CHAPTER 2

FUNGI ASSOCIATED WITH LESIONS ON BANANA FOLIAGE IN SOUTH AFRICA.

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ABSTRACT

A survey was conducted to determine the identity and distribution of fungi associated with banana leaf diseases in South Africa. Banana leaves were randomly collected from the five banana-growing areas in the country. Isolations were made from leaf lesions following surface disinfestation, incubation in moisture chambers, or spores were collected directly from lesions. Single-spore isolates were cultured on half-strength potato-dextrose agar and identified. Four foliar diseases were observed in the different banana-growing areas. Yellow Sigatoka (caused by *Mycosphaerella musicola*) was present in all five areas, Mycosphaerella speckle (caused by *M. musae*) and Cordana leaf spot (caused by *Cordana musae*) in four, and Cladosporium speckle (caused by *Cladosporium musae*) in one. Various other fungi, mostly saprobes, were also isolated. The most common species included (in order of prevalence) *Alternaria alternata, Colletotrichum gloeosporioides, Nigrospora oryzae, N. sacchari, N. sphaerica, Pestalotiopsis* sp., *Phoma glomerata, Selenophoma asterina* and *S. juncea.*

INTRODUCTION

Various fungi are associated with the foliage of banana plants. Diseases caused by foliar pathogens such as *Mycosphaerella musicola* R. Leach ex J.L. Mulder (yellow Sigatoka), *M. fijiensis* M. Morelet (black Sigatoka) and *M. eumusae* P. Crous & X. Mourichon (eumusae leaf spot) are major constraints to production of the crop (Jones 2000). Other leaf pathogens that can be damaging to predisposed plants, or under climatic conditions conducive to disease, include *M. minima* Stahel (leaf speckle), *M. musae* (Speg.) Syd. & P. Syd. (Mycosphaerella speckle), *Acrodontium simplex* Mangenor & de Hoog (leaf speckle), *Cercospora hayi* Calp. (brown or diamond spot), *Chaetothyrina musarum* (Speg.) Theiss. (sooty blotch), *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (sooty mould), *Cladosporium musae* E.W. Mason (Cladosporium speckle), *Colletotrichum musae* (Berk. & M.A. Curtis) Arx (leaf spot, anthracnose, fruit and stem rot), *Cordana musae* (Zimm.) Höhn. (Cordana leaf spot), *Deightoniella torulosa* (Syd.) M.B. Ellis (black leaf spot), *Drechslera gigantea* (Heald & F.A. Wolf) S. Ito (eyespot), *Haplobasidion musae* M.B. Ellis (Malayan or diamond leaf spot), *Hendersonula toruloidea* Nattrass (leaf spot, tip rot), *Periconiella musae* Stahel ex M.B. Ellis and *Veronaea musae* M.B. Ellis (tropical speckle), *Pestalotia leprogena* Speg. (ringspot), *Phyllachora musicola* C. Booth & D.E. Shaw (black-cross leaf spot), *Phyllosticta musae* F. Stevens & E. Young (leaf spot), *Uredo musae* Cummins and *Uromyces musae* Henn. (rust), *Curvularia* sp. (leaf spot), *Helminthosporium* sp. (fine speckle, leaf spot) and *Pyricularia* sp. (pyricularia leaf spot) (Brown *et al.* 1998, Jones 2000).

In addition to the pathogenic associations, numerous fungal taxa have been reported as endo- or epiphytes on *Musa* species, particularly wild banana (*Musa acuminata* Colla). Endophytes isolated most commonly include *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Curvularia* spp., *Fusarium* spp., *Nigrospora* spp., *Pestalotiopsis* spp., *Phomopsis* spp., xylariaceous taxa and sterile species (Brown *et al.* 1998, Photita *et al.* 2001). Some of these endophytes, e.g. *Colletotrichum gloeosporioides*, can cause disease in banana, whereas others may become problematic under environmental stress (Brown *et al.* 1998). However, many asymptomatic endophytic colonisers exist mutualistically with their hosts, benefiting the latter by protecting them from attack by pathogens (Petrini 1993, Dorworth & Callan 1996).

Three foliar diseases have been reported in South Africa on banana, viz, yellow Sigatoka (Van den Boom & Kuhne 1969), Mycosphaerella speckle (Brodrick 1973) and a leaf spot (Roth 1965). However, identification of yellow Sigatoka and Mycosphaerella speckle was based only on observation of symptoms and not on isolation of the causal organisms, whereas leaf spot was ascribed to a complex comprising *Glomerella cingulata* (Stoneman) Spauld & H. Schrenk, *Cordana musae, D. torulosa* and *Helminthosporium* sp., together with two bacterial species belonging to the genera *Pseudomonas* and *Xanthomonas*. Roth (1965) also found *Fusarium* sp., *Nigrospora* sp. and *Verticillium* sp., as well as various bacteria, to occur saprophytically in diseased tissue. From the above it is evident that the identity of fungi associated with banana foliar diseases in South Africa is unclear and that very little is known about endo- and epiphytes occurring in and on the crop. The aim of this study therefore was to isolate and identify the fungi associated with banana foliage, particularly lesioned sections, in the various banana-growing areas of South Africa.

MATERIALS AND METHODS

A total of 517 leaf samples displaying disease symptoms were randomly collected in 1999 and 2000 from Williams, Chinese Cavendish and Grande Naine cultivars in various banana plantations in southern KwaZulu-Natal, Komatipoort, Kiepersol, Levubu and Tzaneen, South Africa. Samples were placed in envelopes and stored at 5 °C until primary isolations were made.

Leaf sections with lesions were excised, submerged in a 2 % sodium hypochlorite for 30 sec, transferred to 70 % ethanol for 1 min, and rinsed twice in sterile distilled water (SDW). Aseptically blot-dried segments (ca. 4 mm^2) were dissected from the periphery of each lesion and plated on half-strength potato-dextrose agar (½ PDA) (Merck) (19 g PDA + 10 g agar (Merck) in 1 l water) supplemented with 200 mg/l Novobiocin in 90-mm Petri dishes. Plates were incubated for 3–7 d at 25 °C and fungi that developed were isolated. Excised lesioned leaf sections were sprayed with 70 % ethanol, placed into a 90-mm Petri dish containing sterile filter paper moistened with SDW, and incubated at 20 °C. After 1–2 d, each leaf sample was examined for fruiting structures and a tiny piece of agar touched to the fruiting structure and cultured on ½ PDA with Novobiocin. Isolates were grown at 25 °C for approximately 2–3 weeks before identification. For direct isolation, spores were collected from lesions in 100 µl SDW pipetted onto a lesion, allowed to stand for 30 sec, transferred to 400 µl SDW in an Eppendorf tube and mixed. The total volume was spread onto a 2 % water agar plate (20 g agar (Merck) in 1 l distilled water) and 24–48 hr later, single spores were collected and cultured on ½ PDA with Novobiocin. Fungal isolates were identified according to morphological characteristics.

Fungal structures were also observed *in situ* by scanning electron microscopy. Leaf lesions were prepared by fixing in 3 % glutaraldehyde for a minimum of 1 hr. Three rinse steps of 15 min each in 0.075 M phosphate buffer were carried out, followed by dehydration of the samples in 50, 70 and 90 % ethanol for 15 min at each concentration, and 3 x 15 min in 100 % ethanol. Samples were mounted on stubs, coated with gold in a Polaron sputter coater and viewed with a Jeol JSM scanning electron microscope at 5 kV.

RESULTS

Four leaf pathogens were identified (Table 1). *Mycosphaerella musicola* was the most prevalent, being isolated from 31 % of all the leaf samples and present in all five regions. *Mycosphaerella musae* and *Cordana musae* were isolated from 18 and 6 % of the samples, respectively. *Cladosporium musae* occurred only in Levubu, where it was isolated from 28 % of the samples. The appearance of yellow Sigatoka lesions conformed to literature (Fig. 1A) (Jones 2000). Sporodochia developed in sub-stomatal air chambers and emerged through stomatal pores (Fig. 1B). Cordana leaf spot was characterised by ellipsoid, brown lesions having distinct, concentric zones, surrounded by a yellow halo one to several centimetres in diameter towards the leaf margin (Fig. 1C). Conidiophores of the pathogen were pale brown, 150 µm long and 4–6 µm in diameter (Fig. 1D). Two types of speckle symptoms were observed. The most prevalent comprised light brown to tan coloured irregular blotches on the abaxial surface appearing as smoky, dark grey patches on the adaxial side (Fig. 1E). These lesions yielded *M. musae* but the fungus could not be observed *in situ*. Less common was a diffuse grey-green blotching of the adaxial surface of older leaves (Fig. 1F), which became yellow-orange and then necrotic with age

and was also found along the midrib of leaves. *Cladosporium musae* occurred in the lesions as conidiophores with terminal or intercalary branches of conidiogenous cells at the apex (Fig. 1G).

Various other fungi were also isolated from banana leaves. About 20 % of these isolates remained sterile and could not be identified. A fairly high presence of xylariaceous taxa amongst them was nevertheless evident. In order of prevalence, the identified taxa were *Nigrospora oryzae* (Berk. & Broome) Petch (isolated from 10.1 % of the leaf samples), *N. sphaerica* (Sacc.) E.W. Mason (3.7 %), *Alternaria alternata* (Fr.: Fr.) Keissl. (2.9 %), *Selenophoma asterina* (Berk. & Broome) B. Sutton (2.3 %), *Pestalotiopsis* sp. (2.1 %), *N. sacchari* (Speg.) E.W. Mason (1.4 %), *Phoma glomerata* (Corda) Wollenw. & Hochapfel (1.4 %), *Coll. gloeosporioides* (1.2 %), *S. juncea* (Mont.) Arx (1.0 %), *A. tenuissima* (Kunze: Fr.) Wiltshire (0.8 %), *Bipolaris cynodontis* (Marigoni) Shoemaker (0.8 %), *Diapothe* sp. (0.6 %), *Epicoccum nigrum* Link (0.6 %), *A.* cf. *citri* Ellis & N. Pierce (0.4 %), *Drechslera dematioidea* (Bubák & Wróbl.) Subraman. & P.C. Jain (0.4 %), *Colletotrichum musae* (0.2 %), *Curvularia lunata* (Wakker) Boedijin (0.2 %), *Drechslera* sp. (0.2 %), *Exserohilum rostratum* (Drechsler) K.J. Leonard & Suggs (0.2 %), *Guignardia mangiferae* A.J. Roy (0.2 %), *Harpographium* sp. (0.2 %), *Myrothecium verrucaria* (Alb. & Schw.) Ditmar (0.2 %) and *Pithomyces sacchari* (Speg.) M.B. Ellis (0.2 %).

Discussion

This study confirmed the presence of yellow Sigatoka and Mycosphaerella speckle in South Africa and validated the original diagnoses of the two diseases (Van den Boom & Kuhne 1969, Brodrick 1973) by isolating the causal organisms and identifying them as *M. musicola* and *M.*

musae, respectively. The pathogen was confirmed to be *Cordana musae* and not the more recently described *Cordana johnstonii* M.B. Ellis, which causes smaller leaf spots (Priest 1990) and appears to be adapted to cooler environments (Jones 2000). *Cladosporium musae* is a new recording for South Africa. *Deightoniella torulosa*, previously reported by Roth (1965) from the Mpumalanga and Limpopo lowveld regions, could not be isolated.

Yellow Sigatoka was the most prevalent of the various diseases and is reported here for the first time from KwaZulu-Natal. The second-most prevalent disease, Mycosphaerella speckle, has previously also been described as widespread in South Africa, but not as serious as yellow Sigatoka (Brodrick 1973). In accordance with Jones (2000), infection by *M. musicola* appeared to predispose plants to attack by *Cordana musae* as the latter pathogen was often isolated from Cordana-like lesions surrounding yellow Sigatoka spots. However, the incidence of *M. musicola* and *M. musae* seemed to be inversely related, a phenomenon that has not been reported before. Currently, banana leaf diseases in South Africa are under control as a result of implemented spray and deleafing programmes. However, due to the severe yellow Sigatoka outbreak in 1999 and 2000, banana leaf disease status in South Africa is tentative and regular surveys should continue.

With the exception of *Colletotrichum gloeosporioides*, *Colletotrichum musae* and *N. oryzae*, which were described by Roth (1965), Doidge (1950) and Jacobs (1973), respectively, the fungal species isolated here represent new entries for banana in South Africa. Although most were probably opportunistic secondary invaders, taxa such as *A. alternata*, *Colletotrichum gloeosporioides*, *Colletotrichum musae*, *Curvularia* spp., *Diaporthe* spp. (as *Phomopsis* spp.), *E. nigrum*, *N. oryzae* and *Pestalotiopsis* spp. have previously been reported as endophytes of banana (Brown *et al.* 1998, Photita *et al.* 2001, Photita *et al.* 2002). The presence of an endophyte

component is supported by the relatively high incidence of sterile isolates (Brown *et al.* 1998, Photita *et al.* 2001) and the regular occurrence of xylariaceous taxa, which are particularly well adapted to an endophytic existence (Whalley 1995) and commonly occur as endophytes in virtually all tropical plants (Rodriques & Samuels 1990, Pereira *et al.* 1993, Rodriques 1994, Brown *et al.* 1998, Photita *et al.* 2001). However, other common banana endophytes such as *Fusarium* spp. (Brown *et al.* 1998, Photita *et al.* 2001) could not be isolated.

Alternaria alternata and *Curvularia lunata*, isolated from four and one of the banana-growing regions in South Africa respectively, have been associated with leaf lesions on banana in China (Qi *et al.* unpublished data). *Guignardia mangiferae*, a cosmopolitan endophyte of woody plants isolated from one leaf sample collected in KwaZulu-Natal, has previously also been reported from lesions on banana leaves in New South Wales, Australia (Baayen *et al.* 2002). The commonly encountered *N. oryzae* and *N. sphaerica* are well known as banana fruit pathogens (Jacobs 1973, Brown *et al.* 1998). *Phoma glomerata*, isolated from three of the banana-growing regions, could easily have been misidentified as *P. jolyana* Piroz. & Morgan-Jones, causal agent of black finger disease (Brown *et al.* 1998). Separation of the two species is based on the presence of *Alternaria*-like chlamydospores borne catenately in *P. glomerata* and laterally in *P. jolyana* (Sutton 1980), though chlamydospores initially are borne terminally in *P. jolyana* and do not always remain single. Nevertheless, Koch's postulates could not be confirmed for any of the species isolated when artificially inoculated onto banana leaves in the present study (data not presented).

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Table 1: Fungi isolated from banana leaves in South Africa.

Species	Incidence[a]				
	Kiepersol	Komatipoort	KwaZulu	Levubu	Tzaneen
Alternaria alternata	5	7	1	2	-
Alternaria cf. *citri*	-	2	-	-	-
Alternaria tenuissima	1	2	-	1	-
Bipolaris cynodontis	2	-	1	1	-
Cladosporium musae	-	-	-	33	-
Colletotrichum gloeosporioides	2	1	3	-	-
Colletotrichum musae	-		-	1	-
Cordana musae	10	8	9	6	-
Curvularia lunata	-	1	-	-	-
Curvularia pallescens	1	-	-	-	-
Diaporthe sp.	-	1	2	-	-
Drechslera dematoidea	-	1	1	-	-
Drechslera sp.	-	-	1	-	-
Epicoccum nigrum	1	2	-	-	-
Exserohilum rostratum	-	-	1	-	-
Guignardia mangiferae	-	-	1	-	-
Harpographium sp.	-	1	-	-	-
Mycosphaerella musae	12	10	36	36	-
Mycosphaerella musicola	37	36	23	29	33
Myrothecium verrucaria	-	-	1	-	-
Nigrospora oryzae	11	2	27	1	11
Nigrospora sacchari	3	1	3	-	-
Nigrospora sphaerica	4	4	9	1	1
Pestalotiopsis guepinii	-	2	7	2	-
Phoma glomerata	1	5	-	-	1
Pithomyces sacchari	-	-	1	-	-
Selenophoma asterina	1	7	2	2	-
Selenophoma juncea	1	2	1	1	-
Sterile	12	17	6	3	1
Total leaf samples	104	110	135	119	47
Total fungal isolates	116	120	163	122	61

[a] Percentage leaf samples from which fungus was isolated.

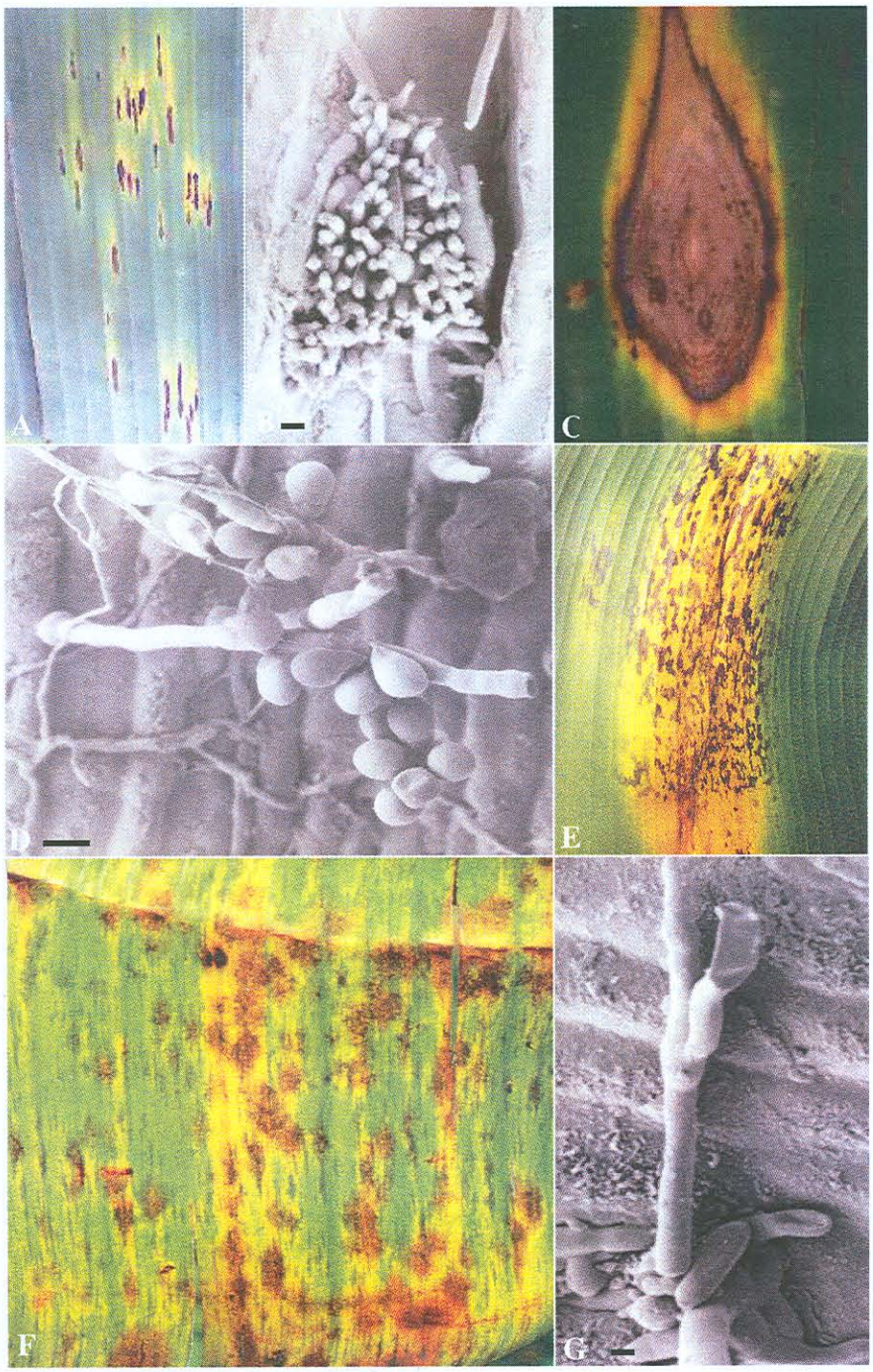

Figure 1: Symptoms and morphology of A. Yellow Sigatoka. B. *Mycosphaerella musicola.* C. Cordana leaf spot. D. *Cordana musae.* E. Mycosphaerella speckle. F. Cladosporium speckle. G. *Cladosporium musae* (scale bars 10 µm).