INCIDENCE AND CHARACTERIZATION OF *FUSARIUM* SPECIES IN CROWN ROT OF BANANAS

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PREFACE

This study represents original work by the author and has not been submitted in any form to another university or institute. Where use has been made of the work of others it has been duly acknowledged in the text.

The research described in this thesis was carried out at the Department of Biological Sciences, M. L. Sultan Technikon, Durban, South Africa, under the supervision of Prof. B. Odhav.

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ABSTRACT

Fusarium species produce toxic mycotoxins that are known to exert adverse health effects in humans and animals. No attempts have been made to establish mycotoxin-producing capabilities of isolates of *Fusarium* species from bananas exhibiting symptoms of crown rot. Crown rot is one of the most serious post harvest problems in banana and the disease is caused by different fungal species, principally *Fusarium* species. Banana, which is of great economic significance in growing countries (i.e. Costa Rica, Cameroon, Ecuador) is seriously affected by crown rot and is a major cause of fruit loss.

Experiments were conducted to determine the incidence of *Fusarium* species occurring in the crown tissue region of infected bananas and thereby to assess the potential pathogenicity of the isolated *Fusarium* species. Five farm holdings in identical geographical locations were used to collect 65 banana hands from the south coast region of Kwa-Zulu Natal during the months of August 2000 and February 2001. Twenty three *Fusarium* isolates were identified to species level by examination of characteristic morphological features. *Fusarium moniliforme* (now referred to as *F. verticilliodes*) (8 isolates) was predominant, followed by *F. oxysporum* (5 isolates), *F. subglutinans* (4 isolates), *F. sambucinam* (3 isolates), *F. solani* (1 isolate), *F. chlamydosporum* (1 isolate) and *F. acuminatum* (1 isolate). Although the presence of *F. verticilliodes*, *F. oxysporum*, *F. subglutinans*, *F. solani* and *F. acuminatum* has been reported previously, this is the first report on the isolation of *F. sambucinum* and *F. chlamydosporum* from banana crown rot infections.

The ability of these *Fusarium* species to produce mycotoxins was determined by extraction and purification of fumonisin B_1, fusaric acid and moniliformin from fungal culture material grown on autoclaved corn extract. Mycotoxins were analysed by thin layer chromatography and high performance liquid chromatography. The mean percentage recoveries of fumonisin B_1, moniliformin and fusaric acid was 82%, 80% and 89%respectively. The detection limits by thin layer chromatography were 200 ng, 100 ng and 50 ng for fumonisin B_1, moniliformin and fusaric acid respectively. High performance liquid chromatography was found to be more sensitive with detection limits of 50 ng, 20 ng and 20 ng for fumonisins B_1, moniliformin and fusaric acid respectively. Mycotoxin concentrations ranged between 0.5 μg/g and 12.4 μg/g for fumonisin B_1; between 0.25 μg/g and 10.7 μg/g for fusaric acid and between 0.18 μg/g and 8.35 μg/g for moniliformin. The highest levels of these mycotoxins were produced by *F. oxysporum* and *F. verticilliodes*.

The effects of fumonisin B_1, moniliformin and fusaric acid on healthy banana fruit were established using a pathogenicity assay. The assay involved the measurement of the mean rot diameter caused by the various *Fusarium* isolates and the percentage weight loss of inoculated banana fruit. After six days of incubation *F. oxysporum* and *F. verticilliodes* caused the greatest rot diameter of 2.5 cm and 2.4 cm respectively and the greatest weight loss of 44% and 38% respectively. These two *Fusarium* species were found to be the most pathogenic. It was found that *Fusarium* isolates which produced more than one mycotoxin also produced a larger mean rot diameter. There was no correlation between weight loss and the *Fusarium* isolates that produced mycotoxins. The presence of *Fusarium* species plays a role in both the mean rot diameter and fruit weight loss.

Crown rot is exacerbated with the currently widespread practice of packing the fruit in boxes as full hands rather than shipping them on a bunch. The severed hand is therefore susceptible to colonization by pathogens. The results of this study showed that the predominant pathogen in crown rot infections were *F. verticilliodes* and *F. oxysporum*. These *Fusarium* species produced fumonisin B_1, fusaric acid and moniliformin. Ingestion of banana infected with these mycotoxins have serious implications for human health, particularly because they are the most widely consumed fruit.

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1.1 BACKGROUND AND MOTIVATION

Banana fruit (*Musa sapientum* L.) is of great economic significance in South Africa with five million tons being produced annually (Mabbet, 1997). After harvesting, the banana fruit is susceptible to crown rot, which occurs widely and is associated with packing the fruits in boxes as full hands, rather than shipping them as bunches (Jimenez *et al.*, 1997). Thus, each hand is severed from the main stalk, which leaves a portion of the crown attached to the fingers, so that the fingers remain intact. The newly exposed tissue lends itself readily to colonization by microorganisms in general and fungi in particular. Crown rot is caused by a complex of fungi with one main pathogen, *Colletotrichum musae* (Ogundero, 1987). Additionally, *Fusarium verticilliodes, F. verticilliodes* var. *subglutinans* Wollenw and Reinking, *Fusarium pallidoroseum* (Cooke) Sacc., *Nigrospora sphaerica* Sacc., and *Botryodiplodia theobromae* Pat have been implicated with pathogenicity (Krause *et al.*, 1998). However, little attention has so far been paid to the potential role of other *Fusarium* species, within and on the fruit. The study of the potential role of such species in banana decay during storage and marketing, and disease-causing potential of the toxins that these fungi produce is important since they have been found frequently in other substrates such as cereals, jimsonweed, and sugar beet (Abbas *et al.*, 1992). These mycotoxins have serious disease implications for humans and animals.

Thus the aim of this study was to determine the incidence of and to characterize the *Fusarium*

species in banana crown rot and to assay for the mycotoxins they produce. This study involved four phases. Phase one involved the collection of the banana hands from various farm holdings. The second phase involved the isolation and identification of different *Fusarium* species. Phase three involved the determination of the mycotoxin-producing potential of the isolated *Fusarium* species and a pathogenicity profile of the isolates. The fourth phase involved a correlation study between mycotoxin production and the pathogenicity of the *Fusarium* isolates. The literature review covers some of the post harvest diseases of bananas with special emphasis on the incidence and characteristics of 'crown rot.' This is followed by a brief review of taxonomy of *Fusarium* species and elaborates some of the important disease causing mycotoxins of importance in the South African context. Finally the literature available on mycotoxins in bananas is presented.

1.2 LITERATURE REVIEW

1.2.1 Importance of Bananas

Bananas are the most popular fruit throughout the world and about 50 million tons are produced annually. Its consumption surpasses that of apples and citrus fruit together (Postharvest News and Information, 1999). Banana and plantain (*Musa spp.*) are among the most important staple food crops for several million people throughout the world. They rank fourth among food crops (after rice, wheat and maize) and also fourth amongst export commodities on a global basis in terms of gross value of production (Graham *et al.*, 1999). Bananas and plantains are a cheap source of carbohydrate (about 35%), potassium, calcium and vitamin C (Harris *et al.*, 2000). They also yield a diverse array of useful secondary products such as fibers, wrappers, confectioneries, chips, beer, wine and vinegar (Stover and Simmonds, 1987). Bananas are generally eaten as fresh fruit, whereas plantains, which contain more starch, are usually consumed after being cooked. Almost six million tons of plantains and cooking bananas produced in Africa is consumed as a staple food. Dessert bananas are exported and they contribute to the Gross National Product (G.N.P.), provide employment and generate foreign exchange to the producing country (Stover and Simmonds, 1987). Apart from their value to the local market, bananas and plantains have great potential for export, especially for South Africa (Mabbet, 1997).

1.2.2 Production of Bananas

Botanically bananas and plantains are relatively simple plants consisting of an underground stem called the corm, from which grows the central bud producing the pseudostem (false stem), leaves, inflorescence, bunch and a series of lateral buds that grow into suckers (Mabbet, 1997). The plant is a tree-like herb with large leaves, which emerge in a rolled position from the centre of the pseudostem (Stover and Simmonds, 1987). It produces a single flowering shoot consisting of several flower clusters, each cluster having 12 to 20 flowers in two rows, covered by a large reddish bract. In cultivated species, the fruits arise without fertilization and are seedless; their weight makes the shoot hang down, so that the developing 'hands' of bananas curve upwards. The hands at the proximal end mature slightly earlier than those towards the distal end of the bunch (Stover and Simmonds, 1987).

The production systems used for plantains and cooking bananas are as varied as the types that are grown. Where there is a large, resident market for these staples, farmers will grow them intensively on small plantations and estates in mono-culture with all the agronomic care and attention associated with cash crops. More commonly, plantains and cooking bananas are grown in mixed cropping stands either with other food crops (groundnuts, maize, taro, cocoyams, cassava and even fruit trees) or with commodity crops such as coffee and cocoa (Mabbet, 1997). The secret of successful cultivation in the so-called 'traditional' mixed food crop planting is to plant a variety of crops that use all the available vertical space (Mabbet, 1997). Thus, the tall plantain and cooking banana plants should be mixed with lower profile crops example, medium-height species such as maize and cassava, low-profile crops such as cocoyam and taro and ground

cover crops such as cucumber and pumpkin (Rodrigo *et al.*, 1997; Rodrigo *et al.*, 2001). In addition, it is vital to include a rich diversity of leguminous crops to continually boost soil nitrogen (Srikul and Turner, 1998).

Incorrect or inappropriate post-harvest handling significantly reduces the shelf-life of the banana fruit. The timing of harvest, maturity and storage conditions are essential in attaining a storage life of up to three weeks (Jiang *et al.*, 1999). The harvesting process involves removal of the stem from the plant, dehanding of the fruit in the field and transport of the fruit on the stems to a central packing facility (Medlicott, 2000). At harvest and subsequently careful handling is essential, firstly to prevent the fruit from being stained by latex (which exudes copiously from cut surfaces), and secondly to minimize the incidence of cuts and bruises, the effects of which will only show when the fruit ripens (Slabaugh and Grove, 1982). Hands should not be removed by lifting with one or two central or end fingers; this may cause finger breakage and will cause pedicel bruising which can lead to problems during ripening. Dirty or blunt knives will increase the levels and development of crown disease, while trimming the crown close to the fingers will result in crown breakage. After dehanding, the fruit is placed with the crown facing downwards onto a layer of leaves to allow for latex drainage. In order to restrict the development of crown disease, a crown pad dipped in a solution of 0.05% thiabendazole can be applied (Medlicott, 2000).

The world's largest producers of bananas are Costa Rica, Gautemala, Ecuador, Honduras, Cameroon, Cote Divoire and Columbia. The major banana growing areas of South Africa are the Ondeberg area, Hazyview, Kwa-Zulu Natal, Tzaneen and Levubu. Since 1996, 98% of bananas grown in the South Africa are derived from tissue culture material (Cavendish genotype) and belong to the Cavendish group (IUF-Towards Respect for Global Rights, 2001).

1.2.3 Post Harvest Diseases of Bananas

Post harvest deterioration of bananas are dependent on the type of cultivars grown, the starting material, the harvesting strategy and methods of disease control used. Deterioration of the fruit can be caused by physical factors and microbial pathogens. Physical disorders of bananas include; i) chilling injury due to exposing bananas to temperatures below 13°C for a few days (cultivar, maturity and temperature dependant); ii) skin abrasions from rubbing against other fruit or surfaces of handling equipment or shipping boxes; iii) finger drop caused by individual fingers becoming detached due to rapid ripening precipitated by too high a temperature in the ripening room (Astorga, 1999; Kader, 2000; Snowdon, 1990; Wade *et al.*, 1993); and iv) high temperature injury at temperatures above 30°C where the the peel may fail to turn yellow but the pulp nevertheless ripens normal. Between 35°C and 45°C ethylene production is inhibited and ripening cannot be induced by ethylene from an external source. If bananas are held at 40°C for several days, they may be irreversibly damaged and fail to ripen on return to a moderate temperature (Akkaravessapong *et al.*, 1998; Snowdon, 1990).

Microbial disorders of bananas are chiefly caused by fungi (Table 1.1), and the most important disease includes anthracnose, cigar end rot, finger rot, pitting disease, black sigatoka and crown rot. The symptoms range from premature ripening leading to abnormal flavour, spots or rotting, which lead to significant losses. Crown rot is considered to be the most serious post harvest disease (Jimenez *et al.*, 1993), because of its frequency of occurrence and economic losses.

Table 1.1 Pathological fungal disorders of bananas (Snowdon, 1990)

Pathological Disorder	Causative Agent	Symptoms	Control
Anthracnose	*Colletotrichum musea*	Small dark circular spots which enlarge and become sunken and leads to premature ripening	Chemical fungicides, hot water dips, modified atmospheric storage
Cigar End Rot	*Verticillium theobromae*	Darkening and wrinkling of the skin, pulp undergo wet rot, resemble ashy end of a burnt cigar	Manual removal of dead flower parts, strict sanitation and fungicide sprays
Finger Rot	*Botryodiplodia theobromae*	Rotting begins at tip of fingers, soft, dark brown and pimpled with minute black bodies, wrinkled skin and semi-liquid pulp	Systemic fungicides, ensure fruit not over-matured when cut and also by prevention of injury
Pitting Disease	*Pyricularia grisea*	Reddish spots on skin of maturing green and shallow black pits on ripe fruits, leading to finger dropping	Apply fungicide spray, protect the emerging bunch with polyethylene sleeve
Black Sigatoka	*Mycosphaerella fijiensis*	Premature ripening and abnormal flavour and aroma development, black spots on leaves, fruit don't mature and bunch size is reduced	Cultural and chemical methods, Spray with oil or an oil-fungicide mix, breed resistant cultivars and by correct spacing of plants
Crown Rot	*Colletotrichum musea* *Fusarium* species	Premature ripening, with white/pink mold on surface of cut crown, infected tissue turns black, fingers drop and rot may advance to finger stalks	Removal of leaf thrash, strict sanitation, apply systemic fungicides and field-pack bananas with reduction in time between cutting and cooling

1.2.4 Crown Rot of Bananas

1.2.4.1 Disease characteristics

The most serious post harvest disease affecting the banana fruit is crown rot (Jimenez *et al.,* 1997, Santchurn, 1999). Bananas can be invaded by various fungal species following harvesting (Shillingford and Sinclair, 1977; Kuthubutheen and Nawawi, 1987). The practice of cutting banana hands from the stalk to package fruit for the commercial market provides an infection court for microorganisms to enter the crown tissue and cause a decay known as crown rot (Marin *et al.,* 1996). Decay is usually confined to the crown but may spread into the pedicels of the fingers (Wallbridge, 1981). Crown rot is unevenly distributed among the hands and healthy and rotted crowns are usually present in the same box. The disease is frequently seasonal, and has been associated with both hot, dry weather and periods of wet weather (Finlay and Brown, 1993). The levels of the disease increases with transit time, and spreads rapidly through the crowns during ripening as fruit are transferred from 14 to 17°C (Marin *et al.,* 1996). Infected tissue turns black and the rot may advance into the finger stalks (Fig. 1.1), causing the fingers to drop off when handled. Severe infection induces premature ripening (Kraus *et al.,* 1998).

In crown rot disease, different organisms predominate according to location, time of year and other factors. Many of the fungi survive on leaf debris in the plantation. Their spores are dispersed either by wind or rain-splash, and impinge on all parts of the developing bunch (Marin *et al.,* 1996). When the harvested fruit is cut into hands and washed, the newly exposed tissue is vulnerable to infection. Spores that have accumulated in the washing water can be drawn several millimeters into the wound. If the site of infection remains beyond the reach of fungicides, decay

ensues and the combined effects of the various organisms can lead to rapid rotting (Snowdon, 1990).

Fig. 1.1 A banana hand showing crown rot. (Snowdon, 1990).

The disease is caused by a complex of fungi with the main pathogen being, *Colletotrichum musae* Berk. & Curt. (Knight, 1982). Additionally, *F. verticilliodes*, *F. verticilliodes* var. *subglutinans* Wollenw and Reinking, *F. pallidoroseum* (Cooke) Sacc., *Nigrospora sphaerica* Sacc., and *Botryodiplodia theobromae* Pat. have been implicated in crown rot in the Windward Islands (Johanson and Blazquez, 1992; Krauss *et al.,* 1998). In Central America and the Caribbean the most prevalent fungi are *Colletotrichum musae* and species of *Fusarium* (Wallbridge, 1981). In Africa and Asia *Botryodiplodia theobromae* and species of *Fusarium* frequently are found instead of or in addition to some of the above mentioned fungi (Marin *et al.,* 1996; Snowdon, 1990).

1.2.4.2 Incidence of crown rot

The prevalence of crown rot of bananas in South Africa is not documented. There is no available literature available to date to indicate if any research on crown rot of bananas is being carried out in South Africa. Jimenez *et al.* (1993) surveyed banana fruit over a six-month period in Ecuador to determine the incidence of some species of *Fusarium* and assess their potential pathogenicity. Among the *Fusarium* species detected in fruits, *F. semitectum* var. *majus* Wollenw. was predominant, followed by *F. verticilliodes*, *F. solani*, *F. oxysporum* Schlecht., *F. proliferatum*, *F. graminearum*, *F. camptoceras*, *F. subglutinans*, *F. dimerum*, *F. acuminatum* and *F. equiseti* (Table 1.2). The lack of noticeable differences in relation to the origin (Panama, Ecuador and Canary Islands) of the different species isolated, indicated that the mycoflora found may be typical of this fruit and does not depend on its origin (Jimenez *et al.*, 1993). Further studies by the same authors showed that *F. subglutinans*, *F. acuminatum* and *F. graminearum* were found to be the most pathogenic species (Table 1.3).

Biochemical changes in banana fruit in response to crown rot pathogens were studied by Alam *et al.* (1993). Two variants of bananas, Sagar and Manik (a local variant),susceptible and resistant to crown rot pathogens respectively were studied. Changes in total phenol, orthohydroxy phenols, reducing and non-reducing sugars and amino-nitrogen in the bananas after inoculation with *Botryodiplodia theobromae* and *Fusarium roseum* were investigated. Fruits of Manik variants were characterized by higher quantities of total and orthohydroxy phenols, and reducing and non-reducing sugars when compared to Sagar, which had relatively higher amino nitrogen. Inoculation with the pathogens led to differences in the accumulation and reduction of various components in

the two banana variants. These differences could be attributed to differences in the nutritional requirements of the pathogens and different degrees of biochemical changes caused by the pathogen (Alam *et al.*, 1993).

Table 1.2 Incidence of *Fusarium* species from bananas of different geographical origins (Jimenez *et al.*, 1993).

***Fusarium* species**	**Percentage of infected samples***			
	Panama (21)*	**Ecuador (15)**	**Canary Island (36)**	**Total (72)**
F. semitectum	90.5	86.7	91.7	90.3
F. verticilliodes	33.3	40	41.7	38.9
F. solani	42.8	46.7	19.4	31.9
F. oxysporum	14.3	20	27.8	19.4
F. proliferatum	23.8	20	16.7	16.7
F. graminearum	14.2	33.3	11.1	16.7
F. camptoceras	19	20	13.9	4.2
F. subglutinans	23.8	26.7	5.5	15.3
F. dimerum	9.5	13.3	11.1	11.1
F. acuminatum	19	13.3	0	8.3
F. equiseti	9.5	20	0	6.9

* The figure in brackets denotes the number of samples studied

Table 1.3 Pathogenicity of *Fusarium* species in banana fruits (Jimenez *et al.*, 1993).

	Rot diameter (cm) [b]			Weight loss (%)
***Fusarium* species**	**2 days**	**4 days**	**6 days**	**Mean ± SD**
F. semitectum	1	1.5	2	30.9 ± 4.2
F. verticilliodes	1.1	2.2	3	27.4 ± 3.5
F. solani	1	2	2.5	27.8 ± 3.7
F. oxysporum	1	1.7	2.5	22.3 ± 4.0
F. proliferatum	1	2	2.5	25.9 ± 4.0
F. graminearum	1.2	2.5	3	85.0 ± 6.5
F. camptoceras	1	1.6	3.1	19.2 ± 3.3
F. subglutinans	1	1.9	3	87.0 ± 7.2
F. dimerum	0.8	1.1	1.5	14.3 ± 3.1
F. acuminatum	1	2.5	3	67.8 ± 7.4
F. equiseti	0.9	1.3	1.7	15.0 ± 3.7

[a] Eighteen fruits were inoculated with each species.

[b] The listed diameters are the average of 72 measurements (18 fruits x 4 inoculations).

1.2.4.3 Control strategies

Control of crown rot ranges from immersion in hot water to the use of fungicides. Many of the pathogens develop resistance to fungicides. Control of crown rot begins in the plantation with regular removal of leaf trash. Hygiene is also important in the packing station and washing water should be changed frequently before it becomes heavily contaminated with spores (Marin *et al.*,

1996). Immersion of banana (*Musa* spp., cultivars 'Santa Catarina Prata' and 'Williams') in hot water was evaluated as a potential control procedure for crown rot (Reyes *et al.,* 1998) and this has the potential to replace chemical fungicides to control crown rot of banana (Reyes *et al.,* 1998). In the Windward Islands, crown rot is controlled commercially by a post-harvest treatment that involves submerging clusters of banana in solutions of the fungicides thiabendazole (TBZ) or imazalil. The latter is more effective, but four times as expensive as the former. High levels of resistance to the fungicides, especially TBZ, have been reported from the Windward Islands (Johanson and Blazquez, 1992). TBZ tolerance is believed to be due to cross-resistance to benomyl, another benzimidazole fungicide, which is regularly used in aerial sprays against the yellow sigatoka disease (Cronshaw, 1984; Santchurn, 1999; Wisniewski and Wilson, 1992).

Control systems practised in many tropical countries that use wet pack-houses involve the use of knap sack sprayers or automatic sprayers. The spraying machines are loaded with the recommended fungicide which could be either prochloraz, imazalil or TBZ. The cut hands are placed in trays and then moved slowly on rollers into enclosures for fungicide treatment. After treatment, the banana hands are placed carefully into boxes and are ready for export (Ploetz, 2000). In some countries both TBZ and imazalil are being used in combination for crown rot control, because neither is sufficiently effective as a sole post harvest treatment (Krauss *et al.,* 1998). In South Africa, the hands are dipped into the fungicide (TBZ) before packing into boxes. Thus there is a need to develop sustainable strategies for crown rot control before the present system collapses.

1.2.5 *Fusarium* Species

Knowledge of *Fusarium* taxonomy and the appropriate procedures for identification are basic to most studies involving this genus. Although the genus *Fusarium* includes some populations, which are quite variable, the identification of most species can be accomplished if consistent and appropriate media, culturing procedures and incubation conditions are adopted (Burgess *et al.*, 1988).

The genus *Fusarium*, which is classified in the class Hypomycetes, is now widely considered an anamorphic genus affiliated with the Hypocreales (Ascomycetes). *Fusarium* is characterized by the production of slimy, hyaline, septate, canoe-shaped conidia (known as macroconidia), that are produced in fruiting-structures called sporodochia. In addition to this, some species also produce distinctly different conidia in the aerial mycelium often referred to as microconidia (Burgess *et al.*, 1988). According to the species and the ecological situation, either macroconidia or microconidia may dominate on the natural substrate. Chlamydospores are also produced by some species (Burgess *et al.*, 1988). Because one must see all of these anamorphic forms in order to identify a *Fusarium* species with certainty, most modern *Fusarium* taxonomy is based on cultural characters. The important cultural characters of *Fusarium* species are used in conjunction with a *Fusarium* taxonomic key for their identification (Burgess *et al.*, 1988).

Identifying cultures of *Fusarium* species requires careful observation and attention to every detail. The *Fusarium* identification manuals presently available provide a series of synoptic keys (Nelson *et al.*, 1983), dichotomous keys (Booth, 1971), tabular keys (Burgess *et al.*, 1988) or no key at all (Gerlach and Nirenberg, 1982). There are basically two types of keys: branched and synoptic

(numerical) keys. Branched keys contain steps that are numbered in sequence. The user follows the numbers according to the features that match the organism being identified. Dichotomous keys are the simplest branched keys, with only two choices at each step (Booth, 1971). Synoptic keys (also known as outline keys) are set out in a tabular form. The illustrated identification manual of Nelson *et al.* (1983) provides synoptic keys to both the sections and species of 30 different *Fusarium* species.

The literature on toxigenic abilities of *Fusarium* species contains significant number of confusions (Burgess *et al.*, 1988), caused by usage of several taxonomic systems, wrong identification of toxigenic isolates, or incorrect identification of mycotoxins. The taxonomy of *Fusarium* species is complex because many of them, even when grown from monospore cultures, exhibit extreme variability in their morphological characteristics (Guarro *et al.*, 1999).

1.2.6 Mycotoxin Production by *Fusarium* species

At one time the taxonomy of *Fusarium* species may have been a subject of purely academic interest. However, since species of this genus have been implicated in grave disorders in man and animals due to the production of toxins, identification of these species and strains has become a matter of extreme importance. The toxic *Fusarium* species have been isolated from various samples of fresh, stored and overwintered cereals (prosomillet, wheat, barley, rye, oats), from vegetable crops, fruits, soil and from feed grains and have also been implicated in alimentary toxic aleukia resulting in death (Jacobsen *et al.*, 1999).

Fusarium species, which may be described as soil fungi, produce a variety of mycotoxins. The members of the genus *Fusarium* are among the most important plant pathogens in the world (Nelson *et al.*, 1983). In recent years, the genus has acquired additional importance since many *Fusarium* species have been shown to produce mycotoxins that have been implicated in both animal and human diseases. *Fusarium* species are a widespread cosmopolitan group of fungi which commonly colonize aerial and subterranean plant parts, either as primary or secondary invaders. Since *Fusarium* species are common in soil, it is rare to find a necrotic plant root in agricultural soils that is not colonized by at least one *Fusarium* species.

The genus *Fusarium* is also known to produce many mycotoxins that attack plant cells. These are called phytotoxins (substances that are toxic to plants), and they may break down the cell walls of the host plant, or help dissolve and release nutrients. If these fungi did not contain such toxins, they would not be able to gain entry into plants i.e., they would not be toxic to the plant, but it is also true that many of these phytotoxins are toxic to humans and other living organisms as well. Phytotoxins that have been isolated from *Fusarium* species are fusaric acid, lycomarasmin, lycomarasmic acid and Nep? (24kDA protein). Fusaric acid is considered to be the most important phytotoxin. It is produced by pathogenic strains of *F. oxysporum* that causes wilt diseases of a great variety of plants (Kuiper-Goodman, 1998).

Fusarium species that cause vascular wilt, attack many important agricultural and horticultural crops, including banana, cabbage, cotton, flax, muskmelon, onion, pea, tomato, watermelon and tulips. These *Fusarium* species have the unique ability to become established systemically in the water-conducting system of their host (MacHardy and Beckman, 1981). Symptoms are quite variable, but include combinations of vein-clearing, leaf epinasty, wilting, chlorosis, necrosis and

abscission. Severely infected plants wilt and die, while plants affected to a lesser degree may become stunted and unproductive (MacHardy and Beckman, 1981). Two roles have been proposed for the involvement of mycotoxins in *Fusarium* wilt: toxins have a casual role in susceptibility and toxins have a primary role in symptom induction. A few studies have indicated that toxins may be involved in pathogenesis. One study showed that vein clearing occurred in tomato 24 hour after inoculation (MacHardy and Beckman, 1981). Changes have also been noted in infected leaf chloroplasts that were absent in plants lacking water and possible involvement of mycotoxins. The changes appeared to accompany chlorosis (MacHardy and Beckman, 1981).

1.2.6.1 *Fusarium* mycotoxicosis

Disease in animals and humans arising from the consumption of mycotoxins are called mycotoxicoses. The effects in domestic animals include allergic reactions, reproductive failure, unthriftiness, loss of appetite, feed refusal, suppression of the immune system, decreased feed efficiency, and mortality (Table 1.4) (Jacobsen *et al.*, 1999). Human mycotoxicoses includes ergot poisoning associated with ingestion of rye flour contaminated with ergot, cardiac beriberi associated with *Penicillium* molds in rice (yellow rice toxins), and alimentary toxic aleukia associated with *Fusarium* molds on over-wintered wheat, millet and barley (Table 1.5). Several mycotoxins have been linked to increased incidence of cancer in humans. These include aflatoxins, sterigmatocystin, zearalenone, patulin, ochratoxin and fumonisins (Jacobsen *et al.*, 1999; Miller, 1994). Certain *Fusarium* fungi are capable of causing a variety of diseases in corn, wheat, maize, potato, vegetable crops and fruits, including seedling disease, stalk rots, and ear rots. In addition

to their effect on corn yields, theses *Fusarium* species sometimes produce mycotoxins in the infected ears and kernels (Bottalico *et al.*, 1989; Vincelli and Parker, 1999). Since many *Fusarium* species such as *F. graminearum* and *F. culmorum* often produce more than one toxin, co-contamination is common (Smith, 1992).

Table 1.4 Toxic effects of some *Fusarium* mycotoxins in humans and animals

Mycotoxin	Symptomatic Effects in Humans and Animals	References
Deoxynivalenol	Nausea, vomiting, visual disturbances, chills, vertigo and feed refusal in animals. Acute gastrointestinal illness in humans	Bhat *et al.* (1989) Childress *et al.* (1990) Luo (1988)
T-2 Toxin	Immunosuppressor, strong inhibitor of protein synthesis in mammalian cells	Rotter *et al.* (1994)
Fusaric acid	Affects brain and pineal neurotransmitters. Hypotensive reaction in dogs and cats	Porter *et al.* (1990) Porter *et al.* (1995)
Fumonisin B_1	Equine leucoencephalomalacia, porcine pulmonary edema. Associated with human esophageal cancer	Marasas (1995) Rheeder *et al* (1992)
Moniliformin	Decreases performances and immune function of broiler chicks	Li *et al.* (2000)

Table 1.5 Selected human and veterinary diseases associated with mycotoxins (Krogh, 1978; Marasas and Nelson, 1987).

Diseases (Affected Species)	Causative Toxin	Food / Feed
Alimentary toxic aleukia (human)	T-2 and other *Fusarium* toxins	Over wintered wheat
Encephalopathy (horses)	Fumonisins	Grains
Facial eczema (sheep)	Sporidesmin	Pasture grass
Hypoestrogenism (swine)	Zearalenone	Corn
Nephropathy (pigs, poultry)	Ochratoxin	Barley, oats
Stachybotryotoxicosis (cattle)	Verrucarin, satratoxin H	Hay, straw
St. Anthony's Fire (human)	Ergot alkaloids	Rye bread
Yellow rice disease (human)	Citrinin, citreovirdin	Rice
Turkey-X disease (poultry)	Aflatoxins	Peanut meal, grains

The toxic effects of mycotoxins can be divided into two broad categories:

1) acute effects which cause rapid and often fatal diseases; and
2) chronic effects which may cause weight loss, immunosuppression, cancer, reduced milk yields and other sublethal effects (Anke, 1999; Anon, 1993).

Since levels of mycotoxins depend on weather conditions, their amounts vary from year to year, from commodity to commodity and from one locality to another. In a similar manner to animals, humans are susceptible to mycotoxins. Exposure originates from the consumption of contaminated plant commodities, but also might occur via a secondary route following the

consumption of meat, milk and eggs containing residual amounts of mycotoxins ingested by food-producing animals (Gremmels, 1999). This carry over-linking animal production to public health has been observed for almost all mycotoxins and is based on their high lipophilicity. However, the concentrations found in animal tissues are considerably lower than those found in plant commodities and thus residues in edible tissues of animals contribute only to a small percentage of mycotoxin exposure to humans. An exception might be the exposure of young children (babies and infants) consuming higher amounts of milk and dairy products in relation to their body weight than adults. Since experimental and epidemiology evidence suggests that certain mycotoxins are associated with different forms of human cancer, mechanistic research has focussed primarily on mutagenic and carcinogenic fungal toxins. Prominent examples of mycotoxins produced by *Fusarium* species in grains are fumonisin B_1, fusaric acid, T-2 toxin, deoxynivalenol, moniliformin and zearalenone (Moss, 1996; Omurtug, 2001). Prominent examples of mycotoxins produced by *Fusarium* species in plants are fumonisin B_1, fusaric acid and moniliformin (Moss, 1996).

1.2.7 Mycotoxins Produced by *Fusarium* Species isolated from Bananas

The ability of *Fusarium* species isolated from bananas to produce mycotoxins was studied by Jimenez *et al.* (1997) with 66 isolates of the following *Fusarium* species: *F. semitectum* (8 isolates), *F. camptoceras* (3 isolates), *F. verticilliodes* (16 isolates), *F. proliferatum* (9 isolates), *F. subglutinans* (3 isolates), *F. solani* (3 isolates), *F. oxysporum* (5 isolates), *F. graminearum* (7 isolates), *F. dimerum*, (3 isolates), *F. acuminatum* (3 isolates) and *F. equiseti* (3 isolates). These *Fusarium* species produced mycotoxins, moniliformin, zearalenone and T-2 Toxin (Table 1.6)

Table 1.6 Production of mycotoxins in corn cultures by *Fusarium* species isolated from bananas (Jimenez *et al.*, 1997).

***Fusarium* species**	**No. of isolates tested**	**No. of mycotoxin-producing isolates**	**Mycotoxin (μg/g)**
F. semitectum	8	0	ND*
F. camptoceras	3	3	ND*
F. verticilliodes	16	13	Moniliformin (50)
F. proliferatum	9	9	Moniliformin (20-400)
F. subglutinans	3	0	ND*
F. solani	3	0	ND*
F. oxysporum	5	5	Moniliformin (10-500)
F. graminearum	7	7	Zearalenone (6-470)
F. dimerum	3	0	ND*
F. acuminatum	3	3	T-2 Toxin (5-15)
F. equiseti	3	3	Zearalenone (5-25)

ND*. Not Detected

The predominant mycotoxins that were isolated included moniliformin, zearalenone and T2 toxin. Further studies by Jimenez and Mateo (1997), of mycotoxins produced by *Fusarium* isolates from banana fruits by capillary gas chromatography and high performance liquid chromatography showed that in addition to the above mycotoxins, fumonisin B_1 is also present. The fungi *F. verticilliodes* Sheldon has been isolated from bananas by several workers throughout the world including India (Peshney and Ghaukar, 1984), the Windward Islands (Wallbridge, 1981) and

Panama, Ecuador and the Canary Islands (Jimenez *et al.,* 1993). These toxins have also been detected in *Musa sapientum* L. (a variety of banana) infected with this species (Chakrabarti and Ghosal, 1986). *F. verticilliodes* isolates have been found to produce three 12, 13-epoxytrichothecene mycotoxins (trichothecolone, diacetoxyscirpenol (DAS), and T-2 toxin), the palmitoyl esters of trichothecolone, scirpenetriol, and T-2 tetraol, and free and palmitic acid conjugate zearalenone, in laboratory cultures. In many cases, the banana fruits exhibited no apparent visible signs of fungal contamination.

1.2.8 Characteristics of the Predominent *Fusarium* Mycotoxins in Bananas

The mycotoxins produced by *Fusarium* species are structurally quite varied. Often, there is a series of closely related compounds that can be identified as a group, such as the trichothecenes that lack nitrogen in their structure and fumonisins and lycomarasmins, which possess amine functions. *Fusarium* mycotoxins may leach into the soil, causing damage to plants and animals through leaching even after the fungus is no longer active. Although *Fusarium* mycotoxins such as T-2 toxin, fumonisins B_1, fusaric acid, moniliformin and zearalenone are found in bananas, this section only will review fumonisin B_1, fusaric acid, and moniliformin mycotoxins.

1.2.8.1 Fumonisin B_1

The most recently discovered *Fusarium* toxin is fumonisin B_1 (FB_1), a representative of the fumonisin group produced predominantly by *F. verticilliodes* (Moss, 1998). *Fusarium verticilliodes* is a serious pathogen of cereals such as maize and rice and was recognised as a mycotoxigenic species over a decade ago (Marasas, 1985). Although members of the *Fusarium* group, which includes *F. verticilliodes*, do not produce trichothecenes, they are associated with the production of a number of toxic metabolites such as the fusarins and moniliformin. Unlike other mycotoxins, FB_1 has a low lipid solubility which led to its discovery in 1988 (Marasas, 1995). Fumonisins (six different derivatives have been described of which FB_1 is recognized as the most toxic) are structural analogues of cellular sphingolipids (Fig 1.2) and thus impair ceramide synthesis by inhibiting sphinganine-N-acetyl-transferase (ceramide synthetase) [Casteel and Braun, 1992, Moss, 1998].

Fumonisin B_1 is detected most frequently and, is most often associated with illness in farm animals or humans. However, the clinical symptoms resulting from exposure to FB_1 in feed show remarkable variation across species. In horses (and other equines) FB_1 causes encephalomalacia (equine leukoencephalomalacia [ELEM]), a disease characterized by the degeneration of neurons ('hole-in-the-head' disease) [Ross *et al.*, 1990]. In pigs, the main symptoms of FB_1 exposure is pleural edema (porcine pulmonary edema) [PPE] impairing respiratory and heart function (Diaz and Boermans, 1994). In ruminants, FB_1 is not degraded by the rumen flora, but similar to other species poorly absorbed from the intestinal lumen. However, despite the low circulating levels of FB_1, accumulation of free sphinganine bases have been found in proximal tubular cells,

accompanied by the impairment of renal function. In laboratory animals including rats, mice and rabbits, FB_1 was found to induce hepatotoxicity and nephrotoxicity and has tumor-promoting activity (Gremmels, 1999). FB_1 is also known to be associated with Panama disease of the banana tree, causing vascular wilting of the trees (Okole 1995).

The American Association of Veterinary Laboratory Diagnosticians has recommended maximum levels of 5, 10, and 50 ppm FB_1 in feed for horses, swine, and beef and poultry, respectively. Although relatively high levels of fumonisins have been detected in corn in some areas of the world with high rates of esophageal cancer in humans, it has not been determined whether fumonisins are causally related to development of this cancer (FRI Briefings, 1999). A recent report from India described an acute but self-limiting foodborne diseases outbreak in villagers consuming moldy corn containing up to 64.7 mg fumonisin/kg (Bhat *et al.*, 1997). It is not known whether lower mycotoxin concentrations, chronically consumed, cause other detrimental effects in humans, and tolerance levels for fumonisins in grains for human consumption have not been set (FRI Briefings, 1999).

From the point of view of human health, the most pressing need is to understand the role of fumonisins in the epidemiology of oesophageal cancer. In the Transkei region of Southern Africa, there is clear evidence of a significantly higher incidence of *F. verticilliodes* as well as of fumonisin contamination of maize in high-risk areas compared to low risk areas (Rheeder *et al.*, 1992). FB_1 is non-mutagenic and non-genotoxic when tested according to standard protocols for genotoxic carcinogens (Gelderblom and Snyman, 1991). However, *in vivo* studies provided evidence for fumonisin-induced carcinogenicity in rodents (Gelderblom *et al.*, 1991). FB_1 exhibits cancer-promoting activity in rats and may play an important role as human carcinogens. In the Transkei

region of South Africa where maize is the human staple food, consumption of FB_1 contaminated maize has been linked to esophageal cancer. In this region, FB_1 levels of up to 117.5 μg/ml were detected in moldy maize used for brewing beer (Chu and Li, 1994). Similar association between consumption of FB_1 contaminated maize and esophageal cancer has been reported in China and North Eastern Italy. Furthermore, FB_1 has been detected in maize and maize based foods marketed in several countries worldwide (Chu and Li, 1994).

With the widespread occurrence of FB_1 in maize and maize products, it is important to understand how stability of fumonisins and the types of food processing can reduce contamination. An initial process of removing the 'fines' (broken kernel fragments and dust) from bulk shipments resulted in a reduction of 26% to 69% of FB_1 present in maize (Desjardins *et al.*, 1997). Fumonisin B_1 are fairly stable compounds and even processes, such as heat treatment under alkaline conditions, only remove the tricarballyic acid residues leaving a molecule which is still toxic (Hopmans and Murphy, 1993).

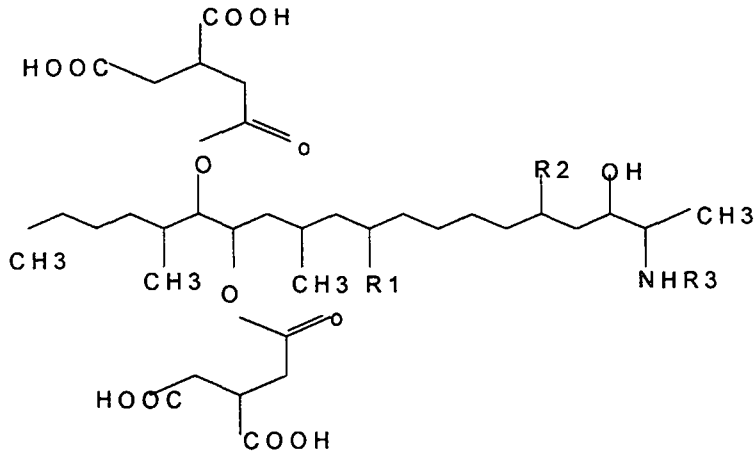

Fig. 1.2 Structure of fumonisin B_1 ($R_1 = R_2 = OH$, $R_3 = H$) [Fink-Gremmels, 1999]

1.2.8.2 Fusaric acid

Fusaric acid is a known phytotoxin, causing wilt diseases of a great variety of plants including banana (Bacon *et al.*, 1996). This mycotoxin has been isolated from *Fusarium*-infected corn, wheat, and suspect toxic feeds and has been suggested to enhance the activity of some of the *Fusarium* mycotoxins. Fusaric acid (5-butylpicolinic acid) [Fig. 1.3] was first discovered during laboratory culture of *Fusarium heterosporum* Nees. This compound was one of the first fungal metabolites implicated as the causal agent of tomato wilt symptoms caused by *F. oxysporum* f. sp. *lycopersici* Schlecht (Burmeister *et al.*, 1985).

Although fusaric acid is not generally regarded as a mycotoxin, some attention was given to fusaric acid production by *F. oxysporum.* Fusaric acid, as well as certain other phytotoxins such as lycomarasmin and lycomarasmic acid produced by *F. oxysporum* are chelating agents and may be involved in certain diseases of abnormal bone development. In addition, fusaric acid is toxic to mice and death caused by lethal doses has been attributed to its hypotensive effect (Ploetz, 1990).

Although not acutely toxic to the developing foetal, neonate, juvenile or adult rat, fusaric acid is passed from the feed of nursing dams (via the milk) to the suckling (Porter *et al.*, 1996). Fusaric acid also alters brain and pineal gland concentrations of tyrosine, dopamine, norepinephrine, serotonin, N-acetylserotonin, and melatonin in the pineal gland of juvenile weanlings from dams maintained on diets containing this mycotoxin (Rimando and Porter, 1997).

Recent studies show that fusaric acid enhances the toxicity of fumonisin B_1 in the fertile leghorn egg test (Smith *et al.*, 1997). Other studies have shown that fusaric acid enhances the toxicity of diacetoxyscirpenol, deoxynivalenol (other *Fusarium* toxins) and selected pesticides to certain insects; the neurooendocrine effects of deoxynivalenol in swine are also enhanced by fusaric acid. Current investigations show that a combination of zearalenone and fusaric acid in the diet of lactating rats increases the passage of both mycotoxins into the milk of the suckling neonate at five-fold than that of their individual concentrations (Porter *et al.*, 1996).

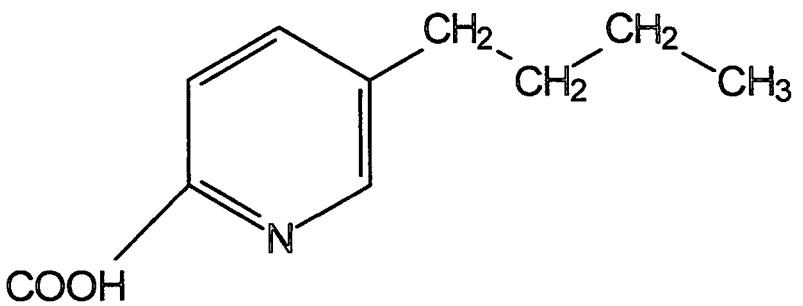

Fig. 1.3 Structure of fusaric acid (Burmeister *et al.*, 1985)

1.2.8.3 Moniliformin

Moniliformin is the sodium or potassium salt of 1-hydroxycyclobut-1-ene-3, 4-dione (Fig. 1.4) produced by *F. verticilliodes* (Cole *et al.*, 1973). It occurs in corn grown in Transkei (Thiel *et al.*, 1982) and the Federal Republic of Germany (Thalmann *et al.*, 1985). Moniliformin has plant-growth regulating and phytotoxic effects (Cole and Cox, 1981). Toxicological analysis disclosed that this mycotoxin also causes rapid death in animals without severe cellular damage. Moniliformin has recently been found to decrease performance and immune response in broiler chicks (Li *et al.*, 2000).

An interesting find was the occurrence of acute degenerative lesions in the myocardium and other tissues, which indicated an involvement of suppressed ATP-derived transmembrane transport mechanisms (Ueno, 1984). At the molecular level, moniliformin does not inhibit eukaryotic protein synthesis and was not mutagenic in the Ames test. This toxin, however, is a potent inhibitor of mitochondrial pyruvate and -ketoglutarate oxidation (Ueno and Shimada, 1974; Faber, 1988).

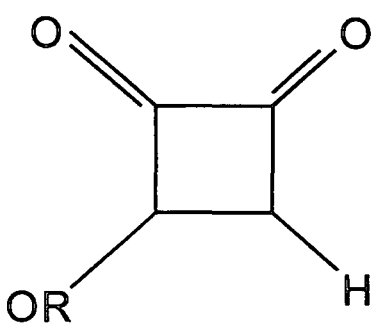

Fig. 1.4 Structure of moniliformin (Cole *et al.*, 1973)

CHAPTER TWO: MATERIALS AND METHODS

2.1 INTRODUCTION

This study involved fungal isolation and identification followed by determining the mycotoxins produced by the various isolates and a correlation of this to pathogenicity. The first phase involved the collection of harvested banana (hands) from various farm holdings along the South Coast of KwaZulu Natal, followed by the isolation of *Fusarium* species, and their subsequent identification. The second part of this investigation involved the determination of the mycotoxin-producing potential of the isolated *Fusarium* species and a pathogenicity profile of the isolates. A correlation was finally determined between mycotoxin production and the pathogenicity of the *Fusarium* isolates.

2.2 ISOLATION OF *FUSARIUM* SPECIES

2.2.1 Sampling

Fifty banana hands of the Cavendish genotype were collected from five farm holdings along the South Coast of Kwazulu Natal following during the harvesting season between August 2000 and February 2001. The five farm holdings are situated along the coastline of Kwazulu Natal. An average of 15 banana hands were collected from each of the five different farm holdings (Farm A, B, C, D, E) and each hand consisted of approximately 4-6 fingers. Three to five fingers from each banana hand were sealed into polyethylene bags, stored at 15°C in commercial packing boxes and incubated for 5-8 days to ripen.

2.2.2 Isolation of *Fusarium* Species from the Crown Tissue of the Banana

Each ripened banana fruit was surface sterilized with 70% ethanol for 45-60 seconds, rinsed in sterile distilled water and blotted dry with sterile filter paper. Tissue fragments from the crown region of each fruit (3-8 mm) were aseptically cut, placed onto Sabouraud dextrose agar (SDA, Saarchem) [Appendix 1] in 9 cm sterile petri-dishes, and incubated at 25°C (± 2°C) for 4-6 days. Fungal colonies exhibiting different morphological characteristics were then inoculated onto *Fusarium*

selective medium (Appendix 1) and dichloran chloramphenicol peptone agar (Sigma) [Appendix 1] to obtain pure cultures for identification. The cultures were incubated at 20°C (± 3°C) for 5-7 days and examined microscopically for future identification studies.

2.3 IDENTIFICATION OF *FUSARIUM* SPECIES

2.3.1 Fungal Cultivation

Pure cultures obtained from the *Fusarium* selective medium (Appendix 1)and dichloran chloramphenicol peptone agar were transferred onto potato dextrose agar (PDA) and carnation leaf agar (CLA). Gross morphological features, characteristics and colony colourations were evaluated after growth on PDA. This medium was prepared according to the method of Nelson *et al.* (1983) using baking-grade, white skinned potatoes. The unpeeled potatoes were washed and sliced then 250 g was added to 500 ml of distilled water and autoclaved for 30 minutes at 121°C. The potato broth was strained through several layers of cheesecloth into a flask containing 4% sterile molten agar. The remaining potato pulp was squeezed through several layers of cheesecloth until half a cup of potato pulp was obtained. The pulp was added to the molten agar and potato broth together with 20 g of dextrose. The final volume was adjusted to one litre by adding distilled water, the mixture homogenized by mixing, autoclaved once again and dispensed into sterile petri-dishes.

Carnation leaf agar (CLA), a minimal nutritional medium that induces sporulation of *Fusarium* species, was prepared according to the method of Nelson *et al.* (1983). Young carnation leaves (*Dianhus cayophyllus L.*) were harvested from actively growing budded plants that were free from pesticide residues. The leaves were cut into 5 mm^2 pieces and dried in an oven (Labcon) at 45°C to 55°C for 2 hours. Loss of green pigmentation was used as an indictor to monitor temperature of drying. The leaf pieces were irradiated with 2.5 megarads from a Cobalt 60 source (Gamwave, Prospecton). Carnation-leaf agar was prepared by placing several sterile leaf pieces in a petri-dish and floating them on 1.5-2 % water agar cooled to 45°C. The medium was left at room temperature for 3-4 days before use to ensure that there was no microbiological growth from the leaf pieces.

Based on colony characteristics suspected *Fusarium* isolates were collected and subcultured onto Sabouraud dextrose agar (SDA) plates to obtain pure cultures. The plates were incubated for 14 days at 28°C. Cultures were freeze-dried and maintained at 4°C until required for species identification.

To obtain single spores, conidial suspensions of *Fusarium*, were harvested from SDA plates by adding 10 ml sterile distilled water to the sporulated *Fusarium* cultures and scraping the surfaces with a sterile blade until a turbid suspension was formed. The conidial suspensions were then overlayed on 2% water agar (Appendix 1). The plates were incubated in an inclined position at room temperature for 24 hours and examined under 100x magnification with a Nikon Dissecting Microscope. Single spores were removed and stored for future work.

2.3.2 Fungal Identification

Small agar squares, each containing a single germinating conidium were cut with a sterile blade and transferred aseptically to PDA and CLA plates. The PDA plates were incubated for 14 days at 25°C, whilst the CLA plates were incubated for 3-5 days at 25°C under a fluorescent lamp with 12 hours per day exposure. After the respective incubation period, the cultures were ready for microscopic examination.

2.3.2.1 Light microscopy

The wet mount method (Nelson *et al.*, 1983) was used to identify *Fusarium* species. Culture material was removed from the CLA plates using a sterile dissecting needle and transferred to a clean glass slide containing a small drop of lacto-phenol cotton blue stain (Appendix 1). The culture material was teased out on the slide using two dissecting needles. A cover-slip was placed over the stained culture material gently, so as to prevent the formation of any air bubbles. For each culture, fungal structures in terms of their size, shape, distribution of macroconidia, microconidia, conidiophores (branched or unbranched) and chlamydospores were observed at 400x magnification with a light microscope (Nikon).

The *Fusarium* isolates were placed into their respective species level using a synoptic key (Pelczar *et al.*, 1993) [Appendix 2]. The characteristics that were used for speciation included surface growth, colour of the undersurface of the colony, conidial colours, colony texture and sulcation (wrinkling), exudates and droplets on the surface of the colony on PDA.

2.3.2.2 Scanning electron microscopy (SEM)

Scanning electron microscopy was carried out at the Electron Microscope (EM) Unit at the University of Natal, Durban. Samples were prepared for SEM according to protocols described by Nelson *et al.* (1983). The first treatment involved primary fixation, where a single piece of carnation leaf containing the *Fusarium* culture was removed from CLA plates using sterile forceps, and transferred to glass vials. Plastic disposable pipettes were used to cover the leaf pieces with buffered 2 % glutaraldehyde solution (Sigma Aldrich) [Appendix 3]. The leaf pieces were fixed twice for 2 hours each.

The leaf pieces were washed three times for 5 minutes each in phosphate buffer (pH 7.0) [Appendix 3]. Once the phosphate buffer was removed from the vials, the leaf pieces were fixed for 1 hour in 0.5% osmium tetroxide (Sigma Aldrich) [Appendix 3]. Thereafter, the leaf pieces were washed three times with distilled water for 5 minutes each.

The leaf pieces were finally dehydrated by successively immersing in 30%, 50%, 75% and 100% ethanol. Each alcohol treatment was performed twice for 5 minutes each except the 100% alcohol treatment which was done twice for 10 minutes each.

The leaf pieces (still submerged in 100% alcohol) were transferred into mesh baskets and placed in a Critical Point Dryer (Hitachi HCP-1) overnight. Drying was achieved by flushing liquid nitrogen through the mesh baskets. After drying, double-sided tape was used to fix the dried leaf pieces onto metal stubs. The CPD works by replacing all the alcohol with liquid carbon dioxide. Over a period of between 30 minutes to three hours, carbon dioxide was fed into the system to drain out the alcohol. Once the alcohol was removed the 'bomb' was sealed and the temperature increased to 45°C. The system was gradually vented to dry the specimens. Once the specimens had dried they were removed and placed on stubs. The metal stubs were then placed in the E5100-Polaron Sputter Coater (SEM Coating Unit) and the leaf pieces coated with colloidal gold. The samples were viewed using the electron microscope unit at the University of Natal. Various magnifications were used to view for the presence and shape of microconidia, macroconidia (including apical and basal cell structures) and chlamydospores.

2.4 MYCOTOXIN ANALYSIS

2.4.1 Culture Conditions

All 23 isolates of *Fusarium*, maintained on SDA at 4°C, were separately and aseptically inoculated onto maize patties that were prepared as outlined by Thiel *et al.* (1993). Thirty grams of the maize mixture was dispensed into sterile glass petri-dishes. The petri-dishes were wrapped in heavy duty aluminum foil and autoclaved for 30 minutes at 121°C. Approximately 5 mm^2 agar pieces containing fungal mycelium of the various *Fusarium* isolates from the SDA plates were excised using a sterile blade, and aseptically transferred to the autoclaved maize patty culture. The cultures were incubated in the dark for 28 days at 25°C to allow for mycotoxin production.

2.4.2 Mycotoxin Extraction and Purification

2.4.2.1 Fumonisin B_1 (FB_1)

Fumonisin B_1 was extracted according to the method of Thiel *et al.* (1993). Fumonisin B_1 was extracted from five grams of the *Fusarium* maize culture by first mixing with 10 ml of methanol/water

(3:1) for 2 hours. The slurry was filtered using Whatman No. 4 filter paper and the pH of the supernatant was adjusted to pH 6 using 1 M hydrochloric acid (HCl) [Appendix 4]. Purification of FB_1 involved preconditioning a strong anion exchange (SAX) resin cartridge (Waters Corporation) with 1 ml of methanol, followed by applying 1ml of methanol/water (3:1) to the SAX cartridges. Two millilitre aliquots of the filtered extract (pH 3) were applied to the SAX cartridge at a flow rate of 1 ml per minute. The cartridge was washed with 1 ml methanol/water (3:1), and FB_1 eluted with 1 ml of methanol. The final eluent was collected with 2 ml of 1% acetic acid in 100% methanol solution [Appendix 4]. The eluents were evaporated to dryness under a stream of nitrogen using a rotary evaporator (Buchi Rotovapour RE 120) and stored at 4°C.

2.4.2.2 Fusaric acid

Fusaric acid was extracted by a modification of the method described by Smith and Sousadias (1993). Five grams of the *Fusarium* culture was weighed into 45 ml centrifuge tubes (Merck) and shaken thoroughly with 25 ml of methanol/1% KH_2PO_4 (50:50, v/v) at pH 3.0 [Appendix 4]. The samples were centrifuged at 10, 000 x *g* for 20 minutes at ambient temperature. The pH of the supernatant was adjusted to pH 3 with 2 M HCl. The acidified supernatant was extracted three times using 20 ml dichloromethane. The dichloromethane extracts were pooled and the volume reduced to less than 10 ml using a rotary evaporator. The dichloromethane fraction was then extracted twice with 5 ml of aqueous 5% $NaHCO_3$ [Appendix 4]. The dichloromethane fraction was discarded and the aqueous $NaHCO_3$ solutions were pooled. The pH of the $NaHCO_3$ fraction was adjusted to pH 3.0 with 5 M

HCl and the solution thereafter extracted twice with dichloromethane. The dichloromethane extracts were combined, and removed *in vacuo* at 40°C using the rotary evaporator. The resulting residue was stored at 4°C for TLC and HPLC analysis.

2.4.2.3 Moniliformin

Moniliformin was extracted from 5 g of the *Fusarium* culture with 10 ml of methanol/water (3:1) for 2 hours as described by Thiel *et al.* (1993). Bond-Elut strong anion exchange (SAX) resin cartridges were used for the extraction of moniliformin. The slurry was filtered using Whatman No. 4 filter paper and the pH of the supernatant was adjusted to pH 6 using 1 M hydrochloric acid (HCl). Purification of the mycotoxin involved preconditioning the SAX cartridges with 1 ml of methanol, followed by applying 1ml of methanol/water (3:1) to the SAX cartridges (Waters). Two millilitres aliquot of the filtered extract (pH 3) was extracted using SAX cartridge as for FB_1 except that moniliformin was collected with 2 ml of 1% HCl in methanol solution (Appendix 4).

2.4.3 Mycotoxins Analysis by Thin Layer Chromatography (TLC)

Thin Layer Chromatography for FB_1 detection was performed according to the method of Thiel *et al.* (1993). Stock solutions of 100 μg of FB_1 standard (Sigma) were prepared by dissolving 0.1 mg of FB_1 standard in 1 ml of acetonitrile/water (1:1, v/v) and stored at 4°C. Working standards of 50 μg/ml, 10 μg/ml, 1 μg/ml and 0.1 μg/ml were prepared by dilution of the stock solution with acetonitrile/water (1:1, v/v). Samples were reconstituted in 1 ml of acetonitrile/water (1:1, v/v). Ten μl of the samples and standard were spotted onto pre-coated 0.25 mm thick Silica Gel 60 TLC plates (Merck) and the components separated using ethyl acetate: glacial acetic acid: water (60: 30: 10, v/v/v) as the solvent system. Detection was achieved by spraying the plates with *p*-anisaldehyde visualizing agent (Appendix 4), and heated at 110°C for 5 min. Migration rates (Rf) of the samples were compared to that of the FB_1 standard.

Thin Layer Chromatography for fusaric acid detection was carried out according to the method of Burmeister *et al.* (1985). Stock solutions of 100 μg of fusaric acid standard (Sigma) were prepared by dissolving 0.1 mg of fusaric acid standard in 1 ml of methanol and storing at 4°C. Working standards of 50 μg/ml, 10 μg/ml, 1 μg/ml and 0.1 μg/ml were prepared by dilution of the stock solution with methanol. Samples were reconstituted in 1 ml of methanol. Ten μl of the samples and standard were spotted onto pre-coated 0.25 mm thick Silica Gel 60 F_{254} TLC plates (Merck) and the components separated using isopropanol/ethyl acetate/water/glacial acetic acid (20: 19: 10: 1, v/v/v/v) as the solvent system. Individual bands were visualized under shortwave UV light (254 nm) and

make with a soft pencil. Migration rates (Rf) of the samples were compared to that of the fusaric acid standard.

Thin Layer Chromatography for moniliformin was modified from the method of Desjardins *et al.* (1997). Stock solutions of 100 μg, of moniliformin standard (Sigma) were prepared by dissolving 0.1 mg of moniliformin standard in 1 ml of methanol and stored at 4°C. Working standards of 50 μg/ml, 10 μg/ml, 1 μg/ml and 0.1 μg/ml, were prepared by dilution of the stock solution with methanol. Samples were reconstituted in 1 ml of methanol. Ten μl of the samples and standard were spotted onto pre-coated 0.25 mm thick Silica Gel 60 F_{254} TLC plates (Merck) and the components separated using acetonitrile/water (85: 15, v/v) as the solvent system. Individual bands were visualized under shortwave UV light (254 nm) and marked with a soft pencil. Migration rates (Rf) of the samples were compared to that of the moniliformin standard.

The detection limits for each mycotoxin (FB_1, fusaric acid and moniliformin) was determined by spotting 10 μl of each of the mycotoxin standards at concentrations ranging from 0.1-10 μg/ml. The lowest concentration of the standard that produced a spot was the detection limit for the technique.

2.4.4 Mycotoxin Analysis by High Performance Liquid Chromatography (HPLC)

Fumonisin B_1, fusaric acid and moniliformin standards were commercially purchased from Sigma Chemicals and diluted in methanol. The following concentrations were prepared for detection by HPLC:

Fumonisins B_1 :	10, 5, 3, 1, 0.5, 0.25, 0.1, 0.05 & 0.01 μg/ml
Fusaric Acid :	10, 5, 3, 1, 0.5, 0.25, 0.1, 0.05 & 0.01 μg/ml
Moniliformin :	10, 5, 3, 1, 0.5, 0.25, 0.1, 0.05 & 0.01 μg/ml

The lowest concentration that produced a reproducible peak area that was three times the signal of the noise level was considered the detection limit for that toxin (Ravindranath, 1989). All samples were analysed in duplicate.

The Merck LaChromm (Hitachi) HPLC system was used for analysis of fumonisin fusaric acid and moniliformin. The HPLC columns were stainless steel, 4 x 250 mm in diameter with 5 μm Lichrospher Reverse Phase - 18 and Lichrospher Reverse Phase particles. The FB_1, fusaric acid and moniliformin extracts were quantified using different HPLC parameters.

2.4.4.1 Fumonisin B_1

Fumonisin B_1 was analyzed according to the method of Thiel *et al.* (1993) with a reverse-phase RP-8 column (Merck). The mobile phase consisted of methanol : 0.1 M sodium dihydrogen phosphate (adjusted to pH 3.3 using orthophosphoric acid) (68:32, v/v) (Appendix 4). The fumonisin B_1 extracts were reconstituted in 200 μl of acetonitrile/water (1:1) and derivatized prior to analysis. The derivatizing reagent was prepared by dissolving 4 mg *ortho*-pthaldialdehyde (OPA) in 1 ml methanol and adding 5 ml 0.1 M sodium tetraborate and 50 μl 2-mercaptoethanol (Appendix 4). Samples were prepared immediately prior to injection by the addition of 450 μl of the OPA-derivatizing reagent to 50 μl of purified toxin extract. The HPLC system was stabilized at a flow rate of 1.2 ml/min. Detection was accomplished using a Merck - L7480 fluorescence detector at an excitation of 365 nm and emission of 425 nm. The final concentration of fumonisin B_1 was calculated from the peak areas using the following equation:

$$A\ (ng) = G/H \times S$$

Where A = ng toxin present in test extract injected into HPLC

G = toxin area in test sample

H = toxin peak area of standard

S = amount of toxin injected into HPLC (Thiel *et al.*, 1993)

The concentration (C) of the toxin present in the test extract (ng/g) was calculated using the following equation:

$$C\ (ng/g) = \frac{A \times T \times D}{I \times W}$$

Where A = calculated above

T = total volume of sample

D = dilution volume used

I = injection volume used

W = test portion equivalent weight (Thiel *et al.*, 1993)

2.4.4.2 Fusaric acid

Fusaric acid was analyzed according to the method of Venter and Steyn (1998) under reverse phase conditions using a Merck RP-18 column. The mobile phase consisted of 40% methanol in 60% of an aqueous solution of 0.62 mM Na_2EDTA and 2% H_3P0_4 (Appendix 4). The system was stabilized at a flow rate of 1.0 ml/min. Peaks were detected at a fixed wavelength of 254 nm using a Merck L7400 UV lamp. Extracts were reconstituted in 1 ml of methanol and 50 μl was injected into the system. The final concentration of fusaric acid was calculated as outlined in 2.4.4.1.

2.4.4.3 Monilformin

Moniliformin was analysed according to the method of Faber (1988) under reverse-phase conditions using a Merck RP-18 column. Extracts were reconstituted in 1 ml of methanol and 50 μl was injected into the system. The mobile phase consisted of 10 mM phosphate buffer with 5 mM tetrabutylammonium bromide (pH 7) / methanol (92:8, v/v) [Appendix 4]. The system was stabilized at a flow rate of 1.0 ml/min. Peaks were detected at a fixed wavelength of 215 nm using a Merck L7400 UV lamp. The final concentration of moniliformin was calculated as outlined in 2.4.4.1.

2.4.5 Recovery Studies

Extraction efficiency was investigated by determining the recovery of FB_1, fusaric acid and moniliformin standards by spiking the maize patty cultures with a concentration of 10 μg/g of each toxin. Experiments were performed in triplicate. The percentage recovery was calculated using the following equation, (Ravindranath, 1989)

$$\% \text{ Recovery} = [C_{calculated} - C_{untreated} / C_{initial\ spiked}] \times f \times 100$$

Where,

$C_{calculated}$ = Detectable concentration of toxin (μg/ml) from the spiked sample

$C_{untreated}$ = Detectable concentration of toxin (μg/ml) from the untreated sample

$C_{initial\ spiked}$ = Initial concentration of toxin (μg/ml) used to spike the sample

f = Dilution factor, where f=1 for the liquid - liquid extraction, and f=5 for the SAX cartridge extraction method (Ravindranath, 1989).

2.5 PATHOGENICITY ASSAYS

The isolated *Fusarium* species were used for pathogenicity assays, as outlined by Jimenez *et al.* (1993). Those isolates that showed the highest mycotoxin-producing capabilities after growth on maize patties were used to inoculate a variety of healthy banana fruit that were obtained from various vendors around central Durban.

Healthy, half-ripened banana fruits were selected, weighed, surface sterilized with 75% (v/v) ethanol and washed twice with standard distilled water. A spore suspension (10^9 spores/ml in 0.01% Tween 80) was prepared from 7 day old cultures of each *Fusarium* isolate cultured on potato-dextrose agar at 25°C. Two light bruises were made on the sterilized banana skins with a sterile needle and 50 μl of the spore suspension was inoculated into each of the bruised spots. In control experiments 50 μl of sterile water, was inoculated into bruised banana fruit.

The inoculated fruit were then incubated at 25°C for 5-6 days. The rot diameter was measured, using a vernier calliper after 2, 4 and 6 days of incubation. The bananas were reweighed and the percentage fruit weight loss was ascertained. The damaged pulps were removed with a scalpel and an attempt was made to extract the mycotoxins using methods described by Jimenez *et al.* (1997).

CHAPTER THREE: RESULTS

3.1 Fungal Populations in the Banana Fruit

Species of *Fusarium*, *Aspergillus* and *Penicillium* and other unidentifiable fungal species were isolated in different proportions. The most predominant fungal species were the *Fusarium* species. This was followed by *Aspergillus* and *Penicillium* species respectively. A high percentage of *Fusarium* species (± 50%) were isolated from farm holdings A, C, D and E. Farm holding E showed the highest percentage of *Fusarium* species, followed by farm holding A, D and C respectively. No *Fusarium* species were isolated in farm holding B (Fig. 3.1).

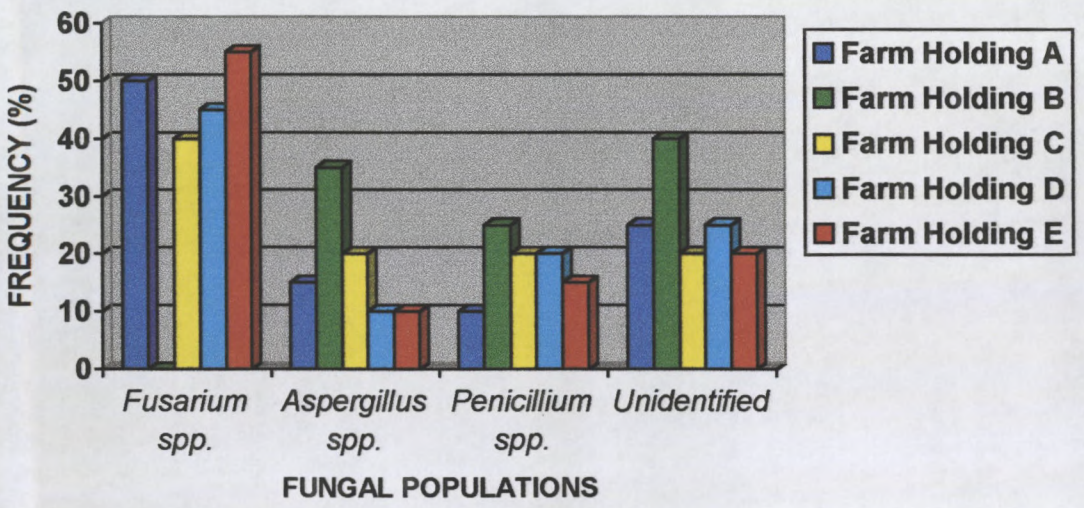

Fig. 3.1 Frequency of occurrence of the different fungal populations isolated from the five farm holdings.

3.2 Isolation and Identification of *Fusarium* Species

3.2.1 Cultural characteristics

Using *Fusarium* selective media and dichloran-chloramphenicol-peptone agar, pure cultures of *Fusarium* species were isolated. Different species of *Fusarium* were isolated from farm holdings A, C, D and E. The *Fusarium* culture isolated from farm holding A showed an abundance of white, aerial mycelium with a tinge of purple (Fig. 3.2). The *Fusarium* culture isolated from farm holding C showed an abundance of white aerial mycelium with a tinge of orange (Fig. 3.3). On the other hand the *Fusarium* culture isolated from farm holding D showed tan brown aerial mycelium with the production of orange exudates (Fig. 3.4), whilst *Fusarium* culture isolated from farm holding E showed an abundance of white to tan brown aerial mycelium (Fig. 3.5).

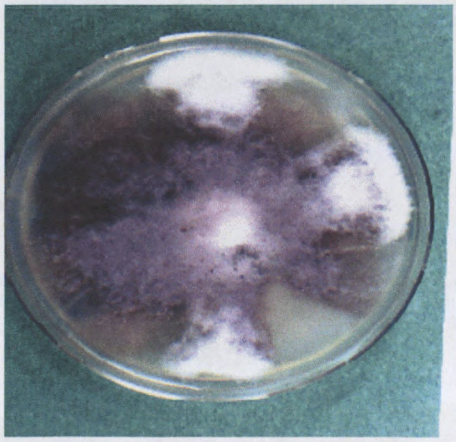

Fig. 3.2 *Fusarium* culture isolated from farm holding A with an abundance of white aerial mycelium with a tinge of purple.

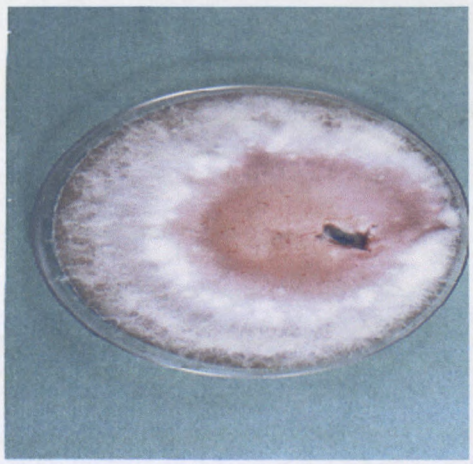

Fig. 3.3 *Fusarium* culture isolated from farm holding C with an abundance of white aerial mycelium with a tinge of orange.

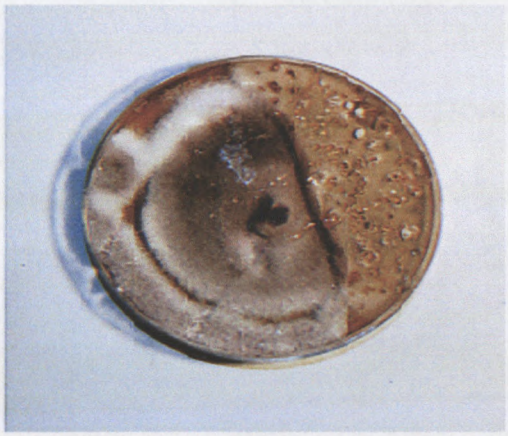

Fig. 3.4 *Fusarium* culture isolated from farm holding D showing tan brown aerial mycelium with the production of exudates.

Fig. 3.5 *Fusarium* culture isolated from farm holding E with an abundance of white to tan brown aerial mycelium.

The characterization of the different isolates of *Fusarium* to species level was done after growth on CLA medium and PDA medium. These media gave rise to different colony morphologies and mycelium pigmentation of the different *Fusarium* species (Fig. 3.6-3.12). *Fusarium verticilliodes* cultures showed an abundance of white aerial mycelium with a tinge of purple (Fig. 3.6). *Fusarium oxysporum* cultures were similar to *F. verticilliodes* cultures, with a tinge of orange sporodochia present (Fig. 3.7). Cultures of *F. subglutinans* showed an abundance of white to tan brown mycelium and orange exudates (Fig. 3.8). *F. sambucinum* cultures showed sparse, tan to reddish brown aerial mycelium (Fig. 3.9). Cultures of *F. solani* showed an abundance of white to cream mycelium, with a tinge of green exudates produced (Fig. 3.10). *Fusarium chlamydosporum* cultures showed dense white to tan yellow aerial mycelium with a tan brown under-surface (Fig. 3.11). *Fusarium acuminatum* cultures showed sparse tan to orange aerial mycelium (Fig. 3.12).

Fig. 3.6 Abundant, white aerial mycelium with a tinge of purple, with colourless to purple under-surface when grown on PDA medium, indicative of *F. verticilliodes*.

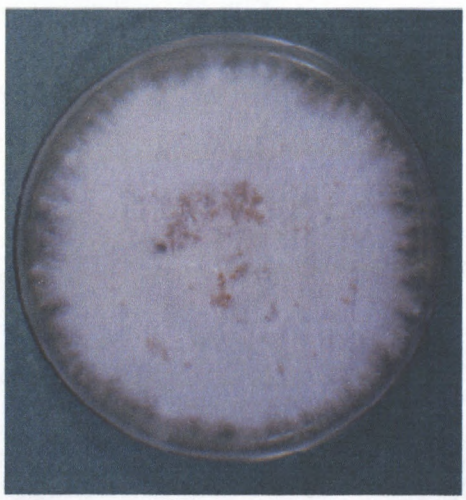

Fig. 3.7 Abundant, white aerial mycelium with a tinge of purple, a tinge of orange sporodochia and colourless under-surface when grown on PDA, indicative of *F. oxysporum*.

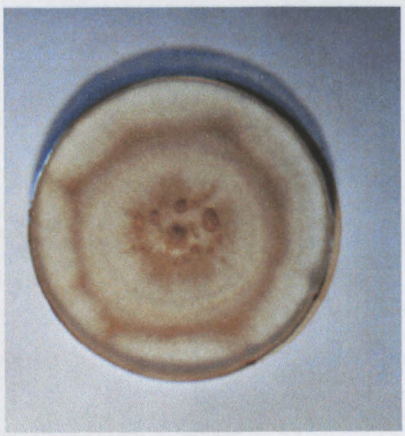

Fig. 3.8 Abundant, white aerial mycelium with a tinge of blue, violet to dark purple under-surface and tan to orange exudates when grown on PDA, indicative of *F. subglutinans.*

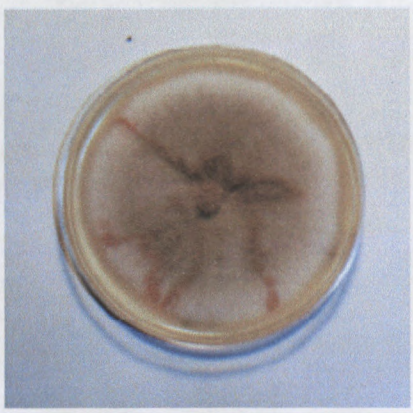

Fig. 3.9 Sparse aerial mycelium with a tan to reddish brown colour, tan to brown under-surface and cream to tan to orange sporodochia when grown on PDA medium, indicative of *F. sambucinum.*

Fig. 3.10 Abundant, white to cream mycelium growth, with cream to colourless under-surface and tinge of green exudates produced when grown on PDA medium, indicative of *F. solani.*

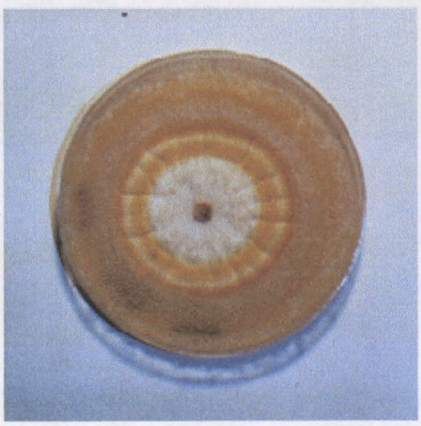

Fig. 3.11 Dense white to tan yellow aerial mycelium and tan to brown under-surface when grown on PDA medium, indicative of *F. chlamydosporum.*

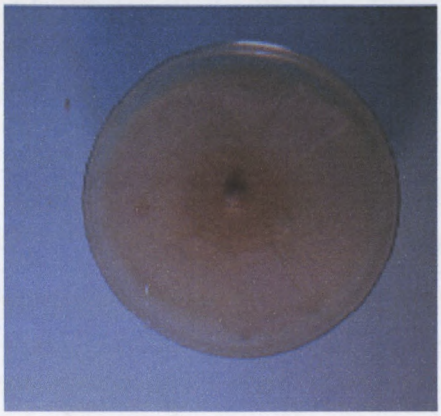

Fig. 3.12 Sparse tan to orange aerial mycelium and carmine red to tan brown under-surface when grown on PDA medium, indicative of *F. acuminatum.*

3.2.2 Morphological characteristics

The morphological characteristics of the 23 isolated *Fusarium* cultures grown on CLA were examined by light microscopy using the wet mount method (Fig. 3.1). *Fusarium verticilliodes and F. sambucinum* cultures contained branched monophialides. *Fusarium oxysporum* cultures contained branched and unbranched, short monophialides. Both branched and unbranched monophialides and polyphialides were observed in cultures of *F. subglutinans. Fusarium solani* cultures contained long, slender, unbranched monophialides. Cultures of *F. chlamydosporum* contained branched monophialides and polyphialides. *Fusarium acuminatum* cultures contained both branched and unbranched monophialides.

Table 3.1 Phialide characteristics of *Fusarium* species isolated from bananas

***Fusarium* species**	**Type of phialides present**
F. verticilliodes	Branched monophialides observed
F. oxysporum	Branched and unbranched, short monophialides observed
F. subglutinans	Both branched and unbranched monophialides and polyphialides observed
F. sambucinum	Branched monophialides observed
F. solani	Long, slender, unbranched monophialides
F. chlamydosporum	Branched monophialides and polyphialides observed
F. acuminatum	Both branched and unbranched monophialides observed

3.2.3 Ultra-structural characteristics

Scanning electron microscopy was used to observe the distinguishing features of the three types of *Fusarium* spores, i.e. microconidia, macroconidia and chlamydospores from the isolates cultured on carnation leaf agar.

Fruiting bodies or spore-bearing structures (sporodochia) were observed in all the *Fusarium* species isolated. The typical foot-shaped basal cell characteristic of *F. verticilliodes*, *F. oxysporum* and *F. solani* macroconidia were observed in 14 isolates (Fig. 3.13 and Fig. 3.16). Macroconidia shape, size and septation were used in the identification of *Fusarium* species (Fig. 3.13). The important features of *Fusarium* macroconidia, which are formed in sporodochia (fruiting bodies) (Fig. 3.17), that distinguishes the different types of species are shape, length and curvature of the basal and apical cells.

Rough-walled chlamydospores typical of *F. chlamydosporum* species were observed in one isolate (Fig. 3.14). The differentiation of *Fusarium* species based on microconidia revealed single-celled, kidney shape microconidia typical of *F. solani* (Fig. 3.18), and also single-celled, double-celled, spindle-shaped and elongated microconidia were observed in most *Fusarium* species (Fig. 3.15).

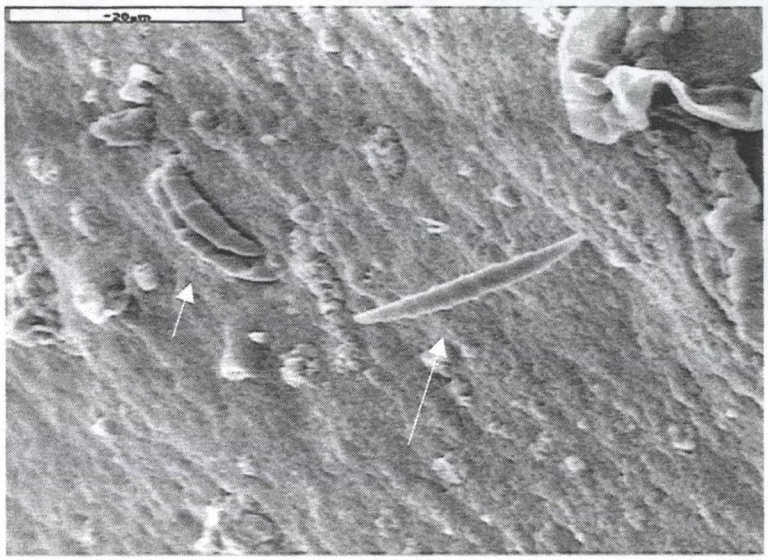

Fig. 3.13 Scanning electron micrograph of a typical septate (2-3) macroconidia of *Fusarium* species. (Bar = 20 μm).

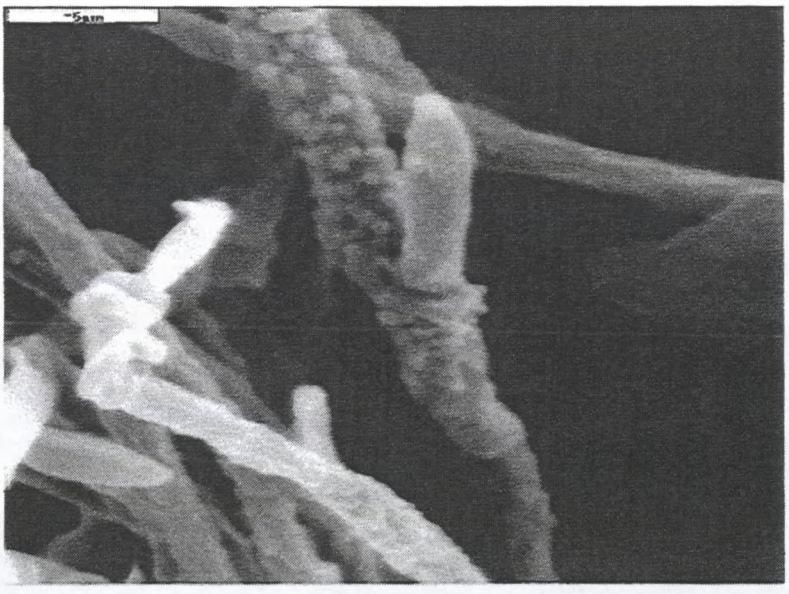

Fig. 3.14 Scanning electron micrograph of rough-walled chlamydospores of *Fusarium* species, used as a secondary identification criterion. (Bar = 5 μm).

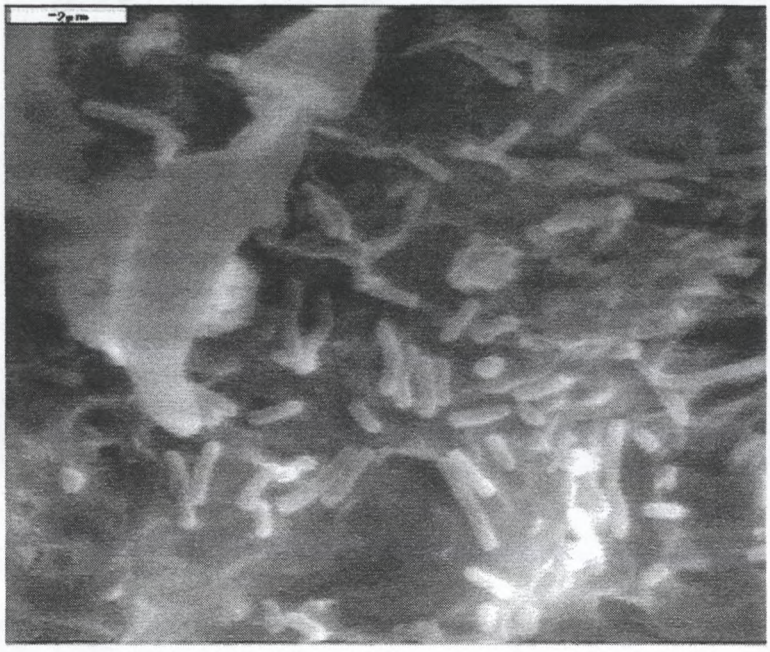

Fig. 3.15 Scanning electron micrograph of single-celled, spindle, kidney-shaped and elongated microconidia common in *Fusarium* species. (Bar = 2 μm).

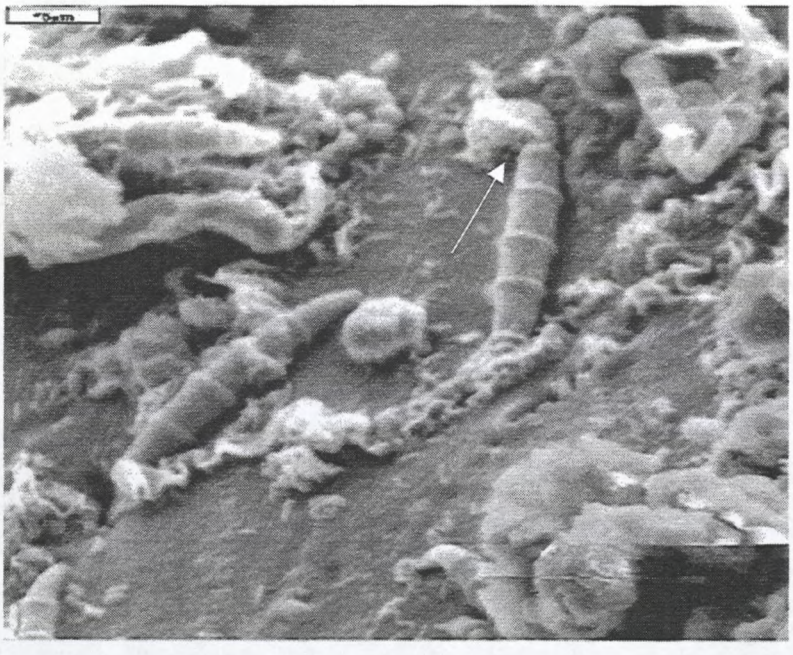

Fig. 3.16 Scanning electron micrograph of a *F. oxysporum* macroconidium. (Bar = 5 μm).

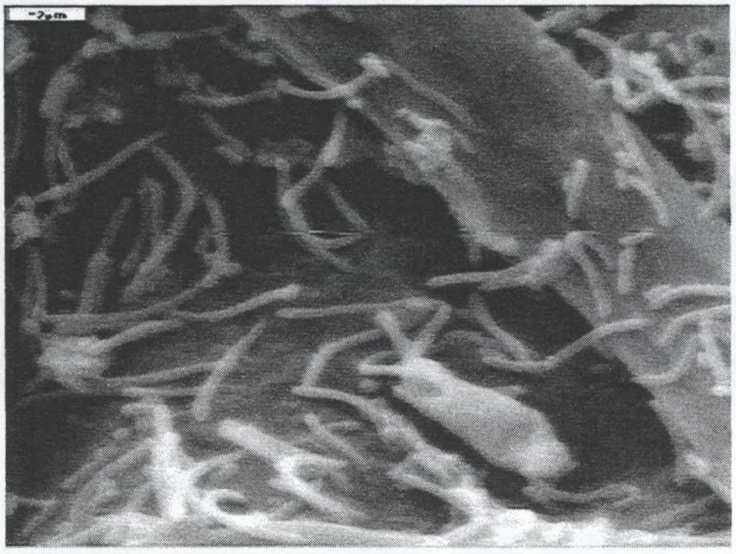

Fig. 3.17 Scanning electron micrograph of fruiting bodies or spore-bearing structures (sporodochia). (Bar = 2 μm).

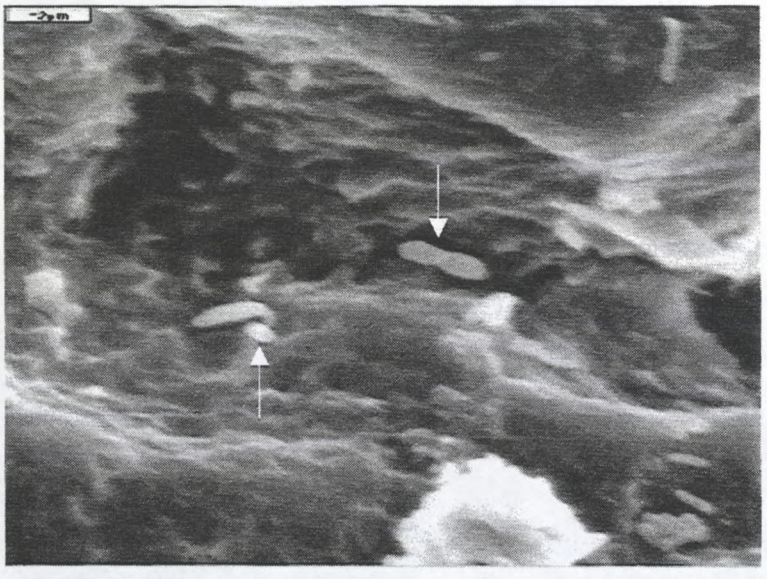

Fig. 3.18 Scanning electron micrograph of single/double-celled, kidney-shaped (arrow) *F. solani* microconidia. (Bar = 2 μm).

3.2.4 Taxonomic Classification

The synoptic key (Appendix) to the sections and species of *Fusarium* revealed that the banana isolates showed similar cultural characteristics of seven *Fusarium* species i.e. *F. verticilliodes, F. oxysporum, F. subglutinans, F. sambucinum, F. solani, F. chlamydosporum* and *F. acuminatum.* Isolates A5, A9, A10, D17, D19, E21, E22 and E2 were identified *as F. verticilliodes* (Table 3.2). Isolates A1, C11, C13, C14 and C15 were identified as *F. oxysporum* (Table 3.3). Isolates A4, A7, A8 and D20 were identified as *F. subglutinans* (Table 3.4). Isolates A2, A3 and A6 were identified as *F. sambucinum* (Table 3.5); isolate C16 as *F. solani* (Table 3.6); isolate D18 as *F. chlamydosporum* (Table 3.7) and isolate C12 as *F. acuminatum* (Table 3.8).

Of the 23 Fusarium isolates, eight (34.7%) were identified as *F. verticilliodes*, five (21.7%) were identified as *F. oxysporum*, four (17.4%) were identified as *F. subglutinans*, three (13.3%) were identified as *F. sambucinum* and one (4.3%) each of *F. solani*, *F. chlamydosporum* and *F. acuminatum* (Table 3.9).

Table 3.2. Synoptic key for section and species for isolates A1, C11, C13, C14 and C15 *(F. oxysporum)*

SYNOPTIC KEY TO SECTION		SYNOPTIC KEY TO SPECIES	
Characteristics used to identify *Fusarium* section	**No. indicating *Fusarium* section**	**Characteristics used to identify *Fusarium* species**	**No. indicating *Fusarium* species in the sections Eupionnotes, Spicarioides and Arachnites**
A. Cultural characteristics		***A. Cultural characteristics***	
1. Rate of growth	2, 4, 5, 6, 7, 8, 10, 11, 12	1. Rate of growth	25, 26, 27, 28, 29, 30
2. Aerial mycelium present or absent	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	2. Aerial mycelium present or absent	24, 25, 26, 27, 28, 29, 30
3. Colour of aerial mycelium	10, 11	3. Colour of aerial mycelium	25, 26, 27, 28, 29, 30
4. Colour of colony from below	1, 3, 4, 5, 6, 7, 8, 10, 11, 12	4. Colour of colony from below	24, 25, 26, 27, 28, 29
5. Colour of spore masses	11, 12		
B. Macrocondidia from sporodochia		***B. Macrocondidia from sporodochia***	
1. Size	1, 4, 5, 6, 7, 8, 9, 10, 11, 12	1. Size	25, 26, 27, 28, 29, 30
2. Shape	2, 6, 8, 9, 10, 11, 12	2. Shape	25, 26, 27, 28, 29
3. Shape of basal and apical cells	2, 4, 5, 6, 7, 8, 9, 10, 11, 12	3. Shape of basal and apical cells	
C. Microconidia from aerial mycelium		***C. Microconidia from aerial mycelium***	
1. Present or absent	2, 4, 7, 8, 9, 10, 11, 12	1. Present or absent	24, 25, 26, 27, 28, 29, 30
2. In chains or false heads	4, 11, 12	2. In chains or false heads	24, 27, 28, 29, 30
3. Shape	2, 4, 7, 8, 10, 11, 12	3. Shape	24, 25, 26, 27, 29, 30
D. Conidiophores		***D. Conidiophores***	
1. Type	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,	1. Type	29
E. Chlamydospores		***E. Chlamydospores***	
1. Present or absent	1, 4, 6, 7, 8, 9, 11, 12	1. Present or absent	29, 30
2. Arrangement	1, 4, 9, 11, 12		

Table 3.3. Synoptic key of section and species for isolates A5, A9, A10, D17, D19, E21, E22 and E23 (*F. verticilliodes*)

SYNOPTIC KEY TO SECTION		SYNOPTIC KEY TO SPECIES	
Characteristics used to identify *Fusarium* section	**No. indicating *Fusarium* section**	**Characteristics used to identify *Fusarium* species**	**No. indicating *Fusarium* species in the sections Eupionnotes, Spicarioides and Arachnites**
A. Cultural characteristics		***A. Cultural characteristics***	
1. Rate of growth	2, 4, 5, 6, 7, 8, 10, 11, 12	1. Rate of growth	25, 26, 27, 28, 29, 30
2. Aerial mycelium present or absent	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	2. Aerial mycelium present or absent	24, 25, 26, 27, 28, 29, 30
3. Colour of aerial mycelium	10, 11	3. Colour of aerial mycelium	25, 26, 27, 28, 29, 30
4. Colour of colony from below	10, 11, 12	4. Colour of colony from below	
5. Colour of spore masses	11, 12		
B. Macrocondidia from sporodochia		***B. Macrocondidia from sporodochia***	
1. Size	1, 4, 5, 6, 7, 8, 9, 10, 11, 12	1. Size	25, 26, 27, 28, 29
2. Shape	2, 6, 8, 9, 10, 11, 12	2. Shape	
3. Shape of basal and apical cells	2, 4, 5, 6, 7, 8, 9, 10, 11, 12	3. Shape of basal and apical cells	
C. Microconidia from aerial mycelium		***C. Microconidia from aerial mycelium***	
1. Present or absent	2, 4, 7, 8, 9, 10, 11, 12	1. Present or absent	24, 25, 26, 27, 28, 29, 30
2. In chains or false heads	2, 10	2. In chains or false heads	25, 26
3. Shape	2, 4, 7, 8, 10, 11, 12	3. Shape	24, 25, 26, 27, 29, 30
D. Conidiophores		***D. Conidiophores***	
1. Type	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,	1. Type	25, 30
E. Chlamydospores		***E. Chlamydospores***	
1. Present or absent	1, 4, 6, 7, 8, 9, 11, 12	1. Present or absent	25, 26, 27, 28
2. Arrangement	1, 4, 9, 11, 12		

Table 3.4. Synoptic key of section and species for isolates A4, A7, A8 and D20 (*F. subglutinans*)

SYNOPTIC KEY TO SECTION		SYNOPTIC KEY TO SPECIES	
Characteristics used to identify *Fusarium* section	**No. indicating *Fusarium* section**	**Characteristics used to identify *Fusarium* species**	**No. indicating *Fusarium* species in the sections Eupionnotes, Spicarioides and Arachnites**
A. Cultural characteristics		***A. Cultural characteristics***	
1. Rate of growth	2, 3, 8, 9	1. Rate of growth	24
2. Aerial mycelium present or absent	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	2. Aerial mycelium present or absent	24, 25, 26, 27, 28, 29, 30
3. Colour of aerial mycelium	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	3. Colour of aerial mycelium	24, 25, 26, 27, 28, 29, 30
4. Colour of colony from below	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12	4. Colour of colony from below	29, 30
5. Colour of spore masses	11, 12		
B. Macrocondidia from sporodochia		***B. Macrocondidia from sporodochia***	
1. Size	1, 4, 5, 6, 7, 8, 9, 10, 11, 12	1. Size	25, 26, 27, 28, 30
2. Shape	2, 6, 8, 9, 10, 11, 12	2. Shape	25, 26, 27, 28, 29
3. Shape of basal and apical cells	2, 4, 5, 6, 7, 8, 9, 10, 11, 12	3. Shape of basal and apical cells	
C. Microconidia from aerial mycelium		***C. Microconidia from aerial mycelium***	
1. Present or absent	2, 4, 7, 8, 9, 10, 11, 12	1. Present or absent	24
2. In chains or false heads	4, 11, 12	2. In chains or false heads	24, 25, 26, 27, 28, 29, 30
3. Shape	2, 4, 7, 8, 10, 11, 12	3. Shape	24, 27, 29, 30
D. Conidiophores		***D. Conidiophores***	
1. Type	4, 6, 7, 10	1. Type	26, 27, 28
E. Chlamydospores		***E. Chlamydospores***	
1. Present or absent	1, 2, 3, 5, 10	1. Present or absent	29, 30
2. Arrangement			

Table 3.5. Synoptic key of section and species for isolates A2, A3 and A6 (*F. sambucinum*)

SYNOPTIC KEY TO SECTION		SYNOPTIC KEY TO SPECIES	
Characteristics used to identify *Fusarium* section	**No. indicating *Fusarium* section**	**Characteristics used to identify *Fusarium* species**	**No. indicating *Fusarium* species in the sections Eupionnotes, Spicarioides and Arachnites**
A. Cultural characteristics		***A. Cultural characteristics***	
1. Rate of growth	2, 4, 5, 6, 7, 8, 10, 11, 12	1. Rate of growth	12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23
2. Aerial mycelium present or absent	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	2. Aerial mycelium present or absent	12, 13, 14, 15, 20, 21, 22, 23
3. Colour of aerial mycelium	4, 7, 8	3. Colour of aerial mycelium	12, 13, 14, 15, 16, 17, 18, 19, 20
4. Colour of colony from below	1, 3, 4, 5, 6, 7, 8, 10, 11, 12	4. Colour of colony from below	16, 17, 18, 19, 20, 21, 22, 23
5. Colour of spore masses	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11		
B. Macrocondidia from sporodochia		***B. Macrocondidia from sporodochia***	
1. Size	1, 4, 5, 6, 7, 8, 9, 10, 11, 12	1. Size	18, 20, 21, 22
2. Shape	2, 6, 8, 9, 10, 11, 12	2. Shape	14, 15, 16, 17, 18, 19, 20, 21, 22, 23
3. Shape of basal and apical cells	2, 4, 5, 6, 7, 8, 9, 10, 11, 12	3. Shape of basal and apical cells	14, 15, 20
C. Microconidia from aerial mycelium		***C. Microconidia from aerial mycelium***	
1. Present or absent	2, 4, 7, 8, 9, 10, 11, 12	1. Present or absent	14, 15, 20
2. In chains or false heads	4, 11, 12	2. In chains or false heads	14, 15, 20
3. Shape	2, 4, 7, 8, 10, 11, 12	3. Shape	24, 25, 26, 27, 29, 30
D. Conidiophores		***D. Conidiophores***	
1. Type	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,	1. Type	14, 16, 17, 18, 19, 20, 21, 22, 23
E. Chlamydospores		***E. Chlamydospores***	
1. Present or absent	1, 4, 6, 7, 8, 9, 11, 12	1. Present or absent	14, 15, 16, 17, 18, 19, 20, 21, 22, 23
2. Arrangement	4, 6, 7, 8		

Table 3.6. Synoptic key of section and species for isolate C16 *(F. solani)*

SYNOPTIC KEY TO SECTION		SYNOPTIC KEY TO SPECIES	
Characteristics used to identify *Fusarium* section	**No. indicating *Fusarium* section**	**Characteristics used to identify *Fusarium* species**	**No. indicating *Fusarium* species in the sections Eupionnotes, Spicarioides and Arachnites**
A. Cultural characteristics		***A. Cultural characteristics***	
1. Rate of growth	2, 4, 5, 6, 7, 8, 10, 11, 12	1. Rate of growth	25, 26, 27, 28, 29, 30
2. Aerial mycelium present or absent	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	2. Aerial mycelium present or absent	24, 25, 26, 27, 28, 29, 30
3. Colour of aerial mycelium	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	3. Colour of aerial mycelium	24, 25, 26, 27, 28, 29, 30
4. Colour of colony from below	1, 3, 4, 5, 6, 7, 8, 10, 11, 12	4. Colour of colony from below	29, 30
5. Colour of spore masses	11, 12		
B. Macrocondidia from sporodochia		***B. Macrocondidia from sporodochia***	
1. Size	1, 4, 5, 6, 7, 8, 9, 10, 11, 12	1. Size	25, 26, 27, 28, 29, 30
2. Shape	2, 6, 8, 9, 10, 11, 12	2. Shape	24, 30
3. Shape of basal and apical cells	2, 4, 5, 6, 7, 8, 9, 10, 11, 12	3. Shape of basal and apical cells	30
C. Microconidia from aerial mycelium		***C. Microconidia from aerial mycelium***	
1. Present or absent	2, 4, 7, 8, 9, 10, 11, 12	1. Present or absent	24, 25, 26, 27, 28, 29, 30
2. In chains or false heads	4, 11, 12	2. In chains or false heads	24, 27, 28, 29, 30
3. Shape	2, 4, 7, 8, 10, 11, 12	3. Shape	24, 25, 26, 27, 29, 30
D. Conidiophores		***D. Conidiophores***	
1. Type	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,	1. Type	25, 30
E. Chlamydospores		***E. Chlamydospores***	
1. Present or absent	1, 4, 6, 7, 8, 9, 11, 12	1. Present or absent	29, 30
2. Arrangement	1, 4, 9, 11, 12		

Table 3.7. Synoptic key of section and species for isolate D18 (*F. chlamydosporum*)

SYNOPTIC KEY TO SECTION		SYNOPTIC KEY TO SPECIES	
Characteristics used to identify *Fusarium* section	**No. indicating *Fusarium* section**	**Characteristics used to identify *Fusarium* species**	**No. indicating *Fusarium* species in the sections Eupionnotes, Spicarioides and Arachnites**
A. Cultural characteristics		***A. Cultural characteristics***	
1. Rate of growth	2, 4, 5, 6, 7, 8, 10, 11, 12	1. Rate of growth	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18
2. Aerial mycelium present or absent	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	2. Aerial mycelium present or absent	8, 9, 11, 12, 13, 14, 15, 20, 21, 22, 23
3. Colour of aerial mycelium	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	3. Colour of aerial mycelium	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19
4. Colour of colony from below	2, 4, 5, 6, 7, 8, 9	4. Colour of colony from below	
5. Colour of spore masses	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11		
B. Macrocondidia from sporodochia		***B. Macrocondidia from sporodochia***	
1. Size	1, 4, 5, 6, 7, 8, 9, 10, 11, 12	1. Size	6, 7, 8, 9, 18, 20, 21, 22
2. Shape	5, 6, 9	2. Shape	14, 15, 16, 17, 18, 19, 20, 21, 22, 23
3. Shape of basal and apical cells	2, 4, 5, 6, 7, 8, 9, 10, 11, 12	3. Shape of basal and apical cells	14, 15, 20
C. Microconidia from aerial mycelium		***C. Microconidia from aerial mycelium***	
1. Present or absent	1, 3, 5, 6, 7, 8, 9	1. Present or absent	6, 7, 8, 9,
2. In chains or false heads		2. In chains or false heads	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18
3. Shape		3. Shape	9
D. Conidiophores		***D. Conidiophores***	
1. Type	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	1. Type	8, 9, 12, 13, 15,
E. Chlamydospores		***E. Chlamydospores***	
1. Present or absent	1, 4, 6, 7, 8, 9, 11, 12	1. Present or absent	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,
2. Arrangement	4, 6, 7, 8		

Table 3.8. Synoptic key of section and species for isolate C12 *(F. acuminatum)*

SYNOPTIC KEY TO SECTION		SYNOPTIC KEY TO SPECIES	
Characteristics used to identify *Fusarium* section	**No. indicating *Fusarium* section**	**Characteristics used to identify *Fusarium* species**	**No. indicating *Fusarium* species in the sections Eupionnotes, Spicarioides and Arachnites**
A. Cultural characteristics		***A. Cultural characteristics***	
1. Rate of growth	2, 4, 5, 6, 7, 8, 10, 11, 12	1. Rate of growth	12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23
2. Aerial mycelium present or absent	1	2. Aerial mycelium present or absent	14, 15, 16, 17, 18, 19, 20, 21, 22, 23
3. Colour of aerial mycelium	10, 11	3. Colour of aerial mycelium	12, 13, 14, 15, 16, 17, 18, 19
4. Colour of colony from below	1, 3, 4, 5, 6, 7, 8, 10, 11, 12	4. Colour of colony from below	16, 17, 18, 19, 20, 21, 22, 23
5. Colour of spore masses	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11		
B. Macrocondidia from sporodochia		***B. Macrocondidia from sporodochia***	
1. Size	2	1. Size	14, 15, 16, 17, 19, 23
2. Shape	1, 5, 10	2. Shape	14, 15, 16, 17,
3. Shape of basal and apical cells	7	3. Shape of basal and apical cells	
C. Microconidia from aerial mycelium		***C. Microconidia from aerial mycelium***	
1. Present or absent	1, 3, 5, 6, 7, 8, 9	1. Present or absent	12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23
2. In chains or false heads	4, 11, 12	2. In chains or false heads	
3. Shape	2, 4, 7, 8, 10, 11, 12	3. Shape	
D. Conidiophores		***D. Conidiophores***	
1. Type	4, 6, 7, 10	1. Type	12, 13, 15
E. Chlamydospores		***E. Chlamydospores***	
1. Present or absent	1, 4, 6, 7, 8, 9, 11, 12	1. Present or absent	12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23
2. Arrangement	4, 6, 7, 8		

Table 3.9 *Fusarium* species from the five farm holdings sampled in this study.

Identity	Number of *Fusarium* isolates per farm holding				
	A	B	C	D	E
F. verticilliodes	3	0	0	2	3
F. oxysporum	1	0	4	0	0
F. subglutinans	3	0	0	1	0
F. sambucinum	3	0	0	0	0
F. solani	0	0	1	0	0
F. chlamydosporum	0	0	0	1	0
F. acuminatum	0	0	1	0	0
TOTAL	10	0	6	4	3

3.3 Mycotoxin Analysis

3.3.1 Method optimization

Autoclaved maize patty cultures were spiked with commercial mycotoxins to assess the extraction efficiency. At n=3, the extraction efficiency for FB_1 was 82%; 80% for moniliformin and 89% for fusaric acid (Table 3.10).

Table 3.10 Percentage recovery of mycotoxins from spiked corn cultures (n = 3)

Mycotoxin	Spike Level (µg/ml)	Mean Recovery (%) ± S.D.
FB_1	10 (µg/g)	82% ± 14
Moniliformin	20 (µg/g)	80% ± 16
Fusaric Acid	20 (µg/g)	89% ± 17

Detection limits for FB_1, moniliformin and fusaric acid were established at 200 ng, 100 ng and 50 ng respectively using TLC. Detection limits for FB_1, moniliformin and fusaric acid using HPLC were 50 ng, 20 ng and 20 ng respectively (Table 3.11).

Table 3.11 Mycotoxin detection limit using TLC and HPLC systems

Mycotoxin	Detection Limits (ng)	
	TLC	HPLC
FB_1	200 ng	50 ng
Moniliformin	100 ng	20 ng at 229 nm
Fusaric Acid	50 ng	20 ng at 254 nm

3.3.2 Detection of mycotoxins produced by *Fusarium* species

3.3.2.1 Thin layer chromatography (TLC)

FB_1 standards produced a dark violet spot when sprayed and heated with the visualizing agent. FB_1 standards exhibited R_f value of 0.65. Dark violet spots with a similar R_f value were observed for the purified extracts obtained from isolates A5, A9, C13, D17, D19, E21 and E23. The similar R_f value for the FB_1 standards and the purified extracts indicate FB_1 production by seven *Fusarium* species.

Moniliformin standard produced a purple spot under shortwave UV light with a R_f value of 0.88. Purple spots with a similar R_f value were observed for the purified extracts of isolates A5, A8 and E23. The R_f value for the moniliformin standards and the purified extracts indicate that moniliformin production was detected for these three isolates (A5, A8 and E23) only.

Fusaric acid standard produced a bright purple spot under shortwave UV light with a R_f value of 0.8. Bright purple spots with a similar R_f value were observed for the purified extracts of isolates

A1, A2, A5, A8, A9, C11, C15, D20 and E23. The R_f value for the fusaric acid standards and the purified extracts indicate that fusaric acid was produced by these nine *Fusarium* species.

3.3.2.2 High performance liquid chromatography (HPLC)

Detection and quantification of fumonisin B_1, moniliformin and fusaric acid by HPLC, was verified by comparing the retention times of the purified compounds to commercial standards. Injection of 100 µg/ml FB_1 standard into the HPLC system revealed a peak at a retention time of 4.70 min (Fig. 3.19a). Purified extracts of isolate A5, when compared to the standard, eluted at a similar retention time as that of purified FB_1 (Fig. 3.19b). In contrast uninoculated maize patty (control) did not exhibited a peak at 4.70 min (Fig. 3.19c). Peaks obtained for the purified extracts of isolates A5, A9, A10, C13, C15, D17, D19, E21, E22 and E23 (Table 3.12), eluted at a retention time of 4.65 (± 0.15).

A peak at a retention time of 3.20 min was obtained after injection of 100 µg/ml moniliformin standard (Fig. 3.20a). Purified extracts of isolate A5, when compared to the standard, eluted at a similar retention time (Fig. 3.20b). Uninoculated maize patty (control) did not exhibit a peak at 3.20 min (Fig. 302c). Peaks obtained for the purified extracts of isolates A5, A8 and E23 (Table 3.12), eluted at a retention time of 3.15 (± 0.15).

A peak at a retention time of 3.50 min was obtained after injection of 100 µg/ml fusaric acid standard (Fig. 3.21a). Purified extracts of isolate A1, when compared to the standard, eluted at a similar retention time (Fig. 3.21b). Uninoculated maize patty (control) did not exhibit a peak at 3.50 min (Fig. 3.21c). Peaks obtained for the purified extracts of isolates A1, A2, A5, A8, A9, C11, C15, D20 and E23 (Table 3.12), eluted at a retention time of 3.4 (± 0.15).

Toxin levels were quantified using sample and mycotoxin standard (FB_1, moniliformin and fusaric acid) peak areas in a formula outlined by Thiel *et al.* (1993). Eight of the 23 *Fusarium* isolates obtained in this study did not produce any of the toxins tested for here (Table 3.13).

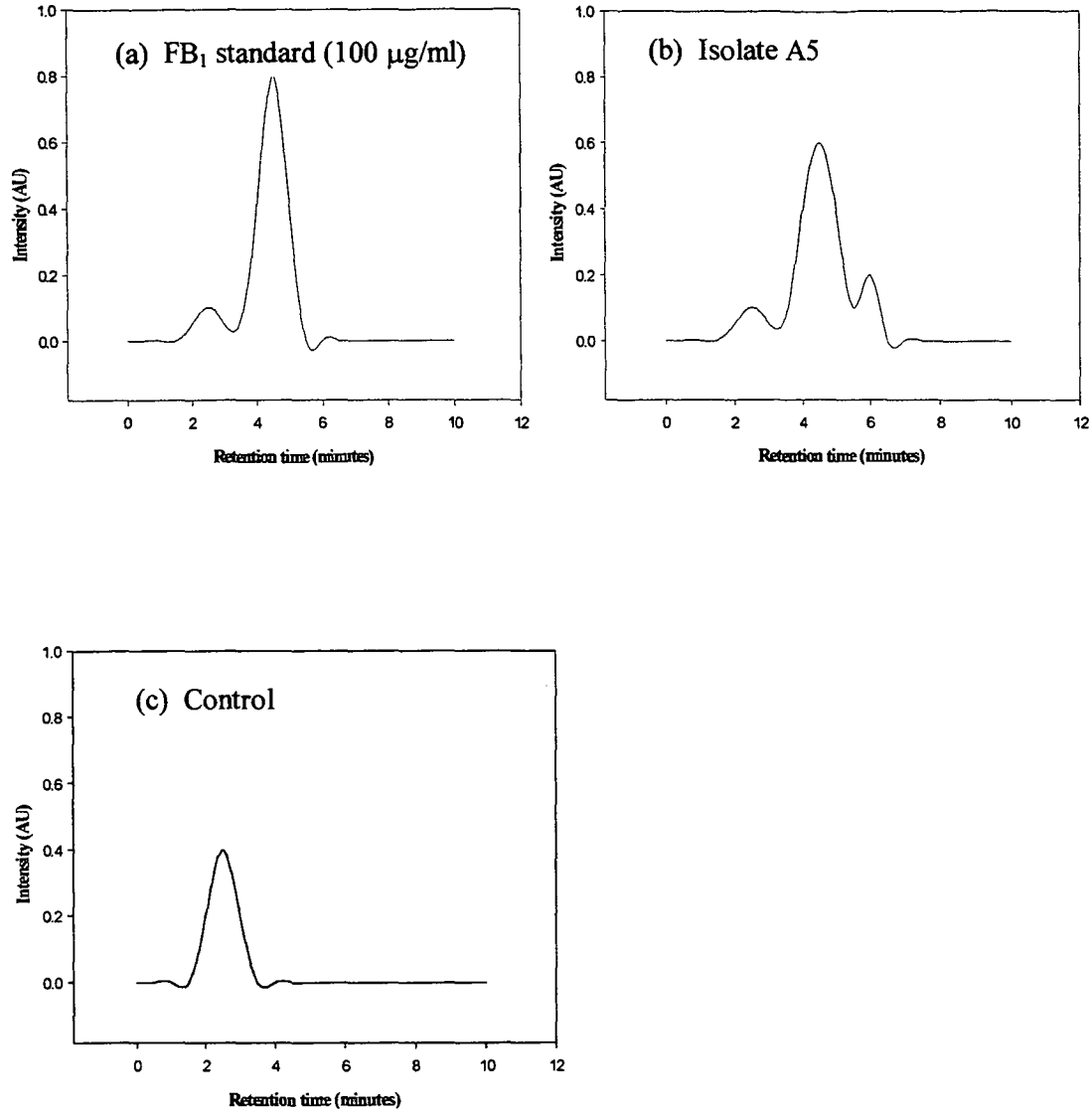

Fig. 3.19 HPLC chromatograms obtained for the analysis of FB_1. Fig. 3.19a shows the chromatogram obtained for toxin standard injected at 100 µg/ml. Fig. 3.19b shows a chromatogram obtained for the purified extracts of isolate A5 and Fig. 3.19c shows a chromatogram obtained for the control (no toxin).

[**Analysis conditions:** Mobile phase – methanol : 0.1 M NaH_2P0_4 (80:20), Merck RP C18 column, OPA – derivatizing reagent (450 µl + 50 µl sample), 50 µl sample injection, fluorescence detection, flow rate 1 ml/min, R_t = 4.5-4.8 minutes].

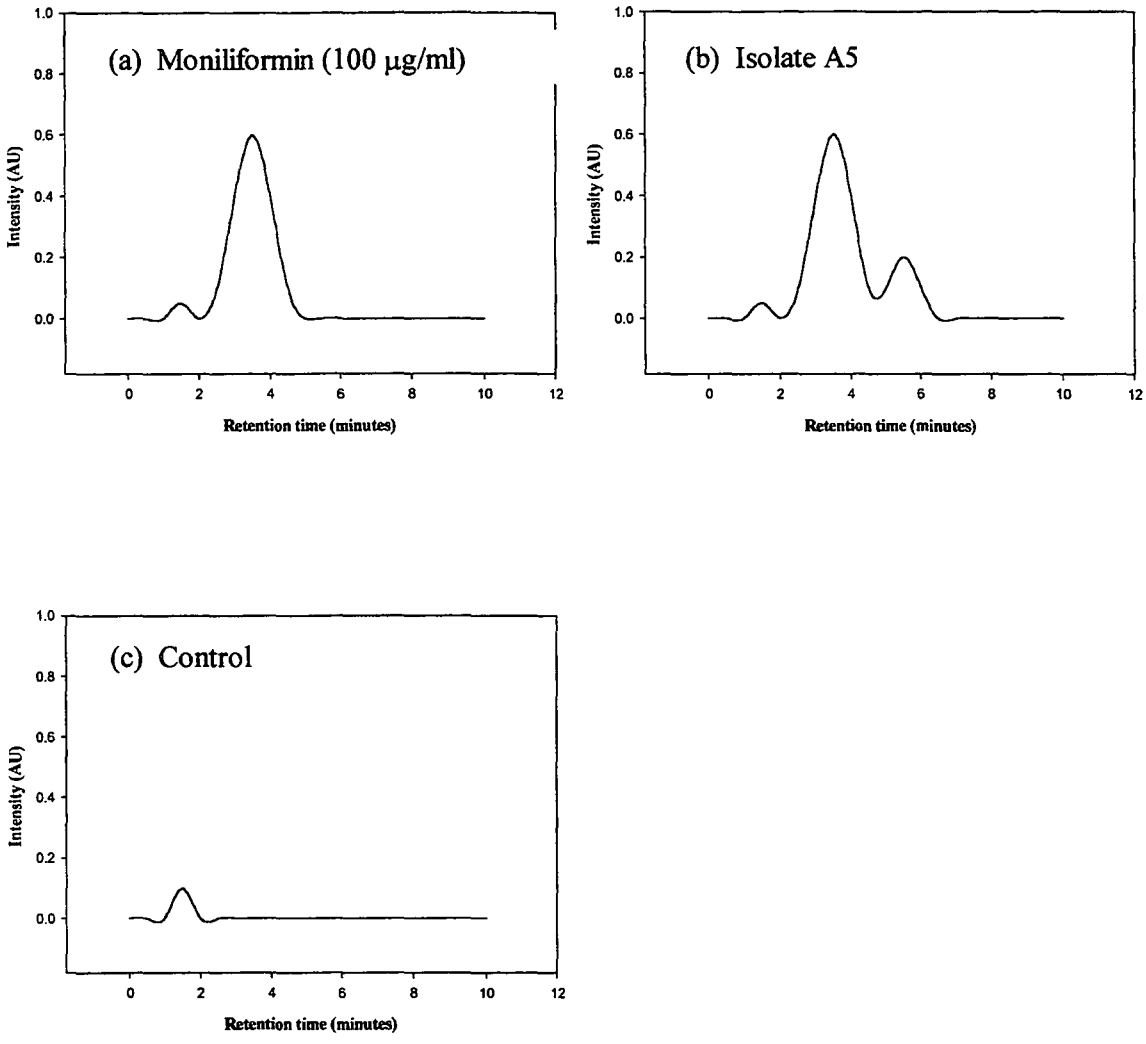

Fig. 3.20 HPLC chromatograms obtained for the analysis of moniliformin. Fig. 3.20a shows the chromatogram obtained for toxin standard injected at 100 µg/ml. Fig. 3.20b shows a chromatogram obtained for the purified extracts of isolate A5 and Fig. 3.20c shows a chromatogram obtained for the control (no toxin).

[**Analysis conditions:** Mobile phase – 10 mM phosphate buffer / 5 mM tetra-butyl-ammonium bromide (pH 7.0) : methanol (92/2, v/v), Merck RP C18 column, 50 µl sample injection, UV – 215 nm detection, flow rate 1 ml/min, R_t = 3.1-3.3 minutes].

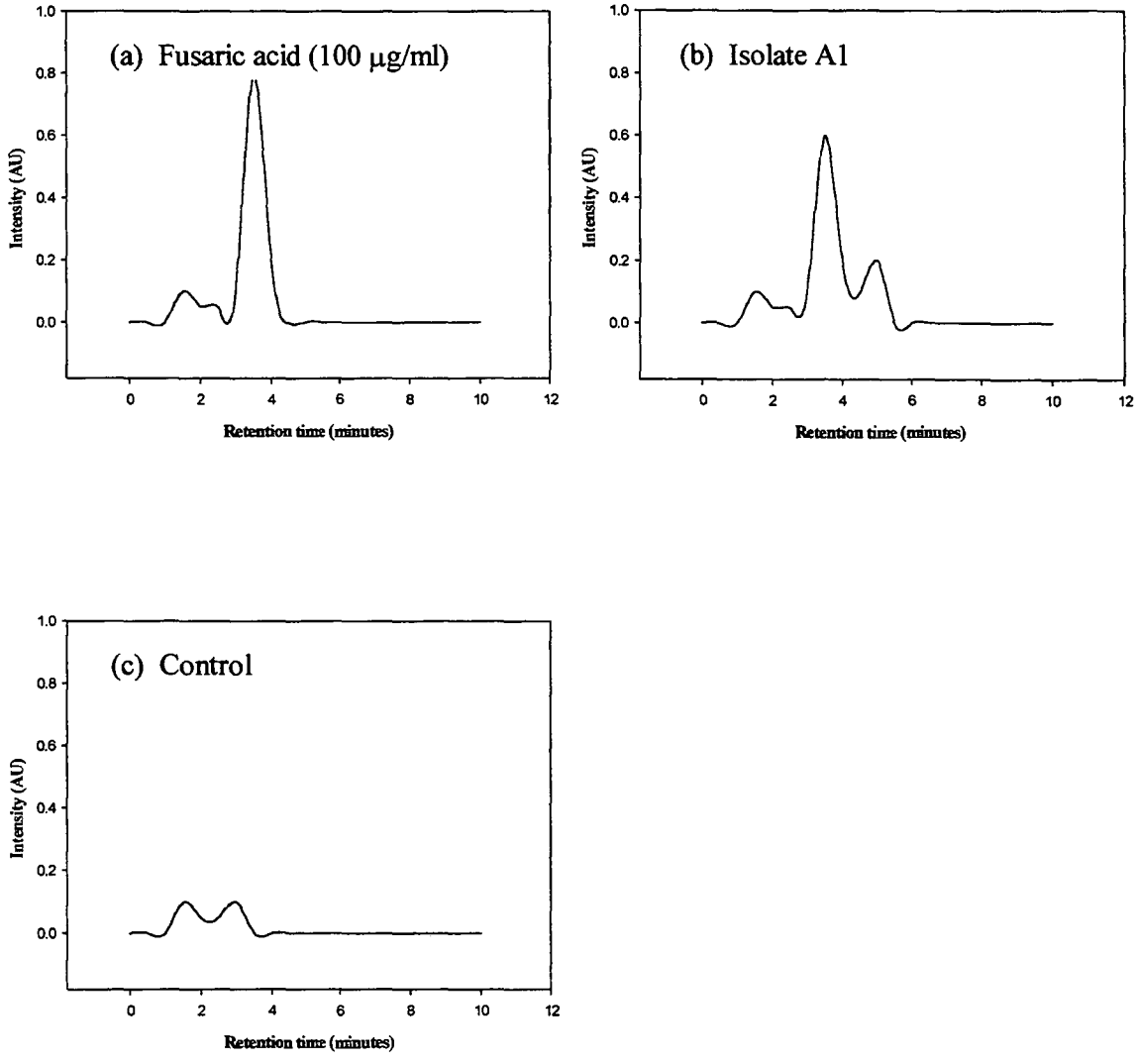

Fig. 3.21 HPLC chromatograms obtained for the analysis of fusaric acid. Fig. 3.21a shows the chromatogram obtained for toxin standard injected at 100 µg/ml. Fig. 3.21b shows a chromatogram obtained for the purified extracts of isolate A1 and Fig. 3.21c shows a chromatogram obtained for the control (no toxin).

[Analysis conditions: Mobile phase – 40% methanol, 60% aqueous 0.62 mM Na_2EDTA, 2% H_3P0_4, Merck RP C18 column, OPA – derivatizing reagent (450 µl + 50 µl sample), 50 µl sample injection, UV – 254 nm detection, flow rate 1 ml/min, R_t = 3.2-3.6 minutes].

Table 3.12 Mycotoxin levels (μg/g maize patty) from the 23 *Fusarium* isolates

Isolate	Identified *Fusarium*	Mycotoxin concentration μg/g detected in maize samples by HPLC (n=2)		
		FB_1	Moniliformin	Fusaric Acid
A1	*F. oxysporum*	ND*	ND*	10.3 (0.25)
A2	*F. sambucinum*	ND*	ND*	0.25 (1.2)
A3	*F. sambucinum*	ND*	ND*	ND*
A4	*F. subglutinans*	ND*	ND*	ND*
A5	*F. verticilliodes*	3.64 (0.80)	8.35 (0.4)	7.5 (0.5)
A6	*F. sambucinum*	ND	ND*	ND*
A7	*F. subglutinans*	ND*	ND*	ND*
A8	*F. subglutinans*	ND*	0.18 (0.67)	1.6 (1.5)
A9	*F. verticilliodes*	5.7 (0.62)	ND*	3.3 (0.74)
A10	*F. verticilliodes*	0.5 (0.7)	ND*	ND*
C11	*F. oxysporum*	ND*	ND*	4.3 (0.82)
C12	*F. acuminatum*	ND*	ND*	ND*
C13	*F. oxysporum*	3.2 (0.58)	ND*	ND*
C14	*F. oxysporum*	ND*	ND*	ND*
C15	*F. oxysporum*	12.4 (2.2)	ND*	10.7 (0.62)
C16	*F. solani*	ND*	ND*	ND*
D17	*F. verticilliodes*	2.1 (1.7)	ND*	ND*
D18	*F. chlamydosporum*	ND*	ND*	ND*
D19	*F. verticilliodes*	3.1 (1.5)	ND*	ND*
D20	*F. subglutinans*	ND*	ND*	0.55 (2.0)
E21	*F. verticilliodes*	0.9 (0.8)	ND*	ND*
E22	*F. verticilliodes*	1.75 (0.7)	ND*	ND*
E23	*F. verticilliodes*	2.7 (0.50	4.8 (1.2)	5.8 (1.6)

Values given are the mean of two replicates. Standard deviation is given in brackets (SD ≤ 2.2).

ND* = Not Detected

Table 3.13 Frequency of mycotoxin production from the *Fusarium* isolates

Fusarium species	No. of isolates tested	No. of mycotoxin producing isolates	Mycotoxins (μg/ml)		
			FB_1	Moniliformin	Fusaric acid
F. verticilliodes	8	8	8	2	3
F. oxysporum	5	4	2	ND*	3
F. subglutinans	4	2	0	1	2
F. sambucinum	3	1	0	0	1
F. solani	1	0	ND	ND	ND
F. chlamydosporum	1	0	ND	ND	ND
F. acuminatum	1	0	ND	ND	ND
TOTAL	23	15	10	3	9

ND* = Not Detected

3.4 Pathogencity Assays

The pathogenicity of the isolated *Fusarium* species was accomplished by inoculating them into healthy banana fruit. *Fusarium* isolates A1, A5, A8, A9, C14, C15, C16, D19, E22 and E23 that were used for this assay, showed an increase in rot diameter ranging from 0.2 cm to 2.5 cm over a 6 day period. The highest increase in rot diameter was observed for *F. verticilliodes*, isolate A9 (2.0 cm increase) followed by isolates E23 (1.6 cm), D19 (1.2 cm), A5 (1.1 cm) and E22 (1.0 cm) respectively. Isolates of *F. oxysporum* (A1, C14 and C15) showed an increase of 0.7 cm, 0.5 cm and 1.1 cm respectively. *Fusarium subglutinans* and *F. solani* also showed an increase of 1.0 cm and 1.1 cm respectively.

All isolates also showed percentage weight loss ranging from 17% to 44%. The percentage weight loss was highest for *F. oxysporum* (isolate C15) followed by *F. verticilliodes* (isolate A5) [Table 3.14]. Fig. 3.22 depicts a healthy banana fruit not inoculated with a *Fusarium* species (control) and Fig. 3.23 depicts a banana fruit when inoculated with *Fusarium* isolate C15 at 4 days incubation.

Table 3.14 Pathogenicity of *Fusarium* species isolated from Banana

Isolate	*Fusarium* species Inoculated	Rot diameter (mean diameter of rot in cm)			Weight loss (%)
		2 days	4 days	6 days	Mean ± SD
A1	*F. oxysporum*	0.6	0.8	1.3	17 ± 3.8
A5	*F. verticilliodes*	1.3	1.8	2.4	38 ± 3.6
A8	*F. subglutinans*	0.8	1.3	1.8	26 ± 4.0
A9	*F. verticilliodes*	1.0	1.5	2.0	34 ± 3.4
C14	*F. oxysporum*	0.3	0.5	0.8	18 ± 3.2
C15	*F. oxysporum*	1.4	2.0	2.5	44 ± 3.0
C16	*F. solani*	0.4	0.7	1.5	25 ± 3.2
D19	*F. verticilliodes*	0.5	0.9	1.7	30 ± 3.9
E22	*F. verticilliodes*	0.3	0.7	1.3	30 ± 4.8
E23	*F. verticilliodes*	0.4	0.9	2.0	32 ± 3.7

Fig. 3.22. A healthy banana fruit inoculated with sterile water (control).

Fig. 3.23. A healthy banana fruit when inoculated with isolate C15.

3.5 Correlation between Toxin Producing and Pathogenicity of the *Fusarium* isolates

Ten of the 23 *Fusarium* isolates were used in the pathogenicity assay. Of the ten isolates, two did not produce any mycotoxins on the maize patty cultures (isolates C14 and C16). Isolates that produced moderately high concentration of mycotoxins, had a high measurement (2.5 cm) of rot diameter and a high percentage (38%) of mean weight loss (isolate C15). Isolates that produced moderately low concentration of mycotoxins, showed a small measurement (1.3 cm) of rot diameter and a low percentage (17%) of mean weight loss (isolate A1). On the other hand the two isolates (C14 and C16) that did not produce mycotoxins did however produce a significant amount of rot diameter and mean weight loss.

Table 3.15 Correlation between mycotoxin production of the *Fusarium* isolates and their pathogenicity

Fusarium isolate	Mycotoxins (μg/ml)			Mean Rot Diameter (cm) [Day 6]	Mean Weight Loss (%)
	FB_1	Moniliformin	Fusaric acid		
F. oxysporum (A1)	ND*	ND*	10.3	1.3	17
F. verticilliodes (A5)	3.64	8.35	7.5	2.4	38
F. subglutinans (A8)	ND*	0.18	1.6	1.8	26
F. verticilliodes (A9)	5.7	ND*	3.3	2.0	34
F. oxysporum (C14)	ND*	ND*	ND*	0.8	18
F. oxysporum (C15)	12.4	ND8	10.7	2.5	44
F. solani (C16)	ND*	ND*	ND*	1.5	25
F. verticilliodes (D19)	3.1	ND*	ND*	1.7	30
F. verticilliodes (E22)	1.75	ND*	ND*	1.3	30
F. verticilliodes (E23)	2.7	ND*	ND*	2.0	32

ND* – Not Detected

CHAPTER FOUR: DISCUSSION

This project was divided into four phases. The first phase involved a survey of the various fungal populations in the banana fruit following harvesting. The origin of the banana hands collected for this study were from the same geographical region. There were no significant variations in the crop genotype (Cavendish genotype) and management practices in the five farm holdings.

This study revealed that the most predominant fungal species isolated were of the genus *Fusarium*, as determined from their colony characteristics on PDA (Nelson *et al.*, 1983) [Fig. 3.2-3.5]. Fig. 3.1 highlights the frequency of the different fungal populations isolated from the five farm holdings. Species of *Fusarium*, *Aspergillus* and *Penicillium* were identified. A high percentage (± 30%) of the fungal population was not identified as belonging to either of the above three fungal groups (Fig. 3.1). Similar results were reported by Postmaster *et al.* (1997) and Kuthubutheen and Nawawi (1987), who also isolated and identified species of *Aspergillus*, *Penicillium* and *Fusarium* from banana fruit. *Aspergillus* and *Penicillium* species occurred at moderately low percentages (± 20%) in all farm holdings except for that of farm holding B. No *Fusarium* species were detected in farm holding B. A high percentage of *Fusarium* species (± 50%) were detected in farm holdings A, C, D and E. Farm holding E showed the highest percentage of *Fusarium* species followed by farm holding A, D and C respectively. Owing to the fact that all farms were very similar, in terms of geographic location and farming

methods employed, one could not find conclusive explanations why this difference in farm holding B occurred. The only explanation stems from the fact that farm holding B had a more improved postharvest management system, when compared to the other four farm holdings.

The second phase of the study included the identification of the 23 *Fusarium* isolates to species level. *Fusarium verticilliodes* was the predominant species and was identified in 34% (8/23) of the isolates. *Fusarium oxysporum* was identified in five isolates (21.7%) *and F. subglutinans* was identified in four isolates (17.4%). *F. sambucinum* was identified in three isolates (13.3%), while there were only one isolate each of *F. solani*, *F. chlamydosporum* and *F. acuminatum* (4.3%). It is evident that *F. verticilliodes* is prevalent in banana crown rot. This is in agreement with previous studies by Jimenez *et al.* (1993), Jimenez *et a*l. (1997), Johanson and Blazquez (1992), Jimenez and Mateo (1997), Knight (1982) and Marin *et al.* (1996). In their studies on the occurrence of *Fusarium* species in banana fruits, they also reported a high frequency of *F. verticilliodes* with crown rot.

While *F. verticilliodes* is often found in association with crown rot of bananas, there are reports that supports the isolation of *F. oxysporum*, *F. solani*, *F. subglutinans* and *F. acuminatum* from crown rot of bananas. Jimenez *et al.* (1993) and Jimenez *et al.* (1997) identified *F. solani*, *F. oxysporum*, *F. subglutinans* and *F. acuminatum*, among other principle causative agents of banana crown rot in Panama and Ecuador. Knight (1982) also isolated *F. oxysporum* from crown rot of bananas in the Windward Islands.

Although *F. sambucinum* and *F.chlamydosporum* has been isolated from a disseminated human *Fusarium* infection (Guarro and Gene, 1995), no literature is available on the isolation of these two *Fusarium* species from crown rot of bananas. Thus, this is the first known report of the isolation of *F. sambucinum* and *F. chlamydosporum* from banana fruit exhibiting symptoms of crown rot.

Fusarium species were identified by the observation of colony morphology, hyphal pigmentation and ultrastructural features using a *Fusarium* species synoptic key (Nelson *et al.*, 1983). *Fusarium verticilliodes* was characterized by the production of white floccose mycelium with a deep purple tinge. The key taxonomic features of this species were the presence of long microconidial chain production from the mycelium, the presence of branched monophialides and the absence of chlamydospores. *Fusarium solani* was identified by the production of white to cream floccose mycelium and confluent bluish-green sporodochia on PDA. *Fusarium solani* is the only known *Fusarium* species that produces this characteristic blue-green pigment (Nelson *et al.*, 1983). Other key characteristics of this species included the abundance of 3-4 septate macroconidia with blunt and rounded apical and basal cells, the presence of single-celled kidney shaped (oval and reniform) microconidia and the formation of long slender, unbranched monophialides. In comparison, the only taxonomic characteristic that enabled the differentiation of from *F. solani* was the formation of short, plump monophialides in *F. oxysporum*. *Fusarium oxysporum* was also identified by the production of white floccose mycelium with a tinge of purple. *Fusarium chlamydosporum* was identified by the characteristic abundant comma-shaped

microconidia produced from polyphialides. Macroconidia have characteristic pointed apical cell and a foot-shaped basal cell. Another taxonomic criterion that aided identification was the abundant production of rough-walled chlamydospores occurring singly, in chains and in clusters. *Fusarium subglutinans* was characterized by the produciton of white floccose mycelium with a deep tinge of reddish brown and confluent tan-orange sporodochia. Other characteristics included the presence of both branched and unbranched monophialides and polyphialides. *Fusarium sambucinum* produced very little reddish brown mycelium with the presence of an orange coloured sporodochium. Only branched monophialides were observed for these organisms. *Fusarium acuminatum* was identified by its characteristic slow growth, with the production of tan to orange mycelium and the presence of branched and unbranched monophialides.

The advantage of using CLA, a minimal media, was that it enhanced sporulation of the *Fusarium* isolates that are vital for the identification of primary taxonomic structures such as macroconidia. Potato dextrose agar, a carbohydrate rich medium, was used because of the distinct colony colours that were produced by the *Fusarium* isolates. Colony colours were a very important secondary criterion for *Fusarium* species identification. Both CLA and PDA was used by Nelson *et al.* (1983) and Sugiura *et al.* (1999) in the identification of human-clinical isolates of *Fusarium* species.

The differentiation of *Fusarium* species from other filamentous opportunistic fungi occurring in crown rot of bananas is important in establishing the correct assessment strategy one needs to undertake, in view of their different susceptibility to fungicides and

antifungal agents. Thus any *Fusarium* species found in association with any plant, especially banana crown rot, are resistant to available fungicides, like thiabendazole, prochloraz and imazalil; thus early diagnosis is of prime importance (Johanson and Blazquez, 1992).

Because of the limited knowledge about the toxigenicity of *Fusarium* species associated with human infections, the *in vitro* production of FB_1, moniliformin and fusaric acid from the 23 *Fusarium* isolates was determined. A percentage recovery of 82% was achieved from maize patties spiked with 10 μg/g FB_1 when using SAX cartridges. Stack and Eppley (1992) recovered 67% FB_1 from maize using SAX cartridges while Thiel *et al.* (1993) reported an 84% recovery of FB_1 from maize spiked with 5 μg/g FB_1. A percentage recovery of 80% was obtained after spiking maize patties with 20 μg/g moniliformin when using SAX cartridges in this study when compared to an average 70% recovery reported by Filek and Lindner (1996) from wheat spiked with between 0.02-0.25 μg/g moniliformin when using a similar extraction method described here. Munimbazi and Bullerman (1998) reported 97.8% recovery of moniliformin from maize spiked with 1 μg/g when using SAX cartridges. A percentage recovery of 89% was achieved after spiking 20 μg/g fusaric acid into maize patties when using an organic extraction method. Venter and Steyn (1998) obtained a recovery of 96.02% of fusaric acid using the identical extraction method.

The detection limits for FB_1, moniliformin and fusaric acid when using thin layer chromatography was 200, 100 and 50 ng respectively whereas the detection limits using

high performance liquid chromatography was 50, 20 and 20 ng respectively. The HPLC methods for the three toxins were four-fold, five-fold and two-fold more sensitive than TLC methods, respectively. This trend was expected, since HPLC is a more advanced technique, using a high-resolution instrumentation.

Fumonisin B_1 produced a dark violet spot when spraying with *p*-anisaldehyde. A R_f value of 0.65 was obtained for an authentic FB_1 standard. A similar R_f value was obtained for the purified extracts of isolates A5, A9, C13, D17, D19, E21 and E23 (7/23). Moniliformin produced purple spots at 254 nm. A R_f value of 0.88 was established for an authentic moniliformin standard. A similar R_f value was revealed for the purified extracts of isolates A5, A8 and E23 (3/23). Fusaric acid produced purple spots under UV-illumination at 254 nm. The R_f value of a fusaric acid standard was 0.8. Purified extracts of isolates A1, A2, A5, A8, A9, C11, C15, D20 and E23 (9/23) revealed a similar R_f value.

High Performance Liquid Chromatography analysis of FB_1 at an excitation and emission wavelength of 335 and 440 nm respectively, revealed a reproducible peak at a retention time of 4.65 min. Peaks that eluted at similar retention times were obtained for the purified extracts of ten *Fusarium* isolates. This confirms the sensitivity of the HPLC instrument, as only seven *Fusarium* isolates producing FB_1 were detected by TLC. The UV detection at 229 nm of a moniliformin standard by HPLC revealed a reproducible peak that eluted at a retention time of 3.15 min. Peaks that eluted at similar retention times were obtained for the purified extracts of three *Fusarium* isolates. High

Performance Liquid Chromatography analysis of fusaric acid at 254 nm revealed a reproducible peak for a fusaric acid standard at a retention time of 3.50 min. Peaks that eluted at retention times ranging from 3.35 to 3.55 min were obtained for the purified extracts of nine *Fusarium* isolates. The purified extracts of an uninoculated maize patty (control) revealed no peak elution at either FB_1, moniliformin or fusaric acid retention times respectively.

Fumonisin B_1 was produced by 10 (43%) isolates of *Fusarium* (Table 3.12). Eight of the isolates were identified as *F. verticilliodes* and the remaining two identified as *F. oxysporum*. Mycotoxin concentrations ranged from 0.5 μg/g to 5.7 μg/g for *F. verticilliodes*, with isolate A9 being the highest producer of FB_1 and isolate A10 being the lowest producer of FB_1. Both *Fusarium* species (*F. verticilliodes* and *F. oxysporum*) are known producers of FB_1 (Nelson *et al.*, 1991). Jimenez *et al.* (1997) encountered FB_1 in corn culture extracts isolated from bananas between a range of 40 and 2700 μg/g.

Moniliformin was produced by three (13%) *Fusarium* isolates. The three isolates were identified as *F. verticilliodes* (2 isolates) and *F. subglutinans*. Mycotoxin concentration ranged from 0.18 to 8. 35 μg/g. The highest mycotoxin producers were *F. verticilliodes* (isolates A5 and E23 respectively), followed by *F. subglutinans* (isolate A8). The two species have been known to produce moniliformin in maize, peanuts, sorghum, millet and soil (Betina, 1989; Cole and Cox, 1981; Marasas *et al.*, 1984; Rabie *et al.*, 1982). Jimenez *et al.* (1997) successfully isolated *F. subglutinans*, from banana, but the species did not produce moniliformin when cultured in corn extracts. Species of *F. verticilliodes*

isolated from banana produced between 10 to 1670 μg/g of moniliformin when cultured in corn extracts (Jimenez *et al.*, 1997).

There is extensive literature citing the production of fusaric acid by *Fusarium* species in contaminated agricultural commodities (cereal grain, maize, wheat, barley and rice) (Porter *et al.*, 1996) and its implications in plant pathogenesis is well documented (Bacon *et al.*, 1996). However, we found no literature on the ability of *Fusarium* species isolated from banana fruit to produce fusaric acid. There are, however, reports by Okole (1995) that *Fusarium* species isolated from banana plants (stem) do produce fusaric acid and cause vascular wilt of the banana plant. In contrast, in this study, fusaric acid was produced by 39% (9/23) of the *Fusarium* isolates. The mycotoxin concentration ranged from 0.25 to 10.7 μg/g maize patty. Of the nine *Fusarium* isolates capable of fusaric acid production, three were identified as *F. verticilliodes*, three were identified as *F. oxysporum*, two were identified as *F. subglutinans* and one as *F. sambucinum*. The highest fusaric acid producer was *F. oxysporum* (10.7 μg/g) and the lowest producer was *F. sambucinum* (0.25 μg/g). Studies by Betina (1989) and Bacon *et al.* (1996) have established that *F. verticilliodes* and *F. oxysporum* as fusaric acid producers. In contrast, reports by Jimenez *et al.* (1997) revealed that *F. sambucinum* and *F. subglutinans* isolated from bananas did not produce fusaric acid. Therefore, this is the first report on the production of fusaric acid by *F. sambucinum* and *F. subglutinans* that were isolated from bananas.

Two isolates of *Fusarium* (A5 and E23) produced all three mycotoxins (FB_1, fusaric acid and moniliformin). Isolate A5 produced the highest concentration of moniliformin, of all 23 isolates (8.35 μg/g) and produced FB_1 and fusaric acid at concentrations of 3.64 and 7.5 μg/g respectively. Isolate E23 produced FB_1, moniliformin and fusaric acid at concentrations of 2.7, 4.8 and 5.8 μg/g respectively. Both isolates were identified as *F. verticilliodes*. Isolate A8 (*F. subglutinans*) produced both moniliformin and fusaric acid at concentrations of 0.18 and 1.6 μg/g respectively, whilst isolate A9 (*F. verticilliodes*) and isolate C15 (*F. oxysporum*) produced the mycotoxins, FB_1 (5.7 μg/g and 12.4 μg/g respectively) and fusaric acid (3.3 μg/g and 10.7 μg/g respectively). Isolate C15 was found to produce the highest concentration of both FB_1 and fusaric acid.

The final phase of this project involved the evaluation of the pathogenic effects of the *Fusarium* species on healthy banana fruit by measuring their mean rot diameter and percentage weight loss following inoculation and subsequent incubation (Table 3.13). The percentage weight loss of banana fruit inoculated with the different *Fusarium* species isolated in this study were between the range of 17% and 44%. The lesion diameters were virtually identical (0.5 cm) after 2 days of incubation, except for that of *F. verticilliodes* (isolate A5) and *F. oxysporum* (isolate C15) whose lesion diameters were 1.3 and 1.4 cm respectively. The size of most lesions became noticeably bigger after 4 days of incubation, while all colonies were 1.5-2.5 cm in diameter by the sixth day. In every case, the fruit behaved as an excellent substrate for development of the mycoflora that were assayed. However, isolates A5 (*F. verticilliodes*), A9 (*F. verticilliodes*) and C15 (*F. oxysporum*) caused the greatest rot diameter of 2.4, 2.0 and 2.5 cm respectively

after 6 days of incubation and 38%, 34% and 44% weight loss respectively. These *Fusarium* species invaded the pulp more extensively than other *Fusarium* species (Fig. 3.17), yet the surface damage they caused was comparable. Jimenez *et al.* (1993) and Knight (1982) also found that *F. verticilliodes* and *F. oxysporum* caused the greatest rot diameter percentage weight loss in crown rot infections, implicating *F. verticilliodes* and *F. oxysporum* as predominant fungal pathogens involved in crown rot of banana.

A correlation between mycotoxin production and pathogenicity of the *Fusarium* isolates was determined (Table 3.15). Only ten of the 23 isolated *Fusarium* species were used for the pathogenicity assay. Isolates C14 (*F. oxysporum*) and C16 (*F. solani*) were used, since they did not produce any mycotoxins when cultured on corn extracts. The remaining eight *Fusarium* isolates that were used, produced either one (A1), two (A8) or three (A5) mycotoxins. It was found that isolates which produced more than one mycotoxin produced a greater mean rot diameter. This could be seen in isolates A5 (*F. verticilliodes*), A9 (*F. verticilliodes*), C15 (*F. oxysporum*) which had mean diameters of 2.4, 2.0 and 2.5 cm respectively. In contrast, there was no correlation between fruit weight loss and isolates that produced mycotoxins. This was verified in isolates C14 (*F. oxysporum*) and C16 (*F. solani*) which did not produce any mycotoxins, but did however cause a significant fruit weight loss of 18% and 25% respectively. This was further verified in isolate A5 (*F. verticilliodes*) which produced all three mycotoxins, but only caused a weight loss of 38%. However, due to insufficient methodology procedures, there is no definite evidence of mycotoxin production in infected banana fruits.

CHAPTER FIVE: CONCLUSIONS

The predominant *Fusarium* species isolated from the crown region of the bananas was identified as *F. verticilliodes*. Other *Fusarium* species identified include *F. oxysporum, F. subglutinans*, *F. solani* and *F. acuminatum,* all of which are common fungal agents of banana crown rot infections. However, this is the first report of *F. sambucinum* and *F. chlamydosporum* as fungal agents of banana crown rot. This therefore highlights the growing trend of *Fusarium* species, normally regarded as common soil saprophytes and plant pathogens, to cause opportunistic infections in fruits and indirectly in humans.

The results of this study confirm the hypothesis that *Fusarium* species play a vital role in the pathogenesis of banana crown rot, and they all produce mycotoxins. Although only five holdings were investigated, 65 banana hands were analysed, of these 23 *Fusarium* species were isolated and identified. Sixty five percent (15/23) of these *Fusarium* species produced one or more mycotoxins i.e. FB_1, moniliformin and fusaric acid. This is the first report of fumonisins B_1, moniliformin and fusaric acid production by *Fusarium* species isolated from banana crown rot infections. This provides compelling evidence that the extent of the infection in crown rot is related to the fungal species. Although many of the *Fusarium* isolates produced more than one mycotoxin, there was no correlation between fruit weight loss and toxins. This study is indicative of the role of *Fusarium* species and their mycotoxins in the severity of banana crown rot. In countries were bananas are considered a staple diet and people will consume bananas with crown

rot infections, the implications of mycotoxins is profound because of the human diseases in which they are implicated.

REFERENCES

Abbas, H.K., Vesonder, R.F., Boyette, C.D., Hoagland, R.E. and Krick, T. 1992. Production of fumonisins by *Fusarium moniliforme* cultures isolated from jimsonweed in Mississippi. *Journal of Phytopathology*. **136:** 199-203.

Akkaravessapong, P., Joyce, D.C. and Turner, D.W. 1998. The relative humidity at which bananas are stored or ripened does not influence their susceptibility to mechanical damage. *Scientia Horticulturae*. Elsevier Science Ltd. **52:** 265-268.

Alam, M.S., Alam, S., Islam, S. and Alam, N. 1993. Biochemical changes in banana fruits in response to crown rot pathogens. *Bangladesh Journal of Botany*. **22:** 143-148.

Anke, T. 1999. Fungal Biotechnology: with 113 Figures and 20 Tables. Chapman and Hall. London. pp. 265-271.

Anon. 1993. *Mycotoxins: Third Report. Food Surveillance Paper No.* 36. London: HMSO.

Astorga, Y. 1999. The Environmental Impact of the Banana Industry: A Case Study of Costa Rica. *International Banana Conference*. Brussels. pp. 1-19.

Bacon, C.W., Porter, J.K., Norred, W.P. and Leslie, J.F. 1996. Production of fusaric acid by *Fusarium* species. *Applied Environmental Microbiology*. **62:** 4039-4043.

Betina, V. 1980. Mycotoxins: chemical, biological and environmental aspects. Elsevier Science Ltd. New York.

Bhat, R.V., Sashidhar, R.B., Ramakrishna, Y. and Munshi, K.I. 1989. Outbreak of trichothecenes mycotoxicoses associated with consumption of mould-damaged wheat-products in Kashmir Valley. *Lancet.* **1:** 35-37.

Bhat, R.V., Shetty, P.H., Amruth, R.P. and Sudershan, R.V. 1997. A foodborne disease outbreak due to the consumption of moldy sorghum and maize containing fumonisin mycotoxins. *Clinical Toxicology.* **35:** 249-255.

Booth, C. 1971. The genus *Fusarium.* Commonwealth Mycological Institute. Key, Surrey, England.

Bottalico, A., Logrieco, A. and Visconti, A. 1989. *Fusarium* species and their mycotoxins in infected cereals in the field and stored grains. pp. 85-119. *In.* J. Chelkowski (ed). *Fusarium*-mycotoxins, taxonomy and pathogenicity. Elsevier Science Ltd. Amsterdam.

Burgess, L. W., Liddell, C. M. and Summercell, B.A. 1988. Laboratory manual for *Fusarium* research. Second Edition. John Wiley and Sons Ltd.

Burmeister, H.R., Grove, M.D., Peterson, R.E., Weisleder, D. and Plattner, R.D. 1985. Isolation and characterization of two new fusaric analogs from *Fusarium moniliforme* NRRL 13, 163. *Applied and Environmental Microbiology.* **50:** 311-314.

Casteel, S.W. and Braun, W.F. 1992. Mycotoxicoses. *Encyclopaedia. of Microbiology.* Academic Press. **3:** 217-227.

Chakrabarti, D.K. and Ghosal. S. 1986. Occurrence of Free and Conjugated 12, 13-Epoxytrichothecenes and Zearalenone in Banana Fruits Infected with *Fusarium moniliforme. Applied Environmental Microbiology.* **51:** 217-219.

Childress, W.L., Krull, I.S. and Selavka, C.M. 1990. *Journal of Chromatographic Science.* **28:** 76-82.

Chu, F.S. and Li, G.O. 1994. Simultaneous occurrence of fumonisin B_1 and other mycotoxins in moldy corn collected from the Peoples Republic of China in regions with high incidence of oesophageal cancer. *Applied Environmental Microbiology.* **60:** 847-852.

Cole, R.J. and Cox, R.H. 1981. Handbook of toxic fungal metabolites. Academic Press. New York.

Cole, R.J., Kirksey, J.W., Cutter, H.G., Doupnik, B.L. and Peckham, J.C. 1973. Toxin from *Fusarium moniliforme*: effects on plants and animals. *Science.* **179:** 1324-1326.

Cronshaw, D.K. 1984. Monitoring of sigatoka disease (*Mycosphaerella musicola*) in the Windward Islands for tolerance to fungicides. *Tropical Pest Management.* **30:** 225-229.

Desjardins, A.E., Plattner, R.D. and Nelson, P.E. 1997. Production of fumonisin B1 and moniliformin by *Gibberella fujikuroi* from rice from various geographic areas. *Applied and Environmental Microbiology.* **63:** 1838-1842.

Diaz, G.J. and Boermans, H.J. 1994. Fumonisin toxicosis in domestic animals: a review. *Vet. Hum. Toxicol.* **36:** 548-555.

Faber, J. 1988. Production of moniliformin by Canadian isolates of *Fusarium. Mycopathologia.* **101:** 187-190.

Filek, G. and Lindner, W. 1996. Determination of the mycotoxin moniliformin in cereals by high-performance liquid chromatography and fluorescence detection. *Journal of Chromatography.* **732:** 291-298.

Fink-Gremmels, J. 1999. Mycotoxins: their implications for human and animal. *The Veterinary Quarterly.* **21:** 115-120.

Finlay, A.R. and Brown, A.E. 1993. The relative importance of *Colletotrichum musae* as a crown-rot pathogen on Windward Island bananas. *Plant Pathology.* **42:** 67-74.

FRI Briefings. 1999. *Fusarium* Mycotoxins. **(www.wisc.edu/fri/fusarium.htm).** pp. 1-5.

Gelderblom, W.C.A. and Snyman, S.D. 1991. Mutagenicity of potentially carcinogenic mycotoxins produced by *Fusarium moniliforme. Mycotoxin Research.* **7:** 46-52.

Gelderblom, W.C.A, Kriek, N.P.J., Marasas, W.F.O. and Thiel, P.G. 1991. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B_1 in rats. *Carcinogenesis.* **12:** 1247-1251.

Gerlach, W. and Nirenberg, H.I. 1982. The genus *Fusarium*-a pictorial atlas. *Mitteilungen aus der Biologischem Bundesanstalt fur Land und Fortwirtschaft Berlin-Dahlem.* **209:** 1-406.

Graham, R, Senadhira, D., Beebe, S, Iglesias, C. and Monasterio, I. 1999. Breeding for micronutrient density in edible portions of staple food crops-conventional approaches. *Field Crop Research.* Elsevier Science Ltd. **60:** 57-80.

Gremmels, J.F. 1999. Mycotoxins: Their Implications for Human and Animal Health. *The Veterinary Quarterly.* **21:** 115-120.

Guarro, J. and Gene, J. 1995. Opportunistic *Fusarial* infections in humans. *European Journal of Clinical Microbiological Infectious Diseases.* **14:** 741-754.

Guarro, J., Gene, J. and Stchigel, A.M. 1999. Developments in fungal taxonomy. *Clinical Microbiology Reviews.* **12:** 454-500.

Harris, D.R., Seberry, J.A., Wills, R.B.H. and Sophr, L.J. 2000. Effect of fruit maturity on efficiency of 1-methylcyclopropene to delay the ripening of bananas. *Postharvest Biology and Technology.* Elsevier Science Ltd. **20:** 303-308.

Hopmans, E.C. and Murphy, P.A. 1993. Detection of fumonisins B_1, B_2 and B_3 and hydrolyzed fumonisin B_1 in corn-containing foods. *Journal of Agricultural and Food Chemistry.* **41:** 1655-1658.

IUF-Towards Respect for Global Union Rights. 2001. (http://www.editorials/banana.htm)

Jacobsen, B.J., Bowen, K.L., Shelby, R.A. and Diener, U.L. 1999. Mycotoxins and Mycotoxicoses. Alabama Cooperative Extension System. Circular ANR - 767.1-12.

Jiang, Y., Joyce, D.C. and Macnish, A.J. 1999. Extension of the shelf life of banana fruit y 1-methylcyclopropene in combination with polyethylene bags. *Postharvest Biology And Technology.* Elsevier Science Ltd. **16:** 187-193.

Jimenez, M., Huerta, T. and Mateo, R. 1997. Mycotoxin Production by *Fusarium* Species Isolated from Bananas. *Applied and Environmental Microbiology.* **63:** 364-370.

Jimenez, M., Logrieco, A. and Bottalico, A. 1993. Occurrence and Pathogenicity of *Fusarium* Species in Banana Fruits. *Journal Phytopathology.* **137:** 214-220.

Jimenez, M. and Mateo, R. 1997. Determination of mycotoxins produced by *Fusarium* isolates from banana fruits by capillary gas chromatography and high performance liquid chromatography. *Journal of Chromatography.* Elsevier Science Ltd. **778:** 363-372.

Johanson, A. and Blazquez, B. 1992. Fungi associated with banana crown rot on field packed fruit from the Windward Islands and assessment of their sensitivity to fungicides thiabendazole, procloraz and imazalil. *Crop Protection.* **11:** 79-83.

Kader, A.A. 2000. Recommendations for Maintaining Postharvest Quality. *In:* Banana Produce Facts. University of California. pp. 1-3.

Knight, C. 1982. Pathogenicity of Some Fungi Associated with Crown Rot of Bananas. *Phytopathology Z.* **104:** 13-18.

Krauss, U., Bidwell, R. and Ince, J. 1998. Isolation and Preliminary Evaluation of Mycoparasites as Biocontrol Agents of Crown Rot of Bananas. Banana Growers Association. *Biological Control.* **13:** 111-119.

Krogh, P. 1978. Causal association of mycotoxic nephropathy. *Acta Pathologica et Microbiologica Scandinavica.* **269:** 1-28.

Kuiper-Goodman, T. 1998. Food safety: Mycotoxins and Phytotoxins in Perspective. *Development in Chemistry, Toxicology and Food Safety.* pp. 25-48.

Kuthubutheen, A.J. and Nawawi, A. 1987. Fungi associated with banana and the potential role of some novel triorganotin (IV) compounds in the control of diseases of banana. *Asean Food Journal.* **3:** 95-100.

Li, Y.C., Ledoux, D.R., Bermudez, A.J., Fritsche, K.L. and Rottinghaust, G.E. 2000. Effects of moniliformin on performance and immune function of broiler chicks. *Poultry Science*. **79**: 26-32.

Luo, X.Y. 1988. *Fusarium* toxin contamination of cereals in China. *Proc. Japan. Assoc. Mycotoxicology Supplement*. **1**: 97-98.

Mabbet, T. 1997. Go Bananas. *African Farming*. pp. 19-21.

MacHardy, W.E. and Beckman, C.H. 1981. Vascular Wilt *Fusaria*: Infection and Pathogenesis. In. *Fusarium* : diseases, biology, and taxonomy. pp. 352-381.

Marasas, W.F.O. 1985. *Fusarium moniliforme*: a mycotoxicological miasma. In. *Mycotoxins and Phycotoxins*. pp. 19-28.

Marasas, W.F.O. 1995. Fumonisins: their implications for human and animal health. *Nat Toxins*. **3**: 193-198.

Marasas, W.F.O. , Kriek, N.P.J. , Fincham, J.E. and van Rensburg, S.J. 1984. Primary liver cancer and oesophageal basal cell hyperplasia in rats caused *by Fusarium monoliforme*. *Int. J. Cancer*. **34**: 383-387.

Marasas, W.F.O. and Nelson, P. 1987. Mycotoxicology, Introduction to Mycology, Plant Pathology, Chemistry, Toxicology, and Pathology of Naturally Occurring Mycotoxicoses in Animals and Man. University Park. The Pennsylvania State University Press.

Marin, D.H., Sutton, T.B., Blankenship, S.M. and Swallow, W.H. 1996. Pathogenicity of Fungi Associated with Crown Rot of Bananas in Latin America on Grande Naine and Disease-resistant Hybrid Bananas. *Plant Disease*. **80:** 525-528.

Medlicott, A. 2000. Postharvest Handling of Plantain. *In:* Product Specifications and Postharvest Handling of Fruits, Vegetables and Root Crops Exported from the Caribbean. *Fintrac.* pp. 1-5.

Miller, J.D. 1994. Fungi and Mycotoxins in Grain: Implications for Stored Product Research. *Plant Research Centre.* Elsevier Science Ltd. **31:** 11-16.

Moss, M.O. 1996. Mycotoxins. *Mycological Research*. **100:** 513-523.

Moss, M.O. 1998. Recent studies of mycotoxins. *Journal of Applied Microbiology Symposium Supplement*. **84:** 62-72.

Munimbazi, C. and Bullerman, L.B. 1998. High performance liquid chromatographic method for the determination of moniliformin in corn. *Journal of AOAC International*. **81:** 999-1004.

Nelson, P.E., Plattner, R.D., Shackelford, D.D. and Desjardins, A.E. 1991. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. *Applied and Environmental Microbiology*. **57**: 2410-2412.

Nelson, P.E., Tousson, T.A. and Marasas, W.F.O. 1983. *Fusarium* species: an illustrated manual for identification. Pennsylvania State University Press: University Park.

Ogundero, V.W. 1987. Crown rot fungi of Nigerian banana, and the effects of benomyl on their exo-enzymes. *Journal of Basic Microbiology*. **27**: 43-47.

Okole, B.N. 1995. Selection of banana and plantain (*Musa spp.*) tissues resistant to toxins produced by *Mycosphaerella* species using tissue culture techniques. Berlin 1-20.

Omurtug, G.Z. 2001. Determination of Fumonisin B_1 and B_2 in Corn and Corn-Based products in Turkey by High Performance Liquid Chromatography. *Journal of Food Protection*. **64**: 1072-1075.

Pelczar, M.J., Chan, E.C.S. and Krieg, N.R. 1993. Microbiology, concepts and applications (Int. ed.). McGraw Hill. New York.

Peshney, N.L. and Ghaukar, K.B. 1984. Black heart of banana caused by *Fusarium moniliforme*. *Indian Phytopathology*. **37**: 682-683.

Ploetz, R.C. 1990. *Fusarium* Wilt of Banana. APS Press, St Paul. pp. 1-10. (http://helios.bto.ed.ac.uk/bto/microbes/panama.htp.).

Ploetz, R.C. 2000. Panama Disease: A Classic and Destructive Disease of Banana. Online. Plant Health Progress. (http://www.planthealthprogress.org/bananapanama/htm).

Porter, J.K., Bacon, C.W. and Norred, W.P. 1990. Effects of *Fusarium moniliforme* and corn associated with equine leukoencephalomalacia on rat transmitters and metabolites. *Proc. Soc. Exp. Biol. Med.* **194:** 265-269.

Porter, J.K., Wray, E.M., Bacon, E.W. and Hagler, W.M. 1995. Fusaric acid in *Fusarium moniliforme* cultures, corn and feeds toxic to livestock and the neurochemical effects in the brain and pineal glands of rats. *Natural Toxins.* **3:** 91-100.

Porter, J.K., Wray, E.M., Rimando, A.M., Stancel, P.C., Bacon, C.W. and Voss, K.A. 1996. Lactational passage of fusaric acid from the feed of nursing dams to the neonate rat and effects on pineal neurochemistry in the F1 and F2 generations at weaning. *J. Tox. Environ. Health.* **49:** 101-115.

Postharvest News and Information. 1999. Research on Postharvest Physiology in South Africa. **10:** 1-10. (http://hort.cabweb.org/POSTHARV/Pni_9902.htm)

Postmaster, A., Sivasithamparam, K. and Turner, D.W. 1997. Enumeration and identification of microorganisms isolated from the surface of banana fruits at three developmental stages. *Scientia Horticulturae.* Elsevier Science Ltd. **69:** 189-197.

Rabie, C.J., Marasas, W.F.O., Thiel, P.G., Lubben, A. and Vleggaar, R. 1982. Moniliformin production and toxicity of different *Fusarium* species from Southern Africa. *Applied and Environmental Microbiology.* **43:** 517-521.

Ravindranath, B. 1989. Principles and practice of chromatography. John Wiley and Sons. New York. pp. 228-456.

Reyes, M.E., Nishijima, W. and Paul, R. E. 1998. Control of crown rot in 'Santa Catarina Prata' and 'Williams' banana with hot water treatments. *Post-harvest Biology and Technology.* Elsevier Science Ltd. **14:** 71-75.

Rheeder, J.P., Marasas, W.F.O., Thiel, P.G., Sydenham, E.W., Shephard, G.S. and Schalkwyk, D.J. 1992. *Fusarium moniliforme* and fumonisin in corn in relation to human oesophageal cancer in Transkei. *Phytopathology.* **82:** 353-357.

Rimando, A.M. and Porter, J.K. 1997. Fusaric acid increases melatonin in serum of weanling rats. *J. Tox. Environ. Health.* **50:** 101-110.

Rodrigo, V.H.L., Stirling, C.M. Teklehaimanot, Z. and Nugawela, A. 1997. The effect of planting density on growth and development of component crops in rubber/banana intercropping systems. *Field Crop Research.* Elsevier Science Ltd. **57:** 97-110.

Rodrigo, V.H.L., Stirling, C.M. Teklehaimanot, Z. and Nugawela, A. 2001. Intercropping with banana to improve fractional interception and radiation-use efficiency of immature rubber plantations. *Field Crop Research.* Elsevier Science Ltd. **69:** 237-249.

Ross, P.F., Nelson, P.E. and Richard, J.L. 1990. Production of fumonisins by *Fusarium moniliforme* and *F. proliferatum* isolates associated with equine leukoencephalomalacia and a pulmonary edema syndrome in swine. *Applied Environmental Microbiology.* **56:** 3225-3226.

Rotter, B.A., Thompsom, B.K., Lessard, M., Trenholm, H.L. and Tryphonas, H. 1994. Influence of low level exposure to *Fusarium* mycotoxins on selected immunological and haematological parameters in young swine. *Fundamental and Applied Technology.* **23:** 117-124.

Santchurn, D. 1999. A study on the common post-harvest diseases in Banana *musae* sp. (http://www.uom.ac.mu/Faculty/FA/Agricultu...esearch/santchurn.html)

Shillingford, C.A. and Sinclair, J.B. 1977. Susceptibility of five banana cultivars to anthracnose and crown rotting fungi. *Plant Disease Reporter.* **61:** 797-801.

Slabaugh, W.R. and Grove, M.D. 1982. Post-harvest diseases of bananas and their control. *Plant Disease.* **66:** 746-750.

Smith, T.K. 1992. Recent advances in the understanding of *Fusarium* trichothecene mycotoxicosis. *Journal of Animal Science.* **70:** 3989-3993.

Smith, T.K., McMillian, E.G. and Castillo, J.B. 1997. Effects of feeding blends of *Fusarium* mycotoxin contaminated grains containing deoxynivalenol and fusaric acid on growth and feed consumption of immature swine. *Journal of Animal Science.* **75:** 2184-2191.

Smith, T.K. and Sousadias, M. 1993. Fusaric acid content of swine feedstuffs. *Journal of Agriculture and Food Chemistry.* **41:** 2296-2298.

Snowdon, A.L., 1990.A Colour Atlas of Post Harvest Diseases and Disorders of Fruits and Vegetables. General Introduction and Fruits. pp. 3-120. Wolfe Scientific Ltd. London.

Srikul, S. and Turner, D.W. 1998. High N supply and soil water deficits change the rate of fruit growth of bananas (cv. 'Williams') and promote tendency to ripen. *Scientia Horticulturae.* Elsevier Science Ltd. **62:** 165-174.

Stack, M.E. and Eppley, R.M. 1992. Liquid chromatographic determination of fumonisins B_1 and B_2 in corn-products. *Journal of AOAC International.* **75:** 834-837.

Stover, R.H. and Simmonds, N.W. 1987. Bananas. Longmans *Scientific and Technical.* Harlow. pp. 468-470.

Sugiura, Y., Barr, J.R., Barr, D.B., Brock, J.W., Elie, C.M., Ueno, Y., Patterson, D.G., Potter, E.M. and Reis, E. 1999. Physiological characteristics and mycotoxins of human clinical isolates of *Fusarium* species. *Mycological Research.* **103:** 1462-1468.

Thalmann, A., Matzenauer, S. and Gruber-Schley, S. 1985. Survey on the occurrence of *Fusario* toxins in cereals. *Berlin Landwirtschaft.* **63:** 257-272.

Thiel, P.G., Meyer, C.J. and Marasas, W.F.O. 1982. Natural occurrence of moniliformin together with deoxynivalenol and zearalenone in Transkei corn. *Journal of Agriculture and Food Chemistry.* **30:** 308-312.

Thiel, P.G., Sydenham, E.W., Sheperd, G.S. and van Shalkwyk, D.J. 1993. Study of the Reproducibility Characteristic of a Liquid Chromatographic Methods for the Determination of Fumonisins B1 and B2 in corn : IUPAC Collaborative Study. *J. of. AOAC. Int.* **76:** 361-365.

Ueno, Y. 1984. Toxicological features of T-2 toxin and related trichothecenes. *Fundamental and Applied Toxicology.* **4:** 224-232.

Ueno, Y. and Shimada, N. 1974. Reconfirmation of the specific nature of reticulocytes bioassay system for trichothecene mycotoxins of *Fusarium* species. *Chem. Pharm. Bull.* **22:** 2744-2746.

Venter, S.L. and Steyn, P.J. 1998. Correlation between fusaric acid production and virulence of isolates of *Fusarium oxysporum* that causes potato dry rot in South Africa. *Potato Research*. **41**: 289-294.

Vincelli, P. and Parker, G. 1999. Mycotoxins in Corn Produced by *Fusarium* Fungi. Cooperative Extension Service. University of Kentucky. College of Agriculture. pp. 1-5.

Wade, N.L., Kavanagh, E.E. and Sepiah, M. 1993. Effects of modified atmosphere storage on banana postharvest diseases and the control of bunch main-stalk rot. *Postharvest Biology and Technology*. Elsevier Science Ltd. **3**: 143-154.

Wallbridge, A. 1981. Fungi associated with crown-rot disease of boxed bananas from the Windward Islands during a two-year survey. *Transactions of the British Mycological Society*. **77**: 567-577.

Wisniewski, M.E. and Wilson, C.L. 1992. Biological control of postharvest diseases of fruits and vegetables. *Hort. Sci.* **27**: 94-98.

APPENDICES

APPENDIX 1 *Fusarium* species culture media and mountant preparation

Sabouraud dextrose agar – 32.5 g of Sabouraud dextrose agar powder was added to 500 ml distilled water in a 1000 ml conical flask. The contents were heated at 55°C on a hot plate to dissolve any particles. The flask was then placed in an autoclave and sterilized at 121°C for 15 min. After cooling to 45°C, 15 ml molten agar was poured aseptically into sterile petri dishes and allowed to solidify before storing the plates at 4°C.

2% Water Agar – 2 g technical agar powder was added to 100 ml distilled water and sterilized in an autoclave at 121°C for 15 min.

***Fusarium* Selective Medium** – 15.0 g Difco Peptone, 1.0 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 20.0 g Agar and 1.0 g Pentachloronitrobenzene was added to 1 litre of distilled water. The medium was adjusted to pH 5.5-6.5, autoclaved, and 20 ml of a streptomycin sulfate and 12 ml of neomycin sulfate was added to the medium after the medium was cooled and just prior to pouring into petri dishes. The petri dishes were allowed to solidify before storing the plates at 4°C.

Lactophenol-cotton blue mountant

phenol (pure crystals)	20.0 g
lactic acid	20.0 g
glycerol	40.0 g
water	20.0 ml
cotton blue	0.5 g/100 ml water

The phenol crystals were dissolved in water heated to 60°C before adding lactic acid and glycerol. A small amount of cotton blue dye was finally added to the lactophenol mixture and thoroughly mixed before use.

APPENDIX 2. Synoptic key to sections and species of *Fusarium*

The 12 *Fusarium* sections as listed by Nelson *et al.* (1983)

Number of *Fusarium* species	Sections of *Fusarium* species
1	Eupionnotes
2	Spicarioides
3	Arachnites
4	Sporotrichiella
5	Roseum
6	Arthrosporella
7	Gibbosum
8	Discolor
9	Lateritium
10	Liseola
11	Elegans
12	Martiella-Ventricosum

The numbers used to identify each section in the synoptic key appear in front of the section name. Numbers printed in italics indicate sections included in more than one description under a given characteristics.

***Fusarium* species and the 12 sections to which each species belong**

Species in sections 1, 2 and 3 **Section Eupiionnotes (1)** *1.* *F. aquaeductum* *2.* *F. merismoides* 3. *F. dimerum* **Section Spicarioides (2)** *4.* *F. decemcellulare* **Section Arachnites (3)** *5.* *F. nivale*
Species in sections 4, 5, 6, 7 and 8 **Section Sporotrichiella (4)** *6.* *F. poae* *7.* *F. tricinctum* *8.* *F. sporotrichiodes* 9. *F. chlmaydosporum* **Section Roseum (5)** *10.* *F. graminum* 11. *F. avenaceum* **Section Arthrosporiella (6)** *12.* *F. semitectum* *13.* *F. camptoceras* **Section Gibbosum (7)** *14.* *F. equiseti* *15.* *F. scirpi* *16.* *F. acuminatum* 17. *F. longipes* **Section Discolor (8)** *18.* *F. heterosporum* *19.* *F. reticulatum* *20.* *F. sambucinum* *21.* *F. culmorum* *22.* *F. graminearum* 23. *F. crookwellense*
Species in Sections 9, 10, 11 and 12 **Section Lateritium (9)** *24.* *F. lateritium* **Section Liseola (10)** *25.* *F. moniliforme* (*F. verticilliodes*) *26.* *F. proliferatum* *27.* *F. subglutinans* 28. *F. anthophilum* **Section Elegans (11)** *29.* *F. oxysporum* **Section Martiella and Ventricosum (12)** 30. *F. solani*

Synoptic key to *Fusarium* species in section 1-3 (species 1-5)

Species in section 1, 2 and 3 (Eupionnotes, Spicarioides and Arachnites)

A. **Cultural characteristics**

1. **Rate of growth**
   - a. Growth slow, less than 2 to 3 cm in diameter after 10 days. **1, 2, 3**
   - b. Growth relatively slow, less than 7 cm in diameter after 10 days. **4,5**

2. **Aerial mycelium present or absent**
   - a. Present. **4, 5**
   - b. Absent and colony surface slimy and yeast-like. **1, 2, 3**

3. **Colour of aerial mycelium**
   - a. White. **4**
   - b. White to light orange. **5**

4. **Colour of colony from below (colour may diffuse into agar)**
   - a. Colourless to cream. **1, 2, 3**
   - **b.** Tan to carmine red. **4**
   - c. Cream to pale or bright orange. **5**

5. **Colour of spore masses (sporodochia)**
   - a. Spore masses distinct, cream to yellow. **4**
   - b. Spore masses distinct, pale to bright orange. **5**
   - **c.** Spore masses making up entire colony surface, cream, tan or orange. **1, 2, 3**

B. **Macroconidia from sporodochia**

1. **Size**
   - **a.** Small, 1-2 septate. **3, 5**
   - b. Small to moderately large, 3-7 septate. **1, 2, 3**
   - c. Very large, 9 septate or more. **4**

2. **Shape**
   - **a.** Spores straight with sides parallel for most of their length or slightly curved. **1, 3, 5**
   - b. Spores curved. 1, 3, 5
   - **c.** Spores cylindrical to robust with the sides parallel for most of their length. **2, 4**
   - **d.** Spores thin, needle-like. **1**

3. **Shape of basal and apical cells**
   - **a.** Pointed, basal cell not distinctly notched. **1, 2, 3, 5**
   - b. Blunt, or with distinctly shaped basal and apical cells. Basal cell distinctly notched. **1, 3, 4**

C. **Microconidia from aerail mycelium**

1. **Present or absent**
   - a. Present. **4**
   - **b.** Absent. **1, 2, 3, 5**

2. **In chains or false beads**
   - a. In chains and false heads. **4**

3. **Shape**
   - **a.** Oval and 0-1 septate. **4**

D. **Conidiophores (Type)**
   - a. Monophialides only (may prodcue either macroconidia or microconidia). **1, 2, 3, 4, 5**

E. **Chlamydospores**

1. **Present or Absent**
   - **a.** Present. **2, 3**
   - b. Absent. **1, 4, 5**

Synoptic key to *Fusarium* species in section 4-8 (species 6-23)

Species in section 4, 5, 6, 7 and 8 (Sporotrichiella, Roseum, Arthrosporiella, Gibbosum and Discolor)

A. Cultural characteristics

1. Rate of growth

a. Relatively slow, less than 7 cm in diameter after 10 to 14 days. **19**

b. Growth rapid, more than 7 cm diameter after 10 to 14 days. **6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23**

2. Colour of aerial mycelium

a. White. **6, 7, 8, 9, 10, 11, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23**

b. Tan, brown. **8, 9, 11, 12, 13, 14, 15, 20, 22, 23**

3. Colour of colony from below (colour may diffuse into agar)

a. Spores in light orange to orange masses scattered over the surface of the colony. **7, 8, 12, 14, 15, 20**

b. Spores in reddish-brown masses concentrated in the center of the colony. **10, 11, 16, 17, 18, 19, 20, 21, 22, 23**

B. Macroconidia from sporodochia

1. Shape

a. Thin, needle-like with thin walls. **10, 11**

b. Stout, with thick walls. **20, 21, 22, 23**

c. Curved but with the walls mainly parallel through most of their length. **6, 7, 8, 9, 18, 20, 21, 22**

d. Strong, dorsi-ventral curvature (hunch-backed). **14, 15, 16, 17, 19, 23**

e. Spindle-shaped and produced on monophialides or polyphialides in the aerial mycelium. **11, 12, 13**

2. Shape of basal and apical cells

a. Basal cell distinctly notched or foot-shaped (pedicellate). **6, 7, 9, 10, 11, 14, 16, 17, 18, 19, 20, 21, 22, 23**

b. Basal cell not distinctly foot-shaped. **8, 21**

c. Apical cell cone-like, elongated or whip-like. **14, 15, 16, 17**

d. Apical cell nipple-like, sometimes strongly curved as a beak. **20, 21, 22, 23**

C. Microconidia from aerial mycelium

1. Present or absent

a. Scarce to none. **10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23**

b. Fairly abundant. **14, 15, 20**

c. Abundant. **6, 7, 8, 9**

2. Shape

a. Oval, ellipsoidal, comma-shaped and club-shaped. **14, 15, 20**

b. Globose (napiform). **6**

c. Lemon-shaped, pear-shaped and spindle-shaped. **7, 8**

d. Mainly spindle-shaped. **9**

D. Conidiophores

1. Type

a. Monophialides only (may produce either macroconidia or microconidia). **6, 7, 10, 11, 14, 16, 17, 18, 19, 20, 21, 22, 23**

b. Monophialides (may produce either macroconidia or microconidia) and polyphialides (produce only microconidia and spindle-shaped macroconidia in the aerial mycelium). **8, 9, 12, 13, 15**

E. Chlamydospores

1. Present or absent

a. Present. **6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23**

b. Absent. **10, 11**

Synoptic key to *Fusarium* species insections 9-12 (species 24-30)

Species in sections 9, 10, 11 and 12 (Lateritium, Liseola, Elegans, Martiella and Ventricosum)

A. **Cultural characterisitcs**

1. **Rate of growth**
   - a. Relatively slow, less than7 cm in diameter after 10 to 14 days. **24**
   - b. Growth rapid, greater than 7 cm diameter after 10 to 14 days. **25, 26, 27, 28, 29, 30**

2. **Colour of aerial mycelium**
   - a. White. **24, 25, 26, 27, 28, 29, 30**
   - b. Tan to carmine red. **24**
   - c. Light purple. **25, 26, 27, 28, 29**

3. **Colour of colony from below (colour may diffuse into agar)**
   - a. Carmine red. **24**
   - b. Tan to orange. **24, 25, 26, 27, 28, 29, 20**
   - c. Light to dark purple pigment diffusing into agar. **25, 26, 27, 28, 29, 30**

4. **Colour of spore masses (sporodochia)**
   - a. Tan to orange. **24, 25, 26, 27, 28, 29**
   - b. Cream. **29, 30**
   - c. Blue-green to blue. **30**

B. **Macroconidia from sporodochia**

1. **Size**
   - a. Long. **25, 26, 27, 28, 30**
   - b. Short. **25, 26, 27, 28, 29, 30**

2. **Shape**
   - a. Thin, with thin walls. **25, 26, 27, 28**
   - b. Relatively thin, with thin walls. **25, 26, 27, 28, 29**
   - c. Stout with thick walls, and walls parallel through most of their length. **24, 30**

3. **Shape of basal and apical cells**
   - a. Apical cell with distinct beak or sharply curved. **24**
   - b. Apical cell not distinctly shaped. **30**

C. **Microconidia from aerial mycelium**

1. **Present or absent**
   - a. Sparse. **24**
   - b. Abundant. **24, 25, 26, 27, 28, 29, 30**

2. **In chains or false heads**
   - a. In chains and false heads. **25, 26**
   - b. In false heads only. **24, 27, 28, 29, 30**

3. **Shape**
   - a. Oval to kidney-shaped (reniform) and clavate. **24, 25, 26, 27, 29, 30**
   - b. Oval, pear-shaped or globose (napiform). **28**

D. **Conidiophores**

1. **Type**
   - a. Medium length monophialides producing microconidia. **24**
   - b. Short monophialides producing microconidia. **29**
   - c. Long monophialides and polyphialides producing microconidia. **26, 27, 28**

E. **Chlamydospores**

1. **Present or absent**
   - a. Absent. **25, 26, 27, 28**
   - b. Sparse. **24**
   - c. Abundant. **29, 30**

APPENDIX 3 Preparation of scanning electron microscopy reagents

2% Glutaraldehyde solution – was prepared by diluting 2 ml glutaraldehyde with 100 ml distilled water.

Phosphate buffer (pH 7.0) – was prepared by dissolving 0.275 g di-sodium hydrogen orthophosphate anhydrous ($Na_2H_2PO_4$) and 0.199 g sodium dihydrogen orthophosphate dihydrate ($Na_2H_2PO_4 : 2H_2O$) in a beaker containing 200 ml Milli-Q water. The pH of the solution was adjusted with 0.1 M HCl to pH 7.0. The solution was transferred to a 250 ml volumetric flask and made up to mark.

0.5% Osmium tetroxide – 0.5 g osmium tetroxide was diluted to 100 ml with water.

APPENDIX 4 Preparation of reagents and stock solutions for mycotoxin extraction and purification (TLC & HPLC)

1 M HCl – 10 ml HCl (99.95% purity) diluted to 100 ml with water.

1% methanolic HCl – 1 ml of concentrated HCl diluted to 100 ml with methanol.

1% methanolic acetic acid – 1 ml of concentrated acetic acid diluted to 100 ml with methanol.

Methanol-1% KH_2PO_4 (100:100 v/v) (pH 3.0) - 1% KH_2PO_4 was prepared by diluting 1 g potassium dihydrogen orthophosphate to 100 ml distilled water. An equal volume of methanol was added to the above solution and the pH adjusted to 3.0 using 0.1 M HCl.

Fumonisin B_1 , moniliformin & fusaric acid stock solutions – 1 g each of pure toxin standard was dissolved in 1 ml methanol (1 g/ml). A volume of 100 µl was transferred into each of 10 eppendorfs (1 ml). The methanol was evaporated under a stream of nitrogen so that a pure toxin residue of 100 µg remained. These stock solution were stored in a fridge at 4°C until required.

ρ-anisaldehyde spray reagent – 5 ml concentrated sulphuric acid was added drop-wise to 70 ml methanol and the mixture allowed to cool for 30 min. Glacial acetic acid (10 ml) and 0.5 ml ρ-anisaldehyde (4-methoxybenzaldehyde) were finally added. The mixture was gently swirled before use.

40% methanol : 60% aqueous 0.62 mM Na_2EDTA with 2% H_3PO_4 (40:60 v/v) – 0.62 mM Na_2EDTA was prepared by diluting 0.231 g Na_2EDTA to 880 ml of Milli-Q water. The solution was then made to mark in a 1000 ml volumetric flask with 20 ml *ortho*-phosphoric acid. Methanol (400 ml) was then added to 600 ml of the above solution.

10 mM phosphate buffer with 5 mM tetra butyl ammonium bromide (pH 7.0) – was prepared by dissolving 0.55 g disodium hydrogen orthophosphate anhydrous NaH_2PO_4, 0.379 g sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$) and 5 mM tetra butyl ammonium-bromide (TBAB) salt (1.511 g) in a beaker containing 200 ml Milli-Q water. The pH of the buffer solution was adjusted with 0.1 M HCl to pH 7.0. The solution was transferred to a 250 ml volumetric flask and made up to mark.

0.1 M NaH_2PO_4 (pH 3.3) – 15.6 g sodium dihydrogen orthophosphate was dissolved in 800 ml Milli-Q water and the pH adjusted to 3.3 with *ortho*-phosphoric acid. The solution was made up to mark in a 1000 ml volumetric flask.

***ortho*-phthaldialdehyde (OPA) derivatizing reagent** – was prepared by dissolving 0.04 mg OPA in 1 ml methanol, and adding 5 ml 0.1 M sodium tetraborate (3.8 g in 100 ml distilled water) and 50 µl of 2-mercaptoethanol.

1% KH_2PO_4 – 1 g potassium dihydrogen orthophosphate was dissolved in 100 ml distilled water.

5% aqueous $NaHCO_3$ – 5 g sodium hydrogen carbonate was dissolved in 100 ml distilled water.