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Pathogen profile

Mycosphaerella fijiensis, the black leaf streak pathogen of banana: progress towards understanding pathogen biology and detection, disease development, and the challenges of control

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SUMMARY

Background: Banana (*Musa* spp.) is grown throughout the tropical and subtropical regions of the world. The fruits are a key staple food in many developing countries and a source of income for subsistence farmers. Bananas are also a major, multibillion-dollar export commodity for consumption primarily in developed countries, where few banana cultivars are grown. The fungal pathogen *Mycosphaerella fijiensis* causes black leaf streak disease (BLSD; aka black Sigatoka leaf spot) on the majority of edible banana cultivars grown worldwide. The fact that most of these cultivars are sterile and unsuitable for the breeding of resistant lines necessitates the extensive use of fungicides as the primary means of disease control. BLSD is a significant threat to the food security of resource-poor populations who cannot afford fungicides, and increases the environmental and health hazards where large-acreage monocultures of banana (Cavendish subgroup, AAA genome) are grown for export.

Taxonomy: *Mycosphaerella fijiensis* M. Morelet is a sexual, heterothallic fungus having *Pseudocercospora fijiensis* (M. Morelet) Deighton as the anamorph stage. It is a haploid, hemibiotrophic ascomycete within the class Dothideomycetes, order Capnodiales and family Mycosphaerellaceae. Its taxonomic placement is based on DNA phylogeny, morphological analyses and cultural characteristics.

Disease symptoms and host range: *Mycosphaerella fijiensis* is a leaf pathogen that causes reddish-brown streaks running parallel to the leaf veins, which aggregate to form larger, dark-brown to black compound streaks. These streaks eventually form fusiform or elliptical lesions that coalesce, form a water-soaked border with a yellow halo and, eventually, merge to cause extensive leaf necrosis. The disease does not kill the plants immediately, but weakens them by decreasing the photosynthetic capacity of leaves, causing a reduction in the quantity and quality of fruit, and inducing the premature ripening of fruit harvested from infected plants. Although *Musa* spp. are the primary hosts of *M. fijiensis*, the ornamental plant *Heliconia psittacorum* has been reported as an alternative host.

New opportunities: Several valuable tools and resources have been developed to overcome some of the challenges of studying this host–pathogen system. These include a DNA-mediated fungal transformation system and the ability to conduct targeted gene disruptions, reliable quantitative plant bioassays, diagnostic probes to detect and differentiate *M. fijiensis* from related pathogens and to distinguish strains of different mating types, and a genome sequence that has revealed a wealth of gene sequences and molecular markers to be utilized in functional and population biology studies.

Useful websites: http://bananas.bioversityinternational.org/, http://genome.jgi-psf.org/Mycfi2/Mycfi2.home.html, http://www.isppweb.org/names_banana_pathogen.asp#fun, http://www.promusa.org/

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INTRODUCTION

Mycosphaerella fijiensis (anamorph *Pseudocercospora fijiensis*) causes black leaf streak disease (BLSD) or black Sigatoka leaf spot of banana (*Musa* spp., which include plantain). It is considered to be the most destructive member of the 'Sigatoka disease complex', which includes *M. musicola* (anamorph *P. musae*), the causal agent of Sigatoka leaf spot disease (also called yellow Sigatoka leaf spot), and *M. eumusae* (anamorph *P. eumusae*), which causes Eumusae leaf spot disease (previously called Septoria leaf spot disease). *Musa* spp. are the primary hosts of *M. fijiensis* and exhibit a range of symptoms that depend on the levels of resistance of individual hosts. The ornamental plant *Heliconia psittacorum* is the only known alternative host of *M. fijiensis* (Gasparotto *et al*., 2005).

Banana is grown in more than 120 countries around the world, primarily in the tropical, rain-fed regions of Africa, Asia

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and Latin America, with almost 104 million tons produced annually (Anonymous, 2009a). *Mycosphaerella fijiensis* is a major constraint to banana production wherever it occurs, and is considered to be the most costly and damaging disease of banana worldwide (Carlier *et al*., 2000a). The disease has been reported in most countries in which banana is grown (summarized in Carlier *et al*., 2000a; Jones, 2009), with recent reports of its spread in the Caribbean to the Bahamas (Ploetz, 2004), Puerto Rico (Irish *et al*., 2006), Grenada (Graham, 2007), Guyana (Solomon, 2010), St. Vincent and the Grenadines (Richards, 2009) and St. Lucia (Anonymous, 2010a). Its arrival appears imminent in Dominica and the French Antilles as predicted by Carlier *et al*. (2000a). Banana production in dry and high-altitude areas is less favourable for BLSD development, although the latter advantage may be lost over time because of the adaptability of the pathogen to higher altitudes (Arzanlou *et al*., 2007), potentially facilitated by climate change and warming global temperatures (Anonymous, 2009a).

Bananas are the fourth most economically important food crop after rice, wheat and maize. India is the world's largest producer of bananas, and Ecuador is the world's largest banana exporting country, selling approximately one-third of the world's exported bananas (Anonymous, 2009b). Because the bulk of world banana production (approximately 85%) comes from relatively small plots and kitchen or backyard gardens, in which bananas are grown for local consumption (Arias *et al*., 2003), the production, consumption and export by developing countries are dangerously impacted by BLSD. Conversely, the 15% of bananas grown for export to developed and developing countries, estimated at 14.6 million tons in 2008 (Anonymous, 2009b) with a value of more than US $5 billion per year (Arias *et al*., 2003), would suffer the same risks as those of smallholder farmers without repeated applications of fungicides to control BLSD.

BLSD seriously affects the world's most popular dessert banana (AAA and some AAB genomes) and plantain (AAB genome) cultivars. The AAA dessert banana cultivars are most commonly from the Cavendish subgroup and are the genomic group typically grown in monoculture. Few clones other than the ABB cooking bananas have strong resistance to BLSD (Jones, 2009). Ploetz *et al*. (2003) have summarized the taxonomy, anatomy, and general attributes of these cultivated, hybrid *Musa* genomes. BLSD does not kill plants immediately, but weakens them by decreasing the photosynthetic capacity of leaves, causing a reduction in the quantity and quality of fruit, and inducing premature ripening of fruit harvested from infected plants. The susceptibility of the crop necessitates the use of multiple fungicides at relatively high frequencies. Such applications are potentially detrimental, not only to the environment, but also to those who live and work in areas in which banana is treated to control the disease. It is even more devastating for those smallholder farmers who cannot afford the use of pesticides and therefore have no practical means to effectively control the impact or spread of the fungus (Bennett and Arneson, 2003; Marín *et al*., 2003; Ploetz, 2000, 2001).

Estimates of losses to BLSD for dessert bananas and plantains are in the range 20–80% in the absence of fungicides (Anonymous, 2009a; Maciel Cordeiro and Pires de Matos, 2003). BLSD control on Cavendish cultivars ranks towards the top of global inputs of agricultural fungicides with approximately US $550 million spent annually (G.H.J. Kema, personal communication). By 2003, the annual cost of controlling the disease in large plantations was estimated at approximately US $1000/ha (Arias *et al*., 2003). As acreage and production have increased, fungicide usage and resistance have risen, necessitating as many as 66 applications per year in some locations (Anonymous, 2009a). More than 30% of total banana production costs are directed towards the chemical control of *M. fijiensis* (Kema, 2006). A working group facilitating a 'pesticide reduction plan for banana' was formed with the goal of reducing pesticide use on banana by 50% in 10 years (Kema, 2006). This is an important effort for environmental sustainability, human health and food security. In addition, the carbon footprint of large-scale banana production is predicted to be high because of intensive pesticide, fungicide and fertilizer inputs, as well as the extensive distances travelled for export purposes.

A comprehensive and extensive review describing the Sigatoka leaf spots of banana has been published previously (Jones, 2000a) and, for BLSD (Carlier *et al*., 2000a), includes factors of worldwide distribution and economic importance (Pasberg-Gauhl *et al*., 2000), symptoms (Jones *et al*., 2000), taxonomy, cultural characteristics, diagnostic methods, pathogenic variability and genetic diversity of the causal agent (Carlier *et al*., 2000b), disease cycle and epidemiology (Gauhl *et al*., 2000), host responses to the disease (Fouré *et al*., 2000), host–pathogen interactions (Mourichon *et al*., 2000) and disease control (Romero, 2000). More recent reviews of the disease were published in 2003 (Marín *et al*., 2003; Ploetz *et al*., 2003). In addition to providing an overview, the present paper focuses primarily on the research progress described for *M. fijiensis* since the latter reviews were published, as well as introducing studies or perspectives not presented therein. Readers are referred to earlier reviews (Carlier *et al*., 2000a; Jones, 2000a; Marín *et al*., 2003; Ploetz *et al*., 2003) for comprehensive listings of references describing substantial work published prior to 2003.

HISTORICAL ASPECTS OF BANANA LEAF SPOT DISEASES AFFECTING PATHOGEN DISTRIBUTION

Three fungi are recognized as the primary agents of the Sigatoka disease complex found on banana: *Mycosphaerella fijiensis*, *M.*

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musicola and *M. eumusae*. Because these fungi are morphologically and symptomatically similar, it is critical to understand their distinguishing characteristics (reviewed in Jones, 2000a) and to acknowledge the potential for their overlapping, coexisting lifestyles with each other and related fungi (Arzanlou *et al*., 2007, 2008; Crous, 2009; Johanson, 1995). *Mycosphaerella musicola* was the first *Mycosphaerella* pathogen to have a significant impact on bananas internationally. Sigatoka leaf spot disease was first recorded on the Indonesian island of Java in 1902 and, within approximately 20 years, had begun to spread globally; by the 1950s and 1960s, it had reached most banana-growing areas of the world (reviewed in Jones, 2000a, 2003, 2009). It has been reported as a significant problem in regions with high altitudes and cool temperatures (Mouliom-Pefoura *et al*., 1996).

Mycosphaerella fijiensis, most typically found at relatively low, warm elevations, was first identified on the island of Fiji in the South Pacific in 1963, but is believed to have been widespread in the Pacific long before then, with some records suggesting its presence in Taiwan as early as 1927 (Stover, 1978). In the 1960s, *M. fijiensis* was determined to be more virulent than *M. musicola* on Cavendish cultivars (AAA genome), causing symptoms 8–10 days earlier than those caused by Sigatoka leaf spot disease (Ploetz *et al*., 2003). *M. fijiensis* also is capable of attacking members of the plantain subgroup (AAB genome), which are resistant to Sigatoka leaf spot disease. Within 40 years, *M. fijiensis* had spread to most banana-growing regions globally, adapting to cooler climates at higher elevations (Arzanlou *et al*., 2007; Gauhl *et al*., 2000) and effectively replacing Sigatoka as the dominant leaf spot disease (Carlier *et al*., 2000a). Johanson (1995) noted the existence of unspecified areas in which *M. fijiensis* and *M. musicola* were thought to coexist, yet we have little knowledge of the extent to which this may be the case (Jones, 2003; Romero, 2003). A pathogen described in the literature as *M. fijiensis* var. *difformis* was later determined as synonymous with *M. fijiensis* (Pons, 1987; Stover and Simmons, 1987).

Mycosphaerella eumusae was first isolated in the early 1990s in Southeast Asia, where it appears to have become the dominant banana leaf spot disease (Carlier *et al*., 2000d; Crous and Mourichon, 2002). It has also been reported in South Asia and West Africa, and there is concern that its distribution, which is still largely unknown, may be greater than currently reported (Carlier *et al*., 2000c). In Southeast Asia, *M. eumusae* appears to have competed effectively with *M. fijiensis* to prevent the latter from displacing it, in contrast with the displacement of *M. musicola* by *M. fijiensis* in other regions worldwide (Jones, 2003; Pérez Vicente *et al*., 2003). Recently, Zandjanakou-Tachin *et al*. (2009) failed to detect *M. musicola* in a population of 95 isolates sampled in Nigeria, suggesting that it had been replaced by *M. fijiensis* and *M. eumusae*, the former being the most prevalent of the two fungi identified. Because the visual symptoms of disease caused by *M. fijiensis* and *M. eumusae* are similar, the impact caused by the latter may often be overlooked. Many more studies are needed to determine the distribution and virulence of *M. eumusae* in regions in which it is found or suspected, especially as it may affect cultivars highly resistant to both *M. fijiensis* and *M. musicola* (Carlier *et al*., 2000c). If *M. eumusae* is indeed competing effectively with *M. fijiensis* for host 'real estate', what genetic mechanisms allow this fungus to do so at the apparent expense of *M. musicola*? Greater documentation of regional pathogen populations and a better knowledge of the competitive abilities of the *Mycosphaerella* leaf spot pathogens that facilitate ecological displacement of each other, and perhaps other banana-associated fungi (Arzanlou *et al*., 2007, 2008; Romero, 2003), are needed to better understand pathogen population dynamics and the environmental and host conditions contributing to changing community structures.

LIFE CYCLE AND DISTINGUISHING TAXONOMIC CHARACTERS

The disease cycle of *M. fijiensis* consists of four distinct stages that include spore germination, penetration of the host, symptom development and spore production (Fig. 1A) (Agrios, 2005). Comprehensive disease cycle descriptions have been published previously (Gauhl *et al*., 2000; Henderson *et al*., 2006; Marín *et al*., 2003). *Mycosphaerella fijiensis* M. Morelet (Morelet, 1969) is a sexual, heterothallic fungus having *Pseudocercospora fijiensis* (M. Morelet) Deighton (Deighton, 1976) as the anamorph stage (Crous, 2009; Crous *et al*., 2003, 2009; Stewart *et al*., 1999). It is a haploid, hemibiotrophic ascomycete with a bipolar, heterothallic mating system within the class Dothideomycetes, order Capnodiales and family Mycosphaerellaceae. It produces multicellular conidia from conidiophores [Fig. 1A (Agrios, 2005) and B (Liberato *et al*., 2009)] in culture and *in planta*, in the latter case arising from stomata primarily on the abaxial (lower) surface of infected leaves. Conidiophores arise from hyphae present in the substomatal chamber and can produce multiple conidia from a single conidiophore. Conidiophores are produced on the lower surface of initial specks (Stage 1; not shown) or at the first streak stage (Stage 2; Fig. 2A) up to the second spot stage (Stage 5; Fig. 2D). Conidia are produced almost continuously between the second streak (Stage 3; Fig. 2B) and second spot (Stage 5; Fig. 2D,E) stages (Meredith and Lawrence, 1969). Rodríguez-García *et al*. (2007) have described the process of asynchronous conidiogenesis of *M. fijiensis* in culture using phase contrast and scanning electron microscopy.

The production of spermatia (Fig. 1C) (Liberato *et al*., 2009), considered as the male gametes, occurs in spermagonia (Fig. 1A), which begin development from the substomatal chambers primarily on the abaxial leaf surface at the second streak

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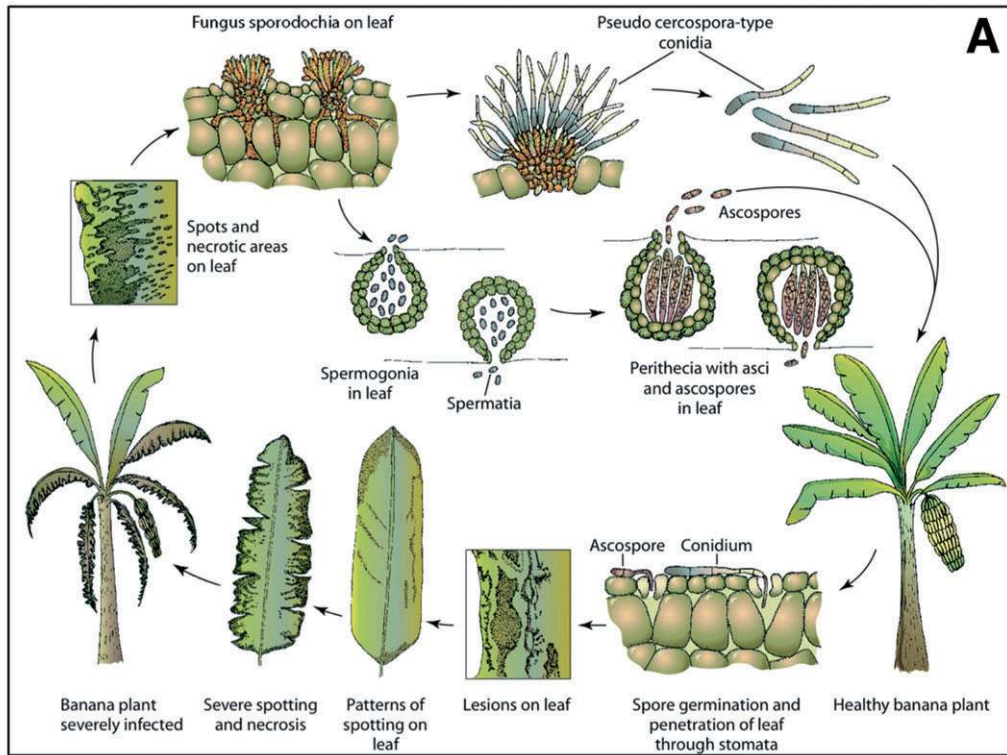

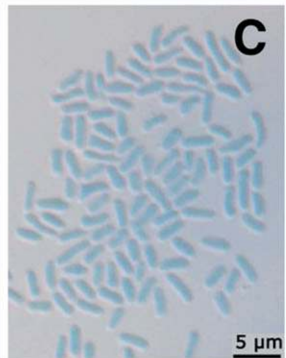

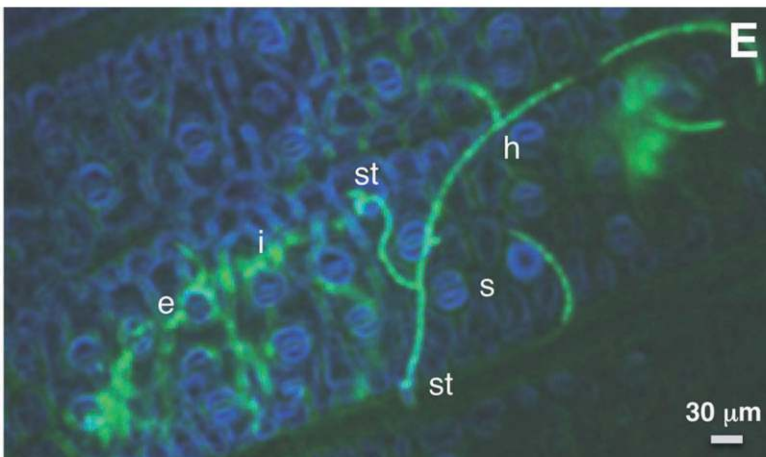

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Fig. 1 Developmental stages of *Mycosphaerella fijiensis* affecting disease progression on banana. (A) Life cycle of development of black leaf streak disease of *Musa* spp., caused by *M. fijiensis*. [Reprinted from Agrios (2005), © Elsevier, with permission.] (B) Conidia and conidiophores. (C) Spermatia. (B and C: Source: José R. Liberato, Department of Primary Industries and Fisheries, Qld, Australia, with permission; Liberato *et al*., 2009.) (D, F) Ungerminated and germinated two-celled ascospores, respectively. (Source: Marie-Françoise Zapater, CIRAD, with permission.) Germination from both polar ends with germ tubes parallel to the long axis of the spore is a diagnostic character for *M. fijiensis* ascospores (Arzanlou *et al*., 2008). (E) Growth of a single epiphyllic hypha (h) of *M. fijiensis* expressing green fluorescent protein, after *Agrobacterium*-mediated transformation, on the abaxial surface of a 'Grande Naine' banana leaf. Infection occurs after a fungal spore germinates and germ tubes penetrate the plant by growth through stomata (s). The germ tube sometimes forms a swollen structure above the stomate, called a stomatopodium (st), prior to penetration and intercellular growth (i). It is not unusual for the fungus to encircle the substomatal cavity during this early phase of growth (e) (see also Balint-Kurti *et al*., 2001).

(Stage 3; Fig. 2B) or first spot (Stage 4; Fig. 2C) stage. Hyaline, single-celled, rod-shaped spermatia are released through the ostiole of the spermagonium, which protrudes through the stomatal pore. It is notable that both conidia and spermatia production occur most abundantly on the abaxial surface of infected leaves, which is consistent with the fact that bananas have a larger number of stomata on this surface in comparison with the adaxial surface. Stomata on the lower surface tend to be smaller in diameter than those on the adaxial surface (Skutch, 1927). The development of both spore-producing structures is first evident inside the substomatal chambers where a growing spermagonium may physically displace conidiophores growing nearby (Meredith and Lawrence, 1969).

The cytological details of spermatization and ascospore development have not yet been described for any *Mycosphaerella* pathogen of bananas. Although ascospores of *M. fijiensis* are produced abundantly in the field, the production of fruiting bodies (Etebu *et al*., 2003; Mourichon and Zapater, 1990) and ascospores (Etebu *et al*., 2003) *in vitro* has met with limited success as methodologies for efficient and reliable sexual crossing are not routine. Now that the mating type idiomorphs of all three primary members of the Sigatoka disease complex have been cloned and characterized (Arzanlou *et al*., 2010; Conde-Ferráez *et al*., 2007, 2010), significant progress towards understanding these critically important developmental processes in sexual reproduction should be forthcoming.

Although the anamorphic states of the three primary agents of the Sigatoka disease complex are recognized as distinct on the basis of their conidial morphologies (reviewed in Jones, 2000a; Zapater *et al*., 2008a), the teleomorphs are believed to be largely similar (reviewed in Carlier *et al*., 2000a, c; Crous and Mourichon, 2002; Jones, 2000b). The early literature refers to the fruiting structures of *M. fijiensis* as perithecia (Jones, 2000a; Meredith and Lawrence, 1969) or, more generally, ascostroma (Pons, 1987). Placement of the *Mycosphaerella* banana pathogens in the family Mycosphaerellaceae is defined, in part, by the production of pseudothecia (perithecia-like structures) containing bitunicate (two-layered) asci (reviewed in Crous *et al*., 2009). The *M. eumusae* teleomorph has been described as producing pseudothecia (Crous and Mourichon, 2002), and the same description would be expected to apply to the teleomorph stages of the closely related *M. fijiensis* and *M. musicola* banana pathogens (Crous *et al*., 2003).

Ascospore germination patterns are a valuable feature that distinguish morphologically similar species of the Mycosphaerellacae (Crous, 2009), and this characteristic applies to the members of the Sigatoka disease complex. The one-septate ascospores of *M. fijiensis* (Fig. 1D,F) are distinguished from those of *M. musicola* and *M. eumusae* by their germination from both polar ends, with each germ tube parallel to the long axis of the spore (Fig. 1F). *Mycosphaerella fijiensis* ascospores also lack the mucoid sheath characteristic of germinating *M. musicola* ascospores, and do not display the germ tube distortions and three to four parallel or lateral germ tubes characteristic of *M. eumusae* (Arzanlou *et al*., 2008).

Both anamorphs and teleomorphs of *M. fijiensis* can be present on infected leaves concurrently. Conidia and ascospores are both infective and will germinate on the leaf surface in the presence of high relative humidity (92%–100%) or free water, respectively (Jacome and Schuh, 1992; Jacome *et al*., 1991). After germination, the fungus typically undergoes a period of epiphytic growth on the leaf surface before it penetrates the plant through one or more stomatal pores, typically on the abaxial leaf surface (Fig. 1A,E) (Carlier *et al*., 2000a). The germ tube may form a swollen structure, called a stomatopodium, just above the stomate prior to entry (Fig. 1E). Once inside, the hypha encircles the substomatal cavity (Fig. 1E) (Balint-Kurti *et al*., 2001) and grows intercellularly through the mesophyll layers and air chambers, without forming haustoria, into the palisade layer with further proliferation into the air chambers between the veins. There is an extended period of biotrophy that can last for several weeks before the first cytological alterations to mesophyll cells are observed (Beveraggi *et al*., 1995). Conidia production occurs after growth of the conidiophores through the stomates as described above.

Conidia and ascospores can become wind-borne within infected plantations and travel up to several tens of kilometres away from disease sources, but ascospores play the most important epidemiological role in the spread of the disease through wind-borne dispersal (Amil *et al*., 2007; Gauhl *et al*., 2000).

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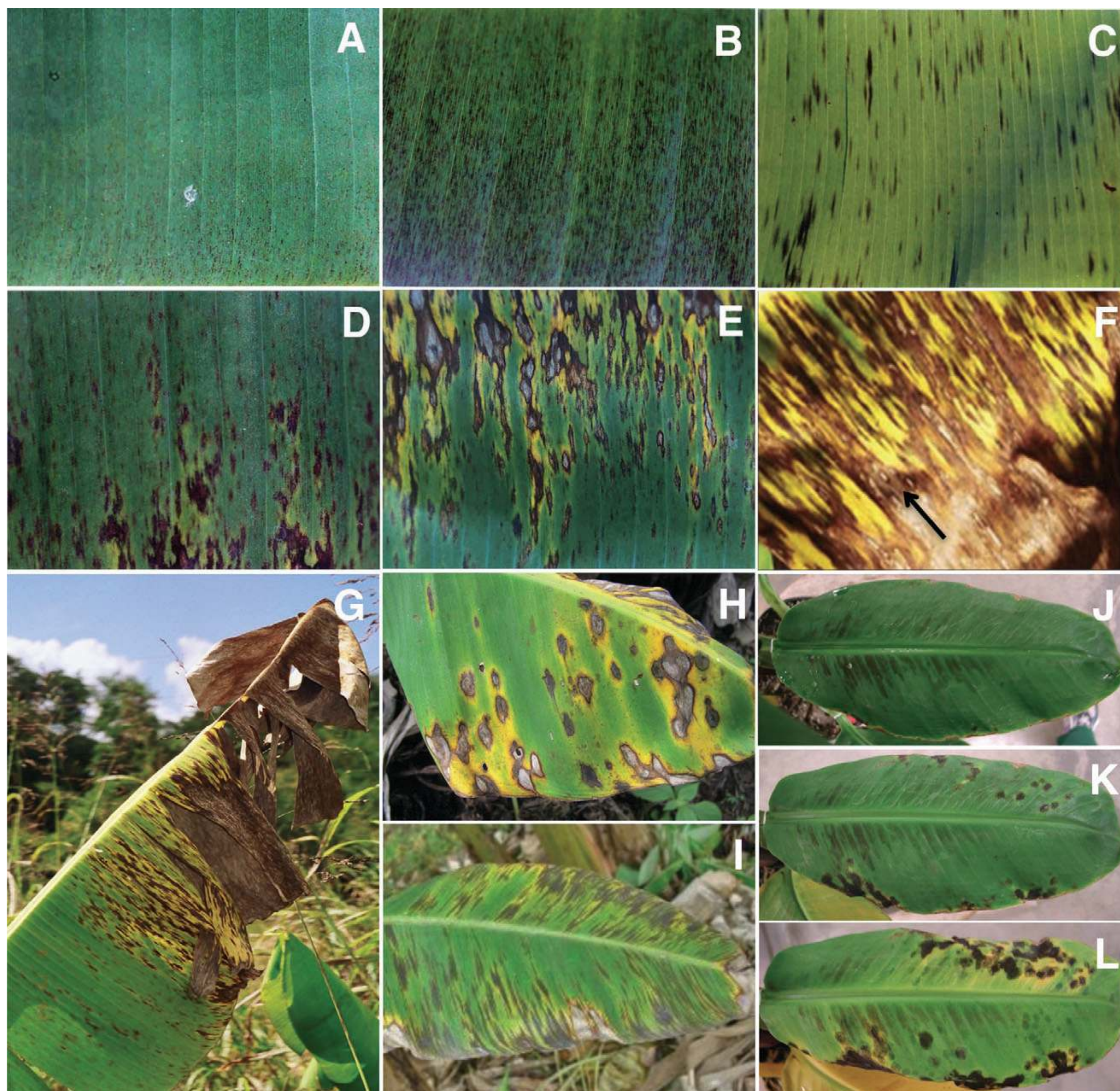

Fig. 2 Stages of development of black leaf streak disease as described by Meredith and Lawrence (1969). Stage 1 (not shown) is the 'initial speck stage' in which specks of less than 0.25 mm are faintly visible on the abaxial leaf surface. (A) Stage 2 is the 'first streak stage'. (B) Stage 3 is the 'second streak stage'. (C) Stage 4 is the 'first spot stage'. (D) Stage 5 is the 'second spot stage'. (E) Stages 5 and 6, where the latter is the 'third or mature spot stage'. [Images A–E are of banana in the Cavendish subgroup, by Ron A. Peterson, © The State of Queensland, Australia (2010) (http://www.dpi.qld.gov.au/4790_7412.htm), used with permission.] (F) Coalescence of streaks and spots on a severely diseased leaf. Note the three distinct spots surrounding the arrowhead, as well as others elsewhere, still visible in the fully necrotic tissue. (G) All stages in streak and spot development are usually present on a single plant or leaf. (H) Rounded, nonstreak lesions found on leaves of young banana 'water' suckers. (Source: Scot C. Nelson, University of Hawaii, Manoa, used with permission.) (I) Narrow leaf streak lesions on 'sword' sucker leaf 2. (Source: Josiane T. Ferrari, Instituto Biologico, Brazil, used with permission.) (J–L) Time course of symptom development on a 'Grande Naine' plant incubated in a growth chamber in upstate New York. Juvenile, tissue culture-derived plants were spray inoculated on the abaxial surface with a suspension of *M. fijiensis* hyphae and conidia, and incubated under broad-spectrum, high-intensity light with high humidity for the full course of disease development, as described previously (Donzelli and Churchill, 2007). First symptoms, especially along the margins of the leaf, were evident by 4 weeks post-inoculation (PI) (J). Thereafter, the round lesions typical of those that develop on 2–3-month-old juvenile plants grown under these conditions expanded and coalesced rapidly between 6 weeks (K) and 7 weeks (L) PI.

However, exposure to ultraviolet radiation in sunlight appears to constrain long-distance airborne dispersal of viable spores. Water, in the form of dew and rain, has been implicated in short-distance dispersal. Further studies on the leaf surface origin, aerobiology and leaf surface run-off of *M. fijiensis* spores are needed in order to fully understand short- and long-distance dispersal and survival of inocula causing BLSD (reviewed in Burt, 2003; Gauhl *et al*., 2000).

DISEASE SYMPTOMS AND FACTORS AFFECTING THEIR DEVELOPMENT

Comprehensive disease symptom descriptions for *M. fijiensis* have been published previously (Henderson *et al*., 2006; Jones *et al*., 2000; Marín *et al*., 2003). All three primary pathogens of the Sigatoka disease complex cause similar symptoms on banana leaves: small specks that become streaks running parallel to the leaf veins, which aggregate to form larger compound streaks that vary in colour from rusty red to dark-brown to black, depending on the pathogen. These streaks eventually form fusiform or elliptical spots that coalesce, form a water-soaked border with variable degrees of a yellow halo and, eventually, merge to cause extensive leaf necrosis (Fig. 2). The specific differences in symptom development that differentiate the diseases caused by *M. fijiensis*, *M. musicola* and *M. eumusae* have been summarized elsewhere (Jones, 2000a; Ploetz *et al*., 2003).

Meredith and Lawrence (1969) provided the first detailed description and photographs of symptom development by *M. fijiensis* on susceptible, mature 'Dwarf Cavendish' plants (AAA genome, Cavendish subgroup), describing six distinct stages. Fouré (1987) also described six stages of symptom development, which included an earlier stage (his 'Stage 1') that preceded the initial speck stage of Meredith and Lawrence (Jones *et al*., 2000; Meredith and Lawrence, 1969). Otherwise, the two approaches to symptom description are comparable and have been summarized elsewhere (see table 1 in Marín *et al*., 2003). The present paper references the Meredith and Lawrence descriptions, paired with reference photographs of these stages on Cavendish banana (Fig. 2A–E).

Although all leaves of a susceptible banana are equally susceptible to *M. fijiensis*, most infections occur on new leaves between emergence and unfurling (Gauhl *et al*., 2000). Ascospores moved by wind currents are typically deposited at the terminal end of the unfolding 'cigar', 'heart', or 'candela' leaf (see Fig. 4 in Ploetz, 2000) leaf and cause an easily visible leaf tip infection (Fig. 1A) (see also fig. 2 in Henderson *et al*., 2006). These early symptoms are monitored frequently to appropriately time fungicide applications. The conidial infection patterns of leaves typically seen with Sigatoka leaf spot are not apparent with BLSD (Gauhl *et al*., 2000; see fig. 2 in Henderson *et al*., 2006). Once the disease has progressed, all stages in streak and spot development are usually present on a single plant or leaf (Meredith and Lawrence, 1969) (Fig. 2G). However, the same stages of BLSD symptoms can vary, depending on leaf age and physiology, developmental stage of the plant and leaves at the time of infection, level of plant stress and disease pressure, environmental conditions and cultivar (Jones *et al*., 2000). Disease symptoms on suckers can develop differently from those on mature plants, and there is symptom variation depending on the origin of the sucker. The leaf in Fig. 2I is from a 'sword' sucker (leaf 2) with symptoms primarily at Meredith and Lawrence Stage 5, except at the leaf margins, where the tissue has begun to collapse and become necrotic. The relatively narrow streaks on this leaf should be noted compared with the broader streaks and spots on the mature leaf in Fig. 2G. In contrast, rounded, nonstreak lesions are typically found on leaves of young banana 'water' suckers (Fig. 2H) in the field and on juvenile (2–3-month-old or less), tissue culture-derived plants (Fig. 2J–L) grown in the laboratory, glasshouse or growth chamber (Alvarado-Capó *et al*., 2003; Donzelli and Churchill, 2007; Jacome and Schuh, 1993a; Jones, 1995; Jones *et al*., 2000). McGahan and Fulton (1965) characterized this difference in lesion morphology as being a result of narrower secondary veins and larger intercellular air spaces in juvenile leaves, which fail to constrict lesion growth to the same extent as in mature leaves, causing elliptical lesions on juvenile leaves and streaks on adult leaves.

The differences in lesion shape between the 'water' sucker leaf (Fig. 2H) and the 'sword' sucker leaf (Fig. 2I) may reflect differences in nutritional or physiological status based on the type of sucker. 'Water' suckers have small rhizomes, an untapered pseudostem and large broad leaves, but are considered weak as planting material. In contrast, 'sword' suckers are more strongly connected to the parent plant, are more vigorous, have large rhizomes, tapered pseudostems and thin, bract-like leaves that broaden once removed from the mother plant as planting material (Robinson, 1995; Stover and Simmons, 1987). This diversity of BLSD symptoms suggests the need for greater awareness and understanding of the effects of the physiological and developmental stages of banana plants on symptom phenotype. This is especially true when bioassays to screen for pathogen virulence or plant resistance are evaluated in artificial growth conditions, such as screenhouse, glasshouse, growth chamber or laboratory.

BLSD development requires stringent environmental conditions for optimal fungal development. These include temperatures around 27 °C for ascospore germination and germ tube growth, as well as a moist leaf surface. Free water is not required for conidial infections as long as humidity is high (reviewed in Gauhl *et al*., 2000). Optimal light quality and intensity are also believed to play an important role, especially in controlled environment settings (Donzelli and Churchill, 2007), although the

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specific effects and requirements are not well understood. Several researchers have noted that shade generally has a negative effect on both Sigatoka leaf spot and BLSD development in the field. Banana grown in full sunlight is more likely to have greater numbers of lesions caused by *M. musicola* or *M. fijiensis* than plants grown under the shade of agroforestry trees, such as cacao, coconut or *Ficus* spp., or with fewer hours of direct sunlight (Karani, 1986; Stover and Simmons, 1987; Thorold, 1940; Thurston, 1992; Wellman, 1972). In growth chamber and glasshouse conditions, failure to provide high-quality and high-intensity light, as well as high humidity, during disease development (after an initial period of high humidity in the dark) (Donzelli and Churchill, 2007) will almost guarantee poor or slow development of disease symptoms. It is unknown whether the effect of light on disease development is caused by the modulation of plant defence responses to the disease or the amplification of pathogen virulence mechanisms, or some combination thereof. Further study of these important effects of light and shade on disease development is warranted.

DETECTION AND DIFFERENTIATION OF *MYCOSPHAERELLA* BANANA PATHOGENS

Because the *Mycosphaerella* leaf spot pathogens cause similar symptoms on banana, can appear morphologically similar to the nonspecialist and can coexist with each other and other fungi on the same leaves (Crous, 2009; Jones, 2000a), molecular tools have been developed for pairing with morphological characteristics to more easily identify and distinguish similar fungi from one other. Johanson and Jeger (1993) first used polymerase chain reaction (PCR) amplification of the internal transcribed spacer 1 (ITS1) region of rDNA to differentiate *M. fijiensis* from *M. musicola* and other banana-associated fungi (Johanson, 1995). This diagnostic tool was refined further when PCR was used to develop random amplification of polymorphic DNA (RAPD) probes to differentiate between the two banana pathogens by Southern analysis or dot-blotting (Johanson *et al*., 1994). Henderson *et al*. (2003, 2006) implemented PCR-based molecular diagnostic assays to complement the morphological identification of *M. fijiensis* during a recent eradication campaign in northern Australia. Nonspecific amplifications and variable efficiencies using conventional PCR prompted the development of improved molecular assays based on real-time PCR to quantitatively detect more diverse populations of isolates. Arzanlou *et al*. (2007) developed real-time PCR TaqMan diagnostic probes based on the β-tubulin gene that differentiate *M. fijiensis*, *M. musicola* and *M. eumusae*, and are used to quantify the amounts of the respective fungi in infected banana tissue.

Additional species of *Mycosphaerella* or their anamorphs have been described previously from *Musa* spp. (Jones, 2000a), but little was known about these taxa until recently. Arzanlou *et al*. (2008) developed DNA barcodes incorporating the ITS region of the rDNA operon, and partial actin, small subunit mitochondrial rDNA and histone H3 gene sequences. These were used to infer the phylogenies of a global set of *Mycosphaerella* spp. from banana using morphological characters and the four-gene dataset. They identified more than 20 species occurring on banana, some of which had been described previously from *Musa* or other hosts, as well as eight new species. The pathological relevance of species outside of the currently defined Sigatoka disease complex is unclear (Aptroot, 2006; reviewed in Crous *et al*., 2003; Jones, 2000a) and warrants further study. These recently developed PCR-based tools, in combination with morphological assessments, cultural growth data and standardized plant bioassays, will help to effectively differentiate Sigatoka disease complex members and related *Mycosphaerella* species, providing additional approaches for the specialist and nonspecialist alike to identify and characterize the diverse assemblage of fungi found in association with banana (Arzanlou *et al*., 2008; Jones, 2000a).

Recent studies have demonstrated that the genus *Mycosphaerella* is polyphyletic (Crous *et al*., 2007), containing members that include plant pathogens that can be facultatively saprobic, as well as symptomless endophytes and mycoparasites (Crous, 2009). As has been reported for banana (Arzanlou *et al*., 2008; Jones, 2000a), surprisingly large numbers of morphologically similar *Mycosphaerella* spp. can occur on the same host (reviewed in Crous, 2009). Furthermore, several reports have described diverse populations of endophytes from *Musa* spp. (Nuangmek *et al*., 2008; Pereira *et al*., 1999; Photita *et al*., 2001, 2002a, b; Surridge *et al*., 2003). These combined observations suggest the need for a greater understanding of the diversity of microbes associated with *Musa*, the roles played by diverse *Mycosphaerella* spp. or their anamorphs in interactions with each other and unrelated microbes on banana, and whether they contribute to disease, mycoparasitism or endophyte-enhanced plant defence, or some combination thereof. A greater knowledge of the microbial consortia associated with banana could provide the basis for the development of integrated disease management schemes that utilize native microbes to promote plant and soil health, and foster disease control, with biological agents used in combination with pesticides as needed.

GENETIC DIVERSITY OF POPULATIONS

Understanding the genetic structure of fungal populations and how pathogens evolve is an important aspect of the development of strategies for plant breeding and the management of disease resistance. Evolutionary studies of *M. fijiensis* have demonstrated that global populations of the fungus maintain a relatively high level of genetic diversity compared with populations

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of other fungi (Carlier *et al*., 2000b). Such high levels of pathogenic variability, if maintained in populations, could allow the adaptation of the fungus to newly introduced resistant host genotypes. As ascospores play a major role in the epidemiology of BLSD and ecological conditions favour their development almost year-round in most banana cultivation areas, recombination is predicted to play an important role in the population structure of *M. fijiensis*. An early study using 19 DNA restriction fragment length polymorphism (RFLP) loci indicated that the Australasia–Southeast Asia region is the probable centre of origin and diversity for *M. fijiensis* (Carlier *et al*., 1996), and that the fungus probably moved relatively recently from this area to other banana-producing regions. The results suggested that random sexual reproduction occurred in populations and that, although founder effects were detected in some regions (Latin America, Africa and the Pacific Islands), leading to an overall reduction in diversity, genetic diversity remained moderately high, suggesting rare *M. fijiensis* migration events between geographical regions. Neu *et al*. (1999) characterized 11 microsatellite markers from a Nigerian isolate of *M. fijiensis* and confirmed the earlier results of Carlier *et al*. (1996), demonstrating comparatively low within-region diversity and strong geographical differentiation between Nigerian and Mexican isolates. Hayden *et al*. (2003) used single-copy RFLP markers to detect a moderate level of genetic differentiation between Pacific Island and Papua New Guinea populations of *M. fijiensis*. This study confirmed earlier results (Carlier *et al*., 1996), suggesting that the Pacific Island population was established from the likely centre of origin of *M. fijiensis* in Southeast Asia.

Müller *et al*. (1997) showed that the genetic variation of *M. fijiensis* in Nigeria exists on a micro- and macro-scale, occurring within one lesion, between lesions on the same plant, between plants and cultivars, and between geographical locations. Studies by Rivas *et al*. (2004) again detected a high level of genetic differentiation of *M. fijiensis* on both a global and local scale, and confirmed that founder effects reflect rare migration events among continents, probably through movements of infected plant material. They also detected a high level of genetic diversity at both the plantation and plant scales, supporting the hypothesis of random mating populations. In Central America, isolates forming dendrogram clusters corresponded to original sampling sites representing discrete populations, supporting the observations of founder effects observed elsewhere (Molina *et al*., 2003). Zapater *et al*. (2004) reported the isolation of 12 PCR-RFLP markers that were used to study *M. fijiensis* population structure within continents (Carlier *et al*., 2003), and described a simple and robust protocol that is especially valuable for conducting such studies in locations not having access to the sophisticated equipment required for other RFLP and microsatellite methodologies.

More recently, microsatellite markers have been developed for all three primary members of the Sigatoka disease complex, including 32 loci for *M. fijiensis*, 15 of which were developed for use in multiplex PCR analyses (Zapater *et al*., 2008b). These genetic markers will be useful for both population structure studies and for further development of a genetic map of *M. fijiensis* (Ferreira *et al*., 2009; Manzo-Sánchez *et al*., 2008). Zandjanakou-Tachin *et al*. (2009) recently used single nucleotide polymorphisms (SNPs) in rDNA to determine the genetic structure of geographically distinct populations of *Mycosphaerella* spp. collected from Nigeria, identifying 14 SNP haplotypes of *M. fijiensis*. Their results also suggested that *M. musicola* had been replaced predominantly by *M. fijiensis*, with a smaller representation of *M. eumusae* in the population. Fahleson *et al*. (2009) recently used PCR-RFLP and microsatellite markers, in combination with benomyl resistance, to characterize a collection of isolates from Uganda, and determined that the Lake Victoria basin population of *M. fijiensis* was essentially homogeneous, probably as the result of a recent founder event.

Significant progress has been made in describing the population structures of *M. fijiensis* and *M. musicola* (Hayden *et al*., 2005) at different geographical scales and, more recently, for a few smaller regions. However, additional knowledge reflecting the population structures of each continent and region impacted by BLSD, as well as by the other banana leaf spot pathogens, is necessary to provide new information relevant to the determination of the durability of resistance of local banana cultivars and for the development of new management strategies that will control disease without the use of fungicides. National and regional knowledge of the variability in pathogenicity within populations of *M. fijiensis*, tested against a broad range of standard reference cultivars, especially resistant and partially resistant cultivars (Fullerton and Olsen, 1995), is necessary. Electrophoretic karyotype analyses of strains from different geographical locations in Mexico demonstrated a surprisingly high degree of chromosome length polymorphisms across 10 isolates, with no two karyotypes being identical (Rodríguez-Garcia *et al*., 2006), suggesting the possibility of variations in virulence as well. Carlier *et al*. (2003) noted as a key priority the need to understand pathogen population diversity in Southeast Asia where the newest member of the Sigatoka disease complex, *M. eumusae*, was first detected, and because this region is the centre of origin for all three *Mycosphaerella* banana pathogens as well as that of the host genus *Musa*.

DISEASE CONTROL AND FUNGICIDE RESISTANCE

Chemical control of BLSD for the export industry utilizes both protectant and systemic fungicides that are alternated as part of a strategy to delay or manage fungicide resistance. The classes of

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fungicide used and the conditions under which each is most effective, as well as the history of the development of resistance, have been reviewed previously (Marín *et al*., 2003; Ploetz, 2000; Romero, 2000).

The seven main classes of fungicide in current use are demethylation inhibitors (DMIs), amines, quinone outside inhibitors (QoIs; strobilurins), anilinopyrimidines (APs), benzimidazoles (BCMs), succinate dehydrogenase inhibitors (SDHIs) and guanidines (Anonymous, 2010b) (http://www.frac.info/frac/work/work_bana.htm). The key strategy, as outlined by the global Banana Working Group of the Fungicide Resistance Action Committee (FRAC), is to apply fungicides with different modes of action in mixtures, and to alternate between noncross-resistant fungicide classes to minimize the risk of the development of resistance. Additional important approaches are to restrict the number of annual applications of each fungicide and use efficient integrated disease management measures in parallel with fungicide application programmes. It is notable that, because BLSD is considered as 'the most important and commercially relevant disease' of banana by the FRAC Banana Working Group (Anonymous, 2008), their biennial review of fungicide sensitivity and use recommendations focuses exclusively on the control of *M. fijiensis* to the exclusion of all other banana pests and diseases, further underscoring the global importance of this disease to the banana trade.

The most recent 2010 FRAC report provides the current recommendations for application of each class of fungicide and indicates that resistance to QoIs (strobilurins) is problematic in at least half of the countries tested (i.e. Columbia, Costa Rica, Guatemala and Panama), and resistance to BCMs continues to be widespread at high levels as described previously (reviewed in Marín *et al*., 2003). The performance of spray programmes for the amines and APs is high as long as they continue to be used as recommended. DMI performance is intermediate in most countries and high in others (i.e. Ecuador, Colombia and Philippines). The use of SDHI and guanidine fungicides for BLSD is too new for data to be reported yet. Sensitivity to fungicides is tested, in part, using ascospore or conidia germination assays, in which the length of germ tubes is measured in response to growth on a range of fungicide concentrations (Chin *et al*., 2001; Marín *et al*., 2003).

Strobilurins are called QoIs because they act by inhibiting the mitochondrial respiration of fungi by binding to the cytochrome bc1 enzyme complex (complex III) at the Qo site (Gisi *et al*., 2002). Resistance to QoIs in *M. fijiensis* and some other fungi is conferred by a point mutation in the mitochondrial cytochrome B gene (*cyt b*) that causes an amino acid change from glycine to alanine at position 143; hence, the mutation is called 'G143A' (Sierotzki *et al*., 2000). G143A does not appear to cause a significant fitness penalty to the fungus and, in random mating populations such as *M. fijiensis*, the overall proportion of sensitive and resistant individuals in unselected populations is predicted to be 1:1. Amil *et al*. (2007) measured the dynamics of QoI sensitivity in *M. fijiensis* populations in Costa Rica, beginning 3 years after the fungicide was first introduced to the country at a time when resistance had already become common. They were unable to determine a direct relationship between the number of spray applications and the frequency of appearance of the G143A mutation on individual farms, and suggested that large-scale mixing of ascospores dispersed by wind effectively created regional averages that masked effects that might otherwise be attributable to the spray regimens of individual farms. In fact, resistant ascospores were detected as far as 6 km away from the main banana production area, both with and against the prevailing winds, clearly demonstrating the regional importance of ascospore dispersal via wind.

The DMIs, e.g. propiconazole, act by interrupting the biosynthesis of ergosterol. DMI resistance of *M. fijiensis* is correlated with one or more of seven distinct point mutations (Y136F, A313G, Y461D, Y463D, Y463H, Y463N and Y463S) in the *CYP51* gene sequence encoding sterol 14α-demethylase (Cañas-Gutíerrez *et al*., 2009; Chong *et al*., 2010), an essential enzyme of the ergosterol biosynthetic pathway. Mutation of this gene has been described in other fungi, including the related wheat pathogen *Mycosphaerella graminicola*, as being correlated with resistance to the systemic azole fungicides (Bean *et al*., 2009). Increased expression of the *CYP51* gene was not correlated with resistance to propiconazole for either *M. fijiensis* or *M. graminicola*, as has been described for some other fungi.

The BCMs, e.g. benomyl, are systemic fungicides that have been widely used since the 1970s for the control of BLSD (Marín *et al*., 2003; Ploetz, 2000; Romero, 2000). Resistance in *M. fijiensis* began to appear within 2–3 years after the fungicide was first used (Stover, 1980). The antifungal activity of BCMs is conferred by their binding to β-tubulin, a subunit of the tubulin protein and component of the microtubules, which prevents microtubule assembly and disrupts fungal cell division. Cañas-Gutiérrez *et al*. (2006) identified a cytosine to adenine change in codon 198 of the β-tubulin genes from all *M. fijiensis* isolates exhibiting medium to high resistance to benomyl, and developed a PCR-based screening method as a tool to efficiently differentiate between susceptible and resistant strains.

A detailed knowledge of the genetic mechanisms conferring tolerance or resistance of *M. fijiensis* to fungicides is of critical importance in a system such as banana, where they play an immense role for the export industry in disease control. With monoculture production, fungicides will continue to play a significant role in disease management. However, a greater understanding of fungal resistance mechanisms may help to elucidate additional target sites for fungicide action, and will facilitate the development of molecular markers for monitoring the generation and movement of resistant fungi. Greater knowledge of

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sexual reproduction and other basic aspects of *M. fijiensis* pathogen biology could contribute to the development of methods or materials that limit fungal recombination, thereby retarding the flow of resistance genes into the population. Finely tuned modelling of inoculum dispersal and disease development under different environmental and cultural conditions could help to predict when minimal amounts of fungicides are best applied to be most effective. Progress in Cuba and the French West Indies in this regard has been reviewed by Pérez Vicente *et al*. (2003) and Côte *et al*. (2009), respectively. Greater progress is needed in the development of novel integrated disease management approaches that increase overall plant health in banana monocultures and reduce the need for high-input fungicide usage.

PLANT BIOASSAYS AND INOCULUM PRODUCTION

BLSD is the most economically important disease of banana worldwide (Carlier *et al*., 2000a). This has been the situation for much of the last 50 years, since *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *cubense*, destroyed an estimated 40 000 ha of 'Gros Michel' banana (AAA genome, Gros Michel subgroup) (Ploetz, 2006). This catastrophic wilt disease (Simmonds, 1966) caused the export industry to shift production to cultivars in the Cavendish subgroup (AAA), which were resistant to *Fusarium* wilt. However, in a few short years, Cavendish cultivars began to succumb to Sigatoka leaf spot and then BLSD. Given the time that has passed since *M. fijiensis* was first identified in 1963, it is surprising how relatively little we understand about the basic biology of the pathogen. This situation is a result, in part, of several difficulties inherent in working with both the pathogen and the plant in controlled settings in which it is challenging to faithfully reproduce optimal environmental conditions supporting natural infections. Aspects of this tropical disease make it especially difficult to work with outside of the tropics. For example, successful disease development on 3–6-month-old plants in growth chambers or glasshouses can take 2–3 months using optimal but stringent environmental conditions, which include high-quality, broad-spectrum, high-intensity lighting and high humidity (95–100%) (Fig. 2J–L) (Donzelli and Churchill, 2007). However, a successful infection can be predicted in the early stages if the first symptoms are achieved by 3–4 weeks post-inoculation.

Mycosphaerella fijiensis can prove challenging to work with, irrespective of location, as it grows relatively slowly *in vitro*, and conidia production in numbers sufficient for routine plant inoculations is generally unreliable. Others have described conditions for conidiation by *M. fijiensis*, which involve 10–14 days of growth on a modified V8 medium at 20 °C with continuous, cool-white fluorescent light (Carlier *et al*., 2002; Jacome and Schuh, 1993b; Mourichon *et al*., 1987). In our hands, the most critical factor positively affecting sporulation under these conditions was an incubation temperature of 18–20 °C, with temperatures above 20 °C repressing conidia production (B.G.G. Donzelli and A.C.L. Churchill, unpublished data). Others have reported increased conidiation at higher temperatures by manipulating light quality, photoperiod, culture medium or harvesting method. Peraza-Echeverría *et al*. (2008) recommended that conidia production can be stimulated following growth at 20 °C ± 2 °C by repeated harvesting of mycelial cultures with a camel hair brush. Sepúlveda *et al*. (2009) recently reported that reduced calcium carbonate levels and continuous red light had the greatest positive effect on conidiation at 26 °C. Great variability in conidiation between and within strains continues to hamper effective and routine production of sufficient amounts of inocula for use in standardized bioassays and other experiments. The development of a robust methodology for highly efficient and reliable conidiation by diverse strains of *M. fijiensis* continues to be needed.

Banana disease bioassays designed to utilize relatively mature juvenile plants that approximate the size and physiological state of young plants exposed to BLSD in the field are challenging to conduct. This is because of the significant time and space needed to first produce sufficient numbers of healthy, potted plants from tissue-cultured plantlets. The whole process of generating and preparing plants for disease bioassays—from tissue culture to acclimatization of rooted plantlets in potting medium to established growth in the glasshouse—can take several months (e.g. Donzelli and Churchill, 2007).

Others have taken the approach of scaling down the plant production aspect of bioassays by utilizing tissue-cultured plantlets in tubes (Twizeyimana *et al*., 2007), small potted plants (e.g. 20 cm in height) (Alvarado-Capó *et al*., 2003) or leaf pieces of approximately 12–36 cm^2 excised from juvenile or mature leaves (also referred to in the literature as 'detached leaves') (Abadie *et al*., 2008; Carlier *et al*., 1999; El Hadrami *et al*., 1998; Mercier *et al*., 2001; Remy *et al*., 1999; Twizeyimana *et al*., 2007) as a means to save space and time. Disease development under each of these conditions has been reported to be similar to that observed in conventional field settings, but some researchers have noted important differences. For example, Alvarado-Capó *et al*. (2003), using 20-cm-tall plants, noted slightly circular lesions similar to those observed on suckers in the field (e.g. Fig. 2H), which would be expected given the age of the plants. All seven cultivars assayed, representing susceptible, partially resistant and resistant genotypes, responded to *M. fijiensis* in a manner similar to that observed in the field, with one exception. The highly resistant cultivar 'Yangambi Km5' (AAA genome) responded as if it was a susceptible genotype. Fullerton and Olsen (1995) reported a similar response in 'Yangambi' using juvenile plants. Such observations suggest that, in some cases, disease development in immature plants or under artificial

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conditions may differ from the response seen in mature plants because of differences in plant physiology and development. However, it is also possible that resistance failed because of the existence of *M. fijiensis* isolates able to overcome 'Yangambi Km5' resistance in artificial bioassay conditions, as has been reported for field observations with this and other cultivars (Daniells, 2009; Fullerton and Olsen, 1995; Mouliom-Pefoura, 1999).

Others have reported inconsistent and unreliable results with the leaf piece assay for *Mycosphaerella* banana pathogens (Arzanlou *et al*., 2007; Donzelli and Churchill, 2007; Townley *et al*., 2001). Using freeze fracture scanning electron microscopy, Townley *et al*. (2001) noted that the development of BLSD in this assay was significantly different from that observed on leaves of intact plants. They concluded that the results obtained with the leaf piece assay could not be assumed to correlate with symptoms that develop on intact plants, and that stringent validation was required to establish this method for each specific objective of BLSD research. Using TaqMan real-time PCR assays, Arzanlou *et al*. (2007) concluded that the leaf piece inoculation method was unsuitable for the quantification of disease development by *Mycosphaerella* spp. because it was inconsistent and unreliable, leading to significant variation between replicates. They suggested that the hemibiotrophic nature of the *Mycosphaerella* banana pathogens requires the host plant to be in an optimal condition for natural disease development.

Although, with current tissue culture methodologies, it is possible to maintain excised banana leaf squares or young detached leaves in a green, nonsenescent state for extended periods (Abadie *et al*., 2008; Twizeyimana *et al*., 2007), it is questionable whether the physiological states can be considered to be comparable with those of leaves on intact plants with a functioning root system, especially given the length of time needed in culture to evaluate the full range of disease symptoms (1–3 months). Liu *et al*. (2007) reported that disease symptom development by hemibiotrophic *Colletotrichum* spp. grown on detached leaves of *Arabidopsis* appeared to be uncoupled from the disease defence pathways expressed in leaves attached to plants, with defence gene expression responses on detached leaves (measured after only 1–5 days post-inoculation) being similar to those of a plant undergoing senescence. Whether comparable results would be observed in the leaf piece assay developed for the *Mycosphaerella*–banana system, where leaf pieces are kept alive for up to a few months to record symptoms, is unknown but would be predicted. Such studies serve to underscore the importance of validating disease bioassays to ensure that controlled environment responses, using plant materials distinct from those found in the field, compare favourably with the symptoms obtained in natural infection settings (Mourichon *et al*., 1987). This is especially critical when choosing a bioassay method for the screening of new experimental host genotypes for disease response to *M. fijiensis* prior to field testing, or for screening of pathogen species or strains across a panel of host genotypes to determine differences in virulence. In addition, there is a need to validate the disease response of each cultivar at different ages of plant development to determine whether young plants respond in a manner comparable with mature plants, and to note exceptions.

As an initial step towards the development of a robust and reliable bioassay for use in glasshouse and growth chambers, Donzelli and Churchill (2007, 2009) utilized a dose–response approach to compare the virulence of multiple strains of *M. fijiensis* on the susceptible cultivar 'Grande Naine' (AAA genome, Cavendish subgroup). Conidia of a known concentration, or fragmented mycelia of a known weight, were applied as slurries to $5 \times 5\text{-cm}^2$ demarcated areas on the abaxial leaf surfaces of 3–6-month-old tissue culture-derived plants. Disease development was assessed over a 2–3-month period, with weekly digital images taken of each replicated inoculation site. Lesion development at each time point was measured using image analysis software. Both fragmented mycelia and conidia provided a dose-dependent assessment of disease symptoms for a set of phenotypically distinct isolates over the full time course of the bioassays, and allowed quantitative determinations of the virulence of each strain. When using mycelium as inoculum, mycelial weight, rather than colony-forming units, provided the most reliable and convenient means to quantify inoculum (Donzelli and Churchill, 2007). The study also revealed the critical importance of standardizing starting inoculum concentrations for plant bioassays as the disease progress curve is significantly impacted by this factor. The use of standardized inocula is especially critical if the data are to be used for comparative analyses with other laboratories to identify variation in pathogen virulence and host resistance. Furthermore, preliminary data suggesting the presence of a self-inhibitor of conidial germination in *M. fijiensis* and *M. eumusae* (Balint-Kurti and Churchill, 2004) reinforce the importance of using standardized inocula to avoid potential complications associated with the application of high spore densities to leaf surfaces.

Others have reported the successful use of mycelial inoculum for banana bioassays (Alvarado-Capó *et al*., 2003; Fouré and Mouliom-Pefoura 1988; Jones, 1995; Mourichon *et al*., 1987; Twizeyimana *et al*., 2007), but our studies were the first to demonstrate that fragmented mycelia provide a reliable dose-dependent development of disease symptoms for multiple, phenotypically distinct isolates of *M. fijiensis*. This methodology is especially valuable when conducting bioassays that require the use of *Mycosphaerella* strains that conidiate poorly *in vitro*. Furthermore, our conclusions of the relative virulence levels of two wild-type strains were directly equivalent to those reported by Fullerton and Olsen (1995), 12 years earlier, when they evaluated the same strains on juvenile 'Grande Naine' plants under

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distinct bioassay conditions in New Zealand. The statistical approaches refined in a second study (Donzelli and Churchill, 2009) allowed the assignment of a single numerical value, representing EC_{50} or EC_{25}, to each strain as a means to reliably measure and compare the virulence of strains. This study also confirmed that the dose–response approach for evaluating infections was applicable to assays using conidia as well as mycelia.

Bioassay development for a plant, such as banana, which first begins to show symptoms of infection at developmental stages considered to be tree-like compared with other agricultural crops, presents several challenges. Probably the most important to consider is in gauging the extent to which the plant materials utilized are sufficiently comparable, physiologically and developmentally, to plants exposed to *M. fijiensis* inoculum in the field. It is probably safest to assume that they are not equivalent, so that the challenge then becomes the identification of a bioassay system that will support symptom development most closely comparable with that observed in nature. In this regard, to what extent do the differences in physiology of 'immature' versus 'mature' plants grown in controlled environments affect the outcome of disease bioassays aimed to assess truly quantitative differences in pathogen virulence or host resistance? How does host nutritional status, which is predicted to be an important factor (Ploetz, 2007), affect disease outcome? Critical factors to consider prior to bioassay implementation are the plant cultivar, age and nutritional status type of inoculum (i.e. conidia, mycelia or a mixture of the two), inoculum age and dose and how it will be accurately quantified and effectively applied to the abaxial leaf surface (which is waxy and tends to shed watery solutions of inocula), and how disease symptoms will be quantified. Arzanlou *et al*. (2007) have developed a TaqMan real-time PCR method to quantify the biomass of *Mycosphaerella* banana pathogens in infected plants. This methodology is especially valuable for monitoring *in planta* growth of the fungus during the early biotrophic stages of infection before disease symptoms are evident on the leaf surface.

DNA-MEDIATED TRANSFORMATION

Balint-Kurti *et al*. (2001) developed the first fungal transformation system for *M. fijiensis*, *M. musicola* and *M. eumusae* using traditional polyethylene glycol (PEG)/protoplast-mediated methods with a plasmid that encoded a green fluorescent protein (GFP) gene and the *hph* gene conferring stable hygromycin B resistance. Use of GFP-expressing strains in plant bioassays confirmed previous studies demonstrating the infection process in susceptible plants (reviewed in Carlier *et al*., 2000a; Meredith and Lawrence, 1969), including the epiphyllic growth of the fungus on the leaf surface and the formation of stomatopodia over stomates prior to penetration. To our knowledge, this was the first study to demonstrate that all three primary members of the Sigatoka disease complex are capable of stomatopodium formation prior to stomate penetration, and that each produces hyphae that encircle the substomatal cavities as part of their early development *in planta* (Fig. 1E) (Balint-Kurti *et al*., 2001). This consistently observed growth pattern within substomatal cavities makes sense given their importance as the location for conidiophore and spermagonium development. Others have noted the importance of the substomatal cavity as the site of early entry and pycnidium formation by the related wheat pathogen *M. graminicola* (Kema *et al*., 1996; Rohel *et al*., 2001). Using GFP-expressing strains, Rohel *et al*. (2001) observed that infection hyphae of *M. graminicola* encircle chlorophyll-containing mesophyll cells in the substomatal cavity in a manner similar to that reported for *M. fijiensis*. Such similarities in host–pathogen development are one reason why *M. graminicola* was chosen by the International *Mycosphaerella* Genomics Consortium in 2003 as the model for further development of genetic and genomic tools for *M. fijiensis* (Goodwin *et al*., 2004; Kema, 2009).

The use of GFP-expressing strains of the *Mycosphaerella* banana pathogens also provided evidence of extensive saprophytic growth of the fungus on dead, necrotic banana leaf tissue (Balint-Kurti *et al*., 2001) and facilitated the observation of fungal development on nonhosts (Balint-Kurti and Churchill, 2004). Conidia of both *M. fijiensis* and *M. eumusae* germinated and formed stomatopodia over stomates of both tomato and tobacco, although subsequent stomatal penetration was not observed. It is intriguing that development occurred to this extent on these nonhosts; active defence responses, such as a classical hypersensitive response (HR), were not evident.

Agrobacterium tumefaciens-mediated transformation of *M. fijiensis*, using both GFP and hygromycin B as markers for the selection of stable transformants (Fig. 1E) and for targeted gene disruption, has also been reported (Donzelli and Churchill, 2003, 2005; B.G.G. Donzelli and A.C.L. Churchill, unpublished data). Other beneficial resources or methodologies that have been developed include the construction of a BAC library for *M. fijiensis* with average insert sizes of 90 kb (Canto-Canché *et al*., 2007) and reports of robust methods for RNA isolation from the fungus (Islas-Flores *et al*., 2006) and infected banana leaf tissue (Rodríguez-García *et al*., 2010). Functional genomic advancements in *M. graminicola*, which include new selectable markers and increased homologous recombination and targeted gene mutation frequencies, are expected to provide new opportunities for valuable tool development in *M. fijiensis* (Bowler *et al*., 2010).

A ROLE FOR SECONDARY METABOLITES AND OTHER EFFECTORS?

One of the diagnostic disease symptoms caused by *M. fijiensis* and other primary members of the Sigatoka complex on banana

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is the yellow halo around lesions that begins at Meredith and Lawrence Stage 5 and becomes much more evident at Stage 6 (Fig. 2D,E). This symptom of chlorosis led scientists to suggest it could be caused by secretion of a toxin from the fungus that kills tissue in advance of fungal growth (Molina and Krausz, 1989). Several efforts were made to identify extracts from 28-day-old *M. fijiensis* cultures that exhibited phytotoxic activity specific to susceptible cultivars of banana (Molina and Krausz, 1989; Stierle *et al*., 1991; Strobel *et al*., 1993; Upadhyay *et al*., 1990). The intent was to identify specific phytotoxins from culture filtrates that could be used to screen tissue-cultured bananas for resistance to *M. fijiensis* (Strobel *et al*., 1993). Phytotoxicity was assayed by dipping cut leaf tips of plants in the extract or using a leaf puncture method on detached banana leaves or small plantlets. Extracts were considered to be phytotoxic if they caused water-soaked necrotic symptoms that were absent when control compounds were used.

The secondary metabolites identified and characterized from culture filtrates of *M. fijiensis* were fijiensin (Upadhyay *et al*., 1990), 2,4,8-trihydroxytetralone (2,4,8-THT), juglone, 4-hydroxyscytalone, 3-carboxy-3-hydroxycinnamic acid and isoochracinic acid (Stierle *et al*., 1991; Strobel *et al*., 1993). Stierle *et al*. (1991) noted that 2,4,8-THT, juglone and 4-hydroxyscytalone are recognized shunt metabolites of fungal melanin biosynthetic pathways (Langfelder *et al*., 2003; Wheeler *et al*., 2008). As 2,4,8-THT was produced *in vitro* at 30 times the rate of the other metabolites in the study, they suggested it was the most important phytotoxin of *M. fijiensis* based on yield, host selectivity (i.e. differential activity against susceptible vs. resistant cultivars) and its high level of bioactivity. Juglone was determined to be more phytotoxic than 2,4,8-THT, but was isolated at only extremely low concentrations *in vitro* and showed no host selectivity. Based on these studies, 2,4,8-THT was identified as the most promising phytotoxin for possible use in plant breeding/selection programmes to identify BLSD-resistant lines (Strobel *et al*., 1993). Attempts to isolate phytotoxins from infected tissues were not successful (Stierle *et al*., 1991).

Further efforts to evaluate the role of toxins in pathogenesis involved the development of new bioassays to quantify the toxic effects of metabolites isolated from *M. fijiensis* culture filtrates (reviewed in Lepoivre *et al*., 2003). The most sensitive and accurate assay developed was based on the measurement of chlorophyll fluorescence. This was considered to be a valid test as the action of some *Mycosphaerella* metabolites found in culture filtrates is light-dependent (Balint-Kurti and Churchill, 2004; Lepoivre and Acuña, 1990; Lepoivre *et al*., 2003), and chlorosis of leaf tissue occurs as a result of disease progression. A 'vitality index' was developed as the most sensitive method to assess the effects of ethyl acetate crude extracts (EaCEs) of *M. fijiensis* on leaves, and as a specific indicator of photosynthetic activity. Further experimental efforts focused primarily on the evaluation of the effects of juglone on plant tissues, as it was reported to be the most phytotoxic of the compounds in extract fractions of four *M. fijiensis* culture filtrates, its toxicity on banana leaves was comparable with that of EaCEs and its activity was light-dependent (Busogoro *et al*., 2004a, b).

A series of experiments identified the chloroplasts as the site of action of juglone and, by extension, they were considered to be a potential target site for *M. fijiensis* toxins (Busogoro *et al*., 2004a, b). Chloroplasts from banana cultivars with partial resistance to BLSD, e.g. 'Fougamou' (ABB genome), were less affected by juglone than chloroplasts of susceptible 'Grande Naine' (AAA genome). Additional analyses comparing these two cultivars determined that, after treatment with juglone, the partially resistant cultivar 'Fougamou' responded more quickly to active oxygen species stress than did the BLSD-susceptible 'Grande Naine' (El Hadrami *et al*., 2005). These studies led to the hypothesis that juglone, a biologically active compound isolated from *M. fijiensis* culture filtrates, acts as a light-dependent, nonhost-specific phytotoxin targeting the photosynthetic apparatus in banana and inducing a differential response in hosts that is consistently correlated with relative levels of susceptibility or partial resistance to BLSD. These latter conclusions of a differential plant response elicited by juglone were in contrast to those reported earlier by others (Stierle *et al*., 1991; Strobel *et al*., 1993). It is unknown whether juglone is produced *in planta* during any stage of disease development, and whether the minimum amount of juglone that induces a differential response in susceptible and resistant banana cultivars (25 μg/mL) (Busogoro *et al*., 2004b) is biologically relevant.

Harelimana *et al*. (1997) concluded that *M. fijiensis* metabolites probably play a secondary role as determinants of pathogenicity, as there is no evidence for the early involvement of phytotoxins in the host–pathogen interaction during the extended biotrophic phase of growth leading up to Meredith and Lawrence Stage 1 symptom development, i.e. chlorosis occurs much later at Stage 5. Nevertheless, *M. fijiensis* metabolites, including juglone (Lepoivre *et al*., 2003) and 2,4,8-THT (Okole and Schulz, 1997), have been used for *in vitro* screening of banana cell suspensions and somatic embryos or micro-cross-sections, respectively, in attempts to select clones resistant to BLSD. Others have screened regenerated survivors of irradiated embryogenic cell suspensions, explant tissues and plantlets to identify clones tolerant to juglone as putative BLSD-resistant candidates (Reyes-Borja *et al*., 2005, 2007; Roux *et al*., 2003, 2009). Although toxin-tolerant plants were generated using each of these strategies, some of which showed increased resistance to *M. fijiensis* in growth chamber studies (Okole and Schulz, 1997), there have been no published reports demonstrating that banana plants tolerant to juglone or any other *M. fijiensis* metabolite show increased field resistance to BLSD. Daub (1986) described numerous limitations of the *in vitro* selection approach and cautioned

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against the use of secondary metabolites to select hosts for pathogen resistance. Based on results to date with banana, as well as with many other host–pathogen systems, this strategy should be discontinued, at least until there is definitive proof of the mode of action of a genetically characterized toxin (Daub, 1986; Yoder and Turgeon, 1996) of *M. fijiensis*.

Hoss *et al*. (2000) identified the pentaketide melanin pathway shunt metabolites flaviolin, 2-hydroxyjuglone, juglone and 2,4,8-THT from *in vitro* cultures of *M. fijiensis*, noting, as did Stierle *et al*. (1991), that 2,4,8-THT was produced in relatively high concentrations over a 6–45-day time course, compared with the relatively low concentrations of juglone detected. Treatment of cultures with the melanin pathway inhibitor tricyclazole increased 2,4,8-THT production significantly, but had relatively little affect on juglone accumulation. Further, they observed that plants inoculated with *M. fijiensis* and treated with tricyclazole—a treatment predicted to stimulate melanin shunt metabolism in the fungus, whilst inhibiting melanin biosynthesis (Woloshuk *et al*., 1980)—developed more substantial disease symptoms more rapidly than did leaves inoculated only with the fungus, as was predicted by Strobel *et al*. (1993). These results provided further support for the hypothesis that melanin shunt metabolites contribute to symptom development. Hoss *et al*. (2000) also reported that intercellular extracts from highly and partially resistant banana cultivars enhanced 2,4,8-THT production in 12-day-old *M. fijiensis in vitro* cultures compared with intercellular extracts from a susceptible cultivar, suggesting a role for plant compounds in modulating shunt metabolism in *M. fijiensis*.

Hoss *et al*. (2000) proposed a model for the role of fungal 2,4,8-THT in the *M. fijiensis*–banana pathosystem as a bivalent compound whose effects *in planta* are dependent on whether its production is: (i) activated early in the interaction by an unknown plant compound, e.g. in an incompatible interaction with a resistant or partially resistant plant in which an HR occurs; or (ii) present at sublethal levels during the biotrophic growth phase of a compatible interaction, with slow accumulation of 2,4,8-THT over time (see Strobel *et al*., 1993), resulting in extensive symptom development at the later stages of pathogenesis. They recommended the characterization of the activating phytochemical from the intercellular spaces of resistant host cultivars to determine the genetic basis for plant induction of fungal 2,4,8-THT, which could lead to new approaches for the induction of resistance mechanisms in susceptible plants. Interestingly, the first report of phytoanticipins (a constitutive phytoprotectant), isolated from healthy 4-month-old susceptible 'Grande Naine' plants, has been published recently (Cruz-Cruz *et al*., 2010), and the authors demonstrated strong antifungal activity of the compound, having a steroidal saponin structure, against *M. fijiensis*.

We hypothesized that, if melanin shunt metabolism is intimately linked to, and dependent on, the first step of melanin biosynthesis (Langfelder *et al*., 2003; Stierle *et al*., 1991; Wheeler *et al*., 2008), disruption of the first gene in the melanin biosynthetic pathway should 'knock-out' both melanin and shunt metabolite production, which would allow a test of the effect of loss of both families of metabolites, including juglone and 2,4,8-THT, on the virulence of *M. fijiensis*. As alternative pathways for the synthesis of melanin shunt metabolites have not been reported in other fungi, this strategy is the most logical genetic approach to evaluate their potential contributions to BLSD. Targeted disruption of the polyketide synthase gene (Mf*PKS1*), predicted to function in the first step of melanin and shunt metabolite biosynthesis in *M. fijiensis*, resulted in the production of cream-coloured, melanin-deficient mutants that were as virulent as the wild-type strain on susceptible 'Grand Naine' plants (Donzelli and Churchill, 2005; B.G.G. Donzelli and A.C.L. Churchill, unpublished data). Our studies demonstrated that melanin is not required for *M. fijiensis* virulence on banana, strongly suggesting—but not confirming without additional analyses—that melanin shunt metabolites do not play a key role in BLSD.

It has long been observed that the symptoms of Sigatoka leaf spot and BLSD are ameliorated in plants grown in the shade (Calpouzos and Corke, 1963; Jones, 2000b; Thorold, 1940), suggesting the possible production of light-activated toxins by these fungi, as suggested for juglone. Fractions of fungal extracts of *M. musicola* that were phytotoxic and light-dependent were identified in an assay used to detect the effects of such compounds on banana suspension cultures (Balint-Kurti and Churchill, 2004). In other experiments, *in vitro* bioassays with photoactivated singlet oxygen-generating dyes demonstrated that all three primary members of the Sigatoka complex exhibit high levels of resistance to several photoactivated dyes, including cercosporin—a highly potent singlet oxygen-producing toxin made by members of the taxonomically related *Cercospora* spp. in the presence of light. The significant degree of resistance detected in the *Mycosphaerella* banana pathogens to the reactive oxygen-generating dyes suggests that they have the potential to synthesize similar compounds that could play roles in pathogen virulence (Daub and Ehrenshaft, 2000). Beltrán-García *et al*. (2009) recently characterized the oxidative stress response of *M. fijiensis* after exposure to hydrogen peroxide and paraquat to assess the potential defensive capabilities of the fungus in response to the predicted oxidative stresses imposed by plant defence mechanisms and fungicide exposure.

Cruz-Cruz *et al*. (2009) have reported the development of a method to purify hydrophilic phytotoxins from the aqueous filtrate of *M. fijiensis*. This is relevant because the majority of secondary metabolites isolated from the fungus to date are lipophilic (Hoss *et al*., 2000; Stierle *et al*., 1991; Upadhyay *et al*., 1990), and would be predicted to have different target sites in the plant than would hydrophilic phytotoxins. The isolation and identification of these new bioactive metabolites are in progress.

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Recent genetic and genomic analyses of a subset of necrotrophic Dothideomycete fungi have revealed the ability of the wheat pathogen *Stagonospora nodorum* and other closely related Pleosporales fungi to produce proteinaceous host-specific toxins (HSTs) involved in pathogenesis (Friesen *et al*., 2008; Hane *et al*., 2007; Pandelova *et al*., 2009). Like most fungi, they also have the genetic potential to produce a suite of secondary metabolite nonhost-specific toxins whose identities and biological roles have yet to be defined. Because *M. graminicola* and *M. fijiensis* are Dothideomycetes within the order Capnodiales, they have the potential to utilize pathogenic strategies similar to their relatives in the Pleosporales (Oliver and Solomon, 2010). There is no evidence to date demonstrating either proteinaceous or secondary metabolite HST production by members of the Capnodiales. However, the recent demonstration of functional homologues of the Avr4 and Ecp2 effectors of the tomato pathogen *Cladosporium fulvum* (also in the Capnodiales) in *M. fijiensis* (Stergiopoulos *et al*., 2010), as well as the understanding that such factors can elicit a plant response comparable with that induced by most HSTs, lends yet further support to the concept that virulence/avirulence determinants can be perceived as selective phytotoxins (Wolpert *et al*., 2002). Clearly, there is much work ahead to clarify the distinct roles of secondary metabolites and proteinaceous effectors, either of which can act as host-specific or nonhost-specific toxins, in the biology of *M. fijiensis* as a banana pathogen.

THE GENOME SEQUENCE OF *M. FIJIENSIS*

Several years ago, an international project was initiated to sequence the genome of *M. fijiensis* plus 40 000 expressed sequence tags (ESTs) (Kema, 2009). The effort began when the *Mycosphaerella* Genomics Consortium, established in 2003, agreed to use the related wheat pathogen *M. graminicola* as the model Dothideomycete to develop more genetic and genomic resources for research on the less well studied but equally important *M. fijiensis* (Goodwin *et al*., 2004). Version 1 of the genome sequence of virulent *M. fijiensis* strain CIRAD86 was released in August 2007. Version 2 was released in May 2010 with approximately 7.11 × coverage, and an estimated genome size of 74.1 Mb, contained in 56 main genome scaffolds. The assembly was improved with the use of markers of a genetic linkage map that were sequenced and aligned to the genome. Approximately half of the genome is contained in five scaffolds, each at least 5.9 MB in length. It is notable that the genome size of *M. fijiensis* is approximately 87% larger than that of *M. graminicola*, and the completed mitochondrial sequence is more than twice as large as that of the wheat pathogen. The increase in genome size over that of *M. graminicola* appears to be primarily a result of the presence of repeated sequences in the *M. fijiensis* genome. The current draft release includes a total of 13 903 gene models structurally and functionally annotated using the JGI annotation pipeline (http://genome.jgi-psf.org/Mycfi2/Mycfi2.home.html). Manual annotation by members of the Dothideomycete Genomics Consortium is in progress. Availability of the genome sequence will provide unprecedented opportunities for research that will contribute to a better understanding of host–pathogen interactions, fungicide resistance, population biology and the basic biology of *M. fijiensis*.

Already the genome sequence is being used for practical research applications. Yang and Zhong (2008) reported the isolation of 14 polymorphic microsatellite markers of *M. fijiensis* based on single sequence repeat (SSR) loci identified from the first draft of the sequenced genome. Additional SSR loci from EST libraries (203) and the genome itself (~4600) were identified (Kema, 2009) and used, together with DArT markers, to generate a denser genetic linkage map to assist with high-quality genome assembly (Ferreira *et al*., 2009). These new tools will also be valuable in the selection of markers for population analyses and isolate genotyping.

Stergiopoulos *et al*. (2010) used the *M. fijiensis* genome sequence to identify and characterize homologues of the *C. fulvum* Avr4 and Ecp2 effectors, which are now believed to be virulence factors (De Wit *et al*., 2009). The Avr4 protein of *M. fijiensis* is functionally homologous to the *C. fulvum* Avr4, acting as a defensive virulence factor to protect fungal cell walls against hydrolysis by plant chitinases by binding to chitin. It is also able to trigger a Cf-4-mediated HR in tomato. In addition to Avr4, three proteins were identified in the genome of *M. fijiensis* that showed homology to *C. fulvum* Ecp2. One of these appears to promote virulence by interacting with a putative host target causing cell necrosis, i.e. it induces an HR in a Cf-Ecp2 tomato line. It is notable that, in an independent study, Cho *et al*. (2008) previously isolated five putative virulence/avirulence factors from 1945 unique sequences from three *M. fijiensis* cDNA libraries representing different culture conditions. Two of these ESTs had significant similarity to genes encoding Avr4 and Ecp2 of *C. fulvum*. Their isolation from three and two cDNA libraries, respectively, generated from *in vitro* culture conditions, indicates that the genes were expressed *in vitro* and were not limited to expression in the intercellular spaces of the plant host. Other putative virulence/avirulence genes they noted are predicted homologues of the pSI-7 gene of *C. fulvum* and the SnodProt1 protein precursor of *S. nodorum*.

Prior to the studies of Stergiopoulos *et al*. (2010), *C. fulvum* was the only fungal pathogen for which effector proteins had been described. The use of comparative genomics to search for effector protein homologues in *M. fijiensis* and other closely related Dothideomycete fungi has opened up a wealth of opportunities for considering novel ways to breed or engineer disease-resistant crops. This is an especially welcome opportunity for

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domesticated banana cultivars, the genetic improvement of which is extremely challenging because of their general sterility.

It should also be noted that efforts to sequence the genome of the wild banana *Musa acuminata* ssp. *malaccensis* 'Pahang HD' are underway (Lescot *et al*., 2008; Roux *et al*., 2008). This subspecies of banana shares a genomic composition with the dessert and cooking banana cultivars. Its genome consists of 600 million base pairs, spread over 11 chromosomes, each present in two identical copies (AA genome). Additional information on this effort can be found at http://www.cns.fr/spip/September-8th-2009-Banana-genome.html

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