CHAPTER 1

Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp *cubense*: A literature review with special reference to the taxonomy and population biology of the pathogen

INTRODUCTION

Soilborne plant pathogens have had an important economic impact on our lives and have significantly influenced agricultural practices. Management of these diseases poses a constant challenge to farmers and plant pathologists. An interesting example is provided by *Fusarium* species that are economically important as pathogens on most agricultural crops grown worldwide.

The soilborne fungal species *Fusarium oxysporum* Schlechtend.: Fr. has a worldwide distribution (Booth 1971). The species has been described based on the morphology of its asexual reproductive structures, such as the shape of the macroconidia, the structure of the microconidiophores, and the formation of chlamydospores (Snyder & Hansen 1940, Booth 1971, Nelson, Toussoun & Marasas 1983). The pathogenic members residing in *F. oxysporum* include those causing highly destructive vascular wilt diseases of many commercially important plant crops (Booth 1984). These pathogens are treated as special forms (*formae speciales*) based on the host species that they infect. At least 120 *formae speciales* exist for *F. oxysporum* (Hawksworth 1995), and these exclude isolates considered as non-pathogenic members of the species. Races within *formae speciales* are determined based on virulence of individual isolates on differential host cultivars (Snyder & Hansen 1940, Armstrong & Armstrong 1981, Booth 1984).

One of the most destructive *formae speciales* of *F. oxysporum* is *F. oxysporum* f. sp. *cubense* (E. F. Smith) Snyder and Hansen (*Foc*), the causal agent of Fusarium wilt (Panama disease) of banana (Stover 1986, Ploetz 1990, Pegg, Moore & Bentley 1996). The damage caused by this pathogen in export banana plantations in Central America between 1900-1960 has made Fusarium wilt one of the most catastrophic plant diseases in the recorded history of

agriculture (Stover 1986, Ploetz 1990, Pegg *et al.* 1996, Ploetz & Pegg 1999). Only the replacement of the highly prized Gros Michel cultivar with resistant Cavendish cultivars at the time of the most severe outbreaks saved the banana export industry from complete collapse (Stover 1962a, Ploetz 1990).

Two of the important traits that need to be considered when managing fungal pathogens are their genetic diversity and reproductive strategy (McDonald & McDermott 1993, Kistler 1997, McDonald 1997, Taylor, Jacobson & Fisher 1999). *Fusarium oxysporum* is an asexually reproducing fungus that includes a number of genetically homogeneous sub-populations (clonal lineages) (Booth 1971). Pathogens including only a few clonal lineages can be controlled more easily than those with a diverse population structure, since diverse fungal populations can relatively easily overcome control strategies such as the use of fungicides or disease resistant plants (McDonald & McDermott 1993, Burdon & Silk 1997). Diversity in fungi is primarily derived through sexual reproduction. The aim of this review is to provide a background to banana cultivation, the importance of Fusarium wilt disease of banana, and to treat the topic of diversity in *Foc*, how it arises, and means whereby it can be determined.

ORIGIN AND HISTORY OF BANANAS

Bananas belong to the genus *Musa* and family *Musaceae*, in the order *Zingiberales* (Simmonds 1962). The genus *Musa* includes five sections, divided into 40 species. *Eumusa* is the largest and best known section and includes *M. acuminata* Colla and *M. balbisiana* Colla, which are the principal progenitors of most edible banana cultivars (Stover 1962a, Waite 1963, Ploetz & Pegg 1999). Inedible, seed-bearing diploids that are still found in Southeast Asia and the western Pacific regions are the ancestors of modern-day bananas and

plantains (Stover 1986, Ploetz & Pegg 1997). Over time, evolution has resulted in the many inedible diploids crossing naturally and resulting in inter – and intraspecific hybrids (Buddenhagen 1990, Robinson 1996).

Musa acuminata crosses were selected, cultivated, propagated and distributed locally in Southeast Asia. In India and the Philippines, wild seeded diploids of *M. balbisiana* grew naturally many years ago (Simmonds 1962, Stover 1962a). Early breeders apparently made crosses to produce hybrids between *M. acuminata* and *M. balbisiana*. In this way, seedless, edible diploid, triploid and tetraploid dessert bananas, cooking bananas and plantains were produced. Dessert bananas mainly contain sets of chromosomes from *M. acuminata*, and include the AA and AAA types. Cooking bananas contain sets of chromosomes from both *M. acuminata* and *M. balbisiana*, for example the AAB and AABB bananas and plaintains (Buddenhagen 1990).

Humans have been responsible for moving vegetative banana planting material (suckers) outside Asia and around the world. The mode and time of introduction of bananas to Africa is believed to be from India around the 1400's. They were then spread across the continent from east to west (Simmonds 1959, Robinson 1996). The Portuguese carried the plant to the Canary Islands some time after 1402 and from that area to the New World (Simmonds 1959). Dessert and cooking varieties were introduced into the Americas from Southeast Asia before 1750 (Wardlaw 1961). Gros Michel was first introduced into Panama before 1866, and with the expanding export industry at the time, was distributed throughout Central America (Stover 1962a). The Silk (ABB) variety was introduced into Australia before 1876 and the Gros Michel cultivar was introduced only around 1910 (Stover 1962a). This set the stage for the cultivation of bananas as a dessert and as a staple crop, around the world.

Fusarium wilt of banana, caused by the soilborne fungus *Foc*, is a classical fungal wilt disease of agricultural crops (Beckman *et al.* 1961, Beckman, Halmos & Mace 1962). *Foc* infects plants through the roots and once in the xylem, progresses by means of conidia carried in the vascular system (Waite & Stover 1960, Beckman *et al.* 1961, 1962). It systematically blocks the xylem of susceptible banana plants and results in a lethal vascular wilt (Stover 1959, 1962a, 1986, 1990, Ploetz & Pegg 1999). Characteristic internal symptoms become visible as reddish to dark brown discolouration of infected roots. These symptoms progress to the rhizome and are most pronounced as a yellow-brown discoloration where the stele joins the cortex (Stover 1962a). Eventually the fungus colonizes the pseudostem, and symptoms are evident as faint brown streaks. Vascular discoloration may extend throughout the pseudostem and into the fruit stalk (Stover 1962a, Beckman *et al.* 1962, Pegg & Langdon 1987). The first external symptoms of Fusarium wilt are yellowing of the oldest leaves, which eventually turn brown, wilt, and die as the disease progresses to the youngest leaves (Beckman *et al.* 1961, Stover 1962a). The pseudostem eventually decays, and its base may split. When the affected parts collapse, the fungus grows out of the xylem into adjacent tissues and produces chlamydospores that are released back into the soil. Chlamydospores can survive under different environmental conditions for long periods and render further production of bananas in infected fields impossible (Stover 1962a).

Chemical, biological and cultural control methods are ineffective in the management of Fusarium wilt of bananas (Jones 1999). The only effective control strategy for the disease is by means of genetic resistance of the plant. Such resistance can be introduced into susceptible cultivars either by classical or non-conventional breeding methods. Since edible bananas are mostly parthenocarpic (seedless), they can be propagated only as suckers or tissue

culture-derived plants (Stover & Buddenhagen 1986, Buddenhagen 1987, Robinson 1996). Fusarium wilt can spread from one field to another by means of non-symptomatic, yet infested suckers. Tissue culture plants eliminate the possibility of introducing *Foc* into non-infested fields because they are produced under sterile laboratory conditions. The disadvantage, however, is that tissue cultured plants are more prone to Fusarium wilt than those generated from suckers (Smith *et al.* 1998).

ORIGIN AND TAXONOMIC HISTORY OF *FOC*

It has been suggested that *Foc* co-evolved with banana in Asia in the distant past (Ploetz 1990). The first discovery of Fusarium wilt, however, was made in Australia in 1874 (Bancroft 1876). This was followed by reports of the disease from tropical America (Costa Rica and Panama in 1890) during the time when Gros Michel was widely planted in the area (Stover 1962a, 1990). The first report of the disease in Africa was approximately around 1924 (Stover 1962a, 1990). Fusarium wilt was first reported from Mexico in 1932, and has more recently been found in Florida, United States (Ploetz & Shephard 1989, Ploetz & Pegg 1999). Stover (1962a) speculated that the movement of the disease in Asia, Africa and Australia has been closely linked to the dissemination of susceptible clones into these areas, since the pathogen is often introduced into new areas on infected rhizomes that are free of visual symptoms (Stover 1962a, Pegg *et al.* 1996). Stover & Buddenhagen (1986) believed that *Foc* might have been introduced into Central and South America and the West Indies with the Silk (AAB) variety that came from South India. Fusarium wilt has now been reported from all countries where bananas are grown, except those bordering the Mediterranean islands in the South Pacific, and Somalia (Stover & Simmonds 1987, Ploetz 1994).

The Fusarium wilt pathogen of bananas was first isolated from Cuban banana plant material and named *Fusarium cubense* (Smith 1910). Ashby (1913) presented the first full description of symptoms and field pathology, while Brandes (1919) made the first extensive studies, proving conclusively that *Foc* was the cause of this disease (Waite & Stover 1960). Reinking (1926) confirmed Brandes's (1919) findings that *Foc* is the cause of banana wilt (Waite & Stover 1960). Between 1926 and 1959, however, no significant research was conducted on the pathogen. The export trade using the Gros Michel variety in South America was well established and successful, but suffered huge losses due to the rapid spread of *Foc* and the susceptibility of Gros Michel. Research efforts then focussed on the importance of the pathogen, when the susceptible Gros Michel was replaced with Cavendish cultivars during the 1960s. Cavendish cultivars, however, were found to be susceptible to Fusarium wilt in the subtropics. Damage to Cavendish clones was first observed in the Canary Islands in the 1920s (Ashby 1926) and, thereafter, from other countries with sub-tropical growing areas such as South Africa (1940s), Australia (1950s), and Taiwan (1970s) (Su, Hwang & Ko 1986). This was followed by reports of a race of the fungus attacking Cavendish varieties in the tropical north of Australia, Indonesia and Malaysia (Pegg, Moore & Sorensen 1994, Ploetz 1994, Bentley *et al.* 1998). The discovery that Cavendish cultivars were susceptible to Fusarium wilt in the subtropics and more recently in some parts of the tropics, has led to greatly increased research efforts on the pathogen.

Various authors have studied the dissemination of *Foc*, and strongly support the theory of its co-evolution with banana in Asia (Stover 1962b, 1990, Vakili 1965, Stover & Buddenhagen 1986). Ploetz & Pegg (1997) conducted an extensive study and suggested that *Foc* evolved to the east of Wallace's line. Wallace, a naturalist, distinguished the two landmasses after studying the fauna and flora found on either side. Evidence points towards an Asian origin for the pathogen (Ploetz & Pegg 1997). Several other studies on the genetic diversity and

ancestry of the pathogen have also confirmed a Southeast Asian origin for *Foc* (Pegg, Moore & Sorensen 1993, Koenig, Ploetz & Kistler 1997, O'Donnell *et al.* 1998). Higher pathogen diversity found within Southeast Asian populations compared to populations in other banana growing regions is further proof of to the pathogen's origin in this region (Ploetz & Pegg 1997, Bentley *et al.* 1998). Most isolates of the pathogen from outside Asia are related to the Asian population (Koenig *et al.* 1997). However, independent evolution could also have occurred outside this centre of origin (Ploetz & Pegg 1997). A variant of the fungus has been isolated in Malawi, which is limited to a specific geographical area and that is genetically distinct from other isolates of *Foc* (Ploetz 1994). Thus, it is suggested that the ability to cause disease on the banana host could have originated within as well as outside the centre of origin of banana (Ploetz 1994, Ploetz & Pegg 1997, Bentley *et al.* 1998).

DEVELOPMENT OF DIVERSITY IN *FOC*

The genus *Fusarium* includes many species that reproduce both sexually and asexually. The section *Elegans*, which includes *F. oxysporum*, has no known teleomorph or sexual stage. There is some potential for non-sexual recombination among isolates that are very similar (Buxton 1962, Tinline & Macneil 1969). Genetic variation that occurs in this species is, therefore, assumed to arise through neutral mutations (Ploetz 1993, Brasier 1995) as well as by parasexuality and heterokaryosis (Buxton 1956, 1962, Parameter, Snyder & Reichle 1963, Tinline & Macneil 1969, Taylor, Jacobson & Fisher 1999).

Parasexuality is a non-sexual mode of genetic exchange, without meiosis or the development of sexual structures, and is unique to some fungi (Buxton 1956, 1962, Parameter *et al.* 1963, Leslie 1993). Heterokaryosis is a form of genetic exchange initiated by fusion of vegetative hyphae (anastomosis) between individuals with very similar genomes. Parasexuality and

heterokaryosis may occur in nature and are considered by some, to contribute towards variability (Buxton 1956, 1962, Parameter *et al.* 1963, Tinline & Macneil 1969). To prove that herokaryosis and parasexuality play an important role, many researchers have attempted to force anastomosis in *F. oxysporum* by pairing complementary auxotrophs (Buxton 1956, Garber, Wyttenbach & Dhillon 1961, Tuveson & Garber 1961). Buxton's (1956) work on heterokaryosis and parasexual recombination in *Foc* is not widely accepted, since he utilized nutritional auxotrophs, highly mutated by UV irradiation. Similar studies to those of Buxton (1956) have been conducted in the laboratory, but much speculation remains as to whether parasexuality has any significant contribution to diversity in *Foc* (Kuhn *et al.* 1995, D'Alessio, Cortes & Kuhn 1998). Ploetz (1993) has argued that the variation observed in *Foc* is rather due to mutation and age of the pathotype.

ANALYSING DIVERSITY IN *FOC*

The first taxonomic work on *F. oxysporum* focused on grouping morphologically similar isolates into specialised forms based on the host species they affect (Booth 1971, Armstrong & Armstrong 1981, Gordon & Martyn 1997, Kistler 1997). Considerable progress has been made over the years to analyse specialised forms making use of different phenotypic and genotypic markers (McDonald & McDermott 1993, Kistler 1997, McDonald 1997). The following section deals with the various phenotypic and genotypic characteristics that have been employed to distinguish and characterise different individuals in *Foc*.

Phenotypic characteristics:

Host and Cultivar specificity (race designation): Virulence to specific host plants has been useful in differentiating strains of *F. oxysporum* into *formae speciales*, although there are some inherent problems associated with characterising isolates based solely on pathogenicity (Armstrong & Armstrong 1981, Kistler 1997). Groupings based on host-pathogen interaction

(virulence) are dictated by the genetic make up of the differential hosts or cultivars used to distinguish strains (Correll 1991). The assumption is that isolates with a shared host range, and thus within the same *formae speciales*, are more similar genetically than isolates with other host specificities. The resulting interpretation from this assumption is that *formae speciales* are monophyletic and that isolates with a shared host range are likely derived from a single, particularly successful, pathogenic genotype (Gordon & Martyn 1997, Kistler 1997). This assumption could be misleading, as it has been shown in *Foc* that there is a polyphyletic rather than a monophyletic origin of the pathogen (O'Donnell *et al.* 1998).

Races in *Foc* are groups of isolates that are pathogenic to certain cultivars in the field. Three pathogenic races of *Foc* are recognised. Race 1 was responsible for the epidemics on Gros Michel (Pegg & Langdon 1987, Stover & Simmonds 1987) and race 2 affects Bluggoe (Stover & Buddenhagen 1986, Stover & Simmonds 1987). Race 4 affects clones that are susceptible to race 1 and race 2 in addition to the Cavendish cultivars (Su *et al.* 1986, Stover & Simmonds 1987, Ploetz *et al.* 1990, Ploetz & Pegg 1997). Race 4 is subdivided into tropical and subtropical race 4.

Foc subtropical race 4 attacks Cavendish bananas in countries such as South Africa, Australia, Taiwan, and the Canary Islands (Brake *et al.* 1990, Ploetz *et al.* 1990, Su *et al.* 1996, Gerlach *et al.* 2000) where cold winter temperatures may predispose Cavendish cultivars to infection (Stover 1962b, Stover and Simmonds 1987, Ploetz *et al.* 1990, Ploetz & Pegg 1997). The tropical strain of *Foc* race 4 attacks Cavendish bananas in the tropics in Southeast Asia and Australia (Pegg *et al.* 1993, Pegg *et al.* 1994, Ploetz 1994, Bentley *et al.* 1998). Race 3 represents *F. oxysporum* isolates that affect *Heliconia* spp., a distant relative of *Musa*. Isolates of *F. oxysporum* that were recovered from wilted heliconias still need additional

testing (using molecular techniques) to determine the genetic relatedness to *Foc* (Waite 1963, Stover & Malo 1972, Ploetz & Pegg 1999).

Cultural Characteristics: *Fusarium* species have a remarkable ability to adapt both their form and colour in response to environmental constraints (Follin & Laville 1966, Booth 1971, Nelson *et al.* 1983, Booth 1984). As a result of their capacity to change rapidly in culture, accurate identification has often been conflicting and confusing. Within *Foc*, isolates may differ in appearance *in vitro*. Based on these characteristics, *Foc* isolates in culture will also differ with respect to type of growth, colony colour, and presence of sclerotia (Waite & Stover 1960). Waite & Stover (1960) proposed that cultural characteristics to identify the different types of growth for *Foc* be divided into sprodochial, sclerotial, cottony, ropy and slimy pionnotal types. All these different forms, however, are simply part of an aging process in *F. oxysporum* on artificial media under laboratory conditions (Follin & Laville 1966). Some researchers have also suggested that races of *Foc* can be distinguished in culture. Sun & Su (1978), for example, found that race 4 of *Foc* can be distinguished from races 1 and 2 on modified synthetic Komada's medium, but not on potato dextrose agar.

Volatile production: Some isolates of *Foc,* when grown on steamed rice, produced volatile substances (Brandes 1919). Stover (1962a) used these volatile compounds to differentiate isolates of the pathogen into volatile and non-volatile groups. The same inexpensive cultural method to determine whether volatile substances are produced on steamed rice was used by Moore *et al.* (1991) and Pegg *et al.* (1993) to distinguish among races of Australian isolates of *Foc*. In that study all race 4 isolates produced volatile odours whereas race 1 and 2 isolates did not produce volatiles. Volatile production could thus be used in resource-poor laboratories to determine and differentiate culturally between races 1 and 2 and race 4.

Vegetative compatibility groups (VCGs): Vegetative compatibility has frequently been used in studies on the diversity and population biology of plant pathogenic fungi. Vegetative compatibility is a fundamental trait for recognising genetic entities in fungi (Puhalla 1985). In *F. oxysporum,* a phenotypic character such as heterokaryon formation between isolates is often used to recognise self and non-self (Leslie 1993). Isolates that have identical alleles at their vegetative compatibility (*vic*) loci will result in heterokaryon formation between them, which indicates the they belong to the same VCG.

Numerous researchers have studied vegetative compatibility in *Foc* (Correll & Leslie 1987, Ploetz & Correll 1988, Brake *et al.* 1990, Hernandez *et al.* 1993, Moore *et al.* 1993, Rutherford *et al.* 1998). *Foc* has 21 VCGs worldwide, of which only a few are common and widespread (Table 1). Of these, nine VCGs belong to race 4, eight VCGs to race 1 and four VCGs in race 2, while races associated with VCGs 01217, 01218, 01219, 01221 have not been determined. Two VCGs are found in tropical race 4, and seven are found in subtropical race 4. VCG 0120 and VCGs 0124-0125 have a worldwide occurrence, and can be found in most subtropical countries. Cross-compatibility between isolates in different VCGs has been demonstrated and described as VCG complexes (Table 1) (Ploetz 1990, Bentley *et al.* 1998).

Fifteen VCGs have been found in Asia, where the pathogen and host probably co-evolved (Pegg *et al.* 1994, Pegg *et al.* 1996). In comparison, few VCGs are found in Africa and the Americas. The small number of VCGs found in these regions suggests that *Foc* has been moved into these areas and that these introductions have been limited in number (Ploetz 1993). Furthermore, this observation suggests that VCG evolution outside Asia is uncommon. VCG analysis has confirmed that some strains of the Panama disease pathogen that occur in Asia have been introduced into Australia with banana planting material (Brake *et al.* 1990, Moore *et al.* 1993). In *Foc*, mutations could have given rise to reproductively

isolated populations through the development of new VCGs. New VCGs arising through mutations could have become independent evolutionary units (Brasier 1995, Kistler 1997, O'Donnell 1993, O'Donnell & Cigelnik 1997, O'Donnell *et al.* 1998).

Populations of *Foc* have been intensively analysed in most banana-growing countries in the world. These analyses, however, are based primarily on the races and VCGs that occur in a particular country. Although cultural growth characteristics and volatile production can give some indication of the physiological characteristics of *Foc* isolates, these tests give no indication of the genetic relatedness or diversity within or between groups of isolates. Since rapid changes can occur on synthetic media, separating races in culture is not always reliable.

Using race designation and VCG analysis as measures of diversity has limitations. Successful disease development relies on the intricate interaction between pathogen and plant genotypes, which, in the case of Fusarium wilt of banana, appear to be strongly influenced by environmental conditions (Moore *et al.* 1993). This has previously been demonstrated when *Foc* VCG 0120 caused disease to Cavendish bananas in the subtropics but not in the tropics (Su *et al.* 1986, Stover & Simmonds 1987). Since Cavendish banana cultivars serve as a differential for race identification, the same fungal phenotype will, therefore, be identified as race 4 in the subtropics and as race 1 in the tropics. Furthermore, tropical and subtropical strains of *Foc* race 4 have been shown to be genotypically different (Bentley *et al.* 1995, 1998). These inconsistencies occur primarily because the grouping of *Foc* isolates in races is loosely determined by their pathogenicity to a limited number of banana differentials, under field conditions. In all other *formae speciales* of *Fusarium oxysporum,* there is a defined genetic basis (cultivars containing certain resistance genes) for race designation. In *Fusarium oxysporum* f. sp. *melonis,* a pathogen of muskmelon, certain cultivars contain genes for resistance against Fusarium wilt and others do not (Punja, Parker & Elmhirst 2001). This is

also true for many other *F. oxysporum* species pathogenic on crucifers, sweet potato, and chickpeas (Bosland & Williams 1986, Clark, Hyun & Hoy 1998, Navas-Cortes, Hau & Jimenez-Diaz 2000). A better understanding of the genetic make-up of both pathogen and host, and the interaction between them under different environmental conditions, therefore, needs to be studied in order to effectively designate races in *Foc*.

VCGs provide a useful means for subdividing *Foc* into genetically isolated groups. They could provide information on pathogen diversity, point of introduction, distribution, phylogeny and dispersal. VCGs can, however, be misleading in terms of true genetic relatedness among isolates (Bentley *et al.* 1995). A study of the population structure of *Foc* using both VCGs and complementary molecular techniques would provide more valuable insights into true genetic relatedness or variation. Once VCGs are clearly defined, they have many applications. Firstly, they can be used to determine whether a new race has arisen from an existing pathogen or from a formerly non-pathogenic predecessor (Correll, Puhalla & Schneider 1986). Also, in isolates that are vegetatively compatible, horizontal transfer of deleterious cytoplasmic infectious agents (dsRNA) (Gobbi *et al.* 1990) and viruses (Anagnostakis & Waggoner 1981) can occur. In *Foc* this may provide an opportunity for biological control through hypovirlence of pathogens without the risk of infecting non-pathogenic strains of *F. oxysporum*.

Genotypic characteristics

DAFs and RAPDs: PCR methods based on the amplification of anonymous DNA fragments include randomly amplified polymorphic DNA analysis (RAPDs) (Williams *et al.* 1990) and DNA amplification fingerprinting (DAFs) (Caetano-Anollés, Bassam & Gresshoff 1991). Bentley, Pegg & Dale (1995) and Bentley & Bassam (1996) used RAPDs to study a worldwide population of *Foc*. They divided isolates of *Foc* into two major groups based on

RAPD fingerprinting. Group 1 contained all isolates in VCGs 0120, 0121, 0122, 0126, 01210, 01211 and 01212. Group 2 contained isolates in VCGs 0123, 0124, 0124/0125 and 0125. Bentley *et al.* (1998) used the DAF system to identify nine clonal lineages among 341 isolates of *Foc* based on DNA fingerprinting analysis. Similar or identical banding patterns were found for isolates within a VCG that was independent of their geographical and host origins. According to these authors, DNA fingerprinting patterns were VCG-specific, with each VCG representing a unique genotype.

Isolates divided into subgroups as defined by Bentley *et al.* (1995) differed only in host genotype. The VCG 0120-01215 complex originated exclusively from the *M. acuminata* banana hybrids and VCG complex 0124-0125-0128-01220 is derived from the *M. acuminata* X *M. balbisiana* hybrids (Pegg *et al.* 1993, Boehm *et al.* 1994, Ploetz & Kistler 1994). This observation led to the hypothesis that the two groups may have co-evolved in the respective centres of diversification of the intra- and interspecific hybrids.

Electrophoretic karyotyping and RFLPs: Based on their electrophoretic karyotype, isolates of *Foc* were divided into two broad groups (Boehm, Ploetz & Kistler 1994). Group 1 contained all isolates in VCGs 0124, 0125, 0124-0125, 01210 and 01214 and group 2 contained isolates in VCGs 0120, 0121, 0122, 0123, 0129, and 01213. Isolates in group 1 had a higher chromosome number and a larger genome size than group 2 isolates. Koenig *et al.* (1997) further differentiated these two major groups using anonymous, single-copy, restriction fragment length polymorphism (RFLP) loci. Seventy-two new haplotypes were identified and most of the isolates were identified by the five most common haplotypes. They found that isolates with identical haplotypes were geographically separated and all isolates within a VCG were found in the same clade and clonal lineage. Ten clonal lineages based on RFLP analysis were identified with the two largest lineages having pantropical distribution.

These authors conclude that the largest lineages, the unique lineage from Africa were genetically distinct and that the ability to be pathogenic on banana were acquired independently.

Foc phylogeny: O'Donnell *et al.* (1998) tested a monophyly hypothesis suggesting that the *formae specialis* concept in *F. oxysporum* is phylogenetically misleading. These authors compared DNA sequences of nuclear and mitochondrial genes of four *formae speciales* of *F. oxysporum*, including *Foc*. Concordant evidence from the respective gene genealogies revealed that *Foc* harbours at least five lineages with independent evolutionary origins. These lineages are as different from each other as they are from other *formae speciales* of *F. oxysporum*. Lineages II and I of *Foc* were genetically more similar to *F. oxysporum* f. sp. *niveum*, a pathogen of watermelon, than they were to each other, and as closely related to each other as they were to *F. oxysporum* f. sp. *lycopersici*, a pathogen of tomato. The five lineages in *Foc* can further be divided into two clades based on phylogenetic analysis (O'Donnell *et al.* 1998). The distant relationship between the two lineages suggests that the major populations of *Foc* evolved independently and provides significant evidence for the geographically separate development of isolates in these clades.

Several DNA-based techniques have been used to analyse the worldwide population of *Foc*, often with confusing and contradicting results. For example, a study by Bentley *et al.* (1995), (1998) divided isolates of *Foc* representing all VCGs into two major groups based on RAPD fingerprinting and nine clonal lineages based on DAF analysis, which in the absence of any sequence identity is reduced to a phenetic technique. RAPDs and DAFs require careful optimisation to ensure reproducibility between laboratories, and they suffer from a lack of portability between laboratories (Gillings & Holley 1997). O'Donnell *et al.* (1998) supported the division of VCGs in *Foc* into two major groups, but found only five clonal lineages after

sequencing of the nuclear and mitochondrial gene regions. Thirdly, Boehm *et al.* (1994) used electrophoretic karyotyping of *Foc* to divide the population into two broad groups containing slightly different VCGs to those described by Bentley *et al.* (1995) and O'Donnell *et al.* (1998). Koenig *et al.* (1997) found ten clonal lineages in *Foc* based on RFLP analysis. The RFLP study is the first significant phylogeny study by focussing on the resolution of subpopulations within *Foc* utilizing cDNA probes. The advantages and limitations of all these techniques can be debated but it is clear that a suitable molecular technique needs to be found to subdivide the worldwide population of *Foc* into appropriate subunits to determine the distribution and relationships of the fungus. I believe that the development of microsatellite markers for *Foc* will provide the best solution to this problem.

Microsatellite markers: Microsatellite markers have rapidly become established as a powerful tool for the analysis of population genetic structure, mating systems, reproductive mode and genetic isolation (Page & Holmes 1998). These markers have several advantages over RFLP or RAPD based techniques. The dominant nature and lack of reproducibility of RAPDs limit their use as routine molecular markers in population studies (McDonald & McDermott 1993, McDonald 1997). RFLPs represent neutral co-dominant markers that provide useful information in population genetic studies. This technique, however, requires large quantities of DNA and the number of enzymes available would ultimately limit the number of loci studied. The high mutation rate of microsatellites resulting in different allele sizes for individuals in the same population, simple pattern of Mendelian inheritance and co-dominance makes microsatellites a powerful molecular marker (Bruford & Wayne 1993, Page & Holmes 1998). Microsatellite markers can, therefore, be used to determine the genetic relationships among and within VCGs from geographically separate origins. The question of gene flow and genotypic diversity between populations of *Foc* from Southeast Asia,

Australia, and South Africa at the locus level could be determined with population genetic studies using microsatellite markers.

In asexually reproducing fungi such as *Foc*, DNA fingerprinting offers a powerful tool for identification of an individual and distinguishing among different clonal lineages in a population with a high degree of certainty. Bentley *et al.* (1998) were thus able to link specific fingerprints to some VCGs in *Foc*. However, a polymorphic marker unique to *Foc* has not been described. Analysing sequence data of nuclear and mitochondrial genes with different evolutionary rates or using microsatellite markers might lead to the development of such a marker for the different races.

Mating type: The capacity of plant pathogens to survive, reproduce, and extend their host range is of fundamental importance in the study and control of these pathogens. The genus *Fusarium* consists of species with a variety of complex reproductive lifestyles. The section *Elegans*, containing *F. oxysporum*, is assumed to reproduce asexually with some potential for nonsexual recombination among isolates that are very similar (Buxton 1962, Tinline & Macneil 1969). However, the relatively large number of VCGs and genetic groupings present in *F. oxysporum* in general, and in *Foc* specifically, justify an investigation into the question whether sexual reproduction has occurred at some stage in the distant past. Since genes coding for opposite mating types in fungi are highly conserved (Glass & Kuldau 1992, Kronstad & Staben 1997) evidence for an early sexual event in *F. oxysporum* should be investigated by searching for the presence of such genes. In a study on *F. oxysporum* f. sp. *lycopersici* Arie *et al.* (2001), the presence of both *MAT*-1 and *MAT*-2 idiomorphs (known to control mating type in ascomycetous fungi) was found in different strains of the pathogen. A study searching for a sexual event in *F. oxysporum*, however should not be limited to a single *formae speciales*, but should eventually be extended to both pathogenic and non-pathogenic

members of the species. Taylor *et al.* (1999) suggested that the sexual cycle might still be active in the *F. oxysporum* complex, although a teleomorph has not been observed.

Investigating the reproductive capabilities, of a worldwide population of *Foc* would result in useful information pertaining to genetic diversity and population structure of *Foc*. Taylor *et al.* (1999) argues that multiple VCGs and races within a given *formae specialis* could have independent origins, with pathogenicity and virulence evolving more than once through mutation or transposition, or spread to distantly related strains through parasexuality or horizontal gene transfer (Brasier 1995, Taylor *et al.* 1999). Baayen *et al.* (2000) suggested that population genetic studies on species in the *F. oxysporum* complex are mostly biased, because isolates investigated originate from agricultural crops. This results in an overestimation of clonality through human dissemination of infected propagative materials. Appel & Gordon (1996) support this view, based on their finding that clonality is less common in putatively non-pathogenic populations of *F. oxysporum*.

CONCLUSIONS

Understanding and measuring diversity and diversification in *Foc* should be an important component of research programmes on Fusarium wilt of banana that focus on disease management. Once the population structure of the pathogen has been analysed, appropriate control measures can be designed for a specific country or region. Measuring diversity in a clonally reproducing fungus such as *Foc*, however, is complicated, and requires a combination of phenotypic and genotypic tools. Whether current tools available are efficient for analysing populations of *Foc* is debatable. But the fact remains that there are several gaps in our knowledge on the worldwide population structure of the pathogen. The full range of

options for disease prevention and management will remain unclear until there is a better understanding of the molecular events controlling pathogen variation.

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Table 1. Vegetative compatibility groups, races and origins of strains of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) (Jones 1999).

VCG[1]	VCG complex[2]	Race	Origins
0120	0120-01215	1, 4	South Africa, Canary Islands, Australia, Brazil, Costa Rica, Honduras, Indonesia, Jamaica, Malaysia, Nigeria, Portugal, Spain, Taiwan, Florida (USA)
0121	none	4	Indonesia, Taiwan,
0122	none	4?[3]	Philippines
0123	none	1	Malaysia, Philippines, Taiwan, Thailand
0124	0124-0125-0128-01220	1, 2	Australia, Brazil, Burundi, China, Cuba, Democratic Republic of Congo, Haiti, Honduras, India, Jamaica, Malawi, Malaysia, Mexico, Nicaragua, Rwanda, Tanzania, Thailand, Uganda, Florida (USA)
0125	0124-0125-0128-01220	1, 2	Australia, Brazil, Burundi, China, Cuba, Democratic Republic of Congo, Haiti, Honduras, India, Jamaica, Malawi, Malaysia, Mexico, Nicaragua, Rwanda, Tanzania, Thailand, Uganda, Florida (USA)
0126	none	1	Honduras, Indonesia, Papua New Guinea, Philippines
0128	0124-0125-0128-01220	1, 2	Australia, Brazil, Burundi, China, Cuba, Democratic Republic of Congo, Haiti, Honduras, India, Jamaica, Malawi, Malaysia, Mexico, Nicaragua, Rwanda, Tanzania, Thailand, Uganda, Florida (USA)
0129	none	4	Australia
01210	none	1	Cayman Islands, Cuba, Florida (USA),
01211	none	4	Australia
01212	none	?[4]	Tanzania
01213	01213-01216	T4	Australia, Indonesia, Malaysia, Taiwan
01214	none	2	Malawi
01215	0120-01215	1, 4	Canary Islands, Australia, Brazil, Costa Rica, Honduras, Indonesia, Jamaica, Malaysia, Nigeria, Portugal, Spain, Taiwan, Florida (USA)
01216	01213-01216	T4[5]	Australia, Indonesia, Malaysia, Taiwan
01217	none	?	Malaysia
01218	none	?	Indonesia, Malaysia, Thailand
01219	none	?	Indonesia
01220	0124-0125-0128-01220	4?	Australia, Brazil, Burundi, China, Cuba, Democratic Republic of Congo, Haiti, Honduras, India, Jamaica, Malawi, Malaysia, Mexico, Nicaragua, Rwanda, Tanzania, Thailand, Uganda, Florida (USA)
01221	none	?	Thailand

[1]Vegetative compatibility groups (VCG) a phenotypic marker used to characterize fungal isolates based on heterokaryon formation (Puhalla 1985).
[2]isolates in a VCG are compatible with isolates in different VCG, forming a VCG complex.
[3]4? Possible race 4.
[4]? Race undetermined.
[5]T4 Tropical race 4.