicity of the end products (Park *et al*., 1996). As with aflatoxin, the use of adsorbent materials, such as activated carbons and cholestyramine, with a high affinity for fumonisin B_1 to tightly bind and immobilise FB_1 in the gastro-intestinal tract of animals, reduced the bioavailability of the toxin (Solfrizzo *et al*., 1998). Cholestyramine had the best FB_1 adsorption capacity followed by activated carbon, bentonite and celite in rats (Solfrizzo *et al*., 1998).

Agricultural practices:

Production practices and HACCP, such as planting hybrids that are adapted to local climatic conditions, use of hybrids with tight husks, control of ear feeding insects, avoiding excessive plant populations, maintaining adequate levels of nitrogen and other essential growth nutrients, sub-soiling in compacted soils and crop rotation to minimise plant stress, are some of the possible means that can reduce the risk of pre-harvest contamination (Vincelli and Parker, 2002). Standard grain storage procedures that prevent the development of fumonisins in stored grain, such as drying maize kernels to a moisture content below 16% within a day or two of harvest is recommended. Stored grain should be aerated regularly to lower moisture content and temperature to desired levels. Adjusting the combine harvester to avoid kernel damage during harvesting reduces mycotoxin contamination (Munkvold and Desjardins, 1997).

Disease resistance:

Resistance to Fusarium ear rot and fumonisin contamination in maize can be achieved by resistance breeding, inserting genes with mycotoxin-degrading ability into plants and detoxification of fumonisin *in planta* (Duvick, 2001). Complete resistance has not been identified in maize (Starr *et al*., 2006). Polygenic sources of resistance to the disease, however, are known to occur in maize (Pérez-Brito *et al*., 2001), but are difficult to incorporate into hybrids. Ear and kernel characteristics such as thick pericarp and tight husk coverage improve resistance to Fusarium ear rot (Warfield and Davis, 1996; Butrón *et al*., 2006). The wider use of partially and insect resistant hybrids, as well as commercialisation of hybrids with novel resistance sources that are based on either native genes or transgenes will form a proactive strategy in the future (Munkvold, 2003).

An aspect that makes maize breeding for resistance to *F. verticillioides* particularly difficult is the fact that *F. verticillioides* is a natural endophyte of maize. Genetically modified organisms, therefore, are a viable alternative to Fusarium ear rot and fumonisin production (Munkvold and Desjardins, 1997). Maize hybrids genetically engineered with *Bt* genes encoding for proteins that are toxic to certain insects are resistant to damage by lepidopteran insects and result in the reduction of symptomless infection, severity of Fusarium ear rot and fumonisin production (Munkvold *et al*., 1997; Munkvold *et al*., 1999; Clements *et al*., 2003). Other approaches include breeding for increased Fusarium ear rot resistance, molecular marker-based breeding, modifying mycotoxin catabolic pathways and gene-for-gene resistance to *Fusarium* spp. (Duvick, 2001).

CONCLUSION

Fusarium and *Aspergillus* spp. produce mycotoxins that exhibit a variety of harmful effects in humans and animals. When food supply is limited due to droughts and poor agricultural practices in subsistence farming systems, the mycotoxin hazard often increases. Food scarcity may then result in fungus-damaged, potentially mycotoxin-containing maize and groundnut being consumed regularly. Determining farming practices that may promote pre- and post-harvest mycotoxin production by *Aspergillus* and *Fusarium* spp. in maize and groundnut, respectively, is an important aspect in managing mycotoxin contamination of food.

Due to poverty, droughts and food shortages in subsistence farming systems, home-grown crops are often the only source of food, irrespective of quality. The damage that fumonisin-contaminated food might cause has been demonstrated in the Transkei region of South Africa where local communities develop high levels of oesophageal cancer. There is, therefore, a compelling need to determine the distribution of Fusarium ear rot and mycotoxin contamination of food in rural areas of South Africa.

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Table 1. Mycotoxins that have a significant impact on human and animal health

Mycotoxin	Fungal producer	References
Aflatoxin	*Aspergillus flavus*	Gourama and Bullerman, 1995
	Aspergillus parasiticus	Gourama and Bullerman, 1995
	Aspergillus nomius	Gourama and Bullerman, 1995
	Aspergillus tamarii Kita	Goto *et al*., 1996
T-2 toxin	*Fusarium sporotrichioides*	Marasas *et al*., 1984
		De Nijs *et al*., 1996
Trichothecenes (deoxynivalenol and nivalenol)	*Fusarium graminearum*	Marasas *et al*., 1984
		De Nijs *et al*., 1996
Zearalenone	*Fusarium graminearum*	Lysøe *et al*., 2006
Ochratoxin A	*Penicillium verrucosum*	Larsen *et al*., 2001
	Aspergillus ochraceus	Larsen *et al*., 2001
Fumonisin B_1, B_2 & B_3	*F. verticillioides*	Bezuidenhout *et al*., 1988
	F. proliferatum	Magnoli *et al*., 1999
	F. thapsinum	Klittich *et al*., 1997

Table 2. Fumonisin-producing *Fusarium* species, analogues produced, and the maximum yields of FB_1, FB_2 and FB_3 reported for each spp. (Rheeder *et al*., 2002)

Fusarium species	Fumonisin analogue(s)z	Maximum fumonisin level (mg/kg)		
		FB_1	FB_2	FB_3
Section *Liseola*				
F. verticillioides[x] MP-A	FA_{1-3}, FB_{1-5}, iso-FB_1, FAK_1, FBK_1, $FC_{1,4}$, FP_{1-3}, PH_{1a-b}	17 900	3 000	2 300
F. sacchari MP-B	FB_1	21	NT[w]	NT
F. fujikuroi	FB_1	7	NT	NT
F. proliferatum MP-D	FA_{1-3}, FB_{1-5}, FAK_1 FBK_1, FC_1, FP_{1-3}, PH_{1a-b}	31 000	17 000	5 700
F. subglutinans MP-E	FB_1	150	NT	NT
F. subglutinans MP-?[v]	FB_1	230	NT	NT
F. thapsinum MP-F	FB_{1-3}	30	5	5
F. anthophilum	FB_{1-2}	610	35	NT
F. globosum	FB_{1-3}	330	4	24
Section *Dlaminia*				
F. nygamai MP-G	FA_{1-3}, FB_{1-5}, FAK_1, FBK_1, FC_1, FP_1, PH_{1a-b}	7 200	530	140
F. dlamini	FB_1	82	NT	NT
F. napiforme	FB_1	480	NT	NT
F. pseudonygamai	FB_{1-2}	Tr[u]	Tr	NT
F. andiyazi	FB_1	Tr	ND[t]	NT
Section *Elegans*				
F. oxysporum	$FC_{1, 3-4}$	NT	NT	NT
F. oxysporum var *redolens*	FB_{1-3}	300	6	0.9
Section *Arthrosporiella*				
F. polyphialidicum	FB_1	500	NT	NT

maximum levels for FB_1, FB_2, and FB_3 are summarised from multiple reports

[w]NT, not tested

F. subglutinans senso lato. These strains are nonfertile with tester strains of mating populations B, E, and H within the *G. fujikuroi* species complex (5)

[u]Tr, trace amounts (1-4 ng/g) detected

[t]ND, not detected (<1 ng/g)

[x]MP-mating population A through G

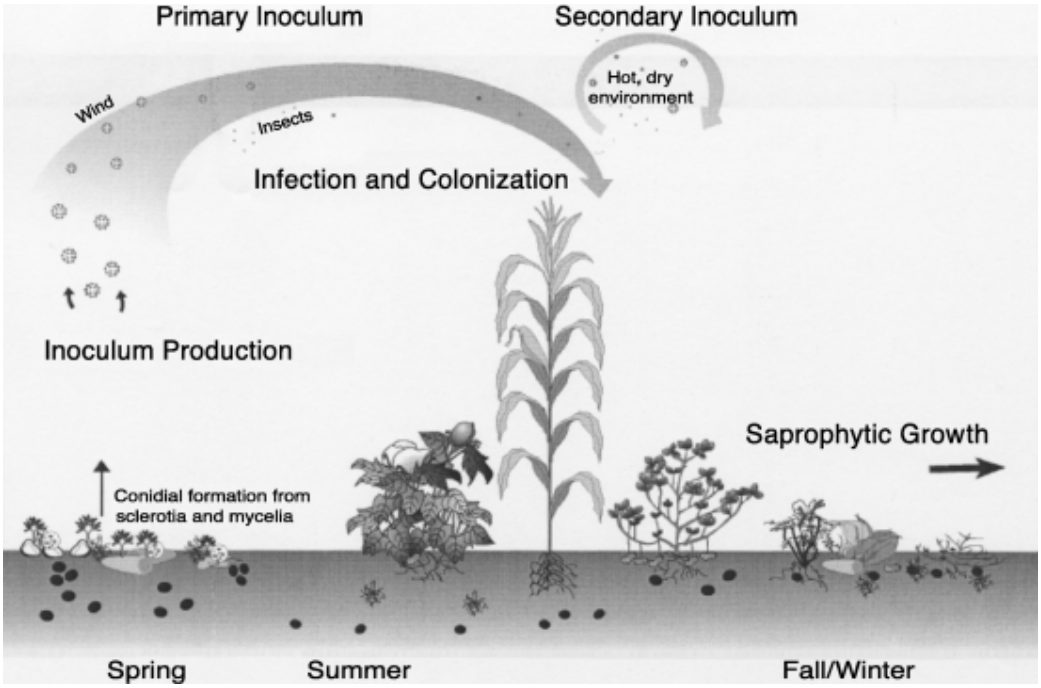

Figure 1. Disease cycle of *Aspergillus flavus* in cotton, maize and groundnut (Scheidegger and Payne, 2003).

Chapter 2

Investigation of agricultural practices in subsistence farming systems in South Africa

ABSTRACT

Mycotoxins, such as the fumonisins produced by *Fusarium verticillioides* in maize and aflatoxins produced by *Aspergillus flavus* and *A. parasiticus* in groundnuts and maize, are harmful to both human and animal health, especially when food supply is limited in subsistence farming systems and people and animals are forced to consume poor quality grain infected with toxin-producing fungi. A questionnaire was compiled to assess the level of mycotoxin awareness and to identify potential agricultural practices that may influence mycotoxin contamination of crops from production through to consumption. The questionnaire was presented to 201 subsistence farmers in the maize production areas of the Eastern Cape (EC), KwaZulu-Natal (KZN), Limpopo (LP) and Mpumalanga (MP) provinces of South Africa during the 2006 planting season. It was also presented to subsistence farmers in the same provinces during the 2007 planting season. In EC and MP, over 74% of the farmers practiced monoculture that may perpetuate the life cycle of the mycotoxin-producing fungi. In all production areas, crop residues were used for cattle feed and stalks were ploughed in, thereby providing primary inoculum for *F. verticillioides* and concomitant fumonisin production in future seasons. Visibly mouldy grain was used as chicken feed in most of the areas, while 30% of the farmers in EC, 17% in MP and 4% in KZN used the mouldy grain for brewing traditional beer, a practice that may increase the level of oesophageal cancer. The most significant finding was that over 90% of the farmers from all the provinces had no knowledge about mycotoxins and their potential effects on animal and human health. Awareness programmes and further research are urgently needed to help subsistence farmers to reduce the deleterious impact of mycotoxins on human health.

INTRODUCTION

About 600 000 households rely on subsistence farming in South Africa (Watkinson and Makgetla, 2002). These households are primarily situated in the Eastern Cape (EC), KwaZulu-Natal (KZN), Limpopo (LP), and Mpumalanga (MP) provinces. In, EC, KZN, LP and MP, respectively, 12, 5, 20 and 8% of households depend on subsistence farming as their main source of food, and 19, 15, 27 and 13%, respectively, depend on it for supplementary food (Watkinson and Makgetla, 2002). Over two thirds of poor households are located in rural areas, where up to 20% of income is spent on maize (*Zea mays* L.) meal, which is the staple food (Watkinson and Makgetla, 2002). An increase in maize prices, such as the raise in 2007 to 50% above the average price for 2006 (Buntrock, 2007), may lead to food insecurity that might result in the consumption of damaged and mycotoxin-contaminated food. The extent of mouldy home-grown maize consumption is not only a matter of food scarcity or drought, but mouldy maize ears are actually preferred by many people in EC for beer making because it is believed that it result in improved flavour (Marasas *et al*., 1979; Shephard *et al*., 2005).

Contamination of maize and groundnut (*Arachis hypogaea* L.) with mycotoxins is often most severe in rural areas where subsistence farmers are unaware of their existence. These people often follow agricultural practices that might contribute to the production of mycotoxins in their crops. Mycotoxin-producing *Aspergillus* spp. and *Fusarium* spp., in particular, are common pathogens or saprophytes of grain exposed to high temperatures, drought stress and insect damage (Miller, 2001; Payne, 1992); conditions common to rural areas in South Africa (www.arc-iscw.agric.za). *Aspergillus flavus* Link ex Fr and *Aspergillus parasiticus* Speare produce aflatoxins in maize and groundnut (Gourama and Bullerman, 1995) while *Fusarium verticillioides* (Sacc.) Nirenberg (syn = *F. moniliforme* Sheldon) and *Fusarium proliferatum* (Matsushima) Nirenberg according to Nelson *et al*. (1983) and Leslie and Summerell (2006), produce fumonisins in maize (Nelson *et al*., 1994).

Food contaminated with aflatoxin can cause fatal acute illness, and is associated with increased risk to developing liver cancer (Maxwell and Wong, 1991; Williams *et al*., 2004; Lanyasunya *et al*., 2005; Nyamogo and Okioma, 2005). Consumption of

fumonisin-contaminated maize has been associated with high levels of human oesophageal cancer and neural tube defects in babies (Sydenham *et al*., 1990; Rheeder *et al*., 1992; Chu and Li, 1994; Yoshizawa *et al*., 1994; Missmer *et al*., 2006). The consumption of aflatoxin-contaminated feed causes oxidative stress, liver necrosis, haemorrhage and death in broiler chickens (*Gullus domesticus* L.), pigs (*Sus scrofa domestica* L.) and cattle (*Bos primigenius* spp. L.) (Sargeant *et al*., 1961; Marasas, 1995; Eraslan *et al*., 2005; Osweiler, 2005), while fumonisin-contaminated feed causes equine leukoencephalomalacia (ELEM), a neurotoxin disease of horses (*Equus caballus* L.), and donkeys (*Equus asinus* L.) (Marasas *et al*., 1988; Kellerman *et al*., 1990) and porcine pulmonary oedema in pigs (Kriek *et al*., 1981; Harrison *et al*., 1990).

Subsistence farming practices during pre- and post-harvest influence the plant's susceptibility to fungal infection and concomitant mycotoxin production. Crop residues left on the field increase the inoculum potential of the pathogenic fungi, while infected stalks partially buried serve as over wintering-sites (Kedera *et al*., 1992). *Aspergillus flavus* can colonise damaged or undamaged maize and groundnut kernels (Hill *et al*., 1983; Cole *et al*., 1984; Munkvold *et al*, 1997; Clements *et al*., 2003). *Fusarium verticillioides* survives as a pathogen and saprophyte on plant debris. It has limited competitive ability in the soil and produces spores on plant debris, thus providing inoculum for the next season's infection (Nyvall and Kommedahl, 1970). *Fusarium verticillioides* is a natural endophyte of maize and may cause kernel or ear rot, root and stalk rot, and seedling blight in maize (Zummo and Scott, 1992), in addition to fumonisin production in maize kernels. Post-harvest practices, such as non-control of insect infestation might increase fungal growth and concomitant mycotoxin production because insects increase grain moisture levels, through respiration, to levels that can support fungal growth, in addition to physical damage to the kernels (Williams *et al*., 2004). A moisture level above 18-20% favours *F. verticillioides* growth and concomitant fumonisin contamination during storage (Munkvold and Desjardins, 1997) and, therefore, maize should be stored at moisture levels below 13-14% if mycotoxin production is to be prevented.

In this study, agricultural practices in rural areas of South Africa that may influence mycotoxin contamination of maize and groundnuts were investigated. A questionnaire

was compiled to assess the level of mycotoxin awareness and to assemble which agricultural practices might influence mycotoxin contamination of maize and groundnuts from production through to consumption. It further addresses aspects related to storage, handling and consumption of food that might be contaminated with mycotoxin-producing *Aspergillus* and *Fusarium* spp.

MATERIALS AND METHODS

Geographic areas surveyed:

Subsistence maize farmers were selected in maize-growing regions in the Eastern Cape (EC), KwaZulu-Natal (KZN), Limpopo (LP) and Mpumalanga (MP) provinces (Figs. 1 and 2) were included in the study. A total of 202 and 127 localities were visited during the 2006 and 2007 planting seasons, respectively, and Global Positioning System (GPS) coordinates recorded at each of the localities. The survey on maize farming practices was carried out in LP province where five districts and 45 households were visited, in Mpumalanga, 13 districts and 64 households were visited while 14 districts and 114 households were visited in KwaZulu-Natal. In the Eastern Cape, 15 districts and 99 households were visited. The survey was also carried out for groundnut farming practices in ten households in three districts, nine households in four districts and 11 households in four districts in KZN, LP and MP, respectively, during the 2006 planting season while 20 households in four districts in KZN were also surveyed during the 2007 planting season. There was, however, a widespread crop failure in 2007, possibly due to drought in LP and MP, resulting in unavailability of groundnuts during the same season. All the households surveyed throughout these provinces were geographically separated from each other, and the climate, rainfall and planting schedule was noted in each area where samples were collected.

Questionnaire:

A questionnaire (Appendix A) was drawn up in English and translated to six local South African languages: Zulu, Xhosa, Sepedi, Venda, Shangaan and Tswana. The questionnaire requested information regarding the crop, crop rotation, land tillage methods, planting dates, seed sources, cultivars planted, maize stalk borer control, pest and fungus disease control, harvesting dates, crop residue disposal, grain sorting and storage, intended use of grain, mouldy grain uses, human consumption of mouldy

grain, mycotoxin and mycotoxicoses awareness. These questions were selected based on factors, conditions and agricultural practices that might favour mycotoxin occurrence on and off the field in rural areas of South Africa. The questions in the questionnaire were placed in a random order to avoid asking leading questions thereby ensuring the adequacy of the questionnaire.

Interviews:

An adult member of the family at each collection site was interviewed to obtain more detailed information on the questionnaire. Data collection was done in collaboration with local extension officers of the Department of Agriculture. At least one person in the research team had a good proficiency of local languages. The extension officers informed the farmers why the survey was carried out and how it would be conducted, and asked them to participate in the survey. A mycotoxin awareness campaign was also carried out at the time of the survey to gain the support of the farmers.

RESULTS

Crop rotation of maize with non-cereal crops such as vegetables, potatoes, beans, cotton and groundnuts were widely used practices in KZN, where 70% of farmers practiced crop rotation (Figs. 3 and 4). In LP, 11% of farmers were intercropping maize with cowpea (Fig. 4), while 16% were rotating maize with vegetables and over 78% were not practicing crop rotation at all (Figs. 3 and 4). During the 2006 planting season, 55% of farmers practiced crop rotation in MP (Fig. 3) while 92% of farmers did not practice crop rotation, with 7% of the farmers rotating maize/beans during the 2007 planting season (Fig. 4). More than 80% of farmers in the high maize production region of the EC province did not practice crop rotation (Figs. 3 and 4). There was an increase in maize production in MP during the 2007 possibly due to the nascent involvement of the Provincial Department of Agriculture in supplying farmers with tractors, fences, seed and fertiliser.

There was a little change in the method of land tillage in the two seasons under review (Figs. 5 and 6). Over 90% of the farmers in EC, LP, and MP were using tractors for ploughing during both seasons while above 60% of the farmers in KZN used tractors. Eighteen % used hand hoes to till the soil possibly due to the terrain, and over 11%

used ox-drawn ploughs. There were over 4% of farmers in KZN and EC that were practising no-till farming.

Over 70% of the farmers included in the survey planted their crops during October to January (Figs. 7 and 8) as maize is a summer crop in South Africa. Two % of the farmers in KZN planted maize during July and August for sale as green mealies (Fig. 8), while approximately 4% of farmers in EC, LP and MP planted between January and March during 2007 season due to late rainfall during that season. Over 70% of the farmers harvested their crops from May to August (Figs. 9 and 10). There was a large variation in harvesting times according to the planting schedule in KZN, LP, and MP (Fig. 9), possibly due to planting hybrids with different maturities and delayed harvesting.

Forty six, 10, 7, and 10% of farmers planted seed retained from the previous plantings in KZN, EC, LP, and MP, respectively, during the 2006 planting season (Fig. 11), while 45, 12, 44, and 21% of the farmers in these respective areas did so during the 2007 planting season (Fig. 12). There was an increase in farmers planting traditional varieties, which was actually seed from previous plantings in EC, LP, and MP possibly due to increased seed prices and reduced provincial agricultural financial support, particularly in the EC.

In the EC, over two thirds of the farmers planted commercial hybrids purchased from seed companies (Figs. 13 and 14). There was an increase in the number of farmers planting seed retained from previous plantings in all provinces (Fig. 14). Hybrids from Pannar® were the most popular. Many farmers in LP did not know the names of cultivars they were planting (Fig. 13 and 14).

Granular insecticides such as Bulldock® were widely used to control maize stalk borers and other insect pests in all the provinces (Figs. 15-18). Over 80% of the farmers in LP did not control the maize stalk borer, while 50, 78 and 58% of farmers in KZN, EC, and MP, respectively, controlled the maize stalk borer (Figs. 15 and 16). In EC, over 70% of the farmers applied fungicides (Figs. 15 and 16). There was a very limited use of pesticides or fungicides during the 2007 planting season in KZN,

LP, and MP (Figs. 17 and 18) possibly due to less visible pest infestation during the season.

Crop residues were widely used as cattle fodder (Figs. 19 and 20). Over 55% of the farmers used crop residues for cattle feed in all the provinces. Thirty, 13, 40 and 17% of farmers ploughed-in their crop residues in KZN, EC, LP, and MP, respectively (Fig. 19). This practice was also common during the 2007 planting season (Fig. 20), since the farmers believe that crop residue enhance their soil properties with nutrient and water holding capacity.

Grain storage invariably mirrored the level of maize production (Figs. 21 and 22). Over 30% of the farmers stored their grain in drums, tanks or bags, while 1% stored their grain in silos or rooftops in KZN (Fig. 21). In EC, over 54% of farmers stored their grain in tanks, 41% in bags and 4% stored their grain in commercial silos (Fig. 21). In LP, 11% of farmers stored their grain in drums while 89% stored their grain in bags. In MP, 7% of farmers stored their grain in drums, 10% in tanks, 34% in bags and over 48% stored their grain in commercial silos. The number of farmers who practiced grain sorting decreased markedly in 2007 planting season in MP while all the farmers sorted their grain before consumption in KZN, EC, and LP (Figs. 23 and 24).

All farmers in all provinces produced maize for their own consumption (Figs. 25 and 26). However, approximately 4% of the farmers in EC and MP traded their surplus grain to millers and other subsistence farmers (Fig. 25). Over 4, 30 and 17% of farmers in KZN, EC, and MP, respectively, used visibly mouldy grain for brewing traditional beer during the 2006 planting season (Fig. 27). There was an increase in farmers using mouldy grain for brewing with over 18, 45, 11 and 28% of the farmers in KZN, EC, LP, and MP, respectively, brewing opaque beer using visibly mouldy grain (Fig. 28). Ten percent of farmers in KZN consumed mouldy whole grain in their traditional dishes such as *istampa* and *umngqushu* (Figs. 29 and 30). None of the farmers, with the exception of 4% in MP, surveyed in the four provinces were aware of mycotoxins and their associated detrimental effects, such as the association of fumonisins with oesophageal cancer, or liver cancer and death resulting from consumption of aflatoxin-contaminated food. However, 8% of the farmers in the EC

were aware of mycotoxins during 2007 planting season due to knowledge imparted to them during sample collection during the previous season (Figs. 31 and Fig. 32).

There was very little variation in groundnut farming practices in KZN, MP and LP (Figs. 3 and 4). Over 66% of farmers in KZN and MP practiced maize-groundnut rotations, while in LP, 57% of the farmers practiced maize-groundnut rotations and 14% were practicing sweet potato-groundnut rotations (Fig. 33). All the farmers surveyed in LP and MP used tractors for land tillage (Fig. 34). In KZN, 67 and 33% of farmers used tractors and hand hoes, respectively, for land tillage (Fig. 34). Over 15% and 40% of the farmers in KZN planted during October and December, respectively (Fig. 35). In LP, approximately, 60% of the farmers planted groundnuts during November while 10% planted in October (Fig. 35). In KZN, 15% of the farmers harvested in January and also another 15% harvested in February (Fig. 36). Over 10 and 58% of the farmers in LP harvested in February and May, respectively, while an equal number of farmers in MP harvested in February, March and April, respectively (Fig. 36).

All the farmers in KZN and 66% in MP, planted groundnut seed retained from previous seasons' plantings, while over 85% of farmers in LP bought the seed (Fig. 37). All the farmers in MP did not know which groundnut cultivars were planted, while 85 and 66% of farmers in KZN and MP, respectively, planted traditional or local varieties (Fig. 38). None of the farmers applied pesticides or fungicides to the groundnut crop in all the three provinces (Fig. 39).

Crop residues were ploughed in by 50, 65 and 37% of the farmers in KZN, MP and LP, respectively, while the rest of the farmers left it in the field for grazing by livestock in all provinces (Fig. 40). All the farmers in KZN and 75 and 67% of farmers in LP and MP, respectively, stored their groundnuts in bags (Fig. 41). All the farmers surveyed hand sorted their groundnuts before consumption (Fig. 42). The groundnuts were solely grown for home-consumption (Fig. 43), while mouldy groundnuts were used as stock feed, mainly chicken feed, in all the provinces (Fig. 44). None of the farmers consumed visibly mouldy groundnuts (Fig. 45) and also, none of them were aware of mycotoxins and their associated dangers to animal and human health (Fig. 46).

DISCUSSION

Subsistence farming is an important way of providing food and income to the poor in the rural areas of South Africa. The farmers in these areas can often not afford pesticides, fungicides and commercial seed required to secure healthy and safe crops. Their biggest problem, however, is the general lack of information on agricultural practices necessary to grow maize that is free of pathogens and mycotoxins. This survey provides the first in-depth investigation into the risk that these farmers and rural communities face by unknowingly consuming food that could be contaminated with mycotoxins. Interviewing an adult member of the family involved with the crop from planting through to consumption in their local language ensured that the farmers understood and answered the questions as honestly as possible. In addition, it was explained to farmers that the work was not meant for political or donor purposes so as to reduce the sample error because a farmer could answer the questions in a way that made that particular farmer to appear as a needy individual.

This investigation has shown that subsistence farmers were unaware of the threat that mouldy maize kernels hold to their health and livestock such as chicken, sheep and cattle that are fed with mycotoxin contaminated feed. To them, pre- and post-harvest practices were often considered according to its financial implications and the cultural practices of the community. For instance, more than 80% of farmers in the EC received substantial support from the Provincial Department of Agriculture for seed, chemical, planting and harvesting in the 2006 planting season. There was, however, a 10% decline in farmers using seed received from the Provincial Department in the 2007 planting season, as farmers were expected to contribute 50% to their input costs and they, therefore, decided to plant maize from the previous harvest. This practice may exacerbate systemic infection and increase *F. verticillioides* populations. Also, over 30% of farmers in EC and MP used visibly mouldy grain for brewing traditional beer because it is suspected of imparting a certain desirable flavour into the beer (Shephard *et al.*, 2005). This practice has previously been associated with high incidences of oesophageal cancer in EC (Rheeder *et al.*, 1992).

Some farming practices are likely to increase the inoculum of *Fusarium* spp. in the soil. Crop residues are often left on the soil surface, as farmers believe that crop

residue enhance their soil properties with nutrient and water holding capacity. This practice results in increased severity of *F. verticillioides* stalk and root rot (Byrnes and Carroll, 1986) and might ultimately reduce the yields and lead to the production of fumonisins by *F. verticillioides*. Flett and Wehner (1991), however, found that tillage had little to no effect on Fusarium ear rot development. No-till farming increases water retention in the soil (Prinsloo, 2007) thereby reducing plant moisture stress and the plant's susceptibility to infection by *Fusarium* spp. and concomitant fumonisin production.

In KZN, over half of the farmers participating in the survey planted maize seeds harvested from previous plantings, suggesting the use of open pollinated maize varieties. Sixty % of these maize varieties were developed locally through natural selection and might be adapted to the local conditions by possessing traits such as tight husks, tolerance to high temperatures and low rainfall. In contrast, farmers in MP and EC planted commercial maize hybrids possibly due to success in marketing strategies of certain seed companies, notably Pannar®. These hybrids may not be adapted to local climatic conditions and agronomic practices and, therefore, may be subjected to an increase in abiotic stresses that may result in higher *F. verticillioides*, *A. flavus* and *A. parasiticus* infections and concomitant fumonisin and aflatoxin contamination (Shelby *et al*., 1994; Doko *et al*., 1995; Munkvold, 2003). The farmers in MP were also planting open pollinated cultivars such as ZM521, which possess good cob tip cover, and that is tolerant to drought stress and low soil fertility conditions in addition to high yield under these conditions (Bänziger and Diallo, 2001). This hybrid might be less susceptible to fumonisin contamination since it is tolerant to conditions that favour fumonisin production. The ZM521 cultivar was developed by a consortium of agricultural organisations that included the National Department of Agriculture, the Departments of Agriculture of Limpopo Province and the Mpumalanga Province, the Grain Crops Institute of the Agricultural Research Council (GCI-ARC), the South African National Seed Organization (SANSOR), universities and agricultural colleges in Limpopo Province, EcoLink Mpumalanga (an NGO), International Maize and Wheat Improvement Centre (CIMMYT), and donors (www.cimmyt.org).

Delayed harvesting occurred in areas where farmers left the crop to dry completely on the field. This may cause a significant increase in mycotoxin contamination if the grain was already infected by *F. verticillioides* and *F. proliferatum*. Contact of the grain with soil during harvest must be prevented and small, shrivelled grain may contain higher levels of mycotoxins than healthy normal grain and must be removed from the food chain (www.ec.europa.eu). The number of farmers that practiced grain sorting in order to reduce exposure to mycotoxins (Desjardins *et al*., 2000; Fandohan *et al*., 2005) decreased markedly in 2007 planting season in MP, probably due to the increased use of combine harvesters or lack of visibly mouldy grain in most production areas. It is also possible that farmers were forced to consume all the available grain due to poor harvests because of the drought during the season. The farmers that hand sorted their grain by removing visibly mouldy grain from their food chain might have reduced their exposure to mycotoxins, however, the use of the visibly mouldy grain as feed exposes the animals to high levels of mycotoxins and might result in the occurrence of animal disease such as ELEM in horses.

Good housekeeping procedures to minimise insect infestation and fungal contamination during storage were not adhered to due to the high costs of purchasing suitable insecticides and fungicides. In LP, 5% of the farmers stored their grain in traditional silos, which may be prone to insect infestation and lack of aeration resulting in fungal growth. The EC farmers produced enough maize to take them to the next harvest and also sold surplus maize in better production seasons. In the 2007 planting season, fewer farmers expected surpluses due to the dry weather conditions during the season.

This study strongly recommends that subsistence farmers in South Africa need to be made aware of potential mycotoxin production in their crops through enhanced involvement of extension services in subsistence farming communities. The extension officers need to be targeted for mycotoxin awareness since they are also unaware of these mycotoxins. The extension officers need to know factors that can result in fungal infection and concomitant mycotoxin production and how mycotoxin contamination of crops can be reduced. The extension officers should in turn advise farmers on good agricultural practices that can reduce mycotoxin contamination of crops, such as, planting hybrids adapted to their climatic conditions, soil and crop

management to minimise plant stress, harvesting and drying grain before storage to moisture levels that can not support fungal growth and storing grain under dry conditions free from insects and pests (www.agriculture.gov.ie). This is particularly important in areas where communities rely on maize as their primary source of food and income, such as the EC, northern KZN and LP. Such knowledge may result in improved economic sustainability, improved public health and enhanced food safety, as well as an increase in trade of surplus products. It is also important for subsistence farmers to realise that good agricultural practices represent the first line of defence in controlling the contamination of maize and other cereals by mycotoxins (www.ec.europa.eu). This should be followed by the implementation of good manufacturing practices during the handling, storage, processing and consumption of cereals.

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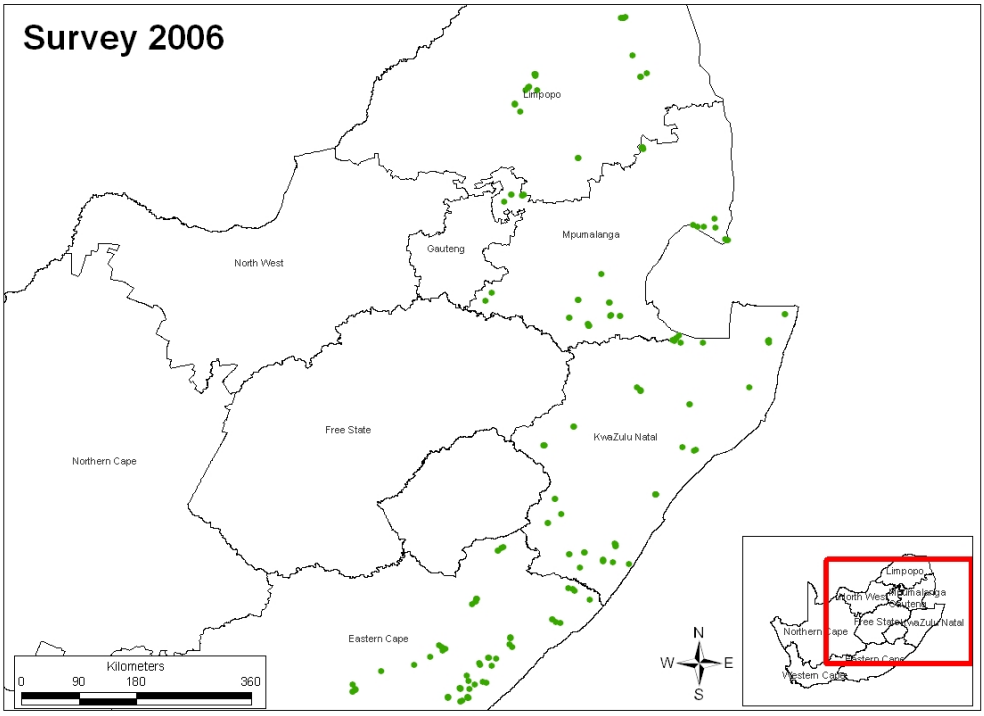

Figure 1. GPS points indicating subsistence farmer fields in South Africa where farming practices were surveyed during the 2006 planting season.

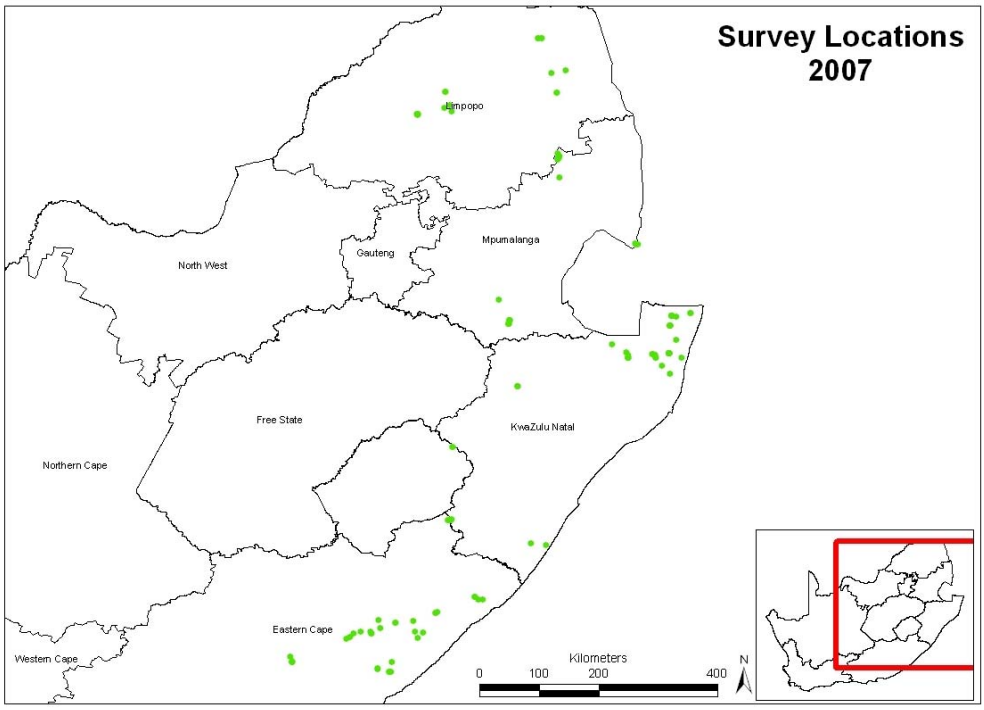

Figure 2. GPS points indicating subsistence farmer fields in South Africa where farming practices were surveyed during the 2007 planting season.

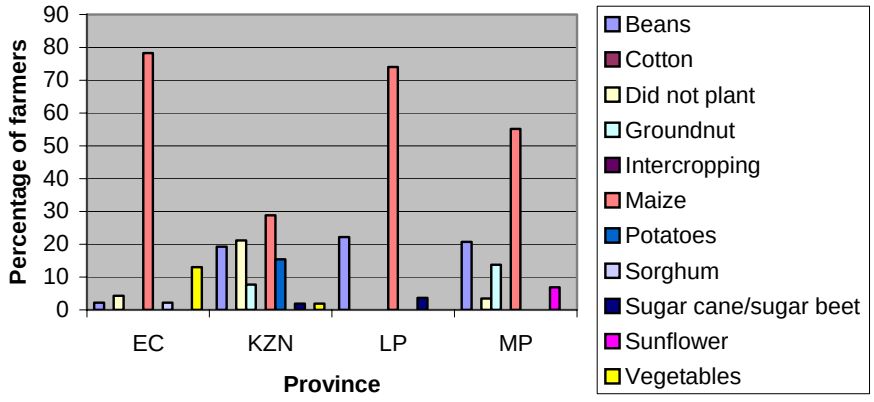

Figure 3. Crop rotation practices adopted by subsistence farmers in South Africa surveyed during the 2006 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

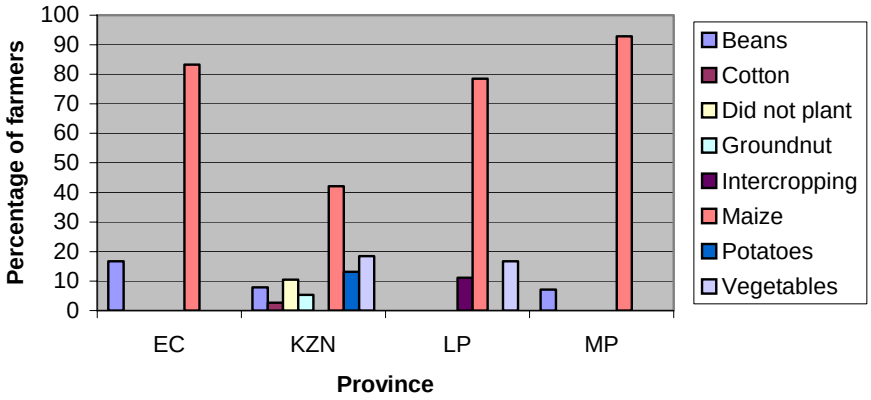

Figure 4. Crop rotation practices adopted by subsistence farmers in South Africa surveyed during the 2007 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

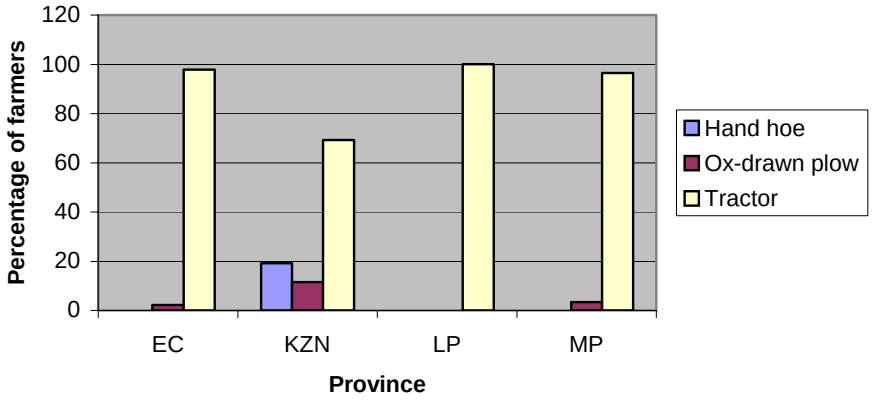

Figure 5. Methods of land tillage applied by subsistence farmers in South Africa surveyed during the 2006 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

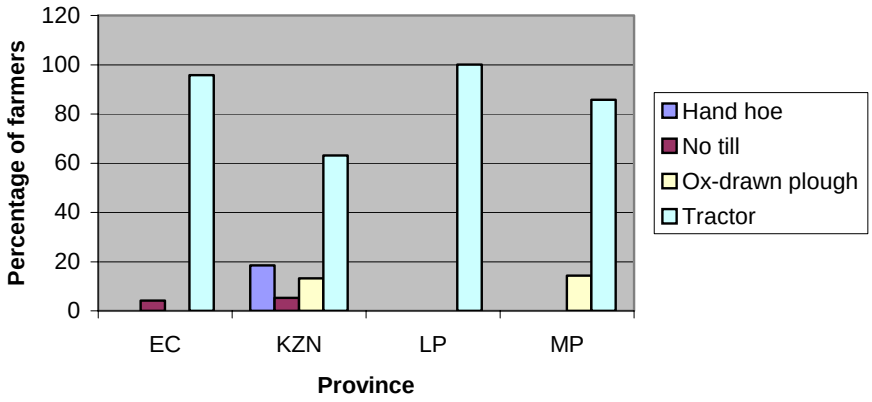

Figure 6. Methods of land tillage applied subsistence farmers in South Africa surveyed during the 2007 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

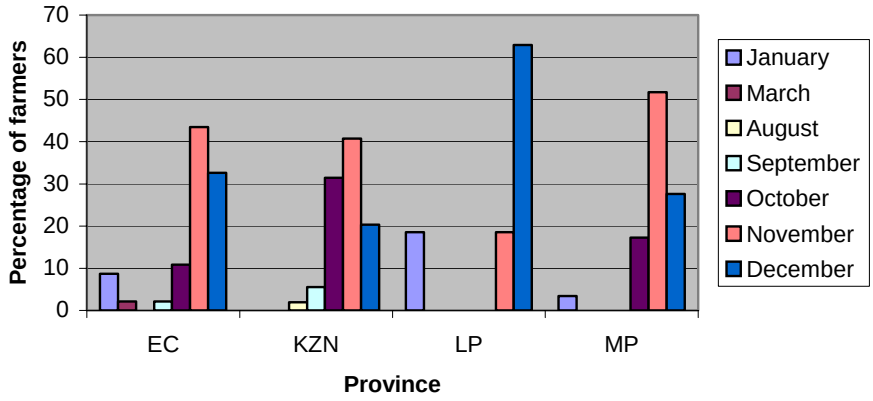

Figure 7. Maize planting dates recorded for subsistence farmers in South Africa during the 2006 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

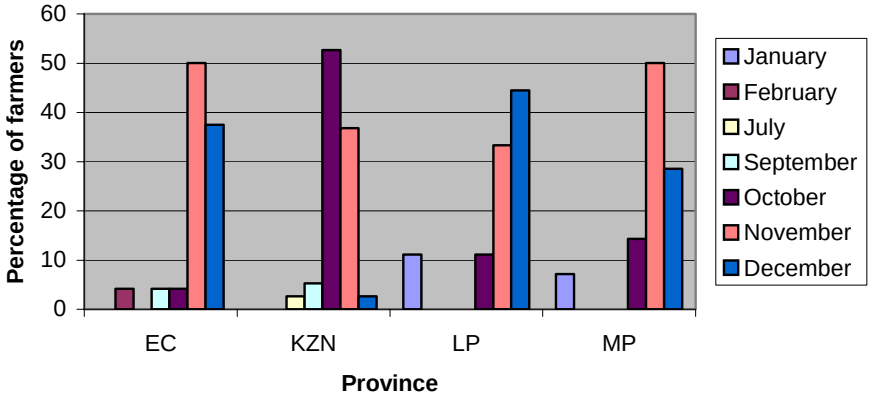

Figure 8. Maize planting dates recorded for subsistence farmers in South Africa during the 2007 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

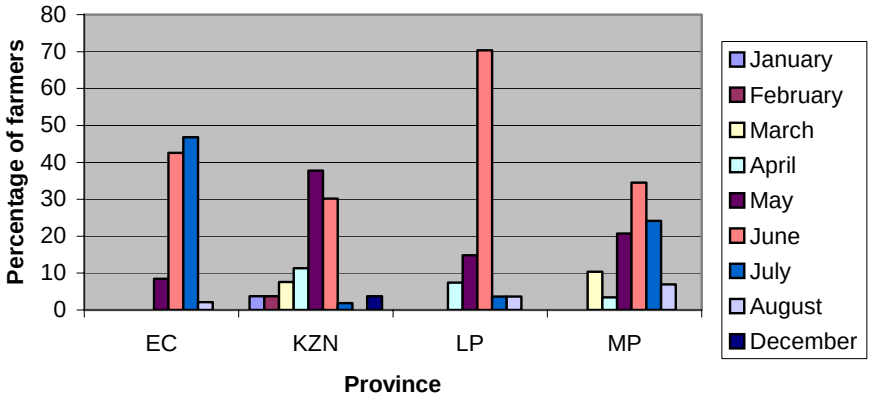

Figure 9. Harvesting dates recorded for subsistence farmers in South Africa during the 2006 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

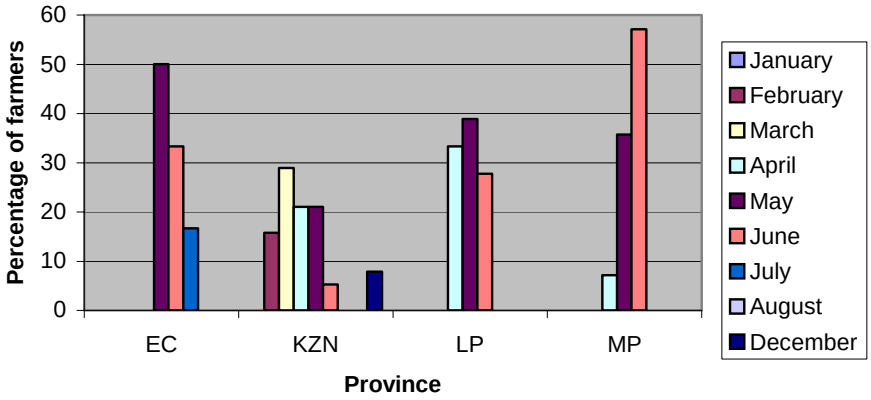

Figure 10. Harvesting dates recorded for subsistence farmers in South Africa during the 2007 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

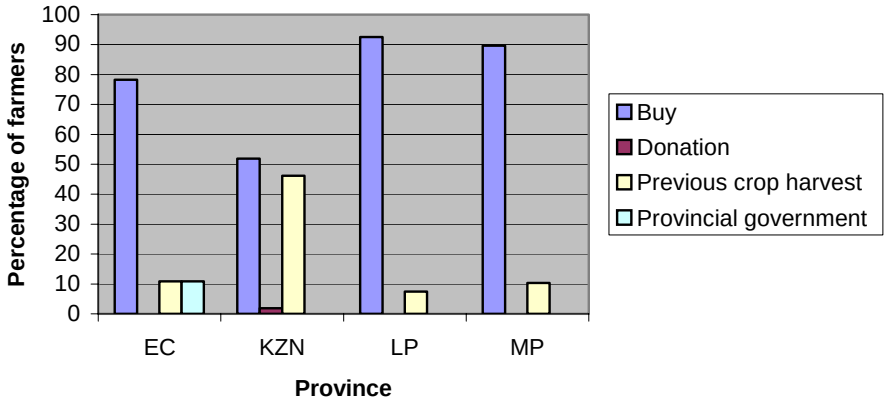

Figure 11. Source of maize seed planted by subsistence farmers in South Africa during the 2006 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

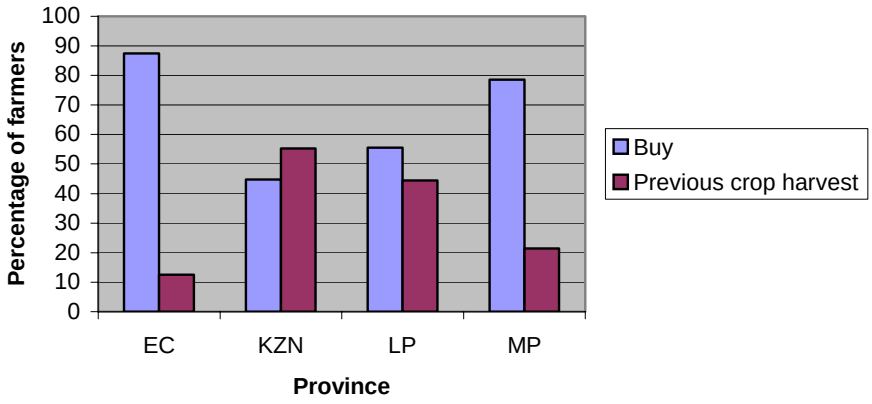

Figure 12. Source of maize seed planted by subsistence farmers in South Africa during the 2007 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

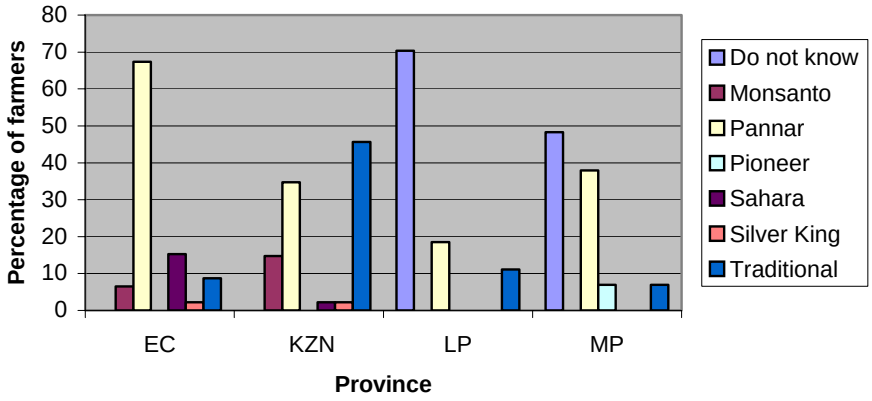

Figure 13. Maize cultivars planted by subsistence farmers in South Africa surveyed during the 2006 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

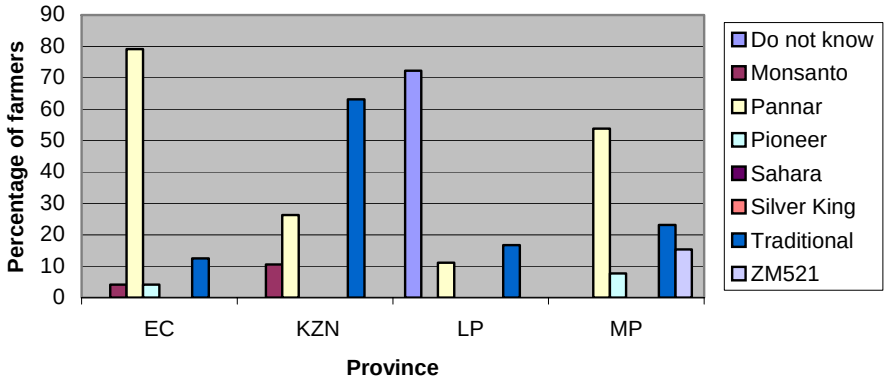

Figure 14. Maize cultivars planted by subsistence farmers in South Africa surveyed during the 2007 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

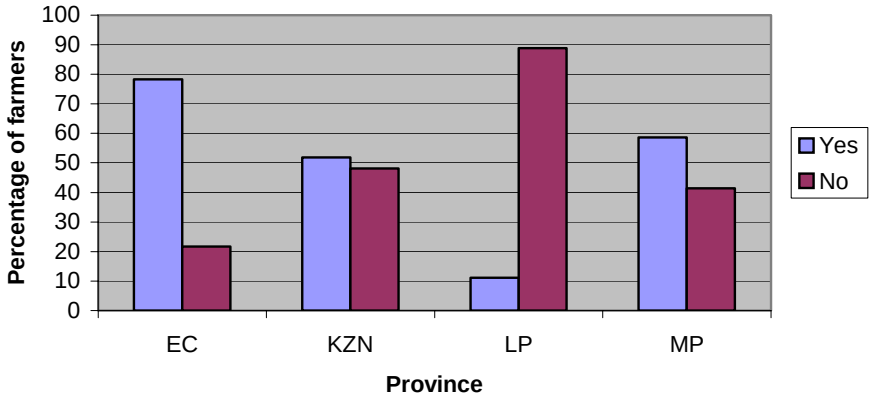

Figure 15. Maize stalk borer control by subsistence farmers in South Africa during the 2006 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

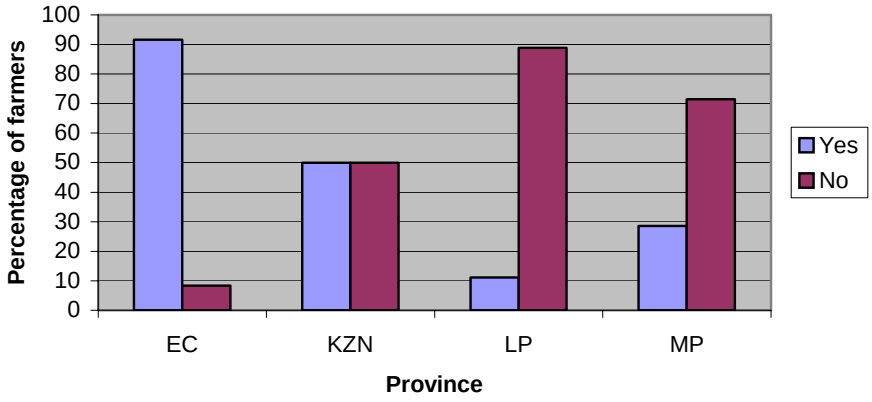

Figure 16. Maize stalk borer control by subsistence farmers in South Africa during the 2007 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

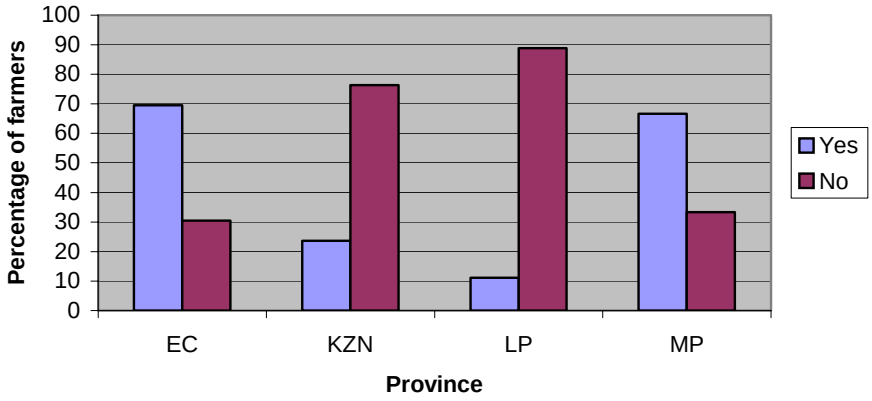

Figure 17. Use of pesticides/fungicides by subsistence farmers in South Africa during the 2006 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

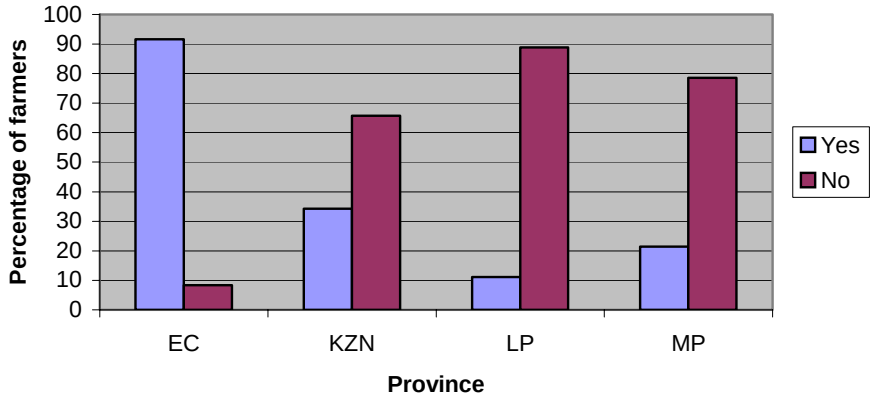

Figure 18. Use of pesticides/fungicides by subsistence farmers in South Africa during the 2007 season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

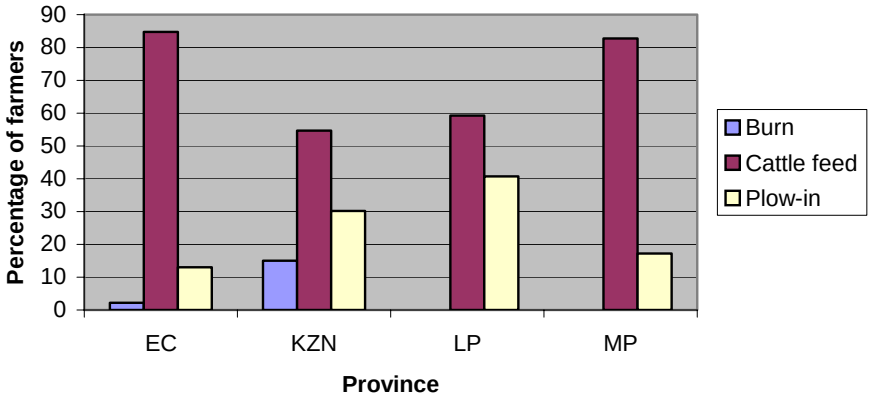

Figure 19. Method of crop residue disposal followed by subsistence farmers in South Africa during the 2006 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

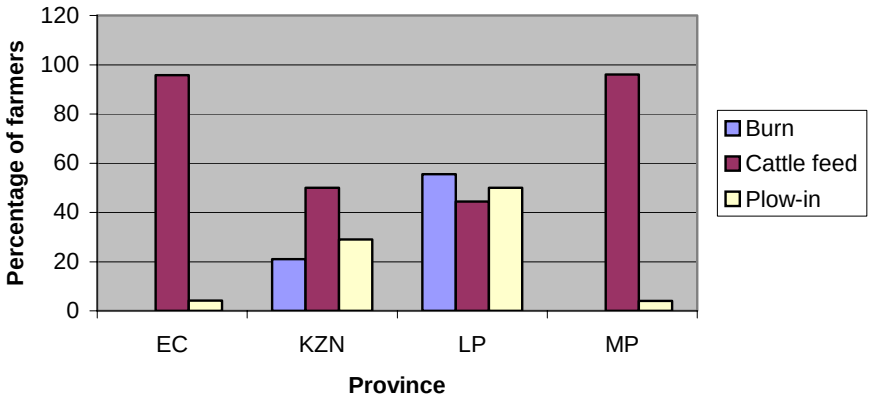

Figure 20. Method of crop residue disposal followed by subsistence farmers in South Africa during the 2007 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

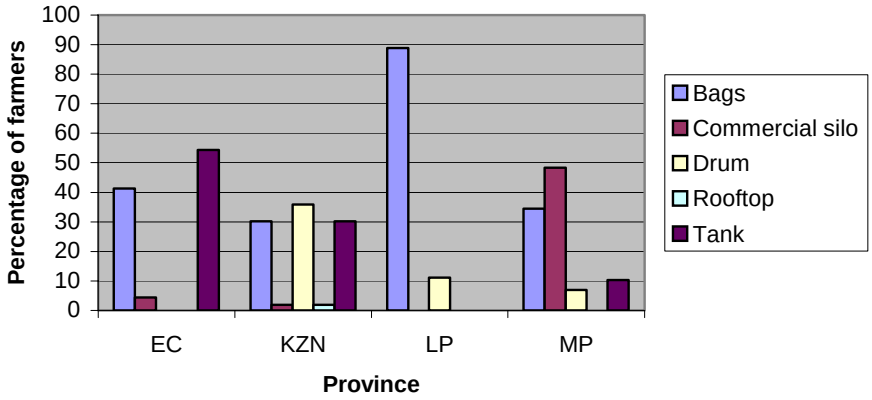

Figure 21. Method of grain storage practiced by subsistence farmers in South Africa during the 2006 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

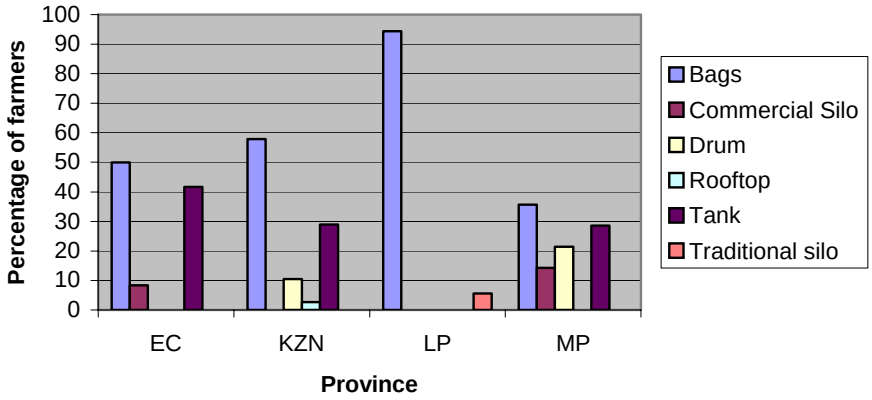

Figure 22. Method of grain storage practiced by subsistence farmers in South Africa during the 2007 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

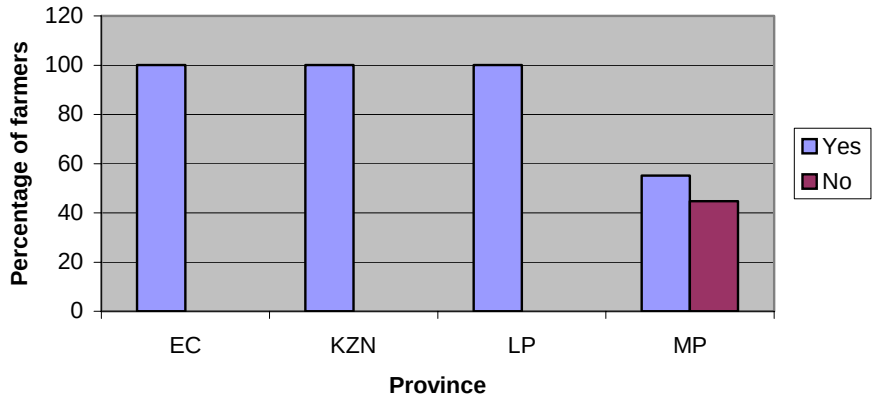

Figure 23. Subsistence farmers in South Africa that sorted ("Yes") or did not sort ("No") their grain after shelling during the 2006 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

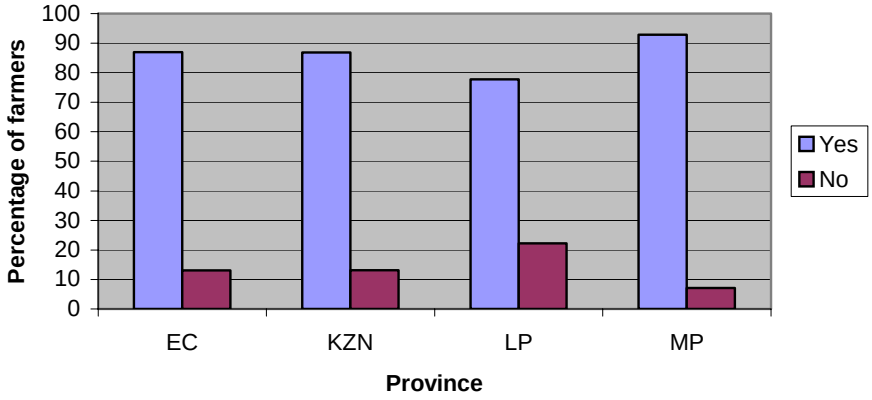

Figure 24. Subsistence farmers in South Africa that sorted ("Yes") or did not sort ("No") their grain after shelling during the 2007 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

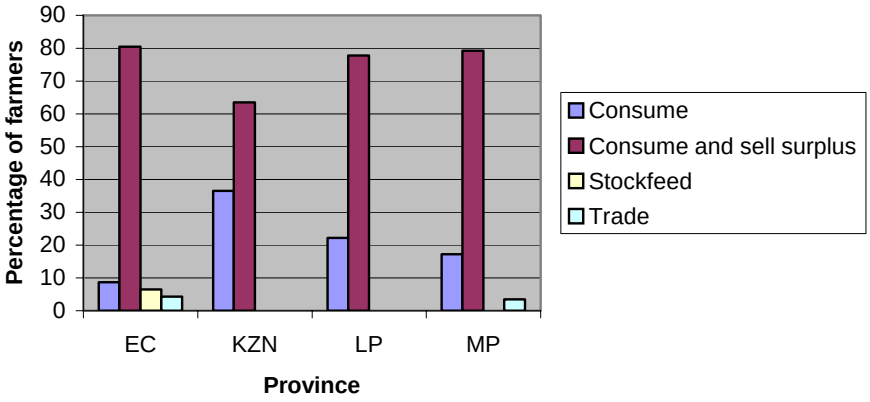

Figure 25. Intended uses of grain harvested by subsistence farmers in South Africa during the 2006 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

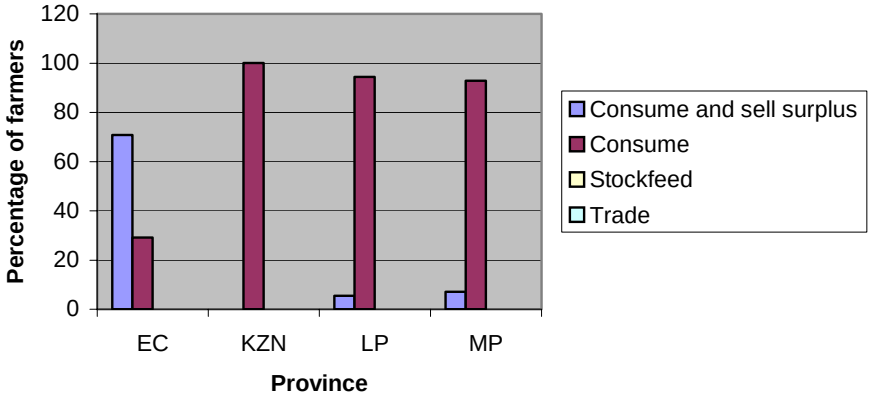

Figure 26. Intended uses of grain harvested by subsistence farmers in South Africa during the 2007 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

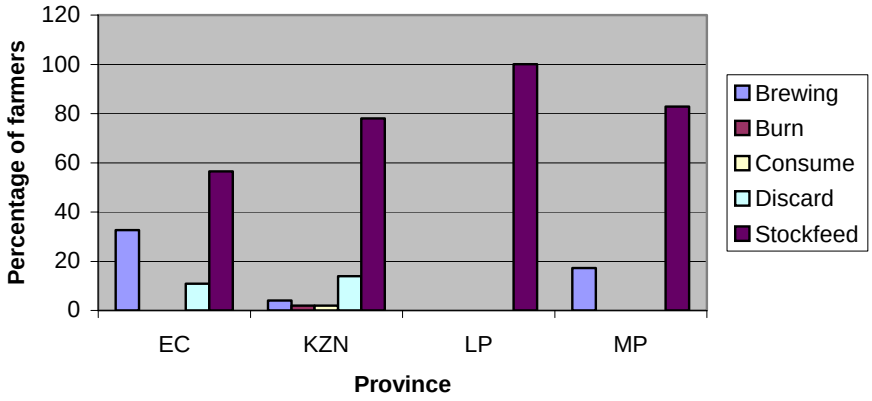

Figure 27. Uses of mouldy grain harvested by subsistence farmers in South Africa during the 2006 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

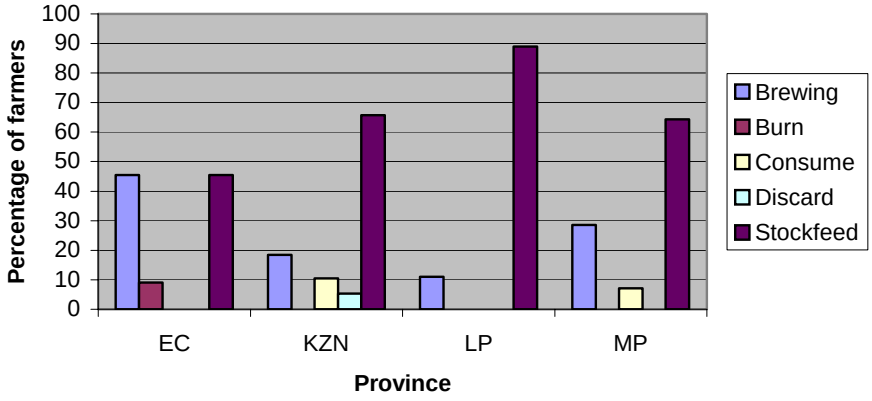

Figure 28. Uses of mouldy grain harvested by subsistence farmers in South Africa during the 2007 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

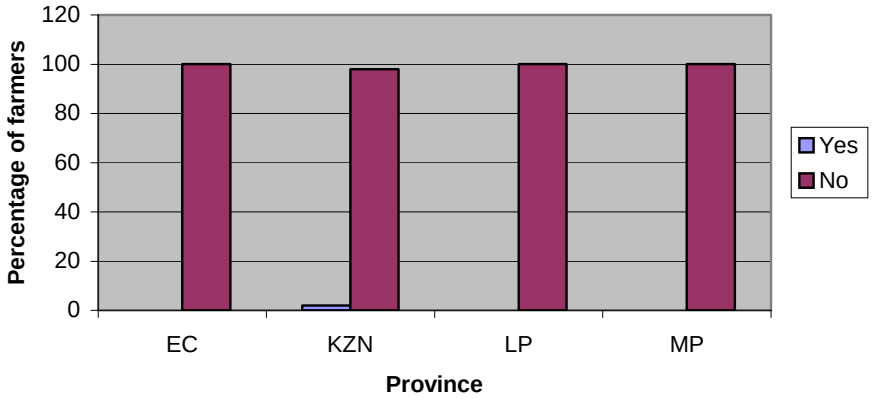

Figure 29. Human consumption of mouldy grain harvested by subsistence farmers in South Africa during the 2006 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

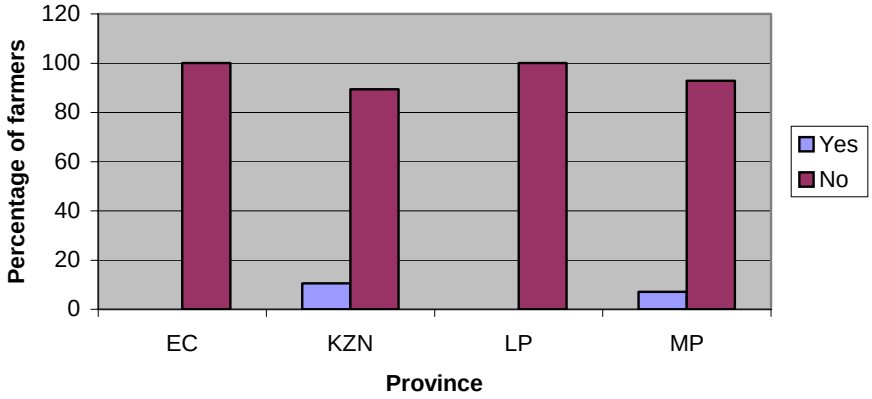

Figure 30. Human consumption of mouldy grain harvested by subsistence farmers in South Africa during the 2007 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

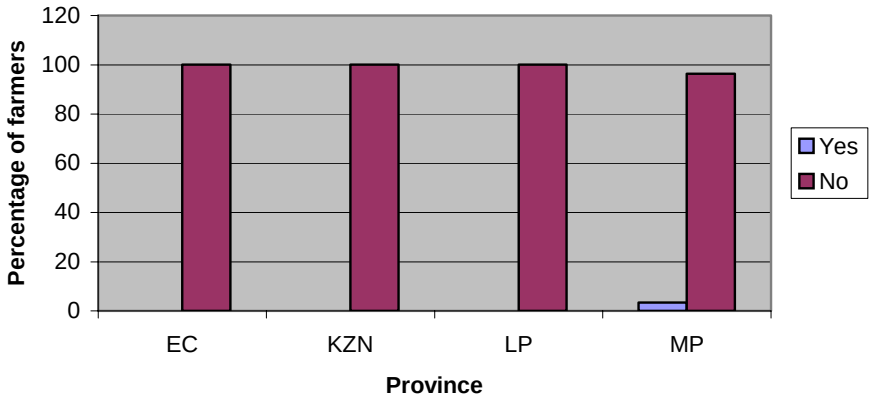

Figure 31. Level of mycotoxin awareness among subsistence farmers in South Africa during the 2006 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

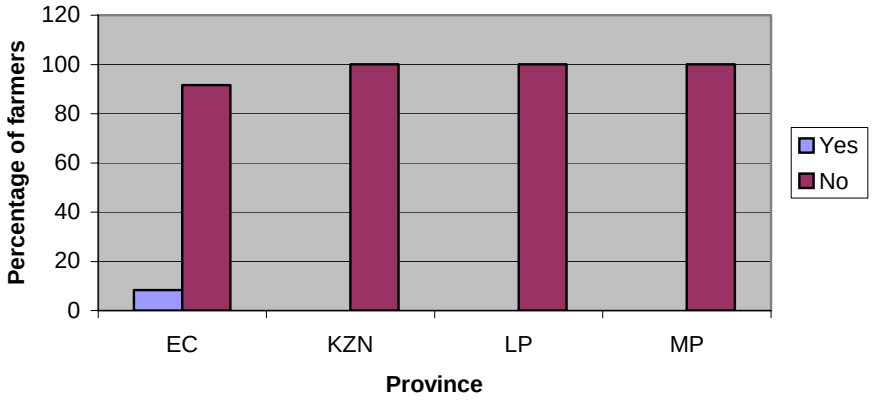

Figure 32. Level of mycotoxin awareness among subsistence farmers in South Africa during the 2007 survey season. EC = Eastern Cape, KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

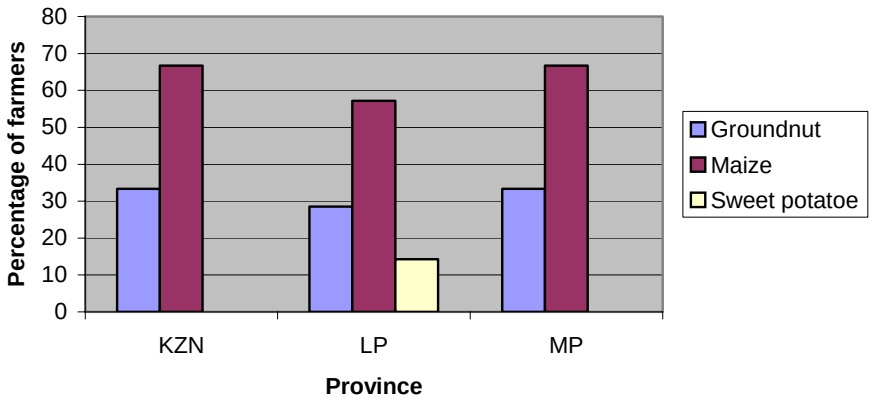

Figure 33. Crop rotation practices adopted by groundnut subsistence farmers in South Africa surveyed during the 2006 season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

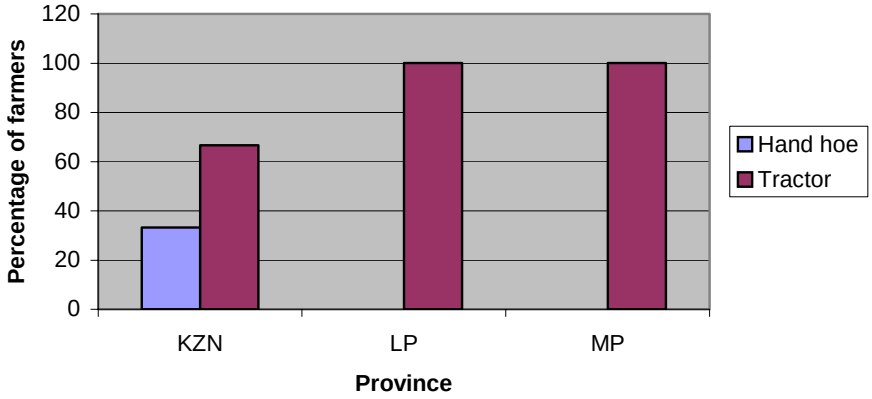

Figure 34. Methods of groundnut land tillage applied by subsistence farmers in South Africa during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

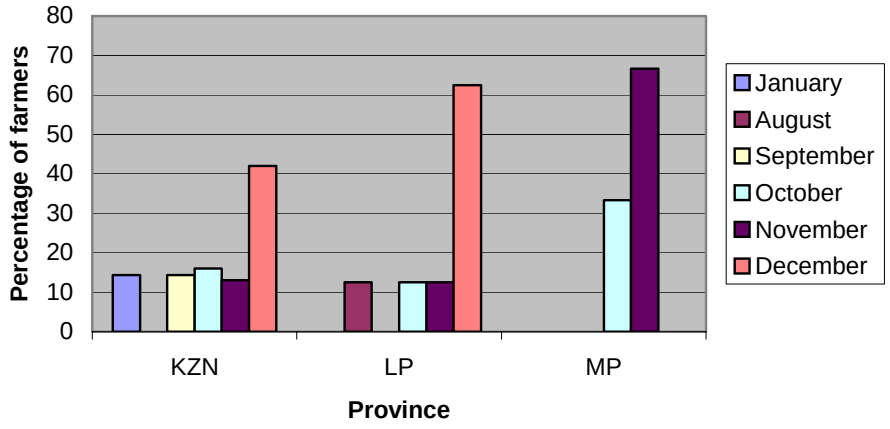

Figure 35. Groundnut planting dates recorded for subsistence farmers in South Africa during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

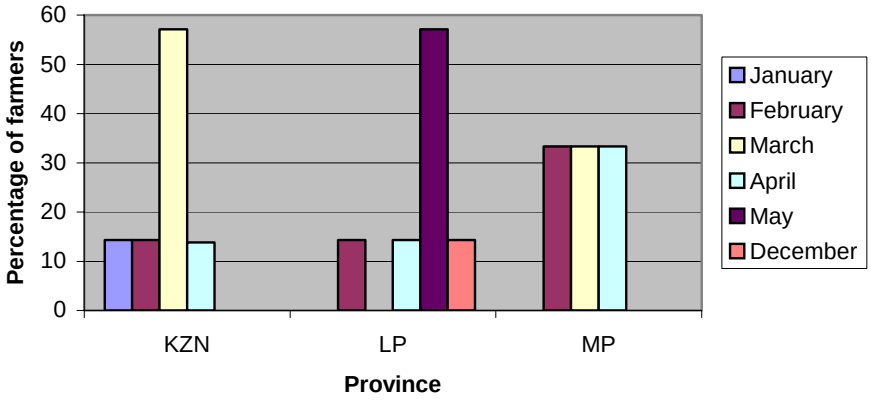

Figure 36. Groundnut harvesting dates recorded for subsistence farmers in South Africa during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

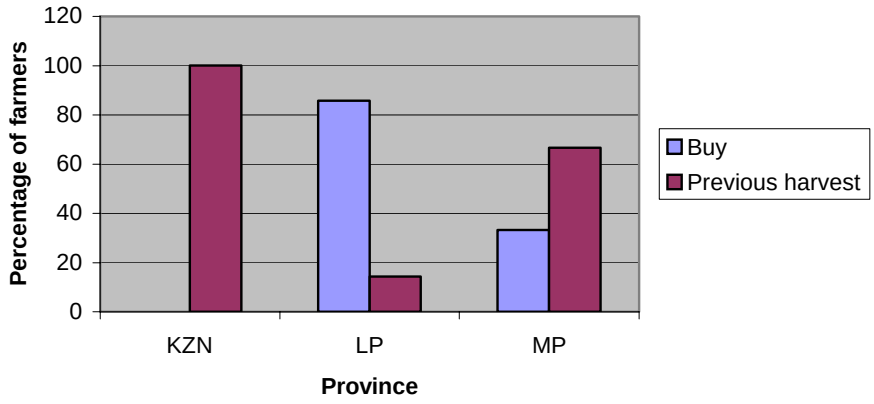

Figure 37. Source of groundnut seed planted by subsistence farmers in South Africa during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

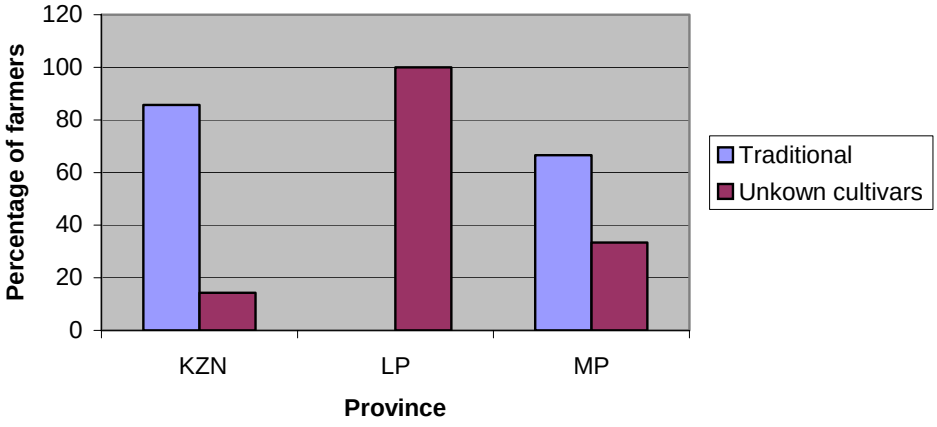

Figure 38. Groundnut cultivars planted by subsistence farmers in South Africa surveyed during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

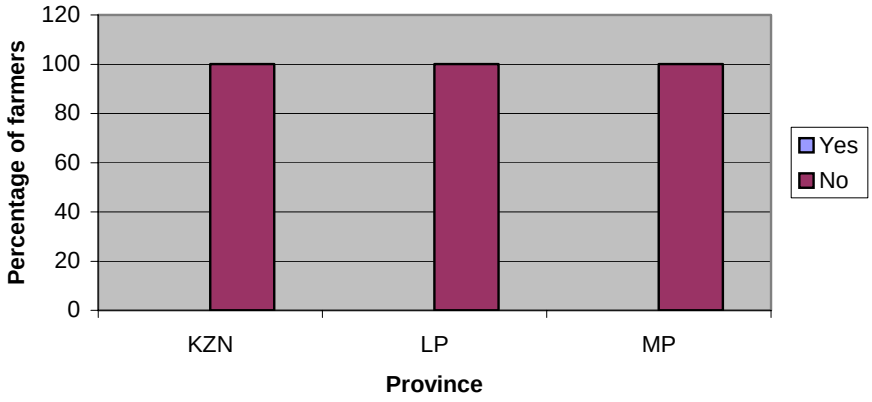

Figure 39. Use of pesticides/fungicides by subsistence farmers in South Africa on groundnuts during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

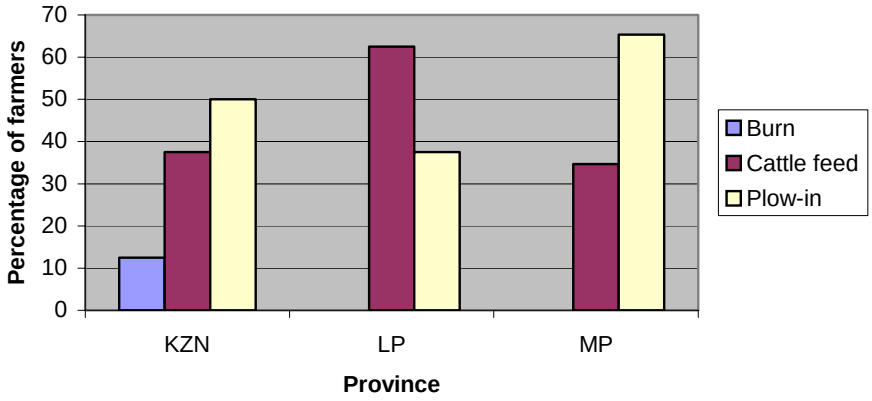

Figure 40. Method of crop residue disposal followed by subsistence in South Africa farmers during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

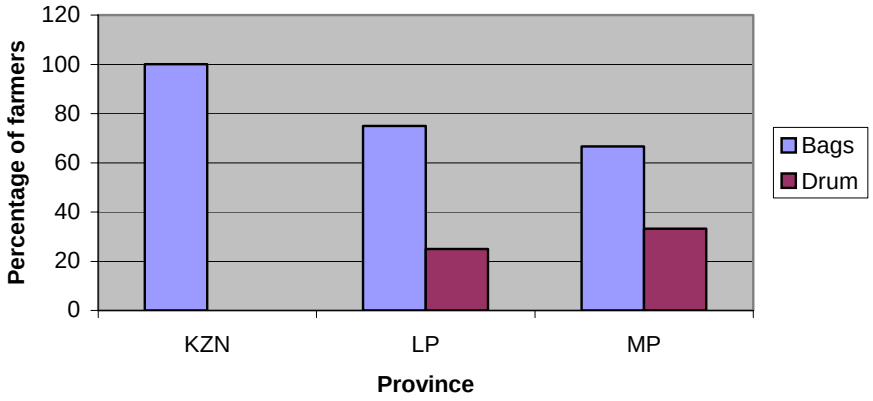

Figure 41. Method of grain storage practiced by subsistence farmers in South Africa during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

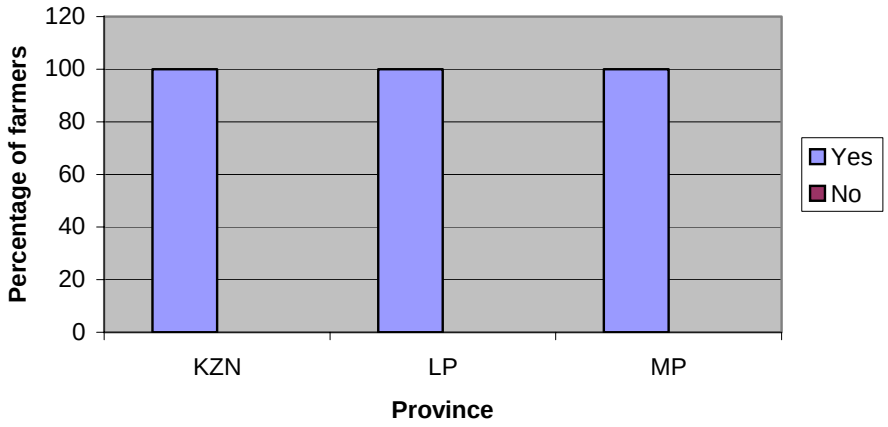

Figure 42. Subsistence farmers in South Africa that sorted ("Yes) or did not sort ("No") their groundnut grain after shelling during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

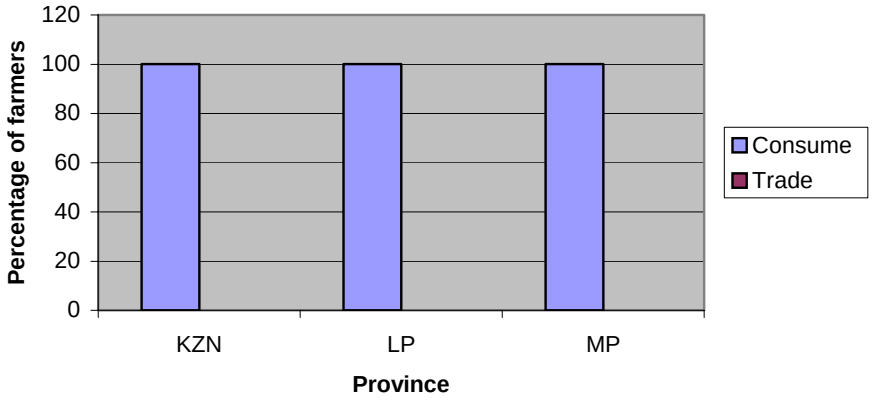

Figure 43. Intended uses of groundnuts harvested by subsistence farmers in South Africa during 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

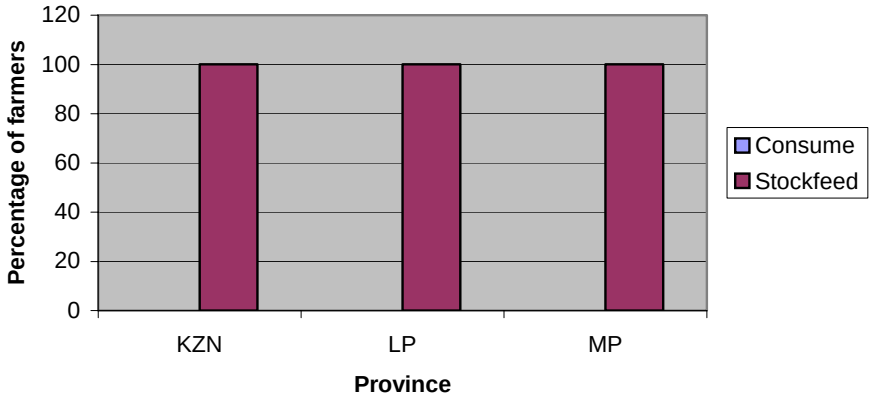

Figure 44. Uses of mouldy groundnuts harvested by subsistence farmers in South Africa during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

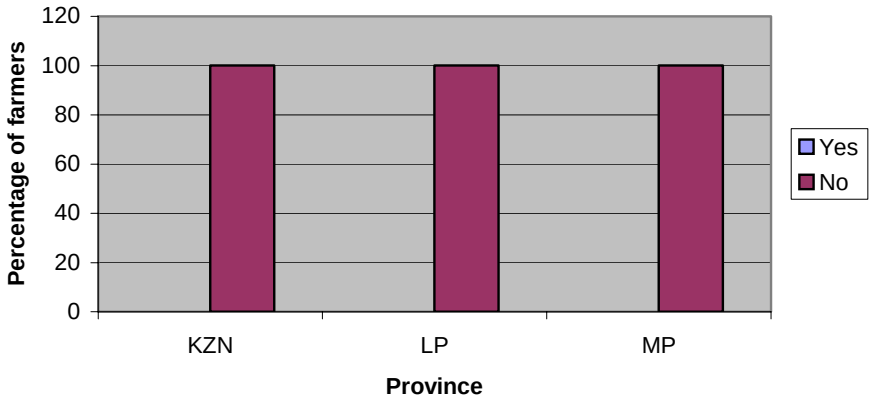

Figure 45. Human consumption of mouldy grain harvested by subsistence farmers in South Africa during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

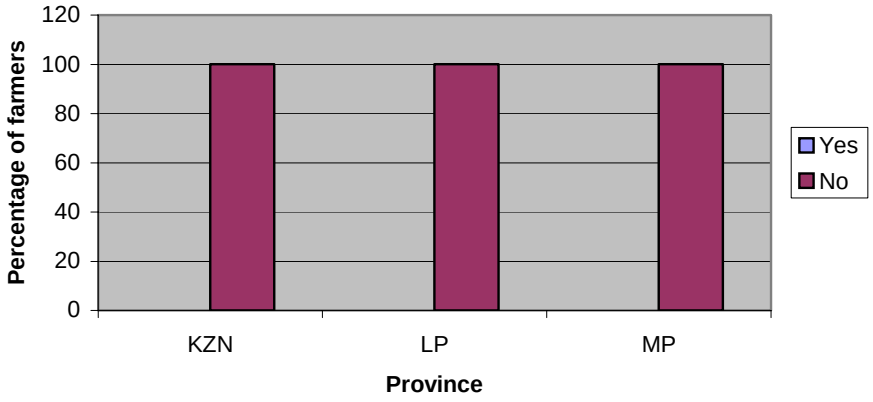

Figure 46. Level of mycotoxin awareness among subsistence farmers in South Africa during the 2006 survey season. KZN = KwaZulu-Natal, LP = Limpopo, and MP = Mpumalanga province.

Chapter 3

Distribution of *Fusarium* species, fumonisins and aflatoxins in maize in subsistence farming systems in South Africa

ABSTRACT

Fusarium verticillioides and *Aspergillus flavus* produce fumonisins and aflatoxins, respectively, in maize kernels. Fumonisins have been correlated with high incidence of human oesophageal cancer, while aflatoxins are mycotoxins with cancer-inducing properties in humans and animals. In this study, maize samples were collected in the Eastern Cape (EC), KwaZulu-Natal (KZN), Limpopo (LP), and Mpumalanga (MP) provinces of South Africa during the 2006 and 2007 planting seasons. The incidence of *Fusarium* spp. in maize was determined by plating out 200 kernels per collection site on *Fusarium* selective medium. Fumonisin-producing *Fusarium* spp. were also quantified by real-time PCR (TaqMan). Fumonisin and aflatoxin levels were determined using ELISA. *Fusarium verticillioides* was the most common *Fusarium* sp. in maize followed by *F. subglutinans* and *F. proliferatum.* Fumonisin contamination levels ranged from 0-21.8 ppm and aflatoxin levels ranged from 0-49 ppb, depending on the region where samples were collected. Regression analyses showed a positive correlation between fumonisin-producing *Fusarium* spp. when determined by real-time PCR and fumonisin concentration (r^2=0.867) in 2007. Samples from northern KZN, Venda and Mokopane districts in LP, Lusikisiki and Engcobo districts in EC, and KwaMhlanga district in MP contained levels of fumonisins that were far in excess of the maximum levels set by the Food and Drug Administration (FDA) and the European Union (EU). Samples from northern KZN also contained levels of aflatoxins that were far in excess of the maximum levels set by the FDA, EU and the National Department of Health in South Africa.

INTRODUCTION

Fusarium verticillioides (Sacc.) Nirenberg and *F. proliferatum* (Matsushima) Nuremberg according to Nelson *et al*. 1983 and Leslie and Summerell 2006, produce toxigenic secondary metabolites called fumonisins in maize (*Zea mays* L.) (Glenn, 2007). Fumonisins have been associated with high rates of human oesophageal cancer worldwide (Sydenham *et al*., 1990; Rheeder *et al*., 1992; Chu and Li, 1994; Yoshizawa *et al*., 1994) and with incidences of neural tube defects in babies of mothers consuming fumonisin-contaminated maize (Missmer *et al*., 2006). Fumonisins also cause equine leukoencephalomalacia (ELEM), a neurotoxin disease of horses (*Equus caballus* L.), donkeys (*Equus asinus* L.) and rabbits (*Oryctolagus cuniculus* L.) (Marasas *et al*., 1988; Kellerman *et al*., 1990), and porcine pulmonary oedema in pigs (*Sus scrofa domestica* L.) (Kriek *et al*., 1981; Harrison *et al*., 1990).

Subsistence farmers, through their cropping systems, may be maintaining an extensive diversity of *Fusarium* spp. associated with maize and other cereals, either as pathogens or symbionts (Leslie, 2005). Of these, *F. verticillioides* can constitute up to 95% of all *Fusarium* species recovered in fields in several African countries (Leslie, 2005). *Fusarium verticillioides* is a ubiquitous facultative endophyte of maize occurring in most maize-producing countries of the world (Marasas *et al*., 1979a; 1979b; Rheeder *et al*., 1995; Galperin *et al*., 2003). It colonises leaves, stems, roots and kernels systemically (Miller, 2001), and has been associated with maize diseases at all stages of plant development (Munkvold and Desjardins, 1997). Other *Fusarium* spp. associated with maize include *F. equiseti* (Corda) Sacc., *F. graminearum* Schwabe, *Fusarium oxysporum* Schlechtend, Emend, Snyder & Hansen, *Fusarium semitectum* Berk. & Ravenel, and *F. subglutinans* (Wollenw. & Reinking) P.E. Nelson, Toussoun & Marasas (Summerell *et al*., 2003).

Fumonisins are not the only mycotoxins associated with maize produced in rural areas of South Africa. Aflatoxin contamination of crops poses a serious health concern because of its known toxicity, mutagenicity and carcinogenicity (Deiner *et al*., 1987). Aflatoxins are produced by *Aspergillus flavus* Link ex Fr, *Aspergillus parasiticus* Speare, *Aspergillus nomius* Kurtzman, Horn & Hesseltine (Gourama and Bullerman, 1995) and *Aspergillus tamarii* Kita (Goto *et al*., 1996). *Aspergillus flavus* and *A.*

parasiticus are the primary aflatoxin producers in maize and groundnut (*Arachis hypogaea* L.). While fumonisins are produced primarily in the field (Headrick *et al*., 1990; Bush *et al*., 2004), aflatoxin contamination occurs both in the field, and during drying, storage and processing of the crop (Deiner *et al*., 1987). Aflatoxin B_1 is the most potent aflatoxin and is carcinogenic to humans. It is classified as a Group 1 carcinogen (www.who.int), and its potency increases substantially in carriers of the hepatitis B virus (Henry *et al*., 2001). Aflatoxins also cause oxidative stress, liver necrosis, haemorrhage and death in broiler chickens (*Gullus domesticus* L.), pigs and cattle (*Bos primigenius* spp. L.) when ingested through animal feed (Sargeant *et al*., 1961; Marasas, 1995; Eraslan *et al*., 2005; Osweiler, 2005).

No statutory maximum levels for fumonisins in maize have been set in South Africa. The commercial grain grading procedures as defined by Act 112 of 1990 are applied only to reduce the risk of mycotoxin contamination of food (www.nda.agric.za). The Food and Drug Administration (FDA) in the USA has set maximum levels of fumonisin in maize intended for direct human consumption at 2 parts per million (ppm) (www.cfsan.fda.gov), while the European Union has a set maximum levels of fumonisin in maize intended for direct human consumption at 1 ppm (www.mycotoxin.de). In South Africa, Act No. 54 of 1972, states that all foodstuffs containing more than 10 parts per billion (ppb) aflatoxin, of which aflatoxin B_1 should not be more than 5 ppb, are regarded as contaminated, impure or decayed (www.doh.gov.za).

Since maize is a staple food in rural areas and the average daily intake by subsistence farmers is approximately 400 g (Shephard *et al*., 2007), *Fusarium*-infected food might result in increased exposure to mycotoxins compared to consumption of non-mouldy grain. Mycotoxicoses has been associated with fumonisin-contaminated maize in the Butterworth and Kentani districts in the Transkei region of South Africa (Marasas *et al*., 1988; Rheeder *et al*., 1992). Very little, however, is known about the occurrence of fumonisins and aflatoxins on maize in other rural areas of the country. The aim of this study, therefore, was to determine the levels of *Fusarium* spp., fumonisin and aflatoxin mycotoxins in maize in all subsistence farming systems in South Africa.

MATERIALS AND METHODS

Field sampling:

Maize samples were collected from selected subsistence farming localities in the Eastern Cape (EC), KwaZulu-Natal (KZN), Limpopo (LP), and Mpumalanga (MP) provinces of South Africa (Appendix B). In total, 201 and 124 maize samples, each totalling approximately 1.5 kg, were collected in the 2006 and 2007 planting seasons, respectively. Fewer samples were collected in 2007 compared to 2006 because farmers did not plant maize in 2007 planting season possibly due to late rainfall during that year. To compensate the farmers and encourage them to participate in the study, grade 1 grain purchased from Senwes Co-op silos in Potchefstroom was exchanged with subsistence farmers' grain. The collected samples were placed in cloth bags to prevent condensation that might promote fungal growth. All samples were properly labelled with the source or locality of the sample, cultivar, farmer and date of collection (Appendix B). Global Positioning System (GPS) coordinates were recorded at each sampling point. Shelled maize or maize ears were collected and stored in a cold room at 6^{o}C and 45% relative humidity until assayed.

Isolation and enumeration of *Fusarium* spp.:

Maize kernels from each locality were used for the identification and quantification of *Fusarium* spp. All kernels were surface-sterilised by dipping them once in 70% ethanol, soaking them for 3 minutes in 1.6% sodium hypo-chlorite (NaOCl) and rinsing them three times in sterile distilled water. The kernels were then plated on *Fusarium* selective medium (Van Wyk *et al*., 1986) in Petri dishes. Two hundred seeds were plated out for each sample, with four kernels being placed in each Petri dish. After 7 days of incubation at 25^{o}C, developing *Fusarium* colonies were identified morphologically to species level according to Nelson *et al*. (1983) and Leslie and Summerell (2006).

Quantitative detection of fumonisin producing-*Fusarium* spp.:

Maize samples collected in EC, KZN, LP, and MP in the 2006 and 2007 seasons were used for real-time PCR analysis of fumonisin-producing *Fusarium* spp. The samples were ground into a fine powder using a Cyclotech sample mill (Foss Tecator, Hoganas, Sweden). DNA was then isolated from 20 mg of each sample using the

Qiagen DNeasy Plant Mini kit (Cat 69106) by Dr Cees Waalwijk at Plant Research International, Wageningen, The Netherlands. TaqMan detection of fumonisin-producing *Fusarium* spp. was also performed by Dr Cees Waalwijk using primers and probes designed for the polyketide synthase gene *fum1* (Waalwijk *et al*., 2008). Real-time PCR was performed using a MicroAmp Optical 96-well reaction plate and MicroAmp Optical Caps (Applied Biosystems, Foster City, USA). An ABI Prism 7700 Sequence Detection System (Applied Biosystems) was used to perform the PCR and assess fluorescence. Each amplification reaction consisted of 2 µl of DNA preparations, 1× real-time PCR buffer (Applied Biosystems), 5 mM $MgCl_2$, 83 nM of the FAM-labeled FUM-probe1, 1.5 U of Hot Gold star DNA polymerase (Eurogentec, Belgium) and 333 nM of forward and reverse primer for the target DNA (Taqfum-2F in combination with Vpgen-3R, VertFum-3R or ProFum-3R). As an internal control, 100 pg of Potato Leaf Roll Virus (PLRV) DNA, forward primer PLRV-F and reverse primer PLRV-R (both at333 nM) were included in the reaction along with 83 nM of the VIC-labeled PLRV probe (Waalwijk *et al*., 2004; Waalwijk *et al*., 2008).

Fumonisin and aflatoxin analysis:

I performed ELISA analysis on 5-g sub-samples taken from the 250-g milled maize samples. The samples were first ground into very fine powder using a Cyclotech sample mill (Foss Tecator). The fumonisins were extracted by mixing the 5-g sub-sample with 25 ml of 70% methanol solution, shaken vigorously for 3 minutes by hand and filtered through a Whatman® #1 (Schleicher & Schuell) filter paper, and collecting at least 5 ml of the filtrate for ELISA analysis. Fumonisin levels were quantified using the Veratox® quantitative fumonisin 5/10 test kit (Neogen Corp, Lansing, MI, USA) according to the manufacturer's instructions. The total fumonisin (FB_1+FB_2+FB_3) levels between 0-6 ppm were accurately determined with this kit, while concentrations above 6 ppm were extrapolated from the standard curve. Aflatoxins were quantified using Veratox® quantitative aflatoxin test kit (Neogen Corp) according to the manufacturer's instructions. The total aflatoxin (B_1+B_2+G_1+G_2) levels between 0-50 ppb were accurately determined using this kit. The Veratox® fumonisin 5/10 test kit and the Veratox® aflatoxin test kit are competitive direct Enzyme-Linked Immunosorbent Assays (CD-ELISA) techniques in a microwell format.

In the ELISA test, the free fumonisin or aflatoxin in the maize samples and controls competed with the enzyme-labelled fumonisin or aflatoxin (conjugate) for antibody binding sites. After a wash step with distilled water, a substrate that reacted with the bound conjugate to produce a blue colour was added. An intense blue colour indicated low fumonisin or aflatoxin concentrations, while a less intense blue colour indicated high fumonisin or aflatoxin concentrations. The test was read in a microwell reader to produce optical densities. In each run, optical densities of a series of standards were included and unknowns were plotted on the standard curve to calculate the exact fumonisin concentration in ppm and the exact aflatoxin concentration in ppb (Neogen Corp). Optical density was scanned with a micro-plate reader (MR 250, Pynatech Laboratories, Chantilly, VA, USA) with a 650 nm absorbency filter. Each analysis was repeated three times to determine reproducibility of the results.

Relationship between *Fusarium* spp. and fumonisin levels:

The relationships between fumonisin levels by CD-ELISA and TaqMan-quantified fumonisin-producing *Fusarium* spp. were determined using simple linear regression on Statgraphics 5 Plus® for the 2006, 2007, and combined seasons. The correlation was done to determine whether the TaqMan technique was a reliable method in quantifying fumonisin-producing *Fusarium* spp. in maize.

Climatic data:

Climatic data for 2006 and 2007 was collected from weather stations (www.arc-iscw.agric.za) situated in several districts in the EC, KZN, LP and MP provinces. These weather stations were not necessarily in the rural areas where maize samples were collected, but were closest to the collection sites. Monthly rainfall and temperature data was obtained from the website of the Agricultural Research Council's Institute of Soil Climate and Water. Climatic data considered was between October of the previous year, and May in the year that maize samples were collected. These specific dates were chosen, as they represent the growing season of maize in South Africa.

RESULTS

Identification and enumeration of *Fusarium* spp.:

Fusarium verticillioides was the dominant *Fusarium* spp. isolated from maize kernels collected from subsistence farmer fields in the KZN and LP provinces, both in 2006 and in 2007 (Table 1). *Fusarium subglutinans*, however, was the dominant species in MP and in the EC in both seasons. Colonisation of maize with *F. verticillioides* was highest in the Venda and Giyani districts of LP, and in the northern KZN districts of Pongola, Makhanisi, Jozini, Vryheid, and Ladysmith (Appendix B). *Fusarium subglutinans* was the dominant species in maize collected from Daggakraal, Balfour and Lieden in MP and in several districts in the EC (Appendix B). Infection by *F. proliferatum* was limited in all the provinces apart from areas in the northern part of KZN.

In all provinces, maize at certain collection sites was free of *Fusarium* spp. (Appendix B). These areas appeared to be mostly situated in the eastern MP and in the EC for *F. verticillioides* (Fig. 1) and in the LP, extreme northern, western and southern KZN, and the EC for *F. subglutinans* (Fig. 2). Several localities in the EC appeared to be affected very little or not at all by any of the *Fusarium* spp. (Figs. 1-3; Appendix B). These include the areas around Idutywa, Libode, Qunu and Sengqu. At most collection sites, kernels would be affected by either *F. verticillioides* or *F. subglutinans* (Appendix B). Only in Matibiti and Mbuzini areas in MP, Ulundi, Port Shepstone and Umzimkhulu areas in KZN, Butterworth, Mqanduli, Kentani, Cofimvaba and Queenstown areas in the EC, did *F. verticillioides* and *F. subglutinans* occurrence appeared to be similar. The distribution and severity of *F. verticillioides* in maize differed between the 2006 and 2007 planting seasons in Mokopane in LP, Ermelo in MP, Ladysmith and Port Shepstone in KZN and Lusikisiki in the EC (Fig. 1; Appendix B). In 2006, the incidence of *F. verticillioides* was substantially higher in both the LP and the EC than in 2007 (Fig. 1; Appendix B). The incidence of *F. proliferatum* infection was below 5% in LP, MP and EC, while in northern KZN high levels in the region of 10-30% infection occurred, except in Highflats where 62% *F. proliferatum* infection occurred (Fig. 3; Appendix B).

Quantitative detection of fumonisin-producing *Fusarium* spp.:

Fumonisin-producing *Fusarium* spp. were most common in maize produced in KZN both in the 2006 and 2007 planting seasons, with the average amount of *fum1* gene DNA more than three times more that of in any of the other provinces (Table 1). The highest concentration of *Fusarium* DNA containing the *fum1* gene was detected in maize kernels collected in the KZN districts of Jozini, Manguzi and Mbazwane in the far northern KZN (Fig. 4; Appendix B). In 2006, maize infected with fumonisin-producing *Fusarium* spp. was second most in MP and in LP and the EC in 2007 (Table 1). Even within the same district, colonisation of maize kernels with fumonisin-producing *Fusarium* spp. differed considerably, ranging from 0 to 1965.5 pg *Fusarium* DNA/mg in Jozini district in KZN province, for instance (Appendix B).

The incidence of the fumonisin-producing Fusaria was more in the 2006 season than in the 2007 season (Fig. 4; Appendix B). Sampling localities yielding *fum1* DNA concentrations of more than 150 pg *Fusarium* DNA/mg also differed substantially between seasons (Appendix B). In 2006, the highest levels of fumonisin-producing *Fusarium* spp. were found in central KZN, while in 2007 it was found in the EC. In most localities in the MP, southern KZN and EC, maize samples free of fumonisin-producers were sampled in 2006, while fumonisin-producing *Fusarium* spp. were absent in maize in many localities in LP, MP and EC in the 2007 season (Fig. 4; Appendix B).

Fumonisin and aflatoxin analysis:

Fumonisin contamination was highest in KZN where infection levels ranged from 0-12 ppm and 0-21.8 ppm, with averages of 1.9 ppm and 1.4 ppm, during the 2006 and 2007 planting seasons, respectively (Table 1). Contamination was particularly severe in samples collected from locations in Mbazwane, Jozini, Pongola, Vryheid and Manguzi in northern parts of the province where fumonisin levels were far in excess of the maximum levels set by the FDA and EU (Fig. 5; Appendix B). The LP province had the second highest fumonisin levels, ranging from 0-6.4 ppm and 0-4.8 ppm during the 2006 and 2007 planting seasons, respectively, with averages of 0.7 and 0.8 ppm. Fumonisin levels in the EC ranged from 0-2.3 ppm and 0-9 ppm for the 2006 and 2007 planting seasons, respectively, with averages of 0.2 ppm and 0.8 ppm. In the LP, locations in Venda and Mokopane districts were most severely affected by

fumonisin production, while locations in the Lusikisiki and Engcobo districts in the EC were worst contaminated with the toxin. Fumonisin contamination was low in MP, with a range of 0-5.3 ppm and 0-0.5 ppm during the 2006 and 2007 planting seasons, respectively, with means of 0.5 ppm and 0.2 ppm. Only the KwaMhlanga district had high fumonisin levels (Fig. 5; Appendix B).

Aflatoxin contamination of maize was higher in the 2006 planting season than in 2007 in all provinces (Table 1). In KZN, aflatoxin levels ranged from 0-29.1 ppb and 0-49 ppb during the 2006 and 2007 planting seasons, respectively (Figs. 6 and 7; Appendix B), while the mean aflatoxin levels were 9 ppb and 1.2 ppb during the same seasons. The high level of aflatoxins produced in maize in locations around Port Shepstone, Highflats, Izingolweni, Ndwedwe and Underberg, in southern KZN and in the Jozini district in northern KZN far exceeded the maximum levels set by the FDA, EU, and the Department of Health in South Africa. The LP province had aflatoxins ranging from 10-16 ppb and 0-8.3 ppb during the 2006 and 2007 planting seasons, respectively (Figs. 6 and 7; Appendix B). Their means of 12.5 ppb in 2006 were the highest in all provinces. In 2007, the mean aflatoxin level in LP was 0.7 ppb. Aflatoxin levels in MP ranged from 3.9-19 ppb (Figs. 6 and 7; Appendix B) with means of 9 ppb and 0.1 ppb during the 2006 and 2007 seasons, respectively, while aflatoxin contamination in EC ranged from 0-12 ppb and 0-6.2 ppb with means of 4 ppb and 1.3 ppb during the 2006 and 2007 planting seasons, respectively (Figs. 6 and 7; Appendix B). There were not enough seeds from the maize samples to enumerate *Aspergillus* spp. and as a result, enumeration of these species was not performed.

Relationship between *Fusarium* spp. and fumonisin levels:

A significant positive correlation was obtained between fumonisin-producing *Fusarium* spp. as quantified by qPCR and fumonisin levels for the 2006 (r^2=0.553, *P*-value = 0.000) (Fig. 8), 2007 (r^2=0.866, *P*-value = 0.000) (Fig. 9), and combined seasons (r^2=0.677, *P*-value = 0.000) (Fig. 10), respectively. The results show that there was a good correlation between fumonisin-producing *Fusarium* spp. and fumonisin levels in 2007 compared to 2006, possibly due to a combination of climatic conditions and agricultural practices followed by different subsistence farmers. A poor correlation, however, was obtained between *F. verticillioides* quantified by plating out, and fumonisin-producing *Fusarium* spp. determined by qPCR (r^2=0.14,

P-value = 0.000). The correlation between the combined occurrence of *F. verticillioides* and *F. proliferatum* was also poor and fumonisin levels (r^2=0.075, *P*-value = 0.000).

Climatic data:

Temperatures in the EC ranged from 4.9-26^{o}C in all districts except in Engcobo where maximum temperatures rose to above 30 ^{o}C in the 2006 planting season. In the 2007 planting season, temperatures ranged from 5.7-26.8 ^{o}C in all districts with the exception of Kentani that had a maximum temperature of 28 ^{o}C (Table 2). In KZN, the Bergville and Underberg districts reported the coolest temperatures with ranges of 8.8-21.9 ^{o}C and 11.8-24.3 ^{o}C, respectively. The coastal district of Port Shepstone in southern KZN, the central and northern KZN, and all the districts in LP reported maximum temperatures above 29^{o}C in the 2007 season (Table 2). The coolest districts in MP were Balfour, Daggakraal, Emersfoort and Ermelo, with temperatures ranging from 9-24.5 ^{o}C, while Boshofontein, Ikhwezi, KwaMhlanga, Mbuzini and Schuzendal districts reported maximum temperatures of 27 ^{o}C and above in both seasons. Temperatures in 2007 were higher than in 2006 in all the provinces (Table 2).

Rainfall data in LP, MP and KZN provinces varied notably among regions. The LP was the driest over both seasons, with the Venda district recording the highest rainfall. In MP, Boshofontein, Phiva, Schoemansdal and Schuzendal received more than 200 mm rain during the 2006 planting season, while most of the districts, with the exception of Aphiring and Matibiti, received less than 100 mm of rain in 2007 (Table 2). The Ladysmith, Underberg and Vryheid districts in KZN received more than 100 mm of rain, while the rest of KZN districts received less than 100 mm during both planting seasons. All the districts in the EC recorded below 100 mm of rain during both seasons (Table 2).

DISCUSSION

Fusarium verticillioides was the most common *Fusarium* sp. associated with maize produced in rural areas in South Africa, followed by *F. subglutinans.* This is in agreement with the findings of Rheeder *et al*. (1993) reported more than a decade ago. The low levels of *F. verticillioides* found in the EC could be a result of the cooler

climatic conditions in the 2006 planting season that may have affected fungal growth. In this province, as in MP, maize kernels were primarily colonised by *F. subglutinans*, a species more adapted to temperate climates (Francis and Burgess, 1975; Marasas *et al*., 1979a). Subsistence farmers in these areas, therefore, might be at risk to moniliformin, which is known to be produced by *F. subglutinans* (Thiel *et al*., 1982). In contrast to the cooler districts in the EC, particularly high fumonisin levels were found in the warm and dry districts of Engcobo and Lusikisiki in EC. It is, however, in the districts of Butterworth and Kentani that high levels of oesophageal cancer have been linked to fumonisin in maize before by Rheeder *et al*. (1992) and Makaula *et al*. (1996).

The high levels of fumonisins in locations of Venda and Mokopane districts in LP, Engcobo district in EC, KwaMhlanga district in MP and Mbazwane, Jozini, Pongola and Manguzi districts in northern KZN, and of aflatoxin in Port Shepstone in southern KZN and Mbazwane, Jozini and Manguzi districts in northern KZN is possibly due to temperatures above 25^{o}C and low rainfall during the two planting seasons. These conditions induce plant stress, thereby predisposing plants to infection by *Fusarium* and *Aspergillus* spp. and concomitant mycotoxin production (Thomson and Henke, 2000). In addition, farmers in northern KZN were planting open pollinated maize seed retained from the previous harvest that may result in a high risk of systemic infection by *F. verticillioides* (Foley, 1962; Munkvold and Carlton, 1997) and concomitant fumonisin production in maize.

In this study, real-time PCR using the TaqMan technique was demonstrated as a reliable method to quantify fumonisin-producing *Fusarium* spp. in maize samples in the 2007 planting season when compared to plating out maize seeds on medium. This shows that the real-time PCR can be used to screen fumonisin-contaminated maize samples by technically skilled personnel, however, it is not an appropriate tool in subsistence agriculture because it requires technical skills and expensive equipment. A poor positive correlation was obtained between the incidence of *F. verticillioides* in plated maize kernels and fumonisin levels, which supports the findings of Rheeder *et al*. (1995) and Kedera *et al*. (1998). A weak, significant positive correlation between the contamination with fumonisin and the occurrence of *F. verticillioides* and *F. proliferatum* in maize kernels indicated that infection of maize kernels with *Fusarium*

spp. did not always result in fumonisin production. Other factors such as growing hybrids outside of their range of adaptation, insect damage, drought and temperature stress significantly affect fumonisin production (Doko *et al*., 1995; Warfield and Gilchrist, 1999; Bankole and Adebanjo, 2003).

The presence of mycotoxins in subsistence farmer crops has previously been reported from maize planted in the EC only (Marasas *et al*. 1988; Sydenham *et al*., 1990; Rheeder *et al*., 1992 and Makaula *et al*., 1996; Shephard *et al*., 2005; 2007). This study, however, showed that mycotoxins occur widely in maize throughout the subsistence farming areas of South Africa. Since maize is cultivated as a major staple food crop in most rural areas in South Africa, the occurrence of high levels of mycotoxins, and their potential toxicity to humans and animals, is a major concern to the health and well-being of these rural communities. In this study, aflatoxin levels higher than the recommended maximum levels of 10 ppb in maize in South Africa are reported for the first time. Low to medium aflatoxin levels ranging from 0.6 to 9 ppb in the 2006 planting season in LP province suggested that subsistence farmers could be exposed to moderate levels of aflatoxin over time, thereby increasing their susceptibility to cancer related to aflatoxin exposure.

Measures to reduce the burden of mycotoxin contamination in rural areas of South Africa are required. These measures should include disease management practices such as avoiding stress, planting adapted maize varieties, reducing insect and bird damage, good harvest and storage procedures, discarding mouldy kernels, farmer education, and social and health considerations of communities. Disease management strategies could include planting early maturing maize hybrids to escape growth-limiting conditions such as hot, dry summers that contribute to aflatoxin production (Betrán and Isakeit, 2004). Local maize varieties should also be planted, as the overall environmental adaptation of maize hybrids appears to have importance in minimising aflatoxin contamination (Betrán and Isakeit, 2004). Identification of critical control points and the use of hazard analysis critical control points (HACCP) in a "farm to fork" system need to be implemented. Awareness programs targeted at extension officers are necessary for the education of farmers. This is particularly important in areas where communities rely on maize as their primary source of food and income irrespective of its quality.

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Table 1. Average percentage maize kernels infected by *Fusarium* spp. and the mycotoxins fumonisins and aflatoxins in four provinces in South Africa over two planting seasons using whole seed agar plate, qPCR and fumonisin and aflatoxin analysis by CD-ELISA

Province	2006 planting season						2007 planting season					
	F. vert	*F. prol*	*F. sub*	qPCR (pg DNA/mg)	Fum (ppm)	Afla (ppb)	*F. vert*	*F. prol*	*F. sub*	qPCR (pg DNA/mg)	Fum (ppm)	Afla (ppb)
LP	[a]27	0.7	0.8	7.8	0.6	7.2	21.6	1.8	0.2	47	0.8	0.7
	[b](0-92)	(0-3.5)	(0-4.50	(0-33)	(0-5.5)	(0.6-9)	(1-77)	(0-9)	(0-1.5)	(0-522.4)	(0-4.8)	(0-8.3)
MP	9.7	0.4	19.3	18.7	0.5	5.4	7.1	1.4	12.5	6.2	0.1	0.1
	(0-55)	(0-5)	(0-77)	(0-232.4)	(0-5.6)	(2.6-10.8)	(0-36.5)	(0-11.5)	(0-28.5)	(0-161.8)	(0-0.5)	(0-1.2)
KZN	32.4	1.5	14.6	65.6	1.9	6.9	28.7	4.8	0.9	147	1.4	1.2
	(0-99)	(0-62)	(0-98)	(0-1057)	(0-12)	(0.2-19)	(0-75)	(0-28)	(0-9)	(0-1965.5)	(0-21.8)	(0-50)
EC	5.8	0.5	13.9	3.32	0.2	2.7	8.4	1.9	12.5	44.6	0.8	1.3
	(0-43)	(0-12.5)	(0-69.5)	(0-62.4)	(0-2.3)	(0.8-7.3)	(0-42.5)	(0-13.5)	(0-56)	(0-558.6)	(0-9)	(0-6.2)

Abbreviations: LP = Limpopo province, MP = Mpumalanga province, KZN = KwaZulu-Natal, EC = Eastern Cape, F. vert = *F. verticillioides*, F. prol = *F. proliferatum*, F. sub = *F. subglutinans*, qPCR = quantitative (q) real-time PCR, Fum = fumonisin, Afla = Aflatoxin.

[a]The top row for each province indicate mean values in all provinces

[b]The bottom row for each province indicate the range in all provinces

Table 2. Temperature and rainfall data in four South African provinces where maize and groundnuts are produced, taken between October and May over 2 years (www.arc-iscw.agric.za)

2006 PLANTING SEASON					2007 PLANTING SEASON			
LOCALITY	Mean Rainfall mm	Mean Max Temp °C	Mean Min Temp °C	Mean Temp °C	Mean Rainfall mm	Mean Max Temp °C	Mean Min Temp °C	Mean Temp °C
EASTERN CAPE PROVINCE								
BIZANA	77.6	23.5	14.1	18.8	88.1	24.2	14.4	19.3
BUTTERWORTH	88	23.6	12.9	18.3	193	24.3	13.6	19
COFIMVABA	30.5	25.9	13.3	19.6	46.4	26.6	13.6	20.1
ELUNDINI	55.3	23.5	10.1	16.8	76.9	25.3	13.5	19.4
ENGCOBO	87.3	31.7	12.1	21.9	70.7	25	13.1	19.1
IDUTYWA	46.5	24.7	12.9	18.8	43.5	25.5	13.3	19.4
KENTANI	58.2	24.7	15	19.9	20.2	28	7.8	17.9
LIBODE	79	21.2	11.4	16.3	87.9	22.1	11.9	17
LUSIKISIKI	75.4	23.6	14.7	19.2	81.3	24.2	15.1	19.7
MALUTI	63.3	18.9	4.9	11.9	69.5	25.2	8.1	16.7
MQANDULI	66.8	22.3	12.3	17.3	71.9	23	12.7	17.9
MT FLETCHER	65.9	24.9	8.6	16.8	175	22.4	10.9	16.7
NGQELENI	71.4	22.9	13.2	18.1	62	16.2	5.7	11
QUEENSTOWN	72.7	24.7	11.7	18.2	75.6	26.4	12.8	19.6
QUNU	66.8	22.3	12.3	17.3	71.9	23	12.7	17.9
TSOLO	57.4	24.6	12.8	18.7	66.4	25.6	13	19.3
WHITTLESEA	44.4	25.4	11.2	18.3	49.6	26.8	11.6	19.2
KWAZULU-NATAL PROVINCE								
BERGVILLE	119	24.3	11.8	18.1	55.8	26.2	11.9	19.1
EMSELENI	79.4	29.7	18.9	24.3	78.4	29	20.9	25
ESHOWE	66.5	29.3	17.6	23.5	89.5	30.5	17.6	24.1
HIGHFLATS	51	29.9	15.3	22.6	65.5	29.1	14.9	22
JOZINI	N/A	N/A	N/A	N/A	61.9	30.3	19.2	24.8
LADYSMITH	142	25.8	14.2	20	99.5	28.3	15.5	21.9
MANGUZI	79.4	29.7	18.9	24.3	78.4	29	20.9	25
MBAZWANE	79.4	29.7	18.9	24.3	78.4	29	20.9	25
NDWEDWE	114	24.5	13.2	18.9	86.6	25.5	14.6	20.1
PONGOLA	57.1	28.6	17.3	23	57.4	29.5	17.6	23.6
PORT SHEPSTONE	53	25.8	17.5	21.7	53.9	26.5	17.8	22.2
ULUNDI	28.3	29.1	19	24.1	72.5	29.6	19	24.3
UMZIMKHULU	12.2	23	12.6	17.8	74.9	23.9	12.9	18.4
UNDERBERG	157	21.9	8.8	15.4	127	23.6	10	16.8
VRYHEID	102	25.8	12.3	19.1	107	27.1	12.1	19.6
LIMPOPO PROVINCE								
GIYANI	56.5	29.1	14.3	21.7	40	32.3	17.9	25.1
JANE FURSE	42.3	27.4	14.7	21.1	35.9	29.3	14.8	22.1
MOKOPANE	35.1	32.3	17.9	25.1	53	30.2	14.7	22.5
PIETERSBURG	42.3	27.4	14.7	21.1	35.9	29.3	14.8	22.1
VENDA	153	28.5	17.6	23.1	82.8	30.1	17.2	23.7

Table 2 (continued): Temperature and rainfall data in four South African provinces where maize and groundnuts are produced, taken between October and May over 2 years (www.arc-iscw.agric.za)

	2006 PLANTING SEASON				2007 PLANTING SEASON			
LOCALITY	Mean Rainfall mm	Mean Max Temp °C	Mean Min Temp °C	Mean Temp °C	Mean Rainfall mm	Mean Max Temp °C	Mean Min Temp °C	Mean Temp °C
MPUMALANGA PROVINCE								
APHIRING	N/A	N/A	N/A	N/A	130	22.1	12.2	17.2
BALFOUR	124	24.6	10.7	17.7	42.4	25.6	8.3	17
BOSHOFONTEIN	218	29.1	18.1	23.6	81.8	30.5	18.4	24.5
DAGGASKRAAL	89.5	24.5	10.3	17.4	59.8	27.2	13.2	20.2
DRIEFONTEIN	128	23.2	11.9	17.6	75.4	24.9	11.8	18.4
EMERSFOORT	89.5	24.5	10.3	17.4	59.8	27.2	13.2	20.2
ERMELO	89.5	24.5	9	16.8	15	25.2	7.7	16.5
GROOTVLEI	124	24.6	10.7	17.7	42.4	25.6	8.3	17
IKHWEZI	218	29.1	18.1	23.6	81.8	30.5	18.4	24.5
KWAMHLANGA	69.1	26.8	13.3	20.1	45.5	28.3	13.3	20.8
LIEDEN	89.5	24.5	9	16.8	15	25.2	7.7	16.5
MATIBITI	N/A	N/A	N/A	N/A	130	22.1	12.2	17.2
MBUZINI	75.8	30.9	19.7	25.3	39.9	31.8	18.6	25.2
PIET RITIEF	56.3	25.5	13.4	19.5	56	27.2	13.5	20.4
PHIVA	218	29.1	18.1	23.6	81.8	30.5	18.4	24.5
SCHOEMANSDAL	218	29.1	18.1	23.6	81.8	30.5	18.4	24.5
SCHUZENDAL	218	29.1	18.1	23.6	81.8	30.5	18.4	24.5

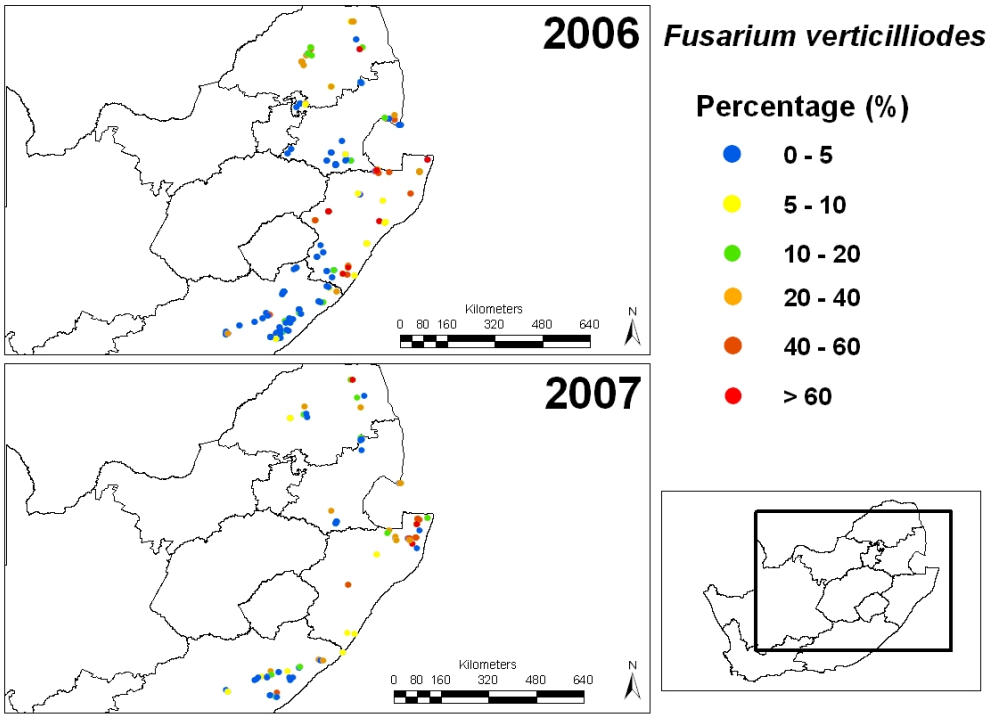

Figure 1. Distribution of *Fusarium verticillioides* in subsistence farmer maize kernels in South Africa during the 2006 and 2007 planting seasons.

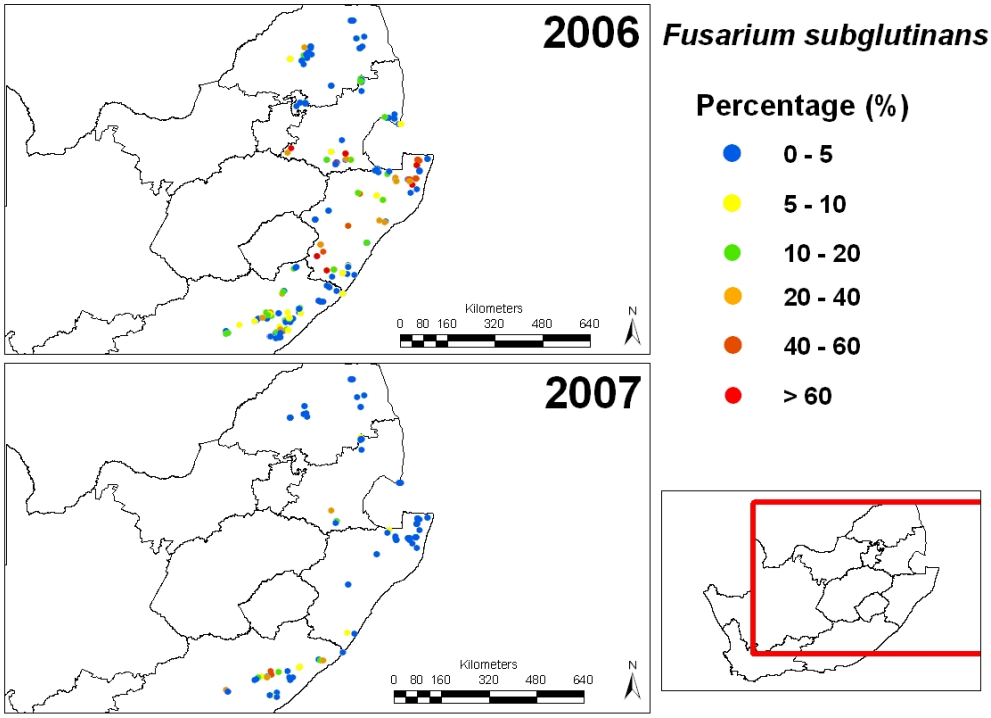

Figure 2. Distribution of *Fusarium subglutinans* in subsistence farmer maize kernels in South Africa during the 2006 and 2007 planting seasons.

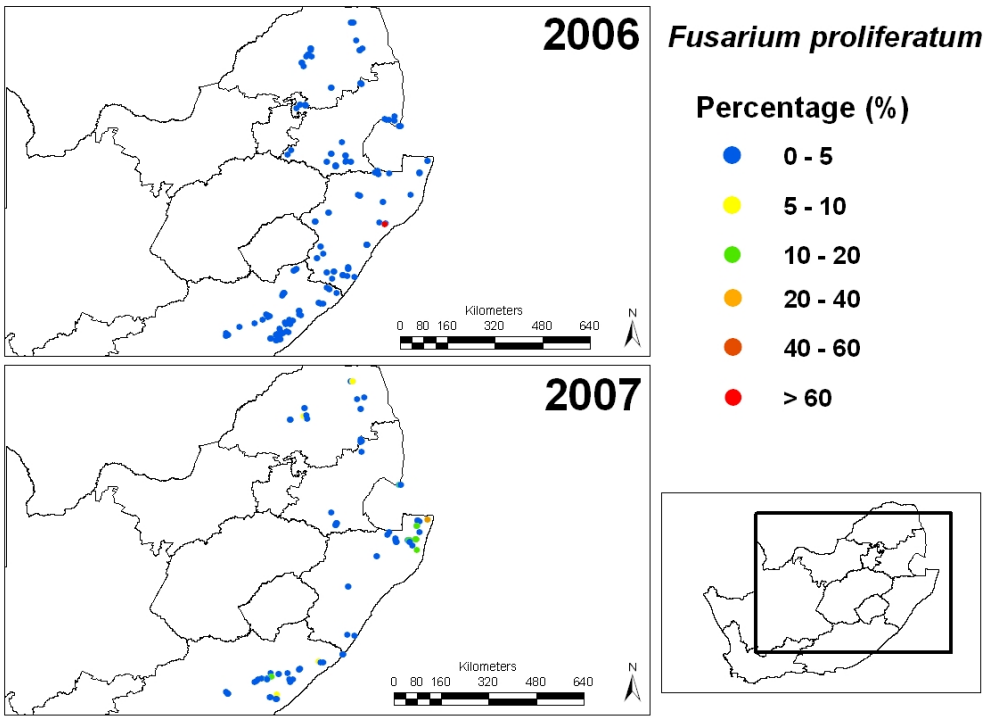

Figure 3. Distribution of *Fusarium proliferatum* in subsistence farmer maize kernels in South Africa during the 2006 and 2007 planting seasons.

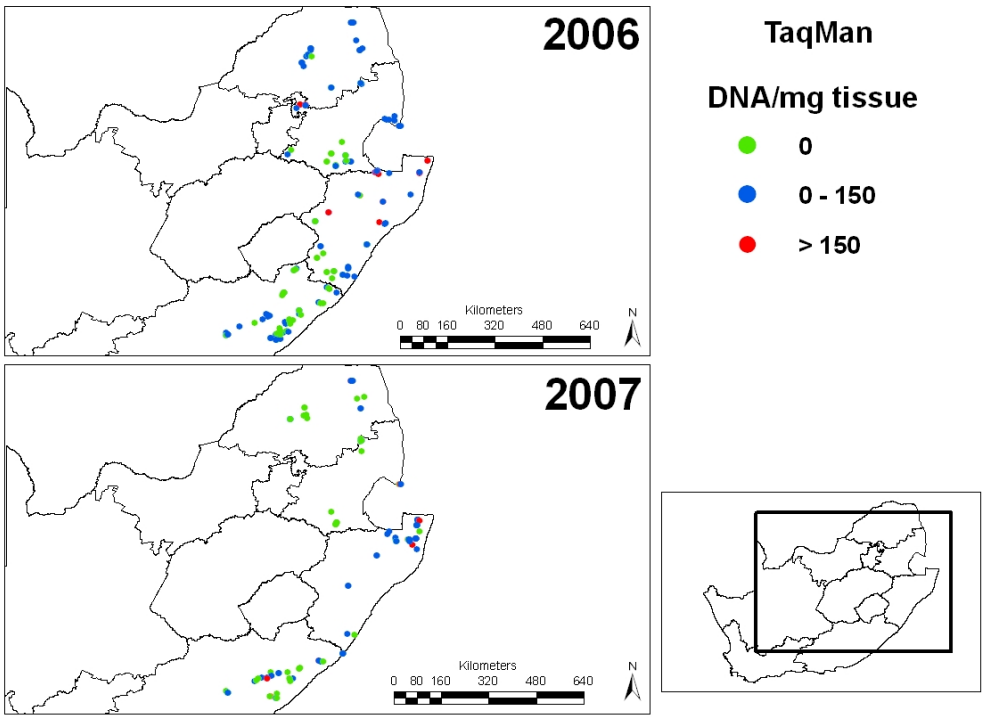

Figure 4. Distribution of fumonisin-producing *Fusarium* spp. in subsistence farmer maize kernels in South Africa during the 2006 and 2007 planting seasons, as analysed by means of real-time PCR.

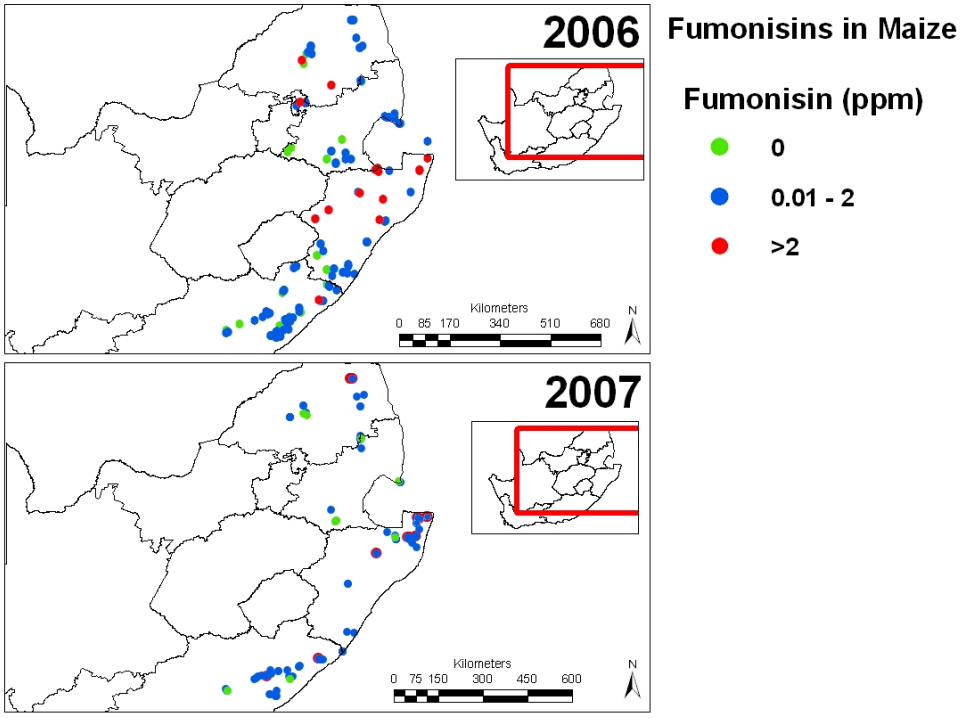

Figure 5. Distribution of fumonisins in subsistence farmer maize kernels in South Africa during the 2006 and 2007 planting seasons.

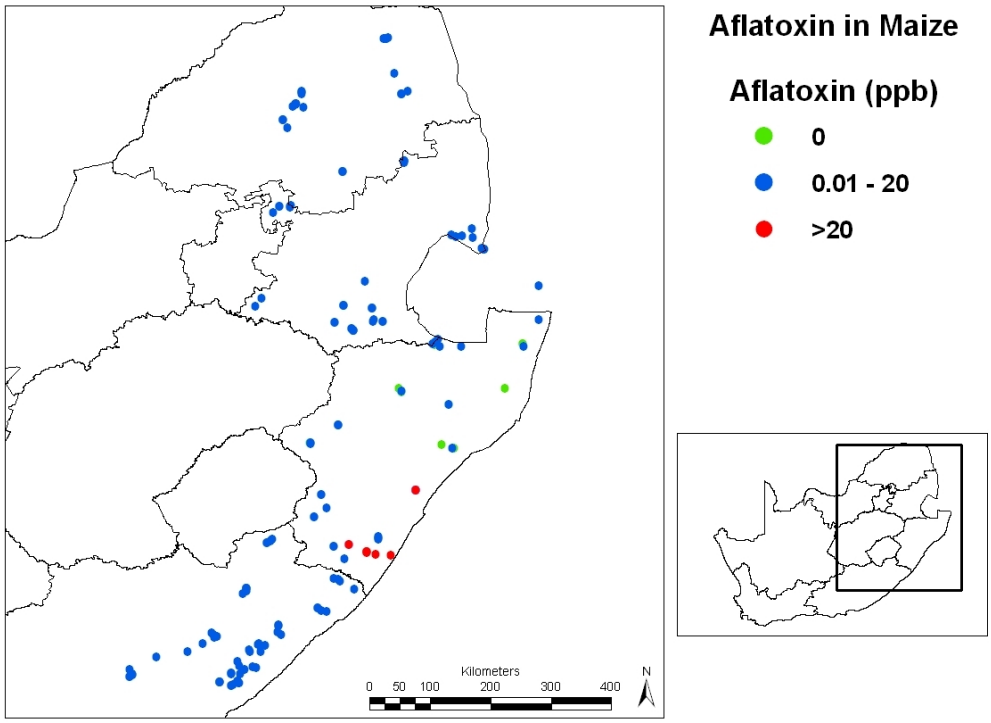

Figure 6. Distribution of aflatoxins in subsistence farmer maize kernels in South Africa during the 2006 planting season.

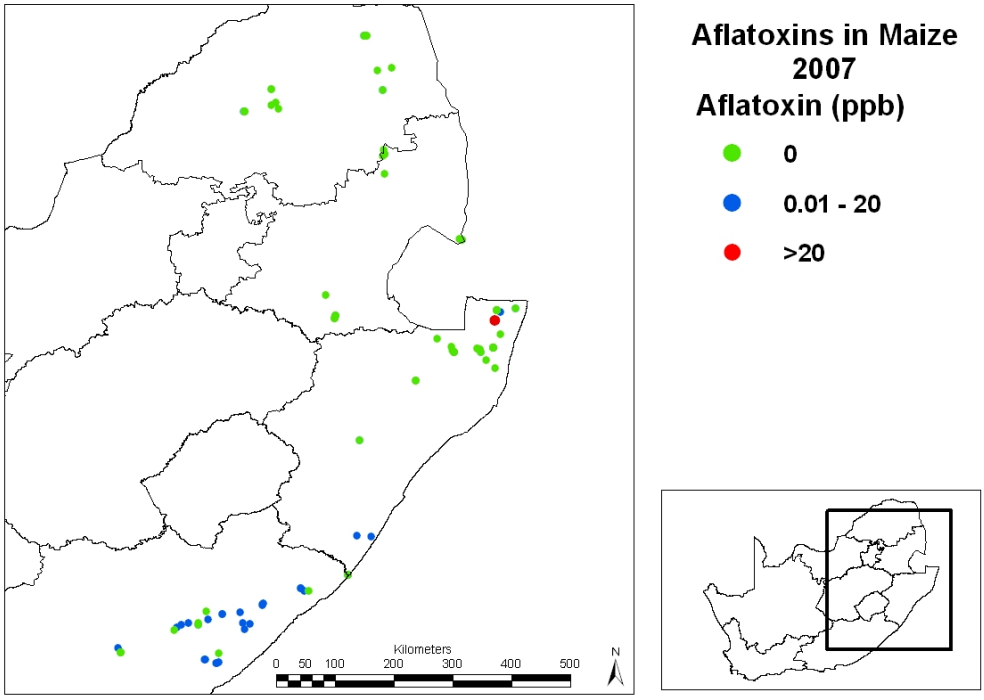

Figure 7. Distribution of aflatoxins in subsistence farmer maize kernels in South Africa during the 2007 planting season.

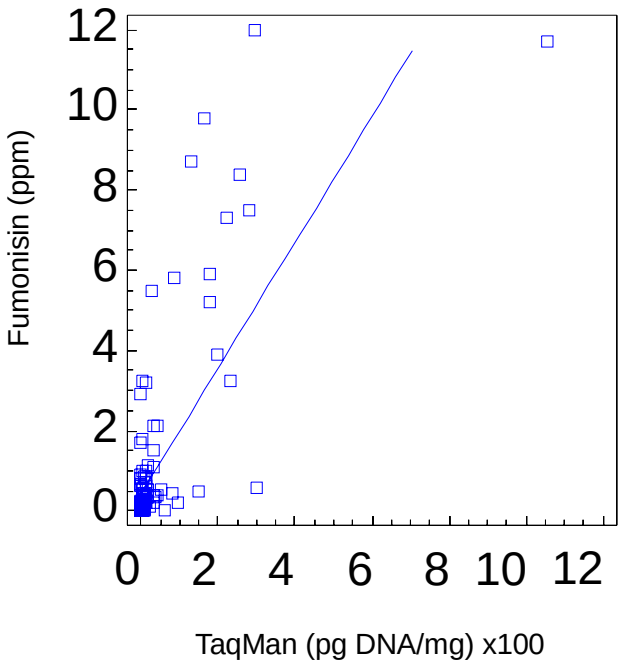

Figure 8. Relationship (r^2 = 0.554, *P*-value = 0.000, n = 167) between real-time PCR analysis of fumonisin-producing *Fusarium* spp. and fumonisin levels (CD-ELISA) in subsistence farming systems in South Africa during the 2006 planting season.

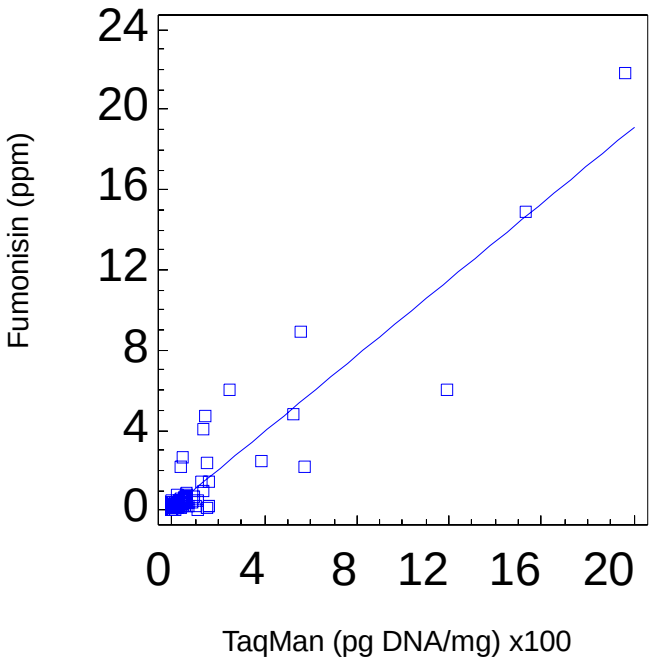

Figure 9. Relationship (r^2 = 0.866, *P*-value = 0.000, n = 124) between real-time PCR analysis of fumonisin-producing *Fusarium* spp. and fumonisin levels (CD-ELISA) in subsistence farming systems in South Africa during the 2007 planting season

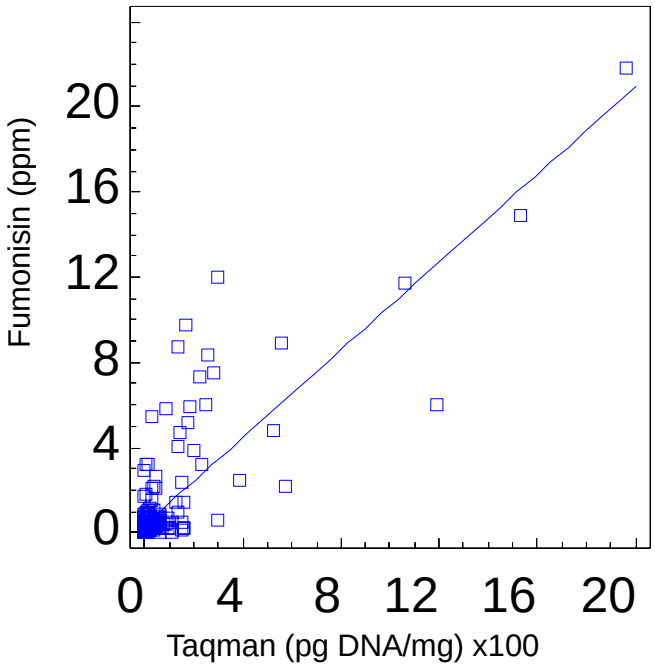

Figure 10. Relationship (r^2 = 0.667, *P*-value = 0.000, n = 291) between real-time PCR analysis of fumonisin-producing *Fusarium* spp. and fumonisin levels (CD-ELISA) in subsistence farming systems in South Africa during 2006 and 2007

Chapter 4

Distribution of aflatoxins and aflatoxin-producing *Aspergillus* spp. in groundnuts in subsistence farming systems in South Africa

ABSTRACT

Aflatoxins are important mycotoxins produced by *Aspergillus flavus* and *A. parasiticus* in groundnut production areas where farmers are unaware of their existence and follow agricultural practices that might contribute to their production. Forty-six groundnut samples were collected from small-scale farmer holdings in three provinces of South Africa, namely KwaZulu-Natal (KZN), Mpumalanga (MP) and Limpopo (LP) provinces, in 2006 and 2007. The incidence of *A. flavus* and *A. parasiticus* was determined by plating out 200 kernels per sample after which the fungi were morphologically identified. Aflatoxin levels of the samples were quantified using ELISA. *Aspergillus parasiticus* was isolated twice more frequently than *A. flavus*, while aflatoxins were mostly found in groundnuts produced in northern parts of KZN, where levels ranged from 0.56-131 ppb. In MP and LP, aflatoxin levels ranged from 0-160.1 ppb and 0-2.6 ppb, respectively. Regression analyses showed a weak, positive correlation between the incidence of *A. flavus* and aflatoxin production in groundnut samples ($r^2 = 0.102$, *P*-value = 0.030), while there was a non-significant correlation between *A. parasiticus* and aflatoxin production in groundnut samples ($r^2 = 0.0237$, *P*-value = 0.306). A positive correlation was also found between *A. flavus* and *A. parasiticus* ($r^2 = 0.537$, *P*-value = 0.000). In the Makhanisi and Mbazwane districts in KZN, and in Boshofontein in MP, aflatoxin levels were in excess of 100 ppb, which is more than the maximum permitted level set by the Food and Drug Administration in the USA (20 ppb), the European Union (6 ppb) and the Department of Health in South Africa (10 ppb) for groundnuts intended for direct human consumption. This study showed that mycotoxin awareness and control programs need to be implemented in rural areas of South Africa.

INTRODUCTION

Aflatoxins are a group of structurally related polyketide mycotoxins that are produced by *Aspergillus flavus* Link ex Fr., *A. parasiticus* Speare, *A. nomius* Kurtzman, Horn & Hesseltine (Gourama and Bullerman, 1995) and *A. tamarii* Kita (Goto *et al*., 1996). *A. flavus* and *A. parasiticus* are closely related and are considered the most important species of the four based on their occurrence in maize and groundnuts. Both *A. flavus* and *A. parasiticus* are well-known contaminants of groundnuts (*Arachis hypogaea* L.) and maize (*Zea mays* L.) (Mirocha and Christensen, 1974).

Studies in developing countries in Africa and Asia have demonstrated strong, significant positive correlations between aflatoxin levels in food samples and the incidence of human liver cancer (Williams *et al*., 2004). A 10% increase in hepatocellular cancer cases was observed in the southeast of the United States, where the estimated average aflatoxin intake is higher than the northern and western areas (http://ntp.niehs.nih.gov). The consumption of aflatoxin-contaminated feed by livestock resulted in oxidative stress, liver necrosis, haemorrhage and death in broiler chickens (*Gullus domesticus* L.) (Eraslan *et al*., 2005), pigs (*Sus scrofa domestica* L.) and cattle (*Bos primigenius* spp. L.) (Marasas, 1995; Osweiler, 2005).

Groundnut production areas in South Africa include the north western Free State, Northwest and Northern Cape Province which accounts for 44.4, 30.4 and 21% of the national commercial production, respectively (www.fao.org). Total groundnut production in the country in 2006 amounted to approximately 74 000 tons. Statistics for groundnut production by subsistence farmers are not readily available. It is common knowledge that subsistence farmers in KwaZulu-Natal, Mpumalanga and Limpopo provinces produce groundnuts for home consumption. The crop is an important source of energy, fats, minerals and proteins (Prathiba and Reddy, 1994), making it an important source of food and nutrition for rural people. Groundnut yields per hectare, however, are low due to unreliable rainfall, non-irrigation of crops, application of traditional farming practices, outbreaks of pests and diseases, and planting low yielding varieties (www.tradeforum.org). In this study, the incidence of aflatoxin-producing *Aspergillus* spp. and the occurrence of aflatoxins in groundnut in rural areas of South Africa were determined.

MATERIALS AND METHODS

Field sampling:

Samples of 1.5 kg were collected from subsistence farmer homes in the KwaZulu-Natal (KZN), Limpopo (LP), and Mpumalanga (MP) provinces, which are the primary subsistence groundnut-producing areas in South Africa. Groundnuts from the Agricultural Research Council's Grain Crops Institute were exchanged with the subsistence farmers' grain. Forty-six groundnut samples were collected, of which 28 were collected during the 2006 and 18 during the 2007 planting seasons. Subsistence farmers in inaccessible areas of Jozini were assembled at a single collection site. The samples were placed in cloth bags to allow air circulation that reduces grain moisture content to levels that limit fungal growth. Samples were marked to include the locality, GPS coordinates, cultivar, farmer and date of collection. All samples were stored in a cold room at 6^{o}C and 45% relative humidity until assayed.

Isolation and enumeration of *Aspergillus* spp.:

Groundnut kernels from each locality were used for the identification of *Aspergillus* spp. All kernels were first surface-sterilised by dipping them once in 70% ethanol, soaking them for 3 minutes in 1.6% sodium hypo-chlorite (NaOCl) and rinsing them three times in sterile distilled water. The kernels were then placed on potato dextrose agar (PDA) in Petri dishes. Two hundred seeds were plated out for each sample, with four kernels being placed in each Petri dish. After 7 days of incubation at 25^{o}C, developing *Aspergillus* colonies were identified morphologically to species level according to Klich and Pitt (1988).

Aflatoxin analysis:

Aflatoxins concentrations in groundnut samples were quantified using Veratox® quantitative aflatoxin test kits (Neogen Corp, Lansing, MI, USA). The Veratox® aflatoxin test kit is a competitive direct Enzyme-Linked Immunosorbent Assay (CD-ELISA) technique in a microwell format. The ELISA tests were done on 5-g sub-samples taken from 250-g milled groundnut samples. The samples were ground into a very fine paste using a Waring commercial blender (Waring Products Division, Torrington, CT, USA). In the ELISA test, the free aflatoxin in the samples competes with the enzyme-labelled aflatoxin (conjugate) for antibody binding sites. After a

wash step with distilled water, a substrate that reacted with the bound conjugate to produce a blue colour was added. Intense blue colour indicated low aflatoxin levels, while a less intense blue colour indicated high aflatoxin levels. In each run, a series of standards were included.

Optical densities of the standards and samples were determined with a micro-plate reader (MR 250, Pynatech Laboratories, Chantilly, VA) with a 650 nm absorbency filter. Optical densities of the standards were then used to plot a standard curve to calculate the exact aflatoxin concentration of the unknown samples in parts per billion (ppb) (Neogen Corp). Aflatoxin levels between 0-50 ppb were accurately determined, and concentrations above 50 ppb were extrapolated from the standard curve. Regression analysis was performed by Statgraphics 5 Plus® and Lotus SmartSuite®. To reduce variation, the analysis of each sample was repeated three times.

RESULTS

Identification and enumeration of *Aspergillus* spp.:

Aspergillus parasiticus was the dominant *Aspergillus* species collected from groundnut in subsistence farmer fields in KZN, LP and MP during the 2006 planting season (Table 1). This species was isolated from groundnuts in all localities sampled in 2006, with the exception of Boshofontein, Schoemansdal and Schuzendal in MP (Appendix C). Only the groundnuts samples from the Piet Retief and Schoemansdal in MP were colonised by *A. flavus* in the same year, while several localities in KZN and Giyani in LP were also free of *A. flavus* contamination. Due to crop failure in the 2006 planting season, and the subsequent unavailability of seed, subsistence farmers in MP and LP did not plant groundnuts in 2007. For this reason, no samples were collected in the two provinces during this planting season. *Aspergillus flavus* and *A. parasiticus* appeared to infect groundnut kernels equally well in KZN during the 2007 planting seasons (Appendix C).

Colonisation of groundnuts by *Aspergillus* spp. was low in all provinces (Figs. 1-3). Groundnuts in Mbazwane and Makhanisi in northern KZN were greatly affected by both *Aspergillus* spp. (Appendix C), while almost all the samples in LP were infected

by *A. parasiticus*, with Giyani and Venda being the districts that had the highest levels of groundnuts colonised by *A. parasiticus* in 2006 (Fig. 2; Appendix C).

Aflatoxin analysis:

Aflatoxin levels appeared to be higher in 2007 than in 2006 in KZN province (Fig. 4; Appendix C). Aflatoxin levels in KZN ranged from 0-2.9 ppb and 0-131 ppb during the 2006 and 2007 planting seasons, respectively, while the mean aflatoxin levels were 1.2 ppb and 22.7 ppb during the same seasons, respectively (Table 1). The LP province had aflatoxins ranging from 0-1.5 ppb during the 2006 planting season. The mean aflatoxin level was 0.52 ppb during the 2006 planting season in LP. In MP, aflatoxin levels ranged from 0-160 ppb and the mean aflatoxin level was 13.5 ppb during the 2006 planting season (Table 1). There was a significant correlation between *A. flavus* and *A. parasiticus* (r^2=0.537, *P*-value = 0.000) (Fig. 5), but a weak significant correlation between aflatoxin levels and *A. flavus* contamination (r^2=0.102, *P*-value = 0.030) determined by simple linear regression analysis (Fig. 6). There was no significant correlation between *A. parasiticus* and aflatoxin contamination (r^2 = 0.0237, *P*-value = 0.306). These correlation suggest that *A. flavus* could be largely responsible for aflatoxin production in groundnuts.

DISCUSSION

Aflatoxin levels in subsistence farming systems in northern KZN were substantially more than the maximum levels allowed for human food set by the Department of Health in South Africa (10 ppb) (www.doh.gov.za), FDA (20 ppb) (www.cfsan.fda.gov), and the EU (6 ppb) (http://eur-lex-europa.eu), respectively. As groundnuts are used primarily as human food in subsistence farming systems in South Africa, the levels recorded in this study are sufficient to lead to serious health defects in both humans and animals (Eraslan *et al*., 2005; Lanyasunya *et al*., 2005).

The high aflatoxin levels in Mbazwane, Jozini and Manguzi districts in northern KZN can possibly be contributed by temperature stress (Chapter 3) in these areas during both 2006 and 2007. Hot, dry conditions that induce plant stress were also prevalent in Boshofontein in MP. Crop rotations of maize and groundnut in the northern coastal region of KZN may also have increased the population of aflatoxin-producing fungi in

the soil (Lisker and Lillehoj, 1991). The very low levels of aflatoxin contamination in groundnut in LP, on the contrary, was possibly due to good farming practices, such as removing weeds, since groundnuts are grown in small gardens in LP.

A second possible explanation for the high aflatoxin levels in groundnuts is that subsistence farmers do not control insects and pests adequately. Their crops, therefore, are at a great risk to damage, which exacerbates levels of infection (Stephenson and Russell, 1974; Beti *et al*., 1995; Dowd, 1998). Insects have been consistently associated with sporulation of the fungus and increased amounts of aflatoxin and they play a major role in aflatoxin contamination in the field (Widstrom, 1979; Lee *et al*., 1986).

The significant correlation between *A. flavus* and *A. parasiticus* was observed in KZN, LP and MP, suggesting that there might be a synergism between the two species, as was earlier suggested by Martins *et al.*, (2000) in Brazil. These findings suggest that both *Aspergillus* spp. tolerated similar environmental conditions. Regression analysis showed a very weak significantly positive correlation between *A. flavus* and aflatoxin contamination, while there was no significant correlation between *A. parasiticus* and aflatoxin contamination, suggesting that *A. flavus* might be largely responsible for aflatoxin production in groundnuts.

Planting disease resistant hybrids to *Aspergillus* spp. and following good agricultural practices, from planting, drying and storage through to consumption, could result in the reduction of aflatoxin contamination of crops in subsistence farming systems. Awareness programmes through effective extension services are advisable in order to maintain acceptable aflatoxin levels. Addition of highly competitive non-toxigenic strains of *A. parasiticus* or *A. flavus* to the soil results in lower concentrations of aflatoxin in groundnut in areas with high aflatoxin concentration (Brown *et al*., 1991) and could be the best approach in reducing aflatoxin contamination in rural areas.

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Table 1. Percentage groundnut kernels infected by *Aspergillus* spp. and aflatoxins in three provinces in South Africa over two planting seasons using whole seed agar plate and aflatoxin analysis by CD-ELISA

Province	2006 planting season				2007 planting season			
	A. flavus	*A. para*	Sum of *A. fla* & *A. para*	Aflatoxin (ppb)	*A. flavus*	*A. para*	Sum of *A. fla* & *A. para*	Aflatoxin (ppb)
LP	[a]1.2	2.6	3.1	0.52	No samples were collected			
	[b](0-1.5)	(2-3.5)	(0-1.5)	(0-2.6)				
MP	0.3	0.8	0.68	13.5	No samples were collected			
	(0-1.5)	(0-3.5)	(2-5.0)	(0-160)				
KZN	1.2	2.7	2.9	1.2	2.3	2.9	4.5	22.7
	(0-7)	(0-7.5)	(0-8)	(0-2.9)	(0-7.8)	(0-7.5)	(0-9.7)	(0.3-131)

Abbreviations: LP = Limpopo province,
MP = Mpumalanga province, KZN = KwaZulu-Natal
A. fla = A. flavus, A. para = A. parasiticus

[a]Top row indicate mean values in all provinces

[b]Bottom row indicate the range in all provinces

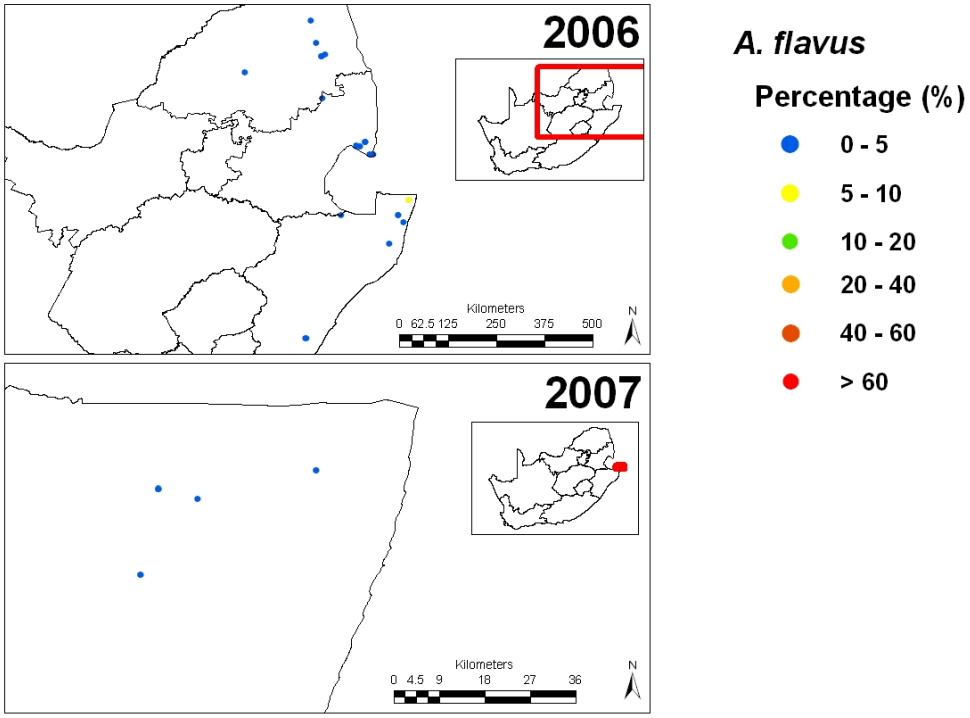

Figure 1. Distribution of *Aspergillus flavus* on groundnuts in South Africa during the 2006 and 2007 planting seasons.

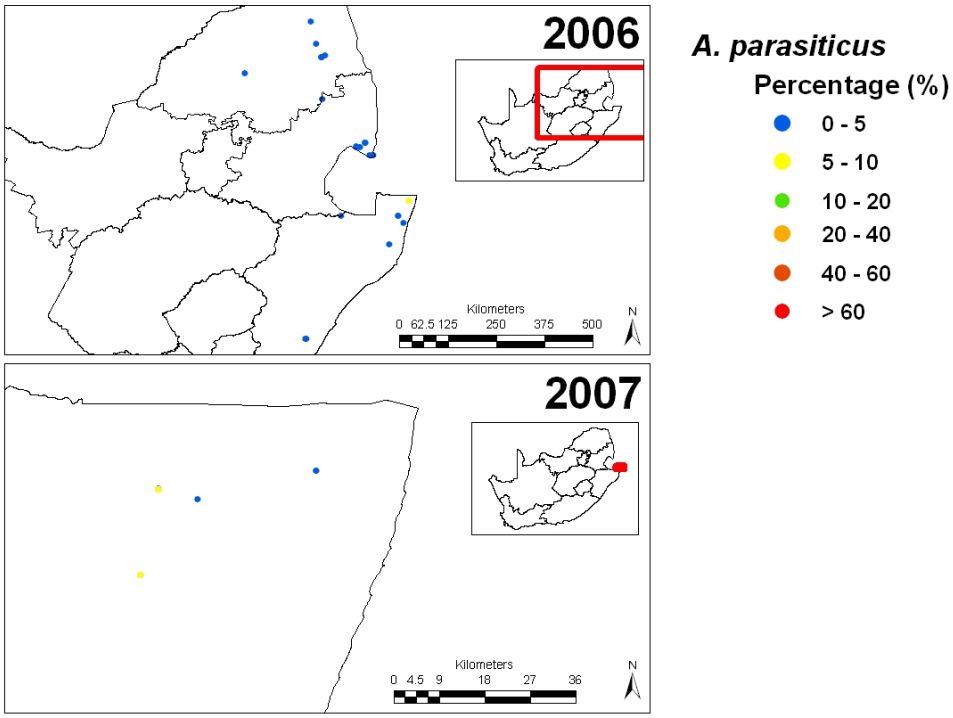

Figure 2. Distribution of *Aspergillus parasiticus* on groundnuts in South Africa during the 2006 and 2007 planting seasons.

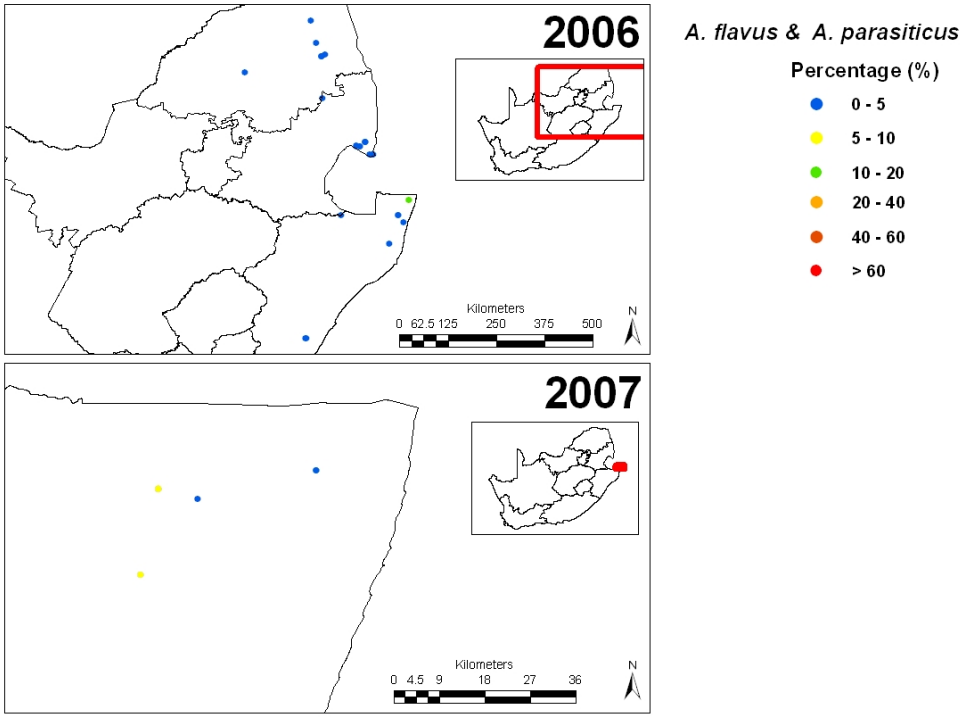

Figure 3. Distribution of *Aspergillus flavus* and *Aspergillus parasiticus* on groundnuts in South Africa during the 2006 and 2007 planting seasons.

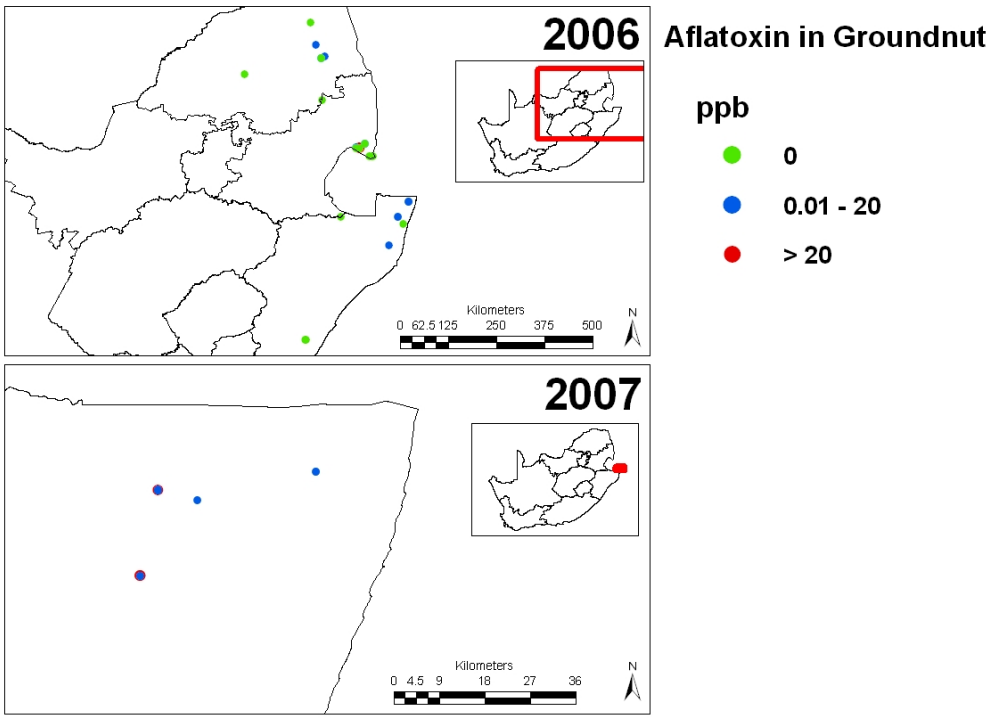

Figure 4. Levels of aflatoxins on groundnuts in South Africa during the 2006 and 2007 planting seasons.

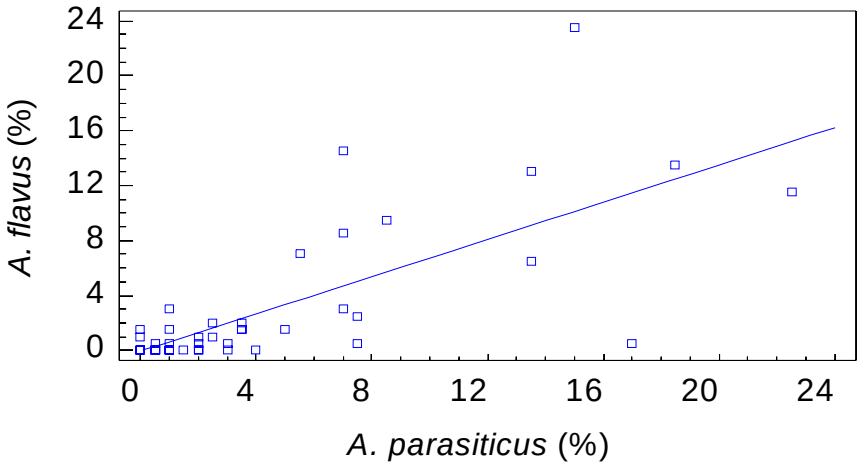

Figure 5. Relationship (r^2 = 0.537, *P*-value = 0.000, n = 46) between *Aspergillus flavus* and *Aspergillus parasiticus* in subsistence farmer groundnuts during both seasons.

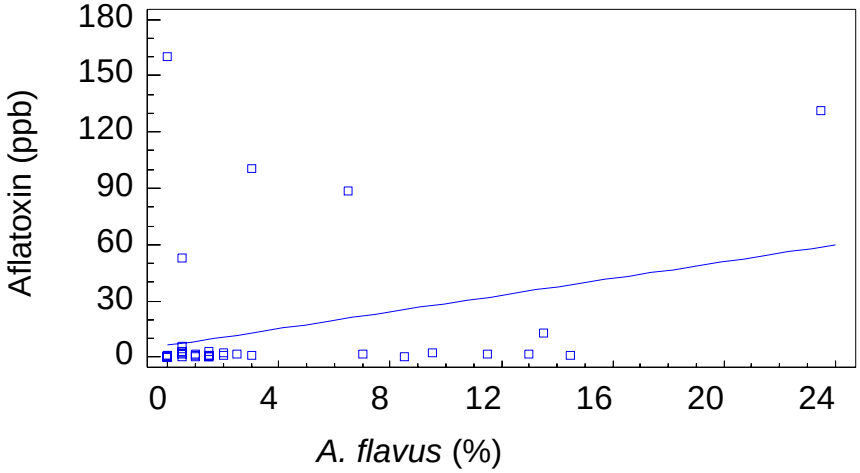

Figure 6. Relationship (r^2 = 0.102, *P*-value = 0.030, n = 46) between *Aspergillus flavus* and aflatoxin levels in subsistence farmer groundnuts during both seasons.

SUMMARY

Fusarium verticillioides (syn = *F. moniliforme*) (Sacc.) Nirenburg is a natural endophyte of maize that can cause Fusarium ear rot and produce fumonisins. A second *Fusarium* spp., *F. proliferatum* (Matsushima) Nirenberg according to Nelson *et al*., (1983) and Leslie and Summerell (2006), also produces fumonisin mycotoxins in maize. Aflatoxins are produced by *Aspergillus flavus* Link ex Fr., and *Aspergillus parasiticus* Speare in maize and groundnut. These mycotoxins are produced in plants that are exposed to drought and moisture stress, insect and physical damage, and planting hybrids outside their range of adaptation. Fumonisins have been associated with human oesophageal cancer and neural tube defects, while the aflatoxins cause liver cancer and fatal aflatoxicoses.

Agricultural practices and mycotoxin awareness of subsistence farmers were investigated in the rural areas of the Eastern Cape (EC), KwaZulu-Natal (KZN), Limpopo (LP), and Mpumalanga (MP) provinces of South Africa. Communities in these areas rely mainly on maize and groundnuts as their primary source of food and income. It was found that most of the farmers practiced monoculture and as a result, high *F. verticillioides* and concomitant fumonisin production occurred in their fields. Good agricultural practices such as the control of insect pests were not adhered to. Almost all the subsistence farmers included in the survey were not aware of the threat that mouldy grain holds to their health, and many use mouldy grain in brewing traditional beer. Animals and birds such as chickens and sheep that are actively fed mouldy grain are also at risk to developing diseases due to mycotoxin exposure in their feeds.

Maize and groundnut samples were randomly collected from subsistence farmers in the EC, KZN, LP, and MP provinces during the 2006 and 2007 seasons. The samples were placed in cloth bags and marked to include the collection source of the sample, global positioning system (GPS) coordinates cultivar, farmer and date of collection. The samples were stored in a cold room at 6^{o}C and 45% relative humidity. Fumonisin and aflatoxin levels were quantified using Veratox® quantitative fumonisin and aflatoxin ELISA kits. The ELISA test was done on a 5-g sub sample taken from a 250g-milled maize or groundnut sample. Each analysis was repeated three times to

determine the reproducibility of the results. The distribution *Fusarium* spp. was determined by plating out on *Fusarium*-selective medium while the distribution of *Aspergillus* spp. was determined by plating out on potato dextrose agar. Real-time PCR was used to quantify the fumonisin-producing *Fusarium* spp.

This study showed that mycotoxins occurred throughout South Africa and were not limited to a particular province. Fumonisin and aflatoxin contamination were highest in areas with hot and dry climatic conditions. Real-time PCR was found to be a reliable method in the quantitative determination of fumonisin-producing *Fusarium* spp. in maize samples when compared to plating out on *Fusarium* selective medium. Data analysis by Statgraphics 5 Plus® showed a significant positive correlation between fumonisin levels determined by ELISA and the quantity of fumonisin-producing *Fusarium* spp. *Fusarium verticillioides* was the most common *Fusarium* spp. on maize kernels followed by *F. subglutinans* in South Africa. High aflatoxin levels occurred in groundnut samples collected in northern KwaZulu-Natal and Mpumalanga provinces. *Aspergillus flavus* and *A. parasiticus* occurred at almost equal levels in KZN, while in Mpumalanga and Limpopo provinces, twice as much *A. parasiticus* colonies were isolated compared with *A. flavus*.

This study strongly recommend that subsistence farmers in South Africa need to be made aware of potential mycotoxin production in their crops through enhanced involvement of extension services in subsistence farming communities. It is important for subsistence farmers to realise that good agricultural practices represent the first line of defence in controlling the contamination of maize and other cereals by mycotoxins. This should be followed by the implementation of good manufacturing practices during the handling, storage, processing and consumption of cereals. Such knowledge may result in improved economic sustainability, improved public health and enhanced food safety, as well as an increase in trade of surplus products.

APPENDIX A

MAIZE QUESTIONNAIRE

Farmer:……

Date:……

Place:……..

Nearest Hospital…….

Climate:……

1. What maize cultivar did you plant?……..

2. Did you apply pesticides to the crop (Y/N)…….
2.1 If yes, what is the name of the pesticide ? ……
2.2 Did you apply fungicides to the crop (Y/N)…….
2.1 If yes, what is the name of the fungicide? ……

3. Planting date……..

4. Harvesting date……..

5. Do you sort, wash, dehull or mill the grain?……

6. Where and how do you store your grain?……..

7. What is the intended use of your grain? (eat/sell/green maize) .

……..

……..

8. How do you dispose of crop residue? (animal feed, burn, left in field, ploughed in)

…….

9. How do you till your land? (hand hoe, tractor, and animal power) ……..

……..

10. What crops did you grow on the same land prior to the current crop?……

……..

11.What are your sources of seed?……...
12. Do you control stalk borer? (Y/N) ……..
12.1 If so how do you control them?…….

13 What are uses of your mouldy grain?……...

13.1 Do you eat mouldy meal??
……..

14. Are you aware of mycotoxins?……...

GROUNDNUT QUESTIONNAIRE

Farmer:……

Date:……

Province:……..

Nearest Hospital…….

Climate:……

1. What groundnut cultivar did you plant?……..

2. Did you apply pesticides to the crop (Y/N)…….
2.1 If yes, what is the name of the pesticide ? ……
2.2 Did you apply fungicides to the crop (Y/N)…….
2.1 If yes, what is the name of the fungicide? ……

3. Planting date……..

4. Harvesting date……..

5. Do you sort, wash, dehull or mill the grain?……

6. Where and how do you store your grain?……..

7. What is the intended use of your grain? (eat/sell) .

……..

8. How do you dispose of crop residue? (animal feed, burn, left in field, ploughed in)

…….

9. How do you till your land? (hand hoe, tractor, and animal power) ……..

……..

10. What crops did you grow on the same land prior to the current crop?……

……..

11.What are your sources of seed?……...
12. Do you control pests? (Y/N) ……..
12.1 If so how do you control them?…….

……..

13 What are uses of your mouldy grain?……...

13.1 Do you eat mouldy meal??
……..

14. Are you aware of mycotoxins?……...

……..

APPENDIX B

Table 1. Distribution of *F. verticillioides, F. proliferatum, F. subglutinans,* fumonisin-producing *Fusarium* spp. (qPCR), fumonisin (ppm) and aflatoxin (ppb) levels in the Eastern Cape province, South Africa, in 2006

	Latitude			latitude	longitude			Longitude	(%)			qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F.vert*	*F.prol*	*F.sub*	(pg DNA/mg)	(ppm)	(ppb)
BIZANA	-30	50	2.5	-30.83403	29	42	52	29.71444	2	0	0	5.48	0	1.527
BIZANA	-30	51	59.2	-30.86644	29	48	2.8	29.80078	8.5	0.5	0.5	0	0.153	0.832
BIZANA	-30	59	30.3	-30.99175	30	1	11.9	30.01997	22	0.5	4	0.01	0.987	1.777
BOLOTWA	-31	59	54.2	-31.99839	27	4	40.3	27.07786	0.5	0	7	8.02	0	1.252
BUTTERWORTH	-32	14	30.8	-32.24189	28	11	0.5	28.18347	31	12.5	4	2.83	0	5.111
BUTTERWORTH	-32	22	9.2	-32.36922	28	1	4.3	28.01786	3	0	4.5	0	0	4.807
BUTTERWORTH	-32	22	3.3	-32.36758	28	0	55.5	28.01542	0	0	38.5	0	0	2.058
BUTTERWORTH	-32	22	0.7	-32.36686	28	0	45.1	28.01253	3.5	0	6.5	13.16	1.462	12.117
BUTTERWORTH	-32	14	1.9	-32.23386	28	11	46.8	28.19633	1.5	0	19	0	0.033	6.944
BUTTERWORTH	-32	22	0.7	-32.36686	28	0	45.1	28.01253	1	0	1.5	0.01	0.707	2.496
COFIMVABA	-31	54	44.2	-31.91228	27	32	9.6	27.53600	1	0	8.5	0	0.04	4.491
COFIMVABA	-31	47	50.6	-31.79739	27	46	7.4	27.76872	4.5	0	29	8	0.105	3.185
ELUNDINI	-30	58	30	-30.97500	28	25	18.4	28.42178	1.5	1	69.5	0	0	2.748
ELUNDINI	-31	0	48	-31.01333	28	24	32.9	28.40914	0.5	0	8.5	4	0	4.857
ELUNDINI	-31	3	16.7	-31.05464	28	21	33.1	28.35919	0	0	33.5	0	0	3.225
ELUNDINI	-30	58	30	-30.97500	28	25	18.4	28.42178	0	0	16	0	0.033	1.647
ELUNDINI	-31	0	48	-31.01333	28	24	32.9	28.40914	0.5	0	17.5	0	0.056	2.753
ENGCOBO	-31	39	48.7	-31.66353	27	55	51.7	27.93103	13	1	38	8.95	0.226	7.153
ENGCOBO	-31	38	18.3	-31.63842	27	53	37.7	27.89381	0.5	0	16.5	0.01	0.071	5.63
ENGCOBO	-31	41	23.6	-31.68989	27	59	2	27.98389	43	1.5	7	3.92	0.423	4.428
ENGCOBO	-31	42	19.4	-31.70539	27	56	34.3	27.94286	0	0	14	3.32	0.404	5.789
IDUTYWA	-32	11	19.1	-32.18864	28	23	33.3	28.39258	15.5	1	42.5	0.91	0.039	2.128
IDUTYWA	-32	3	32.2	-32.05894	28	16	52.1	28.28114	1	0	5.5	0	0	2.427
IDUTYWA	-32	7	55	-32.13194	28	18	39.3	28.31092	1.5	1	31.5	0	0	3.418

Table 1 (continued). Distribution of *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, fumonisin-producing *Fusarium* spp. (qPCR), fumonisin (ppm) and aflatoxin (ppb) levels in the Eastern Cape province, South Africa, in 2006

	Latitude			latitude	longitude			Longitude	(%)			qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F.vert*	*F.prol*	*F.sub*	(pg DNA/mg)	(ppm)	(ppb)
IDUTYWA	-32	8	45.5	-32.14597	28	30	27.1	28.50753	2	0	14	0	0.045	5.606
IDUTYWA	-32	15	14.6	-32.25406	28	19	0.5	28.31681	1	0	1	0	0.075	7.145
IDUTYWA	-31	53	11.9	-31.88664	28	27	27.5	28.45764	17.5	2	16	29.72	0.415	6.446
IDUTYWA	-31	54	51.8	-31.91439	28	28	5.6	28.46822	1.5	0	2	5.2	0.139	5.123
IDUTYWA	-32	9	45.3	-32.16258	28	33	47.7	28.56325	0.5	0	9.5	0.01	0.075	5.239
KENTANI	-32	21	25.3	-32.35703	28	17	17.8	28.28828	35	0	12.5	3.41	0.11	6.426
KENTANI	-32	21	25.3	-32.35703	28	17	17.8	28.28828	0	0	0.5	0	0.05	6.337
KENTANI	-32	24	35	-32.40972	28	13	12	28.22000	0	0	40	0	0.019	4.729
KENTANI	-32	25	6.3	-32.41842	28	11	34	28.19278	13	1	8	0.01	0.037	2.802
KENTANI	-32	22	41.9	-32.37831	28	17	30.2	28.29172	1	0	1.5	0	0	3.046
KENTANI	-32	25	6.3	-32.41842	28	11	34	28.19278	9.5	0	4	1.12	1.932	1.505
LIBODE	-31	31	40.8	-31.52800	28	53	45.2	28.89589	0	1	2	10.5	0.093	3.906
LIBODE	-31	31	40.7	-31.52797	28	53	45.1	28.89586	0	0	16	0.01	0.04	2.452
LUSIKISIKI	-31	19	3.1	-31.31753	29	36	10.7	29.60297	12.5	0.5	5	7.3	2.237	1.176
MALUTI	-30	18	11.6	-30.30322	28	43	1.7	28.71714	7	0	9	0.01	0.859	1.652
MALUTI	-30	18	11.6	-30.30322	28	43	1.7	28.71714	2.5	0	11.5	0	0.126	1.81
MQANDULI	-31	49	18.3	-31.82175	28	41	37.6	28.69378	1	0	2.5	0	0.031	7.389
MQANDULI	-31	55	13.1	-31.92031	28	37	39.6	28.62767	0	0	2	0	0.047	4.999
NGQELENI	-31	37	24.3	-31.62342	28	52	56.2	28.88228	16.5	0	4.5	0.91	0.029	5.084
NGQELENI	-31	39	35.5	-31.65986	28	55	37.2	28.92700	0	0	9.5	0	0	4.424
QUEENSTOWN	-32	17	7.4	-32.28539	26	40	41.6	26.67822	1	0	69.5	62.35	0	5.863
QUNU	-31	48	27.4	-31.80761	28	34	59.2	28.58311	0	0	10.5	0	0.126	4.332
QUNU	-31	48	14.9	-31.80414	28	35	46.7	28.59631	1.5	0	8	0	0	5.23
SENGQU	-30	58	30	-30.97500	28	25	18.4	28.42178	4.5	0	0.5	0.01	0	4.142
WHITTLESEA	-32	15	45.2	-32.26256	26	43	2.1	26.71725	1.5	0	14	0.01	0.382	4.704
WHITTLESEA	-32	11	7.4	-32.18539	26	40	49.7	26.68047	0	0.5	0.5	2.43	0	3.915
WHITTLESEA	-32	17	8.9	-32.28581	26	40	42	26.67833	0	0	11.5	0	0.027	6.748
WHITTLESEA	-32	15	41.5	-32.26153	26	43	0.9	26.71692	20.5	1.5	12	0.01	0.002	6.464

Table 2. Distribution of *F. verticillioides, F. proliferatum, F. subglutinans*, qPCR (pg DNA/mg), fumonisin (ppm) and aflatoxin (ppb) levels in KwaZulu-Natal province, South Africa, in 2006

	Latitude			latitude	longitude			Longitude	(%)			qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F.vert*	*F.prol*	*F.sub*	(pg DNA/mg)	(ppm)	(ppb)
BERGVILLE	-28	49	29.2	-28.82478	29	22	32	29.37556	5.5	0	0.5	0	0.1	2.3
BERGVILLE	-28	49	1.8	-28.81717	29	21	40.6	29.36128	81.5	1	0	1.07	3.1	0
BERGVILLE	-28	49	10.3	-28.81953	29	22	3.3	29.36758	45	3.5	2.5	0	0	0.8
EMSELENI	-27	20	19	-27.33861	32	31	51.9	32.53108	26	0	1.5	14.68	0.1	0
ESHOWE	-28	53	26.1	-28.89058	31	30	29.1	31.50808	8	0	4	2.97	0.1	0
ESHOWE	-28	50	30.3	-28.84175	31	18	47.1	31.31308	69.5	0	20.5	196.75	2.2	0
ESHOWE	-28	54	1.3	-28.90036	31	29	3.1	31.48419	8	62	34	6.89	0.2	0.3
HIGHFLATS	-30	14	27.5	-30.24097	30	22	29.1	30.37475	8.5	0	4.5	0	0	11.473
HIGHFLATS	-30	12	26.7	-30.20742	30	22	17.1	30.37142	40.5	0.5	2	6.95	0.1	11.683
HIGHFLATS	-30	12	56.1	-30.21558	30	22	23.2	30.37311	34.5	0.5	6	5.46	0.318	14.037
HIGHFLATS	-30	14	29.7	-30.24158	30	22	29.8	30.37494	70	0	1.5	5.37	0.301	15.148
IZINGOLWENI	-30	26	18.1	-30.43836	30	12	5	30.20139	89.5	1	4.5	7.64	0	17.3
IZINGOLWENI	-30	26	18.1	-30.43836	30	12	5	30.20139	25.5	0.5	9	0	0	29.1
IZINGOLWENI	-30	26	18.1	-30.43836	30	12	5	30.20139	18.5	0	10.5	4.63	0	20.3
LADYSMITH	-28	33	19	-28.55528	29	46	55.4	29.78206	53	1	19.5	0.01	2.7	0.9
LADYSMITH	-28	33	19	-28.55528	29	46	55.4	29.78206	99	0	0	2.8	0.8	1.4
LADYSMITH	-28	33	19	-28.55528	29	46	55.4	29.78206	78	0	3.5	299.09	0.5	2.4
MANGUZI	-26	59	0.3	-26.98342	32	45	51.2	32.76422	97	0	0	296.53	10.9	2.9
MBAZWANE	-28	0	38.4	-28.01067	32	15	31.8	32.25883	60	0	4	149.03	0.4	0
MBAZWANE	-27	22	39.7	-27.37769	32	32	24.1	32.54003	23.5	0	0	1057	13.2	0.9
MBAZWANE	-27	20	54.3	-27.34842	32	31	39.2	32.52756	28	0.5	2	177.01	5.1	0
NDWEDWE	-29	30	43.9	-29.51219	30	56	27.6	30.94100	44	1.5	3.5	11.95	0.822	11.713
NDWEDWE	-29	30	43.9	-29.51219	30	56	27.6	30.94100	61.5	0	2	14.31	0.901	14.252
NDWEDWE	-29	30	43.9	-29.51219	30	56	27.6	30.94100	56	0	21	5.22	0.313	10.779
NDWEDWE	-29	30	43.9	-29.51219	30	56	27.6	30.94100	10.5	0	2	8.91	0.35	15.638
NDWEDWE	-29	30	43.9	-29.51219	30	56	27.6	30.94100	6.5	0	2.5	0	0.174	21.261

Table 2 (continued). Distribution of *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, fumonisin-producing *Fusarium* spp. (qPCR), fumonisin (ppm) and aflatoxin (ppb) levels in KwaZulu-Natal province, South Africa, in 2006

	Latitude			latitude	longitude			Longitude	(%)			qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F.vert*	*F.prol*	*F.sub*	(pg DNA/mg)	(ppm)	(ppb)
NDWEDWE	-29	30	43.9	-29.51219	30	56	27.6	30.94100	9.5	0	16.5	0.01	0.197	13.654
PONGOLA	-27	22	39	-27.37750	31	36	37.9	31.61053	40.5	2.5	0.5	40.59	0.2	2.9
PONGOLA	-27	23	4.2	-27.38450	31	17	18.7	31.28853	59	0.5	3.5	254.87	9.9	1.8
PONGOLA	-27	16	21.7	-27.27269	31	16	11.3	31.26981	34	1	4	131.42	9.7	1.5
PONGOLA	-27	18	45.1	-27.31253	31	13	34	31.22611	62	0.5	10.5	164.08	10.6	1.5
PONGOLA	-27	21	6.1	-27.35169	31	12	31.4	31.20872	57	0	2	281.87	8	0
PONGOLA	-27	19	8.1	-27.31892	31	13	45.8	31.22939	63.5	2.5	3	85.21	6.4	1.4
PORT SHEPSTONE	-30	26	18.1	-30.43836	30	12	5	30.20139	5	0	1.5	0	0	23.1
PORT SHEPSTONE	-30	26	18.1	-30.43836	30	12	5	30.20139	22.5	0	39	3.45	0	20.7
PORT SHEPSTONE	-30	25	52.1	-30.43114	30	12	8	30.20222	7	0	13	0	0	22.6
PORT SHEPSTONE	-30	28	13.1	-30.47031	30	20	38.3	30.34397	42.5	1	4	9.6	0.3	29
PORT SHEPSTONE	-30	29	14.7	-30.48742	30	33	42.5	30.56181	7.5	0.5	3.5	8.34	0.1	23.9
PORT SHEPSTONE	-30	26	56	-30.44889	30	12	22.2	30.20617	71.5	1	6.5	0.01	0.1	28.4
ULUNDI	-28	14	26	-28.24056	31	25	29.2	31.42478	2	1.5	32	180.2	6	0
ULUNDI	-28	14	21.2	-28.23922	31	25	10.9	31.41969	9.5	0.5	10.5	42.78	1.7	0.9
UMZIMKHULU	-30	21	27.4	-30.35761	29	43	10	29.71944	2.5	0	13.5	0	0	3.4
UMZIMKHULU	-30	21	27.4	-30.35761	29	43	10	29.71944	3	0	61.5	0	0	1.1
UMZIMKHULU	-30	19	25.2	-30.32367	29	56	4.9	29.93469	2	0	98	4.23	0.1	28.5
UMZIMKHULU	-30	19	25.2	-30.32367	29	56	4.9	29.93469	4	0	57	0	0	19.9
UMZIMKHULU	-30	19	25.2	-30.32367	29	56	4.9	29.93469	11.5	0.5	13.5	0	0.093	12.829
UNDERBERG	-29	55	10.5	-29.91958	29	25	30.2	29.42506	3	0	0.5	0	0	13.053
UNDERBERG	-29	55	10.5	-29.91958	29	25	30.2	29.42506	0.5	0.5	60.5	0	0	14.576
UNDERBERG	-29	34	41.6	-29.57822	29	31	47.2	29.52978	7	0.5	42	0.01	0.092	16.97
UNDERBERG	-29	34	41.6	-29.57822	29	31	47.2	29.52978	0.5	0	30.5	2.3	0.079	12.935
UNDERBERG	-29	47	12.7	-29.78686	29	36	43.4	29.61206	1.5	0	53	0	0.184	11.932
VRYHEID	-28	2	57.9	-28.04942	30	43	42.5	30.72847	42	0	9.5	32.25	1.7	0.5
VRYHEID	-28	3	6.3	-28.05175	30	43	42.9	30.72858	68	0	7	222.13	8.4	0
VRYHEID	-28	3	12.7	-28.05353	30	43	37.4	30.72706	4	0	54	0	0.1	0

VRYHEID	-28	0	14.8	-28.00411	30	40	40.2	30.67783	6	0	18	1.42	0.2	0

Table 3. Distribution of *F. verticillioides, F. proliferatum, F. subglutinans,* fumonisin-producing *Fusarium* spp. (qPCR), fumonisin (ppm) and aflatoxin (ppb) levels in Limpopo province, South Africa, in 2006

	Latitude			latitude	longitude			Longitude	(%)			qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F.vert*	*F.prol*	*F.sub*	(pg DNA/mg)	(ppm)	(ppb)
GIYANI	-23	38	14.4	-23.63733	30	43	35.1	30.72642	23	0.5	0	0.01	0.141	12.917
GIYANI	-23	38	14.5	-23.63736	30	43	35.2	30.72644	4	0.5	0	0.86	0.484	11.823
GIYANI	-23	35	32.3	-23.59231	30	48	46.3	30.81286	11	0	0	33	1.231	11.078
GIYANI	-23	19	57.9	-23.33275	30	37	4.2	30.61783	4	0	0	2.74	0.398	13.355
GIYANI	-23	38	14.5	-23.63736	30	43	35.3	30.72647	92	1.5	0	2.34	0.047	12.688
JANE FURSE	-24	46	47	-24.77972	29	50	49.7	29.84714	11.5	0	0	3.89	0.176	12.37
JANE FURSE	-24	46	45.9	-24.77942	29	50	46.5	29.84625	9	3.5	0	0	0.751	12.445
JANE FURSE	-24	46	42.3	-24.77842	29	50	46.1	29.84614	25	0.5	1.5	12.47	4.526	13.65
MOKOPANE	-24	1	21.2	-24.02256	28	57	22.5	28.95625	44	0.5	0.5	26.83	6.4	11.627
MOKOPANE	-24	8	0.7	-24.13353	29	1	48.4	29.03011	28.5	0	4.5	1.1	0	12.4
MOKOPANE	-24	1	16.6	-24.02128	28	57	24.6	28.95683	36	1	2	19.44	0.656	14.625
PIETERSBURG	-23	49	22.3	-23.82286	29	6	26.1	29.10725	20.5	0.5	4.5	3.13	0	12.622
PIETERSBURG	-23	37	51.8	-23.63106	29	14	37.9	29.24386	56.5	0.5	0.5	17.83	1.17	15.118
PIETERSBURG	-23	35	54.7	-23.59853	29	14	34.1	29.24281	21	0	1	0.01	0.16	11.494
PIETERSBURG	-23	35	54.7	-23.59853	29	14	34.1	29.24281	11	1.5	1	31.91	1.46	11.616
PIETERSBURG	-23	47	12.2	-23.78672	29	8	50.8	29.14744	0	0	0.5	0	0.082	12.538
PIETERSBURG	-23	46	59.3	-23.78314	29	8	51.8	29.14772	20	1.5	0	4.63	0.099	13.729
PIETERSBURG	-23	49	33.2	-23.82589	29	16	4.9	29.26803	10.5	1	3.5	0	0.113	10.744
PIETERSBURG	-23	35	53.7	-23.59825	29	14	22.6	29.23961	14	0	0	5.03	0.255	13.139
VENDA	-22	48	21	-22.80583	30	29	43.2	30.49533	70	2.5	0	9.2	0.47	13.775
VENDA	-22	48	14.9	-22.80414	30	29	3.7	30.48436	43	0.5	0	0.01	0.078	10.057
VENDA	-22	48	21.1	-22.80586	30	29	43.2	30.49533	51.5	1	0	3.73	0.1	11.889
VENDA	-22	48	28.2	-22.80783	30	27	4.5	30.45125	9	0.5	1	1.08	0.35	11.911
VENDA	-22	48	14.8	-22.80411	30	29	8.6	30.48572	32	0	0	8.85	0.239	11.044
VENDA	-22	47	59.4	-22.79983	30	31	5.4	30.51817	24	0	0.5	3.85	0.198	10.777

Table 4. Distribution of *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, fumonisin-producing *Fusarium* spp. (qPCR), fumonisin (ppm) and aflatoxin (ppb) levels in Mpumalanga province, South Africa, in 2006

	Latitude			latitude	longitude			Longitude	(%)			qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F.vert*	*F.prol*	*F.sub*	(pg DNA/mg)	(ppm)	(ppb)
BALFOUR	-26	40	23.4	-26.67317	28	38	6	28.63500	3	0	74.5	0	0	10.031
BOSHOFONTEIN	-25	44	55.8	-25.74883	31	32	12.3	31.53675	55	5	2.5	16.42	0.367	7.812
DAGGAKRAAL	-27	6	54.7	-27.11519	29	59	4.8	29.98467	2	0	44	0	0	7.511
DAGGAKRAAL	-27	8	42.5	-27.14514	30	0	5.4	30.00150	0.5	0	18	0	0	7.494
DAGGAKRAAL	-27	8	42.5	-27.14514	30	0	5.4	30.00150	0.5	0	77	0	0	10.821
DAGGAKRAAL	-27	6	57.4	-27.11594	29	59	4.8	29.98467	0.5	0	69	0	0.101	8.47
DRIEFONTEIN	-27	0	26.3	-27.00731	30	26	31.4	30.44206	1.5	0.5	31	1.08	0.61	8.386
DRIEFONTEIN	-27	0	26.3	-27.00731	30	26	31.4	30.44206	16.5	0	17.5	4.17	0.725	5.772
EMERSFOORT	-27	1	18.2	-27.02172	29	43	41.2	29.72811	0.5	0	11.5	0	0	19.129
ERMELO	-26	46	45.6	-26.77933	29	51	23.7	29.85658	0.5	0	18.5	0	0.177	8.8
ERMELO	-26	46	45.6	-26.77933	29	51	23.7	29.85658	0.5	0	8	0	0.033	9.02
GROOTVLEI	-26	47	25.6	-26.79044	28	32	42.6	28.54517	0.5	0	22.5	0.01	0	9.86
IKHWEZI	-25	45	55.5	-25.76542	31	47	7.4	31.78539	50	1.5	3	49.9	0.544	5.776
KWAMHLANGA	-25	18	44.2	-25.31228	29	4	4.04	29.06779	21.5	0.5	1.5	13.33	1.412	14.948
KWAMHLANGA	-25	23	29.6	-25.39156	28	48	38.6	28.81072	5	0	2.5	2.73	0.394	13.475
KWAMHLANGA	-25	18	21.1	-25.30586	29	3	45.2	29.06256	10	0	2	93.41	0.242	6.842
LIEDEN	-26	48	50.2	-26.81394	30	17	17.1	30.28808	0.5	0.5	36	2.19	0	8.346
LIEDEN	-26	48	50.2	-26.81394	30	17	17.1	30.28808	2	0	25.5	0.01	0.098	11.263
LIEDEN	-26	48	50.2	-26.81394	30	17	17.1	30.28808	7	0.5	61.5	0	0	10.643
MATIBITI	-24	39	13.9	-24.65386	30	45	47.4	30.76317	4	0	2.5	1.21	0	9.939
MATIBITI	-25	17	31.6	-25.29211	29	4	50	29.08056	24.5	0	0.5	232.4	1.968	9.406
MATIBITI	-24	37	35.9	-24.62664	30	45	34.3	30.75953	1	0.5	2	1.11	0.172	7.487

Table 5. Distribution of *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, fumonisin-producing *Fusarium* spp. (qPCR), fumonisin (ppm) and aflatoxin (ppb) levels in the Eastern Cape province, South Africa, in 2007

	Latitude			latitude	longitude			Longitude	(%)			qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F. vert*	*F. prol*	*F. sub*	(pg DNA/mg)	(ppm)	(ppb)
BUTTERWORTH	-32	15	55.3	-32.26536	28	13	16.7	28.22131	42.5	7	0	0.0	0.18	0
BUTTERWORTH	-32	22	3	-32.36750	28	0	54.4	28.01511	4	0	12.5	157.6	0.22	1.1
BUTTERWORTH	-32	21	39.3	-32.36092	28	0	21.4	28.00594	0.5	0	0	0.0	0.22	4.23
COFIMVABA	-31	54	43.9	-31.91219	27	32	13.1	27.53697	0	0	4	3.2	0.07	0
COFIMVABA	-31	48	5.6	-31.80156	27	45	31.5	27.75875	5.5	4.5	7	2.6	0.34	0.52
COFIMVABA	-31	49	53	-31.83139	27	38	31.8	27.64217	1.5	1.5	10.5	0.0	0.26	0.89
COFIMVABA	-31	52	43.3	-31.87869	27	35	10.2	27.58617	0	1	3.5	0.0	0.26	1.9
ENGCOBO	-31	40	13.3	-31.67036	28	16	31.8	28.27550	1.5	0.5	8	2.7	0.26	2
ENGCOBO	-31	37	26.9	-31.62414	28	1	28.1	28.02447	22.5	0	50	0.0	0.29	0
ENGCOBO	-31	40	13.6	-31.67044	28	16	31.3	28.27536	2	0	12.5	13.4	0.22	0.33
ENGCOBO	-31	44	58.3	-31.74953	28	3	5.8	28.05161	20	10.5	55.5	127.1	1.44	0.52
ENGCOBO	-31	48	17.6	-31.80489	27	54	13.9	27.90386	3	1.5	2.5	33.7	0.41	0
ENGCOBO	-31	49	44.5	-31.82903	27	54	48.9	27.91358	22	13.5	14	14.7	0.19	0.13
ENGCOBO	-31	49	44.5	-31.82903	27	54	48.9	27.91358	4	0.5	28	248.1	6.05	0
KENTANI	-32	25	7.9	-32.41886	28	11	35.5	28.19319	2.5	0	6	0.0	0.17	0.66
KENTANI	-32	24	58	-32.41611	28	11	8.3	28.18564	17.5	1	7	136.8	0.93	4.53
KENTANI	-32	24	36	-32.41000	28	13	13.9	28.22053	1	0	2	0.0	0.23	6.17
LIBODE	-31	31	36.1	-31.52669	28	53	41.5	28.89486	0.5	0	1	0.0	0.21	1.1
LIBODE	-31	31	10.9	-31.51969	28	53	33.4	28.89261	1.5	0.5	5.5	0.0	0.12	2.17
LIBODE	-31	30	5.4	-31.50150	28	54	39.7	28.91103	11	1	6	0.0	0.15	1.86
LUSIKISIKI	-31	16	29.5	-31.27486	29	28	56.5	29.48236	24	6	11	558.6	8.95	1.15
LUSIKISIKI	-31	16	29.5	-31.27486	29	28	56.5	29.48236	29.5	7	2	63.4	0.9	0.85

Table 5 (continued). Distribution of *F. verticillioides, F. proliferatum, F. subglutinans,* fumonisin-producing *Fusarium* spp. (qPCR), fumonisin (ppm) and aflatoxin (ppb) levels in the Eastern Cape province, South Africa, in 2007

	Latitude			latitude	longitude			Longitude	(%)			qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F. vert*	*F. prol*	*F. sub*	(pg DNA/mg)	(ppm)	(ppb)
LUSIKISIKI	-31	18	48.6	-31.31350	29	32	9.8	29.53606	1.5	0	10.5	36.6	0.17	1.1
LUSIKISIKI	-31	19	1.8	-31.31717	29	36	10.6	29.60294	34	3	25.5	0.0	0.4	0
MBEKWENI	-32	15	49	-32.26361	26	42	44.8	26.71244	0	0.5	29	0.0	0.19	0
MQANDULI	-31	49	6.1	-31.81836	28	41	50	28.69722	1	0	1	20.7	0.4	3.27
MQANDULI	-31	54	16.4	-31.90456	28	36	53.2	28.61478	0	0	0.5	0.0	0	2.8
NGQELENI	-31	38	20.4	-31.63900	28	32	49.2	28.54700	9.5	1	1.5	0.0	0.28	2.37
QUEENSTOWN	-32	15	37.8	-32.26050	26	43	3.9	26.71775	9.5	2	4.5	51.9	0.41	0
QUNU	-31	48	29.1	-31.80808	28	35	6.5	28.58514	2	0	1.5	0.0	0.2	1.58
WHITTLESEA	-32	11	7.4	-32.18539	26	40	49.7	26.68047	0.5	0	34.5	0.0	0.37	0.86
WHITTLESEA	-32	15	14.6	-32.25406	26	43	0.8	26.71689	0	0	0.5	0.0	0	0

Table 6. Distribution of *F. verticillioides, F. proliferatum, F. subglutinans*, fumonisin-producing *Fusarium* spp. (qPCR), fumonisin (ppm) and aflatoxin (ppb) levels in KwaZulu-Natal province, South Africa, in 2007

	Latitude			latitude	longitude			Longitude		(%)		qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F. vert*	*F. prol*	*F. sub*	(pg DNA/mg)	(ppm)	(ppb)
JOZINI	-27	35	20.4	-27.58900	32	25	56.6	32.43239	48.5	1.5	0	153.1	0.16	0
JOZINI	-27	53	44.8	-27.89578	32	27	0.9	32.45025	4.5	13.5	0	2.5	0.25	0
JOZINI	-27	35	20.4	-27.58900	32	25	56.6	32.43239	20.5	3	0	44.9	0.47	0
JOZINI	-27	35	20.4	-27.58900	32	25	56.6	32.43239	21.5	11.5	0	0.0	0.2	0
JOZINI	-27	35	20.4	-27.58900	32	25	56.6	32.43239	51	2	0	9.9	0.17	0
JOZINI	-27	10	6.3	-27.16842	32	27	3.9	32.45108	69	0.5	0	104.6	0.19	0
JOZINI	-27	10	6.3	-27.16842	32	27	3.9	32.45108	9	4	0	43.1	0.27	0
JOZINI	-27	35	20.4	-27.58900	32	25	55.6	32.43211	64	0	0	152.5	2.36	0
JOZINI	-27	35	20.4	-27.58900	32	25	55.6	32.43211	22.5	26.5	0	27.4	0.48	0
JOZINI	-27	10	6.3	-27.16842	32	27	8.9	32.45247	15.5	7.5	0	59.3	0.72	0
JOZINI	-27	10	6.3	-27.16842	32	27	8.9	32.45247	55.5	1	0	34.6	0.59	49.0
JOZINI	-27	35	69.4	-27.60261	32	10	71.7	32.18658	36	0	0	388.3	2.48	0
JOZINI	-27	37	6.2	-27.61839	32	13	19.3	32.22203	2	4.5	0	28.2	0.23	0
JOZINI	-27	37	6.2	-27.61839	32	13	19.3	32.22203	72.8	17.9	0.5	1965.5	21.8	0
JOZINI	-27	46	10.7	-27.76964	32	18	72.4	32.32011	61.5	0	0	162.9	1.48	0
JOZINI	-27	36	45.7	-27.61269	32	12	43.9	32.21219	49.5	12	0	84.9	0.41	0
JOZINI	-27	35	65.1	-27.60142	32	10	72	32.18667	4	0.5	0	0.0	0.05	0
LADYSMITH	-28	4	98	-28.09389	30	74	19.9	31.23886	7.5	0	0	12.7	0.23	0
LADYSMITH	-28	59	84.1	-29.00669	29	82	46.4	30.37956	11.5	0	3	54.4	0.61	0
LADYSMITH	-28	59	84.1	-29.00669	29	82	46.4	30.37956	51	0	0	53.1	0.49	2.03
MAKHANISI	-27	0	55.3	-27.01536	32	28	61	32.48361	8.5	0	0	0.0	0.33	0
MAKHANISI	-27	0	55.3	-27.01536	32	28	61	32.48361	19	21	0	24.7	0.21	0
MAKHANISI	-27	0	55.3	-27.01536	32	28	61	32.48361	32.5	2.5	0	36.4	2.22	0
MAKHANISI	-27	0	55.3	-27.01536	32	28	61	32.48361	39.5	0	4	6.3	0.18	0
MAKHANISI	-27	0	55.3	-27.01536	32	28	61	32.48361	31	21.5	0	3.3	0.4	0
MAKHANISI	-27	0	55.3	-27.01536	32	28	61	32.48361	72.5	1	0	43.0	0.3	0
MAKHANISI	-27	0	54.2	-27.01506	32	28	61	32.48361	50.5	5	0	35.1	0.2	0
MANGUZI	-27	22	39.7	-27.37769	32	32	24.1	32.54003	3.5	1.5	0	0.0	0.05	0

Table 6 (continued). Distribution of *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, fumonisin-producing *Fusarium* spp. (qPCR), fumonisin (ppm) and aflatoxin (ppb) levels in KwaZulu-Natal province, South Africa, in 2007

	Latitude			latitude	longitude			Longitude	(%)			qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F. vert*	*F. prol*	*F. sub*	(pg DNA/mg)	(ppm)	(ppb)
MANGUZI	-26	59	0.3	-26.98342	32	45	51.2	32.76422	66.5	7	0	1191.7	6.06	0
MANGUZI	-26	59	0.3	-26.98342	32	45	51.2	32.76422	16	28	0	1531.3	14.9	1.83
MANGUZI	-27	2	1.1	-27.03364	32	32	15	32.53750	51	0	1.5	571.4	2.18	2.02
MBAZWANE	-27	10	6.3	-27.16842	32	27	8.9	32.45247	75	18	0	56.5	0.7	0
MSELENI	-27	10	6.3	-27.16842	32	27	8.9	32.45247	3	1	0	95.6	0.66	0
MTHELU	-27	35	14	-27.58722	32	25	56.6	32.43239	28.5	11.5	1.5	62.9	0.59	0
MTHELU	-27	35	14	-27.58722	32	25	56.6	32.43239	56	10.5	0	110.8	0.01	1.09
MTHELU	-27	35	14	-27.58722	32	25	56.6	32.43239	41	19	0	49.3	0.22	0.13
PONGOLA	-27	22	39	-27.37750	31	36	37.9	31.61053	21.5	0	7.5	37.8	0.3	1.86
PONGOLA	-27	36	71.7	-27.61992	31	47	65.2	31.80144	0	0	0	0.0	0	0
PONGOLA	-27	34	3.6	-27.56767	31	46	77.8	31.78828	38	0	0	26.0	0.44	0
PONGOLA	-27	27	4.1	-27.45114	31	33	70.7	31.56964	17.5	0	0	31.3	0.38	0
PONGOLA	-27	39	34.2	-27.65950	32	13	82	32.23944	34	0	4.5	109.0	0.5	0
PONGOLA	-27	39	34.2	-27.65950	32	13	82	32.23944	23	0	0	58.3	0.78	0.03
PORT SHEPSTONE	-30	63	87.9	-31.07442	29	74	14.5	30.23736	6	0	1	42.9	0.41	1.11
PORT SHEPSTONE	-30	28	13.1	-30.47031	30	20	38.3	30.34397	9	0.5	6.5	69.9	0.23	2.04
PORT SHEPSTONE	-30	72	1.9	-31.20053	29	94	71.3	30.58647	1	0	1.5	0.0	0.17	0
PORT SHEPSTONE	-30	29	14.7	-30.48742	30	33	42.5	30.56181	8	0	0	0.0	0.43	1.29
PORT SHEPSTONE	-30	63	45.8	-31.06272	29	72	20.5	30.20569	0	0	0	10.0	0.42	0.27
PORT SHEPSTONE	-30	63	45.8	-31.06272	29	72	20.3	30.20564	0	0	9	4.2	0.12	0
PORT SHEPSTONE	-30	63	45.8	-31.06272	29	72	20.7	30.20575	7.5	0	1	3.3	0.13	1.19
ULUNDI	-27	38	72.7	-27.65353	31	48	81.6	31.82267	3.5	0	4	3.2	0.15	0
ULUNDI	-27	38	72.7	-27.65353	31	48	81.6	31.82267	8	0	1.5	0.0	0.11	0
ULUNDI	-27	38	72.7	-27.65353	31	48	81.6	31.82267	30	0	0	62.7	0.06	0
VRYHEID	-28	4	99	-28.09417	30	74	19.9	31.23886	21.5	0	1.5	140.0	4.69	0

Table 7. Distribution of *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, fumonisin-producing *Fusarium* spp. (qPCR), fumonisin (ppm) and aflatoxin (ppb) levels in Limpopo province, South Africa, in 2007

	Latitude			latitude	longitude			Longitude	(%)			qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F. vert*	*F. prol*	*F. sub*	(pg DNA/mg)	(ppm)	(ppb)
BELLINGATE	-23	49	51.9	-23.83108	29	6	4	29.10111	2	0.5	0.5	0.0	0.50833	0.11
GIYANI	-23	38	7.1	-23.63531	30	44	6.3	30.73508	6	0.5	0	12.2	0.50339	0.13
GIYANI	-23	38	7.1	-23.63531	30	44	6.3	30.73508	25.5	1	0	19.0	1.00527	0.78
GIYANI	-23	20	14.2	-23.33728	30	38	59.1	30.64975	14	0.5	0	0.0	0.50000	0.11
JUNO	-23	37	31.9	-23.62553	29	2	0.1	29.03336	6	0	0	0.0	0.00000	0.22
JUNO	-23	37	34.3	-23.62619	29	1	59.1	29.03308	24	1.5	1.5	0.0	1.52500	0.26
JUNO	-23	57	49.8	-23.96383	28	36	33.6	28.60933	9	0	0.5	0.0	0.00833	0.23
JUPITER	-23	52	10.8	-23.86967	29	1	35.1	29.02642	18.5	6	0	0.0	6.00000	0
PIETERSBURG	-23	55	33.9	-23.92608	29	7	54.7	29.13186	3	0	0	0.0	0.00000	0
MOKOPANE	-23	57	54.1	-23.96503	28	36	55.8	28.61550	4.5	1	0	0.0	1.00000	0.11
MOKOPANE	-23	57	56	-23.96556	28	36	59.5	28.61653	65.5	6.5	0.5	44.8	6.52078	0.23
MOKOPANE	-23	58	10.5	-23.96958	28	36	53.7	28.61492	31.5	0.5	0	0.0	0.50000	0.16
MOKOPANE	-23	58	3.5	-23.96764	28	37	3.8	28.61772	8.5	1	0	39.2	1.01090	0.36
VENDA	-22	48	23.4	-22.80650	30	27	5	30.45139	62.5	4	0	522.4	4.14512	4.78
VENDA	-22	48	28.4	-22.80789	30	27	4	30.45111	17.5	0	0.5	132.5	0.04513	4.01
VENDA	-22	48	21.4	-22.80594	30	29	43	30.49528	13	0	0	33.2	0.00921	0.19
VENDA	-22	48	31.4	-22.80872	30	29	38.6	30.49406	77	9	0	42.7	9.01186	2.68

Table 8. Distribution of *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, fumonisin-producing *Fusarium* spp. (qPCR), fumonisin (ppm) and aflatoxin (ppb) levels in Mpumalanga province, South Africa, in 2007

	Latitude			latitude	longitude			Longitude	(%)			qPCR	Fumonisin	Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*F. vert*	*F. prol*	*F. sub*	(pg DNA/mg)	(ppm)	(ppb)
DAGGAKRAAL	-27	5	29	-27.09139	30	0	40.8	30.01133	0.5	0	8	0.0	0	0
DAGGAKRAAL	-27	8	18.7	-27.13853	30	0	11.4	30.00317	0	0	28.5	0.0	0	0
DAGGAKRAAL	-27	5	29	-27.09139	30	0	40.8	30.01133	0	0.5	4	0.0	0.26	1.16
DAGGAKRAAL	-27	5	29	-27.09139	30	0	40.8	30.01133	0.5	0	15.5	0.0	0.12	0
DAGGAKRAAL	-27	8	18.7	-27.13853	30	0	11.4	30.00317	0	0	1	0.0	0	0
DAGGAKRAAL	-27	8	18.7	-27.13853	30	0	11.4	30.00317	1.5	0	7.5	0.0	0.15	0
DAGGAKRAAL	-27	8	18.7	-27.13853	30	0	11.4	30.00317	1	0	0	0.0	0.13	0
ERMELO	-26	46	43.7	-26.77881	29	51	14.8	29.85411	36.5	4	22	0.0	0.09	0
LYDENBURG	-24	37	15.9	-24.62108	30	45	42.4	30.76178	0.5	0	3	24.6	0.1	0
MATIBITI	-24	55	30.9	-24.92525	30	45	52.7	30.76464	4.5	0	3	0.0	0.02	0
MATIBITI	-24	38	46.7	-24.64631	30	44	21.4	30.73928	4.5	3	0	0.0	0.05	0
MATIBITI	-24	33	0.3	-24.55008	30	44	48.6	30.74683	10.5	2.5	9.5	0.0	0.13	0
MATIBITI	-24	37	14	-24.62056	30	45	35	30.75972	5	1.5	2	0.0	0.17	0
MATIBITI	-24	37	15.9	-24.62108	30	45	42.4	30.76178	0.5	0.5	1	0.0	0	0
MATIBITI	-24	36	9	-24.60250	30	46	9.3	30.76925	1	0	1.5	0.0	0.52	0
MBUZINI	-25	55	29.1	-25.92475	31	54	56.8	31.91578	1	0.5	0.5	0.0	0	0
MBUZINI	-25	56	15.8	-25.93772	31	57	13.2	31.95367	16	1.5	2	14.8	0.06	0
MBUZINI	-25	55	50.1	-25.93058	31	56	3	31.93417	26	11.5	0.5	161.8	0.19	0
MBUZINI	-25	56	15.8	-25.93772	31	57	13.2	31.95367	31	3	2	13.0	0.18	0

APPENDIX C

Table 1. Distribution of *A. flavus*, *A. parasiticus* and aflatoxin (ppb) levels in groundnuts in KwaZulu-Natal and Limpopo provinces, South Africa, in 2006

	Latitude			latitude	longitude			Longitude		(%)		Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*A. fla* & *A. para*	*A. flavus*	*A. para*	(ppb)
KWAZULU-NATAL PROVINCE												
HIGHFLATS	-30	12	56.1	-30.21558	30	22	23.2	30.37311	1	0	1	0
HIGHFLATS	-30	12	26.7	-30.20742	30	22	17.1	30.37142	0	0	0	0
HIGHFLATS	-24	1	21.3	-24.02258	28	57	22.8	28.95633	2	0	2	0
MANGUZI	-26	29	0.3	-26.48342	32	45	51.2	32.76422	8	0.5	7.5	3.178
MANGUZI	-26	59	0.3	-26.98342	32	45	51.2	32.76422	12.5	7	5.5	1.864
MANGUZI	-26	59	0.3	-26.98342	32	45	51.2	32.76422	2.5	1.5	1	2.912
MBAZWANE	-28	0	38.4	-28.01067	32	18	31.8	32.30883	4.5	2	2.5	0.944
MBAZWANE	-27	20	54.3	-27.34842	32	31	39.2	32.52756	3	1	2	1.734
MBAZWANE	-27	30	1.1	-27.50031	32	39	9.7	32.65269	0.5	0	0.5	0
MBAZWANE	-27	20	19	-27.33861	32	31	51.9	32.53108	4	0	4	0.035
LIMPOPO PROVINCE												
GIYANI	-23	38	14.5	-23.63736	30	43	35.3	30.72647	3	0	3	0
GIYANI	-23	35	32.3	-23.59231	30	48	46.3	30.81286	3.5	1	2.5	0.181
GIYANI	-23	38	14.5	-23.63736	30	43	35.2	30.72644	2.5	0.5	2	2.606
GIYANI	-23	38	14.3	-23.63731	30	43	35.2	30.72644	5	1.5	3.5	1.073
GIYANI	-23	19	57.9	-23.33275	30	37	4.2	30.61783	2	0	2	0.787
VENDA	-22	48	14.8	-22.80411	30	29	8.6	30.48572	3.5	0.5	3	0

Table 1 (continued). Distribution of *A. flavus*, *A. parasiticus* and aflatoxin (ppb) levels in groundnuts in Mpumalanga province, South Africa, in 2006

	Latitude			latitude	longitude			Longitude		(%)		Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*A. fla* & *A. para*	*A. flavus*	*A. para*	(ppb)
MPUMALANGA PROVINCE												
BOSHOFONTEIN	-25	44	41.9	-25.74497	31	37	26.1	31.62392	0	0	0	0
BOSHOFONTEIN	-25	44	41.9	-25.74497	31	37	26.1	31.62392	1	0	1	160.1
LYDENBERG	-24	37	35.9	-24.62664	30	45	34.3	30.75953	0.5	0	0.5	0
MBUZINI	-25	56	2	-25.93389	31	56	38.9	31.94414	1	0	1	0
MBUZINI	-25	55	29.2	-25.92478	31	54	57.2	31.91589	1.5	0	1.5	0
MBUZINI	-25	56	4.7	-25.93464	31	51	11.5	31.85319	0.5	0	0.5	0
PHIVA	-25	38	21.9	-25.63942	31	45	53.5	31.76486	0.5	0	0.5	0
PHIVA	-25	38	21.9	-25.63942	31	45	53.5	31.76486	0.5	0	0.5	0.31
PHIVA	-25	38	21.9	-25.63942	31	45	53.5	31.76486	0.5	0	0.5	0.488
PIET RITIEF	-27	20	19	-27.33861	31	11	2.3	31.18397	5	1.5	3.5	0
SCHOEMANSDAL	-25	43	3.4	-25.71761	31	33	15.3	31.55425	1.5	1.5	0	1.26
SCHUZENDAL	-25	44	55.9	-25.74886	31	32	12.4	31.53678	0	0	0	0

Table 2. Distribution of *A. flavus*, *A. parasiticus* and aflatoxin (ppb) levels in KwaZulu-Natal province, South Africa, in 2007

	Latitude			latitude	longitude			Longitude		(%)		Aflatoxin
Location	Degree	Min	Sec	Decimal	Degree	Min	Sec	Decimal	*A. fla* & *A. para*	*A. flavus*	*A. para*	(ppb)
MAKHANISI	-27	0	55.3	-27.01536	32	28	61	32.48361	5.5	0.5	5.5	1.6
MAKHANISI	-27	0	54.2	-27.01506	32	28	61	32.48361	2.5	0.7	1.5	2.23
MAKHANISI	-27	0	55.3	-27.01536	32	28	61	32.48361	8	4.3	5.5	1.51
MAKHANISI	-27	0	55.1	-27.01531	32	28	61.2	32.48367	8.5	3.8	7.5	1.8
MAKHANISI	-27	0	55.3	-27.01536	32	28	61	32.48361	7	4.8	2.5	1.25
MAKHANISI	-27	0	55.3	-27.01536	32	28	61	32.48361	1.5	1	1	100.7
MANGUZI	-27	2	0.8	-27.03356	32	33	14.6	32.55406	8	4.5	4.5	12.76
MANGUZI	-27	2	0.8	-27.03356	32	33	14.6	32.55406	1	1	0	1.16
MANGUZI	-26	59	0.3	-26.98342	32	45	51.2	32.76422	6	3.5	2.5	2.55
MANGUZI	-26	59	0.3	-26.98342	32	45	51.2	32.76422	0.5	0	0.5	1.18
MANGUZI	-26	59	0.3	-26.98342	32	45	51.2	32.76422	1.5	0.5	1	5.67
MANGUZI	-27	0	55.2	-27.01533	32	28	61.4	32.48372	3	1.5	3.5	1.55
MBAZWANE	-27	10	6.3	-27.16842	32	27	8.9	32.45247	4.5	1	3	1
MBAZWANE	-27	10	6.3	-27.16842	32	27	8.9	32.45247	9.7	7.8	5	131.03
MBAZWANE	-27	10	6.3	-27.16842	32	27	8.9	32.45247	3.5	2.8	2	0.56
MBAZWANE	-27	10	6.3	-27.16842	32	27	8.9	32.45247	2.5	0.5	1.5	0.26
MBAZWANE	-27	10	6.3	-27.16842	32	27	8.9	32.45247	6	2.2	4	88.3
MBAZWANE	-27	10	6.3	-27.16842	32	27	8.9	32.45247	1	0.5	0.5	52.81