CHAPTER 1

LITERATURE REVIEW:

TAXONOMY AND POPULATION GENETICS OF *FUSARIUM SUBGLUTINANS SENSU LATO* ON PINE AND MANGO

1. INTRODUCTION

The genus *Fusarium* was first described by Link in 1809 (Wollenweber & Reinking, 1925). To simplify identification of *Fusarium* spp., Wollenweber & Reinking (1935) divided the genus into sections based on the presence or absence of microconidia and chlamydospores, as well as the shape of micro- and macroconidia. Thin-walled, falcate macroconidia and the absence of chlamydospores distinguished section *Liseola* from other sections in the genus (Wollenweber & Reinking, 1935; Booth, 1971; Nelson *et al.,* 1983). However, the taxonomy of *Fusarium* has continued to be problematic and the subject of considerable debate (Wollenweber & Reinking, 1935; Booth, 1971; Gerlach & Nirenberg, 1982; Nelson *et al.,* 1983; Nirenberg & O'Donnell, 1998). There is disagreement with respect to the name and number of species in the section *Liseola,* the morphological criteria that are appropriate to distinguish these species (Nelson *et al.,* 1983; Nirenberg & O'Donnell, 1998), as well as the treatment of the section *Liseola* as a monophyletic lineage (O'Donnell *et al.,* 1998; 2000). *F. subglutinans* Wollenweber & Reinking is one of the species that resides in the section *Liseola* (Nelson *et al.,* 1983). The foremost interest in this species, lies in its important role as a plant pathogen.

Fusarium subglutinans is the causal agent of economical important diseases on a wide variety of plants (Hepting & Roth, 1946; Booth, 1971; Hsieh *et al.,* 1977; Rohrbach & Pfeiffer, 1976; Kuhlman, 1982; Ventura *et al.,* 1993). Previously, it was not possible to use morphology to distinguish *F. subglutinans* strains, from different hosts. Pathogenicity tests and mating studies were thus used to make these distinctions (Hsieh *et al.,* 1977; Correll *et al.,* 1991; Elmer & Ferrandino, 1992; Elmer, 1995; Leslie, 1995; Viljoen *et al.,* 1997a). Recently, *F. subglutinans* isolates associated with different host plants have been elevated to species level based on molecular evidence (O'Donnell *et al.,* 1998) and morphological characteristics (Nirenberg & O'Donnell, 1998). However, for the purpose of this review *Fusarium* spp. morphologically similar to *F. subglutinans* will be referred to as *F. subglutinans sensu lato.*

Pitch canker is one of the most important diseases caused by *F. subglutinans* (Hepting & Roth, 1946). This disease is destructive on pines and was first observed in the southeastern United States (Hepting & Roth, 1946). More recently, the pathogen has

been reported from California, Japan, Mexico and South Africa (McCain *et al.*, 1987; Kobayashi & Muramoto, 1989; Santos & Tovar, 1991; Viljoen *et al.*, 1994). Pitch canker has thus become a pathogen of international importance that threatens pine plantations, orchards, natural stands and nurseries, globally (Blakeslee *et al.*, 1980; Dwinell & Barrows-Broaddus, 1981; Viljoen *et al.*, 1994).

Another economically important disease caused by *F. subglutinans* is mango malformation. This disease occurs in mango producing countries throughout the world (Kumar *et al.*, 1993). The causal agent has been characterized using sequences of the β-tubulin and histone *H3* genes (Steenkamp *et al.*, 2000a). However, the taxonomy of the fungi involved in this disease, has yet to be defined.

In this review I will discuss various aspects pertaining to the taxonomy of *F. subglutinans* (section *Liseola*). More specifically, the various characteristics used in the taxonomic classifications for the genus *Fusarium* will be considered. Mating populations have been defined for species in the section *Liseola* and the emerging biological species will also be considered. Additionally, studies have been undertaken to distinguish *Fusarium* spp. in *Liseola* and related sections as phylogenetic species based on multilocus sequences, due to the inability to distinguish these morphologically similar species. Due to the economic importance of *Fusarium* spp. in the section *Liseola,* the population genetics of several of these fungi have been studied. The various techniques used to study the population genetics will be treated. Two important diseases caused by *F. subglutinans sensu lato,* namely pitch canker and mango malformation will also be discussed in detail. Special emphasis is placed on, the influence of the various classification systems (morphology, phylogeny and biological species) used for the identification of these pathogens. Furthermore, the geographical distribution, spread, and molecular characterization of strains influencing the genetic and population diversity of the pitch canker and mango malformation pathogens, will be considered.

2. TAXONOMY

2.1 *Morphology*

Wollenweber & Reinking (1935) first recognized *F. moniliforme* Sheldon as the primary representative of the section *Liseola*. These authors later proposed variety status for *F. moniliforme* (Wollenweber & Reinking, 1935). The variety, *F. moniliforme* var. *subglutinans* Wollenweber & Reinking was the first of these varieties to be described and was first observed on bananas in Honduras (Wollenweber & Reinking, 1925) (Table 1). *F. subglutinans* var. *subglutinans* was distinguished from *F. moniliforme* by the absence of microconidia in chains and the presence of polyphialides in *F. moniliforme* var. *subglutinans* (Wollenweber & Reinking, 1935; Booth, 1971).

In 1955, Bilai proposed a new classification system for *Fusarium* (Bilai, 1955). In this classification, the sections *Liseola* and *Elegans* were combined as one section *Elegans* (Bilai, 1955; 1970). *F. moniliforme* and *F. moniliforme* var. *subglutinans* were, therefore, included in the section *Elegans*. *F. neoceras* Wollenweber & Reinking was included as a synonym of *F. moniliforme* var. *subglutinans* (Table 1).

Booth (1971) transferred *F. moniliforme* and *F. moniliforme* var. *subglutinans* back to the section *Liseola*. This decision was based on the fact that Booth (1971) used morphological characteristics such as colony colour, spore morphology, presence and absence of chlamydospores, as well as mono- and/or polyphialides to divide the genus into different sections. Species in the section *Liseola* were characterized by the absence of chlamydospores, presence of mono- and polyphialides, whereas species in the section *Elegans* produced chlamydospores and monophialides. Conidiophores and conidiogenous cells as well as spore morphology and colony morphology were used to distinguish *F. moniliforme* var. *moniliforme* and *F. moniliforme* var. *subglutinans* in the section *Liseola*.

F. moniliforme var. *subglutinans* was reported as the causal agent of seedling blight, root stalk and kernel rot of maize (*Zea mays* L.), as well as pokkah boeng disease of sugarcane (*Saccharum officinarum* L.) (Booth, 1971). However, Gerlach & Nirenberg (1982) described *F. moniliforme* var. *subglutinans* strains that were

associated with pokkah boeng disease of sugarcane as *F. sacchari* (Butler) Gams var. *sacchari*. *F. moniliforme* var. *subglutinans* strains causing stalk and cob rot of maize were described as *F. sacchari* var. *subglutinans* (teleomorph *G. fujikuroi* var. *subglutinans*) (Gerlach & Nirenberg, 1982). Gerlach & Nirenberg (1982) proposed that narrower microconidia and rarely produced sporodochia observed in *F. sacchari* var. *sacchari* strains, distinguished them from those of *F. sacchari* var. *subglutinans*. *F. moniliforme* var. *moniliforme* that was not pathogenic to sugarcane or maize was described as *F. sacchari* var. *elongatum* Nirenberg by Gerlach & Nirenberg (1982). Furthermore, *F. tricinctum* var. *anthophilum* (=*F. moniliforme* var. *anthophilum*), that was previously included as a synonym of *F. moniliforme* var. *subglutinans* (Booth, 1971), was described as *F. anthophilum* (Braun) Wollenweber (Gerlach & Nirenberg, 1982) based on its pyriform microconidia. These authors also included *F. neoceras* as a synonym of *F. sacchari* (Gerlach & Nirenberg, 1982) (Table 1).

The classification system of Nelson *et al.* (1983), was based on the work of Wollenweber & Reinking (1935). Nelson *et al.* (1983) distinguish *Fusarium* spp. based on colony colour on potato dextrose agar (PDA) and morphological characteristics such as shape and size of macroconidia, presence or absence of microconidia and chlamydospores as well as the type of conidiophores on carnation leave agar (CLA). *F. subglutinans, F. proliferatum* (Matsushima) Nirenberg, *F. moniliforme* and *F. anthophilum* were the species included in the section *Liseola*.

2.2 *Pathogenicity*

Fusarium subglutinans occurs on a wide variety of hosts causing diseases on economically important plants, such as mangos (*Mangifera indica* L.), pineapples (*Ananas comosus* L.), maize (*Z. mays*), sugarcane (*S. officinarum* L.) and sorghum (*Sorghum caffrorum* Beauv.) (Singh *et al.*, 1961; Booth, 1971; Hsieh *et al.*, 1977; Gerlach & Nirenberg, 1983; Ventura *et al.*, 1993) and the pitch canker complex on pine trees (*Pinus* spp.) (Hepting & Roth, 1946; Correll *et al.* 1991). A significant problem has been that *F. subglutinans* associated with specific hosts cannot be distinguished easily based on morphology. Pathogenicity was, therefore, used to distinguish host specific *F. subglutinans* strains. This led to the establishment of *formae speciales* status for some species.

Of the pathogenic strains of *F. subglutinans sensu lato,* only two *formae speciales* have been proposed. These include *F. subglutinans* f. sp. *ananas* Ventura *et al.* (=*F. guttiforme* Nirenberg & O'Donnell), which is pathogenic to pineapples (Ventura *et al.,* 1993) and *F. subglutinans* f. sp. *pini* Correll *et al.* (=*F. circinatum*), which is pathogenic to pines (Hsieh *et al.,* 1977; Correll *et al.,* 1991; Viljoen *et al.,* 1997a).

2.3 *Phylogeny*

Recently, 44 *Fusarium* spp. in the section *Liseola* and related sections (= *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura complex) were shown to represent distinct phylogenetic species using multilocus sequences (O'Donnell *et al.,* 1998; 2000). Nirenberg & O'Donnell (1998) re-evaluated the morphological characteristics of species in this complex, including *F. subglutinans*, and described morphological species that supported their phylogenetic species concept. *Fusarium* spp. in the *G. fujikuroi* complex were distinguished based on conidial shape, production of chlamydospores, origin of conidiophores from the substrate, degree of conidiophore branching and production of mono and/or polyphialides (Nirenberg & O'Donnell, 1998). Twelve of the 44 *Fusarium* taxa in *Liseola,* previously identified as *F. subglutinans sensu lato* were described as distinct species (Nirenberg & O'Donnell, 1998). The pitch canker fungus, *F. subglutinans* f. sp. *pini* was given the name *F. circinatum* Nirenberg & O'Donnell (teleomorph, *G. circinata* Nirenberg & O'Donnell). Other *F. subglutinans sensu lato* species described by Nirenberg & O'Donnell (1998), include *F. begoniae* Nirenberg & O'Donnell ex *Begonia elatior* hybrid, *F. bulbicola* Nirenberg & O'Donnell ex *Nerine bowdenii, Vallota* and *Haemanthus* sp. (= *F. sacchari* var. *elongatum*), *F. concentricum* Nirenberg & O'Donnell ex *Musa sapientum* (banana), *F. guttiforme* ex *Ananas comosus* (pineapple) (= *F. subglutinans* f. sp. *ananas*) and *F. pseudocircinatum* O'Donnell & Nirenberg ex *Solanum* sp., *Pinus kesiya* and *Heteropsylla incisa* (Table 1).

The classification systems proposed for *Fusarium* differ from each other and thus recognize varying numbers and names of species in their various manifestations of the section *Liseola.* The morphological criteria used to distinguish these species also differ in all the proposed systems. Sexual compatibility (mating studies) and phylogenetic analysis have thus been promoted in recent years to differentiate

morphologically similar species in the *G. fujikuroi* complex. The following sections deal with each of these characteristics in more detail.

3. BIOLOGICAL SPECIES (MATING POPULATIONS)

An alternative approach to morphology, when attempting to differentiate *Fusarium* spp. in the section *Liseola* is sexual compatibility. Species in this section are heterothallic and, therefore, governed by two alleles, designated *MAT*-1 and *MAT*-2, at a single mating type locus (Fincham *et al.*, 1979; Yoder *et al.*, 1986). Strains of opposite mating type that belong to the same mating population are potentially sexually fertile, leading to the formation of sexual structures (perithecia). Traditionally, the mating type alleles have been arbitrarily designated as '+' and '-' after a cross has been attempted with each of two tester isolates, which are known to represent different mating types (Leslie, 1991; 1995; 1996).

Eight mating populations (MP) have been identified in the *G. fujikuroi* complex (indicated by the letters A to H) using sexual compatibility. Sexual compatibility in this complex is determined in the laboratory on artificial media under specific conditions. Several factors, including light, medium and temperature influence sexual compatibility (Nelson, 1996). Exposure to near ultra violet and fluorescent light following fertilization, has been reported to be essential for the development of perithecia (Kuhlman *et al.*, 1978). V-8 agar is generally considered to be the best medium for the formation of the sexual stage in MP-A and B, but was unfavorable for perithecium production in MP-C (Hsieh *et al.*, 1977; Kuhlman *et al.*, 1978). A method established by Leslie (1991) resolved this problem by using carrot agar. A lower incubation temperature has been reported to increase the fertility of sexual crosses between isolates of MP-H in the laboratory (Covert *et al.*, 1999).

Each of the eight mating populations residing in the *G. fujikuroi* complex is associated with a single *Fusarium* sp. These species are *Fusarium verticillioides* (Sacc.) Nirenberg (= *F. moniliforme*), *F. sacchari* (= *F. subglutinans*), *F. proliferatum* (Matsushima) Nirenberg, *F. fujikuroi* Nirenberg (= *F. proliferatum*), *F. subglutinans sensu stricto* and *F. thapsinum* Klittich *et al.*, *F. nygamai* Burgess & Trimboli and *F. circinatum* (*F. subglutinans* f. sp. *pini*) (Hsieh *et al.*, 1977; Burgess & Trimboli, 1986; Leslie, 1991, 1995; Klaasen & Nelson, 1996; Klittich *et al.*, 1997; Britz *et al.*,

1999). All but *F. nygamai* belong in the section *Liseola*. This *Liseola*-like species produces chlamydospores (Burgess & Trimboli, 1986), and could, therefore, not be included in the section *Liseola*. A new section *Dlaminia* was proposed in 1991 to accommodate *Liseola*-like species producing chlamydospores like *F. nygamai* (Waalwijk *et al.*, 1996; O'Donnell *et al.*, 1998; 2000).

The differentiation of the mating populations in the *G. fujikuroi* complex was changed with the introduction of the various taxonomic schemes for this group. At first, *F. moniliforme* was identified as the anamorph of MP-A, B and C of *G. fujikuroi* associated with maize, sugarcane and rice, respectively (Hsieh *et al.*, 1977). Kuhlman (1982) later included additional isolates from a variety of hosts and described four varieties in *G. fujikuroi* complex. The varieties included *G. fujikuroi* var. *fujikuroi* Wollenweber from rice, *G. fujikuroi* var. *moniliformis* (Wineland) Kuhlman from maize, *G. fujikuroi* var. *subglutinans* Edwards primarily from sugarcane and *G. fujikuroi* var. *intermedia* Kuhlman, primarily from maize.

In 1991, Leslie reported the presence of six biological species in the *G. fujikuroi* complex, that could not be clearly differentiated based on morphology (Leslie, 1991). Members of MP-A and F shared the *F. moniliforme* anamorph, members of the MP-B and E shared the *F. subglutinans* anamorph and members of the MP-C and D mating population shared the *F. proliferatum* anamorph (Leslie, 1991; Xu *et al.*, 1995). MP-F, known as the second mating population with a *F. moniliforme* anamorph (Klittich & Leslie, 1992), was later described as a distinct species, *F. thapsinum* (*G. thapsina* Klittich *et al.*) (Klittich *et al.*, 1997). Two other mating populations have also been described. MP-G that is based on *F. nygamai*, has been formally described as *G. nygamai* Klaasen & Nelson (Leslie & Mansuetus, 1995; Klaasen & Nelson, 1996). *F. subglutinans* f. sp. *pini* (= *F. circinatum*), the causal agent of pitch canker disease represents a distinct mating population in *G. fujikuroi*, known as MP-H (Viljoen *et al.*, 1997a; Britz *et al.*, 1999).

The defined mating populations of *G. fujikuroi* can be distinguished based on host specificity (Leslie, 1991; Leslie & Mansuetus, 1995), mycotoxin production (Leslie & Mansuetus, 1995) and a range of molecular techniques (DuTeau & Leslie, 1991; Xu *et al.*, 1995; Voigt *et al.*, 1995; Huss *et al.*, 1996; Amoah *et al.*, 1996; O'Donnell *et*

al., 1998; Steenkamp *et al.,* 1999; O'Donnell *et al.,* 2000). Members of the MP-A (*F. verticillioides* anamorph) usually occur on maize and members of the MP-F (*F. thapsinum* anamorph) are generally from sorghum (Klittich & Leslie, 1992). Strains belonging to these two mating populations can also be separated based on their ability to produce the mycotoxin fumonisin B_1. Strains belonging to the A population produce high levels and strains belonging to the F population produce low levels of this mycotoxin (Leslie *et al.,* 1992). Isolates residing in MP-C are primarily isolated from rice (Booth, 1971; Sun & Snyder, 1981), produce high levels of gibberellic acid (Kuhlman 1982; Hsieh *et al.,* 1977; Leslie, 1996) and have a *F. fujikuroi* (= *F. proliferatum*) anamorph (Leslie, 1996) (Table 2). Isolates residing in MP-D occur on various host plants, including asparagus (Elmer & Ferrandino, 1992; Elmer, 1995; Leslie, 1996) that produce the mycotoxin fumonisin (Leslie *et al.,* 1992) and have a *F. proliferatum* anamorph (Table 2).

Isolates residing in MP-B, E, and H represent distinct biological species with *F. subglutinans* anamorphs. These can be distinguished based on their isolated sexual outcrossing (Leslie, 1991, 1995, 1996; Viljoen *et al.,* 1997a; Britz *et al.,* 1999). MP-B strains have been reported to occur primarily on sugarcane, MP-E strains occur on maize and MP-H strains occur on *Pinus* spp. (Kuhlman, 1982; Leslie, 1991; 1995, Viljoen *et al.,* 1997a; Britz *et al.,* 1999) (Table 2). One isolate of *F. circinatum* (=*F. subglutinans* f. sp. *pini*) (ATCC 38479) has been reported to be sexually fertile with mating tester strains of MP-B (Kuhlman, 1982), but Correll *et al.* (1992) and Viljoen *et al.* (1997a) were not able to repeat this cross and queried the validity of the fertile cross. Britz *et al.* (1999) provided an explanation for these results. These authors showed that homothallism exists in MP-B of *G. fujikuroi* complex (Leslie *et al.,* 1986; Britz *et al.,* 1999), leading to the inaccurate conclusion that mating populations with *F. subglutinans* anamorphs are cross fertile with one another (Kuhlman, 1982).

Molecular tests enable designation of mating type alleles as *MAT*-1 and *MAT*-2 using PCR amplification. The *MAT*-2 idiomorph (Covert *et al.,* 1999; Kerenyi *et al.,* 1999; Steenkamp *et al.,* 2000b) and both *MAT*-1 and *MAT*-2 (Steenkamp *et al.,* 2000b; Wallace & Covert, 2000) can be amplified. The PCR technique developed by Steenkamp *et al.* (2000b) can be used to determine the mating type of *Fusarium* strains in the whole *G. fujikuroi* complex. The PCR technique developed by Wallace

& Covert (2000) specifically focuses on mating studies of *F. circinatum* in the *G. fujikuroi* complex.

Morphology, pathogenicity and mating studies indicate that *F. subglutinans sensu lato* strains residing in MP-B, E and H represent separate species (Nirenberg & O'Donnell, 1998; Viljoen *et al.*, 1997a; Leslie, 1995; Britz *et al.*, 1999). However, Desjardins *et al.* (2000) described a genetically isolated population of isolates identified as *F. subglutinans* from domestic maize (*Z. mays* ssp. *mays*) and its teosinte relatives (*Zea* spp.) in Mexico and Central America. These isolates, from the two closely related hosts, were cross fertile. One *F. subglutinans* isolate was, however, cross fertile with a strain of MP-H. This finding prohibited Desjardins *et al.* (2000) from describing isolates identified as *F. subglutinans* from maize and teosinte as a separate mating population of the *G. fujikuroi* complex. Sexual compatibility and phylogenetic analysis of isolates identified as *F. subglutinans sensu lato* from teosinte, maize (MP-E) and pine (MP-H) indicated that isolates from teosinte reside in the existing MP-E (Steenkamp, 2000).

In the past, sexual compatibility was used to differentiate morphologically similar species in the section *Liseola*. The recent classification system proposed by Nirenberg & O'Donnell (1998) has led to consistency between proposed morphological species and mating populations in the *G. fujikuroi* complex. Morphology and sexual compatibility can thus both be used to differentiate *Fusarium* spp. in the section *Liseola*.

4. PHYLOGENETIC SPECIES CONCEPT

More accurate and rapid techniques to discriminate among mating populations and morphological species in the *G. fujikuroi* complex, have been actively sought in recent years. This is mainly due to the importance of these fungi as pathogens and the need for diagnostic tools. A study conducted by Xu *et al.* (1995) determined that strains of each mating population could be distinguished using electrophoretic karyotyping, but this technique has been considered unduly cumbersome for use in routine diagnostics. Electrophoretic karyotypes can only be useful as a supplementary tool that provides insight into the genome organization and evolution of these fungi.

Huss *et al.* (1996) used isozyme analysis on seven mating populations of *G. fujikuroi* (MP A to G). The C and D mating populations were found to be the most similar of all the mating populations. Furthermore, the distinction of MP-B from the other six mating populations is difficult with isozyme analysis. Isozyme analyses can, thus, not be used to resolve all the morphological species in the *G. fujikuroi* complex (Huss *et al.*, 1996).

Random amplified polymorphic DNA (RAPD) (DuTeau & Leslie, 1991; Voigt *et al.*, 1995; Amoah *et al.*, 1996; Viljoen *et al.*, 1997a) and restriction fragment length polymorphisms (RFLPs) (Leslie & Mansuetus, 1995; Steenkamp *et al.*, 1999) have been used as a relatively rapid method to discriminate the mating populations from one another. RFLPs are technically complicated and did not give adequate results using mitochondrial DNA and the internal transcribed spacer (ITS) regions of the ribosomal DNA operon. However, histone-RFLPs can be used as a diagnostic tool in the *G. fujikuroi* complex (Steenkamp *et al.*, 1999). RAPDs have been used to distinguish the different mating populations from one another (Voigt *et al.*, 1995; Amoah *et al.*, 1996; Viljoen *et al.*, 1997a). Unfortunately, RAPDs have low repeatability. RAPDs, mtDNA-RFLP and ITS-RFLP are thus unsuitable for diagnostic purposes.

The ITS region (ITS1 and ITS2) has been used extensively in molecular systematic studies of the genus *Fusarium* (O'Donnell, 1992; Waalwijk *et al.*, 1996). O'Donnell & Cigelnik (1997) found that most *Fusarium* species have two highly divergent ITS2 sequences. These have been designated ITS2 type I and II (Waalwijk *et al.*, 1996). The ITS2 amplifies with conserved ITS primers (White *et al.*, 1990) and has been defined as the major type. The opposite type known as the minor ITS2 type, can be amplified with type-specific forward PCR primers (O'Donnell & Cigelnik, 1997). The gene consensus of ITS2 types I and II data is misleading and inadequate for parsimony analyses (O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 1998). Phylogenetic relationships should, therefore, not be attempted using only the ITS regions (O'Donnell *et al.*, 1998).

Recently, β-tubulin, calmodulin and translation elongation factor 1α (EF-1α) gene

sequences have been used in phylogenetic analyses of *Fusarium* spp. in the *G. fujikuroi* complex (O'Donnell *et al.*, 1998; 2000). These authors recognized 44 *Fusarium* taxa (phylogenetic lineages) in the *G. fujikuroi* complex. Of the 44 *Fusarium* lineages recognized in the *G. fujikuroi* complex, more than 30 lineages have no known teleomorphs. O'Donnell *et al.* (1998; 2000) referred to the 44 phylogenetically distinct species as *Fusarium* spp. in the *G. fujikuroi* complex, despite the fact that the genus *Gibberella* was established to encompass only *Fusarium* spp. that produce perithecia (teleomorph) with septate ascospores (Booth, 1971). Phylogenetic analyses of DNA sequences from multiple loci of the 44 *Fusarium* lineages in the *G. fujikuroi* complex, reside in the sections *Liseola* and *Dlaminia*. The *G. fujikuroi* complex was proposed as a monophyletic lineage, because the 44 *Fusarium* lineages reside in polyphyletic sections (O'Donnell *et al.*, 1998; 2000).

The 44 *Fusarium* lineages in the *G. fujikuroi* complex are separated into three distinct groups, which O'Donnell *et al.* (1998) referred to as 'African', 'American' and 'Asian' clades (O'Donnell *et al.*, 1998; 2000). O'Donnell *et al.* (1998) proposed a phylogeographic hypothesis for these fungi based on this phylogenetic clustering pattern and the geographic origins of the hosts. The hypothesis suggests that the evolutionary histories of *Fusarium* lineages in this complex are consistent with the species origins in Africa, South America and Asia respectively, following the fragmentation of the ancient Gondwanaland super-continent (O'Donnell *et al.*, 1998). However, the phylogenetic placement of some fungi in this scheme is open to criticism and requires further investigation (Steenkamp *et al.*, 1999; 2000a).

Steenkamp *et al.* (1999; 2000a) successfully used histone *H3* gene sequences to distinguish *Fusarium* spp. in the *G. fujikuroi* complex from one another. Phylogenetic analyses with *Fusarium* histone *H3* gene sequence data generated a phylogram similar to those of O'Donnell *et al.* (1999; 2000). The results of the studies of Steenkamp *et al.* (1999, 2000a) and O'Donnell *et al.* (1998; 2000) have thus identified conserved genes that are useful for phylogenetic and taxonomic studies for species of *Fusarium* in the *G. fujikuroi* complex. *Fusarium* spp. previously considered unduly closely related, can now be separated based on histone, EF-1α or β-tubulin gene sequence.

Phylogenetic trees generated from partial *MAT*-1 and *MAT*-2 sequences of eight mating populations of the *G. fujikuroi* complex gave similar phylogenetic clustering patterns, as those generated by O'Donnell *et al.* (1998) and Steenkamp *et al.* (1999) using β-tubulin and histone *H3* gene sequences. However, the *MAT*-2 tester of MP-B grouped with the C and D mating populations as seen with the β-tubulin and histone *H3* gene sequences. The *MAT*-1 sequences indicated that the MP-B grouped equidistant from the other seven mating populations.

5. POPULATION GENETICS

Various evolutionary processes influence the population genetic structure of pathogenic fungi. Genetic variability depends on the mode of reproduction, and thus, the relative contributions of sexual and asexual reproduction, outcrossing and selfing mechanisms and hyphal anastomosis (Brasier, 1992; Glass & Kuldau, 1992; Leslie, 1993; Milgroom, 1996). Many factors other than mating systems contribute to genetic evolution within fungal populations such as as gene flow (migration), population size and selection (McDonald & McDermott, 1993; Burdon & Silk, 1997; McDonald, 1997).

Vegetative compatibility has been broadly applied to provide insight into the genetic structure of fungal populations (Glass & Kuldau, 1992). Individuals of the same fungal species are vegetatively compatible when they fuse asexually to form a stable heterokaryon (Glass & Kuldau, 1992; Leslie, 1993). Vegetative compatibility is controlled by vegetative incompatibility (*vic*) loci and strains that are identical at all *vic* loci are able to form a stable heterokaryon and belong to the same vegetative compatibility group (VCG) (Glass & Kuldau, 1992; Leslie, 1993). Heterokaryon formation between anastomosing nitrate non-utilizing (*nit*) mutants are used to determine the VCGs (Puhalla, 1985; Glass & Kuldau, 1992; Leslie, 1993). Unfortunately, VCGs are not useful for inferences pertaining to evolutionary processes affecting population genetics (McDonalds, 1997). However, the emergence of molecular markers has made it possible to investigate evolutionary processes, similarities and differentiation in various fungal populations. (Williams *et al.*, 1990; McDonald *et al.*, 1989; Levy *et al.*, 1991; Fry *et al.*, 1992; Drenth *et al.*, 1993;

Mitchell & Brasier, 1994; Milgroom, 1995; Milgroom *et al.*, 1996; Bonfante *et al.*, 1997; McDonald, 1997; Moon *et al.*, 1999; Burgess *et al.*, 2001). The following markers have been extensively used in population genetics of several pathogenic fungi:

RAPDs: These are short arbitrary oligonucleotide primers targeting unknown sequences in the genome and low annealing temperatures are used in RAPD-PCR to amplify PCR products consisting of different size (Williams *et al.*, 1990). Often, size polymorphisms are identified within species, which are the result of mutations at specific binding sites (Welsh & McClelland, 1990). RAPD is a fast and relatively economical method for screening large numbers of isolates. RAPDs have been used to distinguish *Fusarium* spp. in the *G. fujikuroi* complex from one another (Voigt *et al.*, 1995; Amoah *et al.*, 1996; Viljoen *et al.*, 1997a). Viljoen *et al.* (1997a) could distinguish *F. subglutinans sensu lato* from one another by including isolates identified as *F. subglutinans* from maize, mango, pine, pineapple and sugarcane. Interestingly, he found that two isolates of *F. subglutinans* from mango (MRC 2802 and MRC 2370) grouped into two separate groups (Viljoen *et al.*, 1997a). However, the low temperatures used for amplification can cause problems with reproducibility, which together with the dominant nature of RAPDs (two alleles for each amplicon locus), limit the use of RAPDs as molecular markers in population studies (McDonald & McDermott, 1993; McDonald, 1997).

Isozymes: Isozyme analyses have been extensively used in the past for population studies of a variety of fungal pathogens (Old *et al.*, 1984, Burdon & Roelfs, 1985; McDermott *et al.*, 1989; Fry *et al.*, 1992; Goodwin *et al.*, 1992; Huss *et al.*, 1996). Isozyme analyses are inexpensive, selectively neutral, codominant and easy to assay (McDonald & McDermott, 1993). Huss *et al.* (1996) used isozymes to distinguish seven mating populations (MP-A to G) from one another. In this study, *F. sacchari* isolates (MP-B) could not be distinguished from the six other mating populations in the *G. fujikuroi* complex and isozymes cannot be used as a diagnostic tool to distinguish species in the *G. fujikuroi* complex. Furthermore, the quantification of isozyme markers is difficult and time-consuming (McDonald & McDermott, 1993; McDonald, 1997).

RFLPs: RFLPs of mitochondrial and nuclear DNA have provided useful genetic markers in various fungal population genetic studies (McDonald *et al.*, 1989; McDonald & Martinez, 1990; 1991; Gordon & Okamoto, 1991; Levy *et al.*, 1991; Kohn *et al.*, 1991; Drenth *et al.*, 1993; Milgroom *et al.*, 1996). The increased variability and number of loci obtained with RFLPs has the advantage of being more useful than isozyme markers (Engel *et al.*, 1996; McDonald, 1997). Furthermore, RFLPs have the additional advantage of being neutral and codominant. However, the number of enzymes available, limit the number of loci that can be examined. Another constraint of this technique is that large quantities of DNA are required. However, RFLPs of PCR products eliminate these problems. Histone *H3*-RFLPs have been used to distinguish *Fusarium* spp. residing in the *G. fujikuroi* complex from one another (Steenkamp *et al.*, 1999).

Simple sequence repeats (SSR) / microsatellites: SSRs, also known as microsatellite repeats, are short DNA repeats that are inherited in a single locus, codominant manner (Tautz, 1989; Weber & May, 1989). SSRs are highly polymorphic between individuals due to slippage of the DNA polymerase during synthesis and DNA repair (Tautz & Renz, 1984; Levinson & Gutman, 1987; Engel *et al.*, 1996; Rafalski *et al.*, 1996; Moon *et al.*, 1999). SSRs can, therefore, be used as a powerful tool for genetic analysis of population structure. SSR markers have been developed using either probing of genomic libraries with di- or trinucleotide repeats (Ostrander *et al.*, 1992; Smith & Devey, 1994; Tenzer *et al.*, 1999), sequencing of fragments amplified by RAPD-PCR (Burt *et al.*, 1994; Desmarais *et al.*, 1998; Dusabenyagasani *et al.*, 1998) or internal short sequence repeats (ISSR)-PCR (Brady *et al.*, 1996; Dusabenyagasani *et al.*, 1998; van der Nest, 2000; Burgess *et al.*, 2001). In different fungal studies, SSR markers have been used in genotypic assessment (Geistlinger *et al.*, 1997; Groppe & Boller, 1997; Longato & Bonfante, 1997; Neu *et al.*, 1999) and as polymorphic markers (Bonfante *et al.*, 1997; Moon *et al.*, 1999; Burgess *et al.*, 2001). Microsatellites can thus be used as ideal markers for fungal populations studies, because these markers are easy to analyze and highly polymorphic. They are, however, somewhat laborious and expensive to produce (Rafalski *et al.*, 1996).

6. PITCH CANKER AND MANGO MALFORMATION

Two diseases caused by *F. subglutinans sensu lato* are pitch canker and mango malformation. The taxonomic history, spread, geographical distributions as well as the sexual compatibility, molecular characteristics and population genetics of each associated pathogen will be discussed in the following sections.

6.1 *Pitch canker*

6.1.1 *Taxonomic history*

As discussed earlier, *F. subglutinans sensu lato* strains are specific to particular host plants and cause a variety of plant diseases (Singh *et al.*, 1961; Booth, 1971; Hsieh *et al.*, 1977; Gerlach & Nirenberg, 1982; Correll *et al.*, 1992; Ventura *et al.*, 1993). Pitch canker of pines is one of the most significant of these diseases. Pitch canker was first reported in 1946 on *Pinus virginiana* Mill. (Virginia pine) in North Carolina, USA (Hepting & Roth, 1946). The causal agent of the disease was recognized as an unidentified *Fusarium* sp. (Hepting & Roth, 1946). Despite interest in this fungus, there was very little known about its identity. Snyder *et al.* (1949) identified the pitch canker fungus as *F. lateritium* Nees. These authors found that isolates of *F. lateritium* recovered from pines, were pathogenic only to pines. A *forma specialis* was proposed for isolates pathogenic to pines and designated *F. lateritium* (Nees) emend. Snyder & Hanson f. sp. *pini* Hepting (Snyder *et al.*, 1949). The subsequent spread and epidemic nature of this pathogen, therefore, renewed interest and highlighted the importance of its taxonomy.

Dwinell (1978) and Kuhlman *et al.* (1978) identified the pitch canker fungus as *F. moniliforme* var. *subglutinans,* rather than *F. lateritium* f. sp. *pini.* However, when Nelson *et al.* (1983) reviewed the taxonomy of the genus *Fusarium*, they elevated *F. moniliforme* var. *subglutinans* to species level as *F. subglutinans.* Correll *et al.* (1991) proposed that the pitch canker pathogen should be recognized as a *forma specialis, F. subglutinans* f. sp. *pini,* based on host specificity and restriction fragment patterns of mtDNA, indicating that pine isolates differed from non-pine isolates. Viljoen *et al.* (1997a) found that isolates of *F. subglutinans* f. sp. *pini* were reproductively isolated from other mating populations of the *G. fujikuroi* complex and confirmed that this fungus has specific pathogenicity to pine seedlings.

DNA sequences of conserved genes distinguished *F. subglutinans* from different hosts as unique phylogenetic lineages (O'Donnell *et al.*, 1998). Morphological species were described that supported the phylogenetic lineages (Nirenberg & O'Donnell, 1998). The pitch canker fungus, was thus assigned the name *F. circinatum* (teleomorph: *G. circinata*) (Nirenberg & O'Donnell, 1998). A serious problem with this description by Nirenberg & O'Donnell (1998) was that it was based on only four isolates for the anamorph and the teleomorph description relied on a single cross between two *F. circinatum* isolate. There was thus virtually no consideration of variability in the teleomorph characteristics, and the availability of a well defined population of isolates was ignored (Correll *et al.*, 1991; 1992; Viljoen *et al.*, 1994; Gordon *et al.*, 1996; 1997a, b). Subsequently, the Index of Fungi (1999: vol. 6: 980) reported the new name *G. circinata* invalid according to Article 37.3 of the International Code of Botanical Nomenclature (ICBN, Greuter *et al.*, 1994).

6.1.2 *Geographical distribution*

Subsequent to the first report of the pitch canker fungus in North Carolina, the fungus was found throughout the southeastern United States where it has caused significant losses on a wide variety of pine species (Hepting & Roth, 1946; Schmidt, 1976; Dwinell & Phelps, 1977; Dwinell, 1978; Dwinell *et al.*, 1985). Pitch canker was also reported on *P. occidentalis* Swartz (Western Indian pine) in Haiti (Hepting & Roth, 1953). However, Dwinell *et al.* (1985) suggested that pitch canker is endemic to the southeastern United States, due to the epidemic nature of the disease in this area.

In 1986, pitch canker was found for the first time on *P. radiata* D. Don (Monterey pine) in Santa Cruz county, California (McCain *et al.*, 1987). The disease is now found throughout California and affects many other pine species as well as Douglas-fir (*Pseusotsuga menziesii* (Mirb.) Franco) (Storer *et al.*, 1994), although *P. radiata* is the most seriously affected (Gordon *et al.*, 1997). Subsequent to 1992, pitch canker was recognized as a threat to native *P. radiata* stands in California (Storer *et al.*, 1994; 1997).

Although pitch canker is most serious and of greatest concern in California, it is also in Japan on native *P. luchuensis* Mayr. (Ryukyu pines) (Muramoto *et al.*, 1988;

Kobayashi & Kawabe, 1992) and in Mexico, where several pine species are native (Santos & Tovar, 1991). In South Africa, the fungus was first reported from a single forestry nursery, where it resulted in serious losses to *P. patula* Schlechtend & Cham. (Mexican weeping pine) (Viljoen *et al.*, 1994). Stem cankers on larger trees such as those found in the United States (Hepting & Roth, 1946; Dwinell *et al.*, 1977) have, thus far, not been seen in South Africa (Viljoen *et al.*, 1995a, b; Wingfield *et al.*, 1999), although damage in nurseries has become increasing more serious in recent years.

6.1.3 *Symptoms*

The pitch canker pathogen is responsible for a complex of symptoms on vegetative and reproductive structures, at different stages of maturity on pines (Dwinell *et al.*, 1985). The vegetative symptoms caused by pitch canker include resin flow from infected branches, shoots, cones and/or the bole of pine trees. This resinous cankers and pitch- soaked wood (Hepting & Roth, 1946; Dwinell *et al.*, 1977; Dwinell & Phelps, 1977) (Dwinell & Phelps, 1977; Dwinell *et al.*, 1985; McCain *et al.*, 1987), often penetrating through to the pith (Dwinell & Phelps, 1977). In severe cases, these cankers girdle trees leading to shoot die-back and to tree mortality (Dwinell & Phelps, 1977; Dwinell *et al.*, 1977; 1981). Infection of the reproductive structures, such as female strobili and mature cones, results in mortality of strobili and poor germination of seeds (Dwinell *et al.*, 1977; Miller & Bramlett, 1978; Miller *et al.*, 1984; Dwinell *et al.*, 1985; Barrows-Broaddus, 1986). Cones on infected branches often abort before reaching full size and remain closed (Miller & Bramlet, 1978; Dwinell & Barrows-Broaddus, 1981; Miller *et al.*, 1984).

The pitch canker fungus has been reported to be a primary pathogen associated with seedlings in nurseries causing lesions on the root collar or upper root and the cotyledonary node region of the seedlings (Barnard & Blakeslee, 1980; Dwinell *et al.*, 1985; Viljoen *et al.*, 1994). Tip die-back, damping-off, chlorotic or reddish brown needle discoloration and wilting are observed on diseased seedlings (Barnard & Blakeslee, 1980; Rowan, 1982; Dwinell *et al.*, 1985; Huang & Kuhlman, 1990). *F. circinatum* is the only *Fusarium* spp. that is pathogenic to pine seedlings as well as mature trees (Viljoen *et al.*, 1995a, b).

6.1.4 *Spread*

Infection of pine trees by *F. circinatum* occurs through wounds (Hepting & Roth, 1946; Dwinell & Phelps, 1977; Dwinell & Barrows-Broaddus, 1981; Dwinell *et al.,* 1985; Storer *et al.,* 1997). Wounds are caused by mechanical damage (Dwinell & Phelps, 1977; Dwinell *et al.,* 1977; Blakeslee *et al.,* 1980; Dwinell & Barrows-Broaddus, 1981; Dwinell *et al.,* 1985; Barrow-Broaddus, 1986), weather-related injuries (Kelly & Williams, 1982; Dwinell & Barrows-Broaddus, 1983) or insects (McGraw *et al.,* 1976; Blakeslee *et al.,* 1980; Dwinell *et al.,* 1985; Fox *et al.,* 1991; Storer *et al.,* 1994; Dallara *et al.,* 1995; Storer *et al.,* 1997).

The deodar weevil (*Pissodes nemorensis* Germar) has been implicated in disease incidences on slash pines (*P. elliottii* Engelm. var. *elliottii*) in Florida (Dwinell & Phelps, 1977; Blakeslee *et al.,* 1980; Dwinell *et al.,* 1985). The tip moth (*Rhyacionia subtropica* Miller) may create wounds that can be infected by air-borne conidia of *F. circinatum* (McGraw *et al.,* 1976; Dwinell & Phelps, 1977; Blakeslee *et al.,* 1980). In California, infection of the pitch canker fungus appears to result from feeding activities of twig beetles (*Pityophthorus* sp.), cone beetles (*Conophthhorus radiatae* Hopkins), spittlebug (*Aphrophora canadensis* Walley) and engraver beetles (*Ips* spp.) (Fox *et al.,* 1991; Correll *et al.,* 1991; Storer *et al.,* 1994; Dallara *et al.,* 1995; Hoover *et al.,* 1996; Storer & Wood, 1998). The fungus has been isolated from *Ips* spp. emerging from pitch canker infected *P. radiata* trees (Fox *et al.,* 1991; Storer *et al.,* 1994; 1997). The association of the pitch canker pathogen with native and introduced insects can potentially have a dramatic impact on the spread of this pathogen.

Fusarium circinatum is thought to have been introduced into new areas on infected seed. In the past, *F. circinatum* has been isolated from both disinfested and non-disinfested seed (Barrows-Broaddus, 1987; Runion & Bruck, 1988) and pitch canker disease symptoms have also been initiated from infected seed (Huang & Kuhlman, 1990). Storer *et al.* (1998) isolated *F. circinatum* from pine seedlings originating from seed collected from cones on diseased as well as asymptomatic branches. They found that *F. circinatum* can be carried inside seeds and may remain dormant until germination, which increases the possibility of seedling infections. Infected plant material can also lead to the spread of the fungus into new areas (Gordon *et al.,* 1996).

6.1.5 *Sexual compatibility*

Before morphological and molecular characteristics were available, the most reliable technique for distinguishing *F. circinatum* from closely related *Fusarium* spp., was sexual compatibility (Correll *et al.*, 1992; Viljoen *et al.*, 1997a). Sexual cross-fertility was not observed between *F. circinatum* isolates from California and Florida (Correll *et al.*, 1992). However, Viljoen *et al.* (1997a) reported sexual compatibility between *F. circinatum* isolates and suggested that the pitch canker fungus represented a distinct biological species. This was verified with fertile crosses reported amongst *F. circinatum* strains from South Africa, California and Florida (Britz *et al.*, 1999). *F. circinatum* was thus shown to represent a unique mating population (MP-H) in the *G. fujikuroi* complex (Coutinho *et al.*, 1995, Viljoen *et al.*, 1997a, Britz *et al.*, 1998; 1999).

6.1.6 *Molecular characteristics*

Distinguishing *F. circinatum* from *Fusarium* spp. in the *G. fujikuroi* complex initially required pathogenicity or sexual compatibility tests (Correll *et al.*, 1991; 1992; Viljoen *et al.*, 1997a; Britz *et al.*, 1999). These tests are time-consuming, labour-intensive and not always reliable. Molecular techniques such as random amplification of polymorphic DNA (RAPD) (DuTeau & Leslie, 1991; Voigt *et al.*, 1995; Viljoen *et al.*, 1997a), mitochondrial restriction fragment length polymorphisms (RFLP) (Correll *et al.*, 1991), and ribosomal DNA (rDNA) internal transcribed spacer (ITS1 and ITS2) sequences (Waalwijk *et al.*, 1996; O'Donnell & Cigelnik, 1997) have been tested for their efficacy in differentiating *F. circinatum* isolates from those in the great *F. subglutinans sensu lato* group. However, these techniques had various problems such as being slow, non-repeatable and some did not differentiate all *F. subglutinans sensu lato* isolates from one another. Sequences of the histone (Steenkamp *et al.*, 1999), β-tubulin (O'Donnell *et al.*, 1998) and EF-1α (O'Donnell *et al.*, 2000) genes separate *F. subglutinans sensu lato* into distinct groups. A PCR-RFLP technique was thus developed from the histone *H3* gene sequence and is used by some groups as a diagnostic tool to distinguish *F. circinatum*, from other *Fusarium* spp. residing in *G. fujikuroi* complex (Steenkamp *et al.*, 1999).

6.1.7 *Population genetics*

Vegetative compatibility has been widely used to provide insight into the genetic structure of *F. circinatum* in Florida, California, South Africa, Japan and to a lesser extend in Mexico. Before the start of the pitch canker epidemic in the 1970s in the southern United States, the disease occurred sporadically (Schmidt *et al.*, 1976; Dwinell & Phelps, 1977). The disease has been well established for at least 27 years in Florida. This was verified by the high VCGs diversity in the Florida population with 45 VCGs amongst 117 *F. circinatum* isolates tested (Correll *et al.*, 1992). In contrast, the Californian population consisted of only 5 VCGs among 209 isolates, with one VCG accounting for 70% of the population collected between 1987 and 1989 (Correll *et al.*, 1992). After a later collection between 1993 and 1995 (Gordon *et al.*, 1996) eight VCGs were identified in the Californian population. This limited diversity is consistent with a recent introduction of the pathogen and the absence or rare occurrence of sexual reproduction in the population (Gordon *et al.*, 1996; Wikler & Gordon, 1999). However, a recent study conducted by Wikler *et al.* (2000) has shown that outcrossing is possible with Californian isolates in the laboratory on artificial media. This might indicate that conditions in California probably support asexual rather than sexual reproduction.

The genotypic diversity of the initial *F. circinatum* population in South Africa was determined using vegetative compatibility tests (Viljoen *et al.*, 1997b). A high genotypic diversity was found in this population where 23 VCGs were identified among 69 *F. circinatum* isolates. This high level of genotypic diversity led Viljoen *et al.* (1997b) to suggest that sexual reproduction was occurring within the South African population. Britz *et al.* (1998) considered the effective population number (N_e) (Wright, 1931; Leslie & Klein, 1996) that estimates the relative contribution of the sexual and asexual cycle to the population. The initial *F. circinatum* population in South Africa had a low frequency of hermaphrodite strains (Britz *et al.*, 1998). If the number of hermaphrodites continues to fall, the *F. circinatum* population in South Africa should ultimately become asexual (Britz *et al.*, 1998).

The Japanese population, where *F. circinatum* is affecting native *P. luchuensis* (Ryukyu pine), and the Californian population have a common VCG (C7) (Wikler & Gordon, 2000). This VCG was the only genotype found among five *F. circinatum*

isolates collected in Japan. The Mexican population, which is thought to represent the origin of pitch canker, had seven VCGs among 10 isolates indicating the population to be diverse (Viljoen *et al.,* 1995b; Wikler & Gordon, 2000).

Wikler & Gordon (2000) estimated the genetic relationships among populations of *F. circinatum* in different geographical areas, using eight polymorphic markers. These authors showed that the California and Japanese populations of *F. circinatum* share lineages with the Florida population. The Mexican population had the highest genetic diversity using eight polymorphic markers (Wikler & Gordon, 2000).

6.2 Mango malformation

Mango trees are grown commercially for their fruit, in the tropical and subtropical areas of the world. A fungal disease known as mango malformation threatens the mango industry. Despite the economic impact of mango malformation, various aspects such as taxonomy, spread, pathogenicity and genetic diversity needs further investigation.

6.2.1 *Taxonomic history*

In 1891, it was postulated that mango malformation was caused by excessive moisture in the soil (Kumar *et al.,* 1993). This disease was, therefore, initially believed to be a physiological disorder. Later, altered C/N ratios in shoots bearing mango malformation, reduced availability of copper and zinc in subsurface soil layers, poor soil and water management were thought to cause malformation (Kumar *et al.,* 1993).

In 1946, Sattar suggested that mango malformation might be of viral origin (Sattar, 1946), because of the virus-like symptoms of the disease, the association of some insects with symptomatic tissue and failure to isolate any obvious pathogenic agent from diseased tissue (Kumar *et al.,* 1993). However, electron microscopy, transmission, cultural and serological studies disproved the involvement of a virus in the disease (Kishtah *et al.,* 1985; Kumar *et al.,* 1993).

Mite infestations were amongst the suspected causal agents of mango malformation in 1946 (Varma, 1983). *Aceria* (*Eriophyes*) *mangiferae* Sayed, also known as the mango bud mite, has been observed to be associated with vegetative and floral

malformation. The cause of the disease was initially attributed to the feeding injuries of this mango bug mite (Narasimhan, 1954). A correlation was observed between mite populations and disease incidence, however, several other studies have shown no correlation between mite populations and disease incidences (Prasad *et al.*, 1965; Bindra & Bakhetia, 1971). It thus appears that *A. mangiferae* might play a role in the malformation disease cycle, but it is certainly not the sole cause of the disease (Wahba *et al.*, 1986).

Previous failures to isolate a pathogenic agent from malformed mango tissue have confused researchers. However, Summanwar *et al.* (1966) showed that the fungus identified as *F. moniliforme* (= *F. subglutinans*) was commonly associated with disease mango tissue. Isolates identified as *F. subglutinans* from malformed mango tissue were used to complete Koch's postulates to induce mango malformation symptoms (Summanwar *et al.*, 1966; Varma *et al.*, 1974; Chakrabarti & Ghosal, 1989; Manicom, 1989; Ploetz & Gregory, 1993). However, controversy regarding the inoculation methods used for induction of mango malformation symptoms remained (Kumar *et al.*, 1993). Freeman *et al.* (1999) eliminated any uncertainty regarding the cause of the disease by inducing typical mango malformation symptoms on mango trees with an isolate originally collected from malformed mango tissue in Israel and identified as the pathogen *F. subglutinans* (MRC 7559 = 506/2).

O'Donnell *et al.* (1998) indicated that a *Fusarium* isolate from mango malformation in India represented a unique lineage in the *G. fujikuroi* complex. However, these authors did not describe the species formally. Two phylogenetically distinct groups of isolates, currently referred to as *F. subglutinans* are associated with mango malformation in South Africa (Steenkamp *et al.*, 1999; 2000a). The one group of isolates represents an undescribed lineage in the *G. fujikuroi* complex and the second group of isolates is conspecific with *F. subglutinans* strains that were previously reported to be the causal agent of mango malformation (Steenkamp *et al.*, 2000a). The precise taxonomic classification of the mango malformation fungi remains undefined.

6.2.2 *Symptoms*

Mango malformation is a disease that affects both vegetative and floral tissue of mango (Varma, 1983; Kumar *et al.*, 1993). The malformation symptoms on mango have been referred to as abnormal inflorescence, bunchy top, die-back and blossom blight (Varma, 1983; Chakrabarti & Ghosal, 1989; Kumar *et al.*, 1993; Ploetz, 1994a). However, the two distinct symptoms of mango malformation are blossom (floral) and vegetative malformation. This disease causes a reduction in yield, because affected panicles are sterile (Kumar *et al.*, 1993; Ploetz, 1994a). Vegetative malformation was first described in 1953 (Kumar *et al.*, 1993) and is more pronounced on young seedlings. Typical symptoms are the swelling of the vegetative buds at the tip and the seedlings then produce small shootlets bearing scaly leaves with a bunch-like appearance. Symptoms of floral or blossom malformation appear with the emergence of inflorescences. Floral malformation is characterized by thick, fleshy branched panicles that are crowded by enlarged flowers (Varma, 1983; Kumar *et al.*, 1993; Ploetz, 1994a).

Darvas (1987) indicated that the mango malformation pathogen is not uniformly or widely distributed in affected trees in South Africa. This author recovered *F. subglutinans* most frequently from malformed panicles and less frequently from non-malformed blossoms. *F. subglutinans* was not recovered from the remaining parts of the affected mango trees (Darvas, 1987).

6.2.3 *Geographical distribution*

Mango malformation was first observed in India (Varma, 1983; Kumar *et al.*, 1993). Since this first report, the disease has been found in many mango producing countries throughout the world. These include America, Bangladesh, Brazil, Cuba, Egypt, Israel, Mexico, Pakistan, South Africa, Sudan, USA and the United Arab Emirates (Varma, 1983; Kumar *et al.*, 1993; Noriega-Cantú *et al.*, 1999; Ploetz, 1994a). Mango malformation was first reported in Mexico in 1958 (Noriega-Cantú *et al.*, 1999). Vegetative malformation is the dominant symptom observed in this country. In Florida, malformation was first reported in 1972 and was not regarded as an important disease, despite increases in incidences and severity of mango malformation (Ploetz & Gregory, 1993). A survey in South Africa, showed that the

disease is present on 73% of South African mango farms and that the severity varies between 1-70% of the trees being affected (Crookes & Rijkenberg, 1985).

6.2.4 *Spread*

Mango malformation is systemic and also spreads slowly from infected seedlings and trees to healthy plants in orchards and nurseries (Varma, 1983). The disease symptoms have been reproduced on mango plants by transferring the mango bug mite (*A. mangiferae*), which is commonly associated with mango plants and carries *F. subglutinans* on its body parts (Varma, 1983; Summanwar & Raychaudhuri, 1968). However, the small percentage of mites carrying the fungus has indicated that other factors are involved in the spread of this disease. One of the most important factors is the distribution of malformed plants into new areas, resulting in the distribution of this disease (Varma *et al.*, 1971; Varma, 1983).

6.2.5 *Sexual compatibility*

No teleomorph has been reported for isolates identified as *F. subglutinans* from mango (Steenkamp *et al.*, 2000a).

6.2.6 *Molecular characteristics*

O'Donnell *et al.* (1998) distinguished a single malformation isolate (NRRL 25226) from India in a unique phylogenetic lineage in the section *Liseola* based on β-tubulin gene sequences. In South Africa, mango malformation is associated with two phylogenetically distinct groups of isolates using β-tubulin and histone gene sequences (Steenkamp *et al.*, 1999; 2000a). The one group of these isolates represents an undescribed lineage in the *G. fujikuroi* complex. The second group of isolates is conspecific with strains identified as *F. subglutinans,* that were previously reported to be the causal agent of mango malformation (Steenkamp *et al.*, 2000a).

As previously mentioned (section 3 of this Chapter), O'Donnell & Cigelnik (1997) found two highly divergent ITS2 sequences in *Fusarium* spp. in the *G. fujikuroi* complex. The mango isolate (NRRL 25226) from India has an ITS2 type II sequence (O'Donnell *et al.*, 1998) and mango isolate (MRC 2802 = NRRL 25623) from South Africa has an ITS2 type I sequence (O'Donnell *et al.*, 2000). This further indicated that two groups of isolates are associated with mango malformation.

6.2.7 *Population genetics*

Very little is known regarding the population genetics of the mango malformation pathogen, globally. Ploetz (1994b) found one VCG to be dominant amongst isolates collected in Florida. Vegetative compatibility analysis of mango malformation populations collected in Egypt indicated that 4 VCGs are present and the most dominant VCG was also found in India (Zheng & Ploetz, 2001). Because mango originated in India, it seems probable that mango malformation was introduced into Egypt from India.

7. CONCLUSIONS

Fusarium subglutinans sensu lato are specific to particular plants and cause a variety of diseases. Morphology provides a very poor system to distinguish *F. subglutinans sensu lato* strains from each another. However, some of these strains can be distinguished based on pathogenicity and sexual compatibility.

Distinct phylogenetic lineages have been described for *Fusarium* spp. in the *G. fujikuroi* complex, which includes *F. subglutinans sensu lato*. This has led to a re-evaluation of morphological characteristics used to identify *Fusarium* spp. in the *G. fujikuroi* complex. Thus, morphological species have been described that are consistent with the phylogenetic species.

The pitch canker fungus has been described as *F. circinatum* (teleomorph: *G. circinata*) based on morphology and molecular phylogeny. However, the name *G. circinata* was declared invalid because insufficient information was supplied for the type specimen in the description. The name *G. circinata,* therefore, requires validation. A more detailed description, particularly encompassing the understanding that species are represented by populations of strains and not single isolates would also be desirable.

Isolates identified as *F. subglutinans* from malformed mango tissue have unequivocally been shown to cause mango malformation. Two phylogenetically distinct groups, defined by β-tubulin and histone H3 sequences, have also been shown to be associated with mango malformation. However, the taxonomic position within

the genus *Fusarium* and distinguishing morphological features of these fungi has yet to be defined.

Co-dominant molecular markers are increasingly being used to determine the genetic relationship of fungal populations. The pitch canker pathogen has been reported in various counties including the southern United States, California, Mexico, Japan and South Africa. Development of molecular markers for *F. circinatum* would be extremely useful as they could be used to determine relationships within and between different geographical populations of this important pathogen.

The species representing the two distinct, phylogenetic lineages associated with mango malformation in South Africa urgently require characterization. The distribution of these two species also needs to be investigated in South Africa. The determination of the vegetative compatibility diversity as well as the distribution of these two lineages will contribute towards the understanding of the biology of mango malformation in South Africa.

Morphological, pathogenicity, sexual compatibility and molecular studies have, in the last decade, contributed significantly towards our knowledge of *Fusarium* spp. in the section *Liseola*. Isolates previously identified as *F. subglutinans* can be identified as different species based on pathogenicity, mating studies (MP-B, E and H), morphology and molecular techniques. However, many questions remain regarding the taxonomy and population genetics of the *F. subglutinans sensu lato* from pine and mango. The aim of this thesis is to present studies to address some of these questions, particularly relating to these pathogens in South Africa.

8. REFERENCES

Amoah, B. K., MacDonald, M. V., Rezanoor, N. & Nicholson, P. (1996) The use of random amplified polymorphic DNA technique to identify mating groups in the *Fusarium* section *Liseola*. *Plant Pathology* **45:** 115-125.

Barnard, E. L. & Blakeslee, G. M. (1980) Pitch canker of slash pine seedlings: a new disease in forest tree nurseries. *Plant Disease* **64:** 695-696.

Barrows-Broaddus, J. B. (1986) A cone and seed disease of southern pines caused by the pitch canker fungus. In *Current topics in forest research: emphasis on contributions by women scientists:* 150-154. Proceedings of National Symposium, Gainsville Florida. General Technical Report SE-46.

Barrows-Broaddus, J. B. (1987) Pitch canker. In *Cone and seed diseases of North American conifers* (J. R. Sutherland, T. Miller & R. S. Quinard, eds): *North American Forestry Commission Publication* **1:** 42-49. Victoria, B.C., Canada.

Bilai, V. I. (1955) The Fusaria (Biology and Systematics). Kiev: P Akad. Nauk. Ukr. SSR. 320 pp.

Bilai, V. I. (1970) Experimental morphogenesis in the fungi of the genus *Fusarium* and their taxonomy. *Annales Academiae Scientiarum Fennicae Series A IV Biologica* **168:** 7-18.

Bindra, O. S. & Bakhetia, D. R. C. (1971) Investigations on the etiology and control of mango malformation. *Indian Journal of Horticulture* **28:** 80-85.

Bonfante, P., Lanfranco, L., Cometti, V. & Genre, A. (1997) Inter- and intraspecific variability in strains of the ectomycorrhizal fungus *Suillus* as revealed by molecular techniques. *Microbiology Research* **152:** 287-292.

Blakeslee, G. M., Dwinell, L. D. & Anderson, R. L. (1980) Pitch canker of southern pines: Identification and management considerations. *USDA Forest Report SA-FR*11.

Booth, C. (1971) The genus *Fusarium.* Commonwealth Mycological Institute. Kew, Surrey, U. K. 237 pp.

Brady, J. L., Scott, N. S. & Thomas, M. R. (1996) DNA typing of hop (*Humulus lupulus*) through application of RAPD and microsatellite marker sequences converted to sequence tagged sites (STS). *Euphytica* **91:** 277-284.

Brasier, C. M. (1992) Evolutionary Biology of *Phytophthora.* Part I. Genetic system, sexuality and the generation of variation. *Annual Review of Phytopathology* **30:** 153-171.

Britz, H., Coutinho, T. A., Wingfield, M. J., Marasas, W. F. O., Gordon, T. R. & Leslie, J. F. (1999) *Fusarium subglutinans* f. sp. *pini* represents a distinct mating population in the *Gibberella fujikuroi* species complex. *Applied Environmental Microbiology* **65:** 1198-1201.

Britz, H., Wingfield, M. J., Coutinho, T. A., Marasas, W. F. O. & Leslie, J. F. (1998) Female fertility and mating type distribution in a South African population of *Fusarium subglutinans* f. sp. *pini.* *Applied and Environmental Microbiology* **64:** 2094-2095.

Burdon, J. J. & Roelfs, A. P. (1985) The effect of sexual and asexual reproduction on the isozyme structure of populations of *Puccinia graminis.* *Phytopathology* **75:** 1068-1073.

Burdon, J. J. & Silk, J. (1997) Sources and patterns of diversity in plant-pathogenic fungi. *Phytopathology* **87:** 664-669.

Burgess, L. W. & Trimboli, D. (1986) Characterization and distribution of *Fusarium nygamai,* sp. nov. *Mycologia* **78:** 223-229.

Burgess, T., Wingfield, M. J. & Wingfield, B. D. (2001) Simple sequence repeat (SSR) markers distinguish between morphotypes of *Sphaeropsis sapinea.* *Applied and Environmental Microbiology* **67:** 354-362.

Burt, A., Carter, D. A., White, T. J. & Taylor, J. W. (1994) DNA sequencing with arbitrary primer pairs. *Molecular Ecology* **3:** 523-525.

Chakrabarti, D. K. & Ghosal, S. (1989) The disease cycle of mango malformation induced by *Fusarium moniliforme* var. *subglutinans* and the curative effects of mangiferin-metal chelates. *Journal of Phytopathology* **125:** 238-246.

Correll, J. C., Gordon, T. R. & McCain, A. H. (1992) Genetic diversity in California and Florida populations of the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. *Phytopathology* **82:** 415-420.

Correll, J. C., Gordon, T. R., McCain, A. H., Fox, J. W., Koehler, C. S., Wood, D. L. & Schultz, M. E. (1991) Pitch canker disease in California: pathogenicity, distribution, and canker development on Monterey pine (*Pinus radiata*). *Plant Disease* **75:** 676-682.

Coutinho, T. A., Viljoen, A., Wingfield, M. J. & Marasas, W. F. O. (1995) Selection of sexually fertile parents of *Fusarium subglutinans* f. sp. *pini*. *South African Journal of Science* **91:** 17.

Covert, S. F., Briley, A., Wallace, M. M. & McKinney, V. T. (1999) Partial *MAT*-2 gene structure and the influence of temperature on mating success in *Gibberella circinata*. *Fungal Genetics and Biology* **28:** 43-54.

Crookes, C. A. & Rijkenberg, F. H. J. (1985) Isolation of fungi associated with blossom malformation of mangoes. *South African Mango Grower's Association Research Report* **5:** 10-14.

Dallara, P. L., Storer, A. J., Gordon, T. R. & Wood, D. L. (1995) Current status of pitch canker disease in California. Sacramento, Californian Department of Forestry and Fire Protection. *Tree Note* **20**.

Darvas, J. M. (1987) Control of mango blossom malformation with trunk injection. *South African Mango Grower's Association Yearbook* **7:** 21-24.

Desjardins, A. E., Plattner, R. D. & Gordon, T. R. (2000) *Gibberella fujikuroi* mating population A and *Fusarium subglutinans* from teosinte species and maize from Mexico and Central America. *Mycological Research* **104:** 865-872.

Desmarais, E., Lanneluc, I. & Lagnel, J. (1998) Direct amplification of length polymorphisms (DALP) or how to get and characterize new genetic markers in many species. *Nucleic Acids Research* **26:** 1458-1465.

Drenth, A., Goodwin, S. B., Fry, W. E. & Davidse, L. C. (1993) Genotypic diversity of *Phytophthora infestans* in the Netherlands revealed by DNA polymorphisms. *Phytopathology* **83:** 1087-1092.

Dusabenyagasani, M., Lecours, N. & Hamelin, R. C. (1998) Sequence-tagged sites (STS) for studies of molecular epidemiology of scleroderris canker of conifers. *Theoretical and Applied Genetics* **97:** 789-796.

DuTeau, N. M. & Leslie, J. F. (1991) RAPD markers for *Gibberella fujikuroi* (*Fusarium* section *Liseola*). *Fungal Genetic Newsletter* **38:** 37.

Dwinell, L. D. (1978) Susceptibility of southern pines to infection by *Fusarium moniliforme* var. *subglutinans*. *Plant Disease Reporter* **62:** 108-111.

Dwinell, L. D. & Barrows-Broaddus, J. B. (1981) Pitch canker in seed orchards. In *Proceedings of the 16th Southern Forest Tree Improvement Conference*: 234-240. Blacksburg, Virginia, USA.

Dwinell, L. D. & Barrows-Broaddus, J. B. (1983) Pine wilt and pitch canker of Virginia pine in seed orchards. In *17th Southern Forest Tree improvement Conference*: 55-62. Athens, Georgia, USA.

Dwinell, L. D., Barrows-Broaddus, J. B. & Kuhlman, E. G. (1985) Pitch canker: a disease complex of southern pines. *Plant Disease* **69:** 270-276.

Dwinell, L. D. & Phelps, W. R. (1977) Pitch canker of slash pine in Florida. *Journal of Forestry* **75:** 488-489.

Dwinell, L. D., Ryan, P. L. & Kuhlman, E. G. (1977) Pitch canker of loblolly pine in seed orchards. In *Proceedings of the 14th Southern Forest Tree Improvement Conference*: 130-137. Gainesville, Florida, USA.

Elmer, W. H. (1995) A single mating population of *Gibberella fujikuroi* (*Fusarium proliferatum*) predominates in asparagus fields in Connecticut, Massachusetts, and Michigan. *Mycologia* **87:** 68-71.

Elmer, W. H. & Ferrandino, F. J. (1992) Pathogenicity of *Fusarium* species (section *Liseola*) to asparagus. *Mycologia* **84:** 253-257.

Engel, S. R., Linn, R. A., Taylor, J. F. & Davis, S. K. (1996) Conservation of microsatellite loci across species of Artiodactyls: implications for population studies. *Journal of Mammalogy* **77:** 504-518.

Fincham, J. R. S., Day, P. R. & Radford, A. (1979) Fungal Genetics. 4th ed. University of California Press, Berkley, USA.

Fox, J. W., Wood, D. L., Koehler, C. S. & O'Keefe, S. T. (1991) Engraver beetle (Scolytidae: *Ips* species) as vector of the pitch canker fungus, *Fusarium subglutinans*. *Canadian Entomologist* **123:** 355-367.

Freeman, S., Maimon, M. & Pinkas, Y. (1999) Use of GUS transformants of *Fusarium subglutinans* for determining etiology of mango malformation disease. *Phytopathology* **89:** 456-461.

Fry, W. E., Goodwin, S. B., Matuszak, J. M., Spielman, L. J., Milgroom, M. G. & Drenth, A. (1992) Population genetics and intercontinental migrations of *Phytophthora infestans*. *Annual Review of Phytopathology* **30:** 107-129.

Geistlinger, J., Weising, K., Kaiser, W. J. & Kahl, G. (1997) Allelic variation at a hypervariable compound microsatellite locus in the ascomycete *Ascochyta rabiei*. *Molecular Genetic Genetics* **256:** 298-305.

Gerlach, W. & Nirenberg, H. I. (1982) The genus *Fusarium* – a pictorial atlas. *Mitteilungen aus der Biologischen Bundesanstalt für Land-und Forstwirtschaft Berlin-Dahlem* **209:** 1-406.

Glass, N. L. & Kuldau, G. A. (1992) Mating type and vegetative incompatibility in filamentous ascomycetes. *Annual Review of Phytopathology* **30:** 201-224.

Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N. & Fry, W. E. (1992) Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. *Phytopathology* **82:** 955-961.

Gordon, T. R. & Okamoto, D. (1991) Population structure and the relationship between pathogenic and nonpathogenic strains of *Fusarium oxysporum*. *Phytopathology* **82:** 73-77.

Gordon, T. R., Storer, A. J. & Okamoto, D. (1996) Population structure of the pitch canker pathogen, *Fusarium subglutinans* f. sp. *pini,* in California. *Mycological Research* **100:** 850-854.

Gordon, T. R., Wikler, K., Storer, A. J. & Wood, D. L. (1997) Pitch canker and its potential impacts on Monterey pine forests in California. *Fremontia* **25:** 5-9.

Greuter, W., Barrie, F. R., Burdet, H-M., Chaloner, W. G., Demoulin, V., Hawksworth, D. L., Jorgensen, P. M., Nicolson, D. H., Silva, P. C., Trehane, P. & McNeill, J. (eds.) (1994) International Code of Botanical Nomenclature (Tokyo Code). [Regnum Vegetabile No. 131]. Koeltz Scientific Books, Königstein.

Groppe, K. & Boller, T. (1997) PCR assay based on a microsatellite-containing locus for detection and quantification of *Epichloë* endophytes in grass tissue. *Applied and Environmental Microbiology* **63:** 1543-1550.

Hepting, G. H. & Roth, E. R. (1946) Pitch canker, a new disease of some southern pines. *Journal of Forestry* **44:** 742-744.

Hepting, G. H. & Roth, E. R. (1953) Host relations and spread of the pine pitch canker disease. *Phytopathology* **43:** 475.

Hoover, K., Wood, D. L., Storer, A. J., Fox, J. W. & Bros, W. E. (1996) Transmission of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini,* to Monterey pine by cone and twig infesting beetles. *Canadian Entomologist* **128:** 981-994.

Hsieh, W. H., Smith, S. N. & Snyder, W. C. (1977) Mating groups in *Fusarium moniliforme. Phytopathology* **67:** 1041-1043.

Huang, J. W. & Kuhlman, E. G. (1990) Fungi associated with damping-off of slash pine seedlings in Georgia. *Plant Disease* **74:** 27-30.

Huss, M. J., Campbell, C. L., Jennings, D. B. & Leslie, J. F. (1996) Isozyme variation among biological species in the *Gibberella fujikuroi* species complex (*Fusarium* section *Liseola*). *Applied and Environmental Microbiology* **62:** 3750-3756.

Index of Fungi. (1999) Index of Fungi. Vol. 6 part 18: 979-980. CABI publishing.

Kelly, W. D. & Williams, J. C. (1982) Incidence of pitch canker among clones of loblolly pine in seed orchards. *Plant Disease* **66:** 1171-1173.

Kerényi, Z., Zeller, K., Hornok, L. & Leslie, J. F. (1999) Molecular standardization of mating type terminology in the *Gibberella fujikuroi* species complex. *Applied and Environmental Microbiology* **65:** 4071-4076.

Kishtah, A. A., Nyland, G., Nasr El-Din, T. M., Tolba, M. A., Lowe, S. K., Khalib, E. M., Tadros, M. R. & El-Amrely, A. (1985) Mango malformation disease in Egypt. I. Electron microscopy, effect on antibiotics, cultural and serological studies. *Egyptian Journal of Phytopathology* **17:** 151-157.

Klaasen, J. A. & Nelson, P. E. (1996) Identification of a mating population, *Gibberella nygamai* sp. nov., within the *Fusarium nygamai* anamorph. *Mycologia* **88:** 965-969.

Klittich, C. J. R. & Leslie, J. F. (1992) Identification of a second mating population within the *Fusarium moniliforme* anamorph of *Gibberella fujikuroi.* *Mycologia* **84:** 541-547.

Klittich, C. J. R., Leslie, J. F., Nelson, P. E. & Marasas, W. F. O. (1997) *Fusarium thapsinum* (*Gibberella thapsina*): a new species in the section *Liseola* from sorghum. *Mycologia* **89:** 643-652.

Kobayashi, T. & Kawabe, Y. (1992) Tree diseases and their causal fungi in Miyako Island. *Japanese Journal of Tropical Agriculture* **36:** 195-206.

Kobayashi, T. & Muramoto, M. (1989) Pitch canker of *Pinus luchuensis,* a new disease in Japanese forsets. *Forest Pests* **38:** 2-6.

Kohn, L. M., Stasovski, E., Carbone, I., Royer, J. & Anderson, J. B. (1991) Mycelial incompatibility and molecular markers identify genetic variability in field populations of *Sclerotinia sclerotiorum.* *Phytopathology* **81:** 480-485.

Kuhlman, E. G. (1982) Varieties of *Gibberella fujikuroi* with anamorphs in *Fusarium* section *Liseola.* *Mycologia* **74:** 759-768.

Kuhlman, E. G., Dwinell, L. D., Nelson, P. E. & Booth, C. (1978) Characterization of the *Fusarium* causing pitch canker of southern pines. *Mycologia* **70:** 1131-1143.

Kumar, J., Singh, U. S. & Beniwal, S. P. S. (1993) Mango malformation: one hundred years of research. *Annual Review of Phytopathology* **31:** 217-232.

Leslie, J. F. (1991) Mating populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). *Phytopathology* **81:** 1058-1060.

Leslie, J. F. (1993) Fungal vegetative compatibility. *Annual Review of Phytopatology* **31:** 127-151.

Leslie, J. F. (1995) *Gibberella fujikuroi:* available populations and variable traits. *Canadian Journal of Botany* **73:** S282-S291.

Leslie, J. F. (1996) Introductory biology of *Fusarium moniliforme.* In *Advances in Experimental Medicine and Biology* vol. 392 (L. S. Jackson, J. W. deVries & L. B. Bullerman, eds): 153-164. Plenum Press, New York, USA.

Leslie, J. F., Hwang, F. & Doe, F. J. (1986) Homothallic and heterothallic isolates of *Fusarium subglutinans. Phytopathology* **76:** 1142.

Leslie, J. F. & Klein, K. K. (1996) Female fertility and mating type effects on effective population size and evolution in filamentous fungi. *Genetics* **144:** 557-567.

Leslie, J. F. & Mansuetus, A. S. B. (1995) Biological species and vegetative compatibility group as population descriptors in *Fusarium.* In *Disease analysis through genetics and biotechnology – interdisciplinary bridges to improved sorghum and millet crops* (J. F. Leslie & R. A. Frederiksen, eds): 277-288. Iowa State University Press, Iowa, USA.

Leslie, J. F., Plattner, R. D., Desjardins, A. E. & Klittich, C. J. R. (1992) Fumonisin B_1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). *Phytopathology* **82:** 341-345.

Longato, S. & Bonfante, P. (1997) Molecular identification of mycorrhizal fungi by direct amplification of microsatellite regions. *Mycological Research* **101:** 425-432.

Levinson, G. & Gutman, G. A. (1987) Slippage-strand mispairing: a major mechanism for DNA sequence evolution. *Molecular Biology Evolution* **4:** 203-221.

Levy, M., Romao, J., Marchetti, M. A. & Hamer, J. E. (1991) DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. *Plant Cell* **3:** 95-102.

Manicom, B. Q. (1989) Blossom malformation of mango. *South African Mango Grower's Association Yearbook* **10:** 11-12.

McCain, A. H., Koehler, C. S. & Tjosvold, S. A. (1987) Pitch canker threatens California pines. *California Agriculture* **41:** 22-23.

McDermott, J. M., McDonald, B. A., Allard, R. W. & Webster, R. K. (1989) Genetic variability for pathogenicity, isozyme, ribosomal DNA and colony color variants in populations of *Rhynchosporium secalis*. *Genetics* **122:** 561-565.

McDonald, B. A. (1997) The population genetics of fungi: tools and techniques. *Phytopathology* **87:** 448-453.

McDonald, B. A. & Martinez, J. P. (1990) DNA restriction fragment length polymorphisms among *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolates collected from a single wheat field. *Phytopathology* **80:** 1368-1373.

McDonald, B. A. & Martinez, J. P. (1991) DNA fingerprinting of the plant pathogenic fungus *Mycosphaerella graminicola* (anamorph *Septoria tritici*). *Experimental Mycology* **15:** 146-158.

McDonald, B. A. & McDermott, J. M. (1993) Population genetics of plant pathogenic fungi. *Bioscience* **43:** 311-319.

McDonald, B. A., McDermott, J. M., Goodwin, S. B. & Allard, R. W. (1989) The population biology of host-pathogen interactions. *Annual Review of Phytopathology* **27:** 77-94.

McGraw, J. R., Wilkinson, R. C., Schmidt, R. A. & Underhill, E. M. (1976) Tip moths and pitch canker in Florida. *Institute for Food and Agricultural Science University of Florida Progress Report* **76-1**.

Milgroom, M. G. (1995) Population biology of the chestnut blight fungus, *Cryphonectria parasitica*. *Canadian Journal of Botany* **73:** S311-S319.

Milgroom, M. G. (1996) Recombination and the multilocus structure of fungal populations. *Annual Review of Phytopathology* **34:** 457-477.

Milgroom, M. G., Wang, K., Zhou, Y., Lipari, S. E. & Kaneko, S. (1996) Intercontinental population structure of the chestnut blight fungus, *Cryphonectria parasitica*. *Mycologia* **88:** 179-190.

Miller, T. & Bramlett, D. L. (1978) Damage to reproductive structures of slash pine by two seed-borne pathogens: *Diplodia gossypina* and *Fusarium moniliforme* var. *subglutinans*. In *Proceedings of Flowering and Seed Development in Trees* (F. Bonner, ed): 347-355. USDA Forest Service, Southern Forest Experimental Station, New Orleans, USA.

Miller, T., Dwinell, L. D., Barrows-Broaddus, J. B. & Alexander, S. A. (1984) Disease management in southern pine seed orchards. In *Proceedings of Integrated Forest Pest Management Symposium* (S. J. Branham & G. D. Hertel, eds): 179-186. Athens, Georgia, USA.

Mitchell, A. G. & Brasier, C. M. (1994) Contrasting structure of European and North American populations of *Ophiostoma ulmi*. *Mycological Research* **98:** 576-582.

Moon, C. D., Tapper, B. A. & Scott, B. (1999) Identification of *Epichloë* endophytes *in planta* by a microsatellite-based PCR fingerprinting assay with automated analysis. *Applied and Environmental Microbiology* **65:** 1268-1279.

Muramoto, M., Minamihashi, H., Mitsuishi, K. & Kobayashi, T. (1988) Occurrence of resiniferous damage on *Pinus luchuensis* Mayr - symptom and analysis of the damage. In *Trans 99th annual meeting of Japan Forestry Society*: 513-514.

Narasimhan, M. J. (1954) Malformation of panicles in mango incited by a species of *Eriophyes*. *Current Science* **23:** 297-298.

Nelson, M. A. (1996) Mating systems in ascomycetes: a romp in the sac. *Trends in Genetics* **12:** 69-74.

Nelson, P. E., Toussoun, T. A. & Marasas, W. F. O. (1983) *Fusarium* species: an illustrated manual for identification. Pennsylvania State University Press, University Park, USA.

Neu, C., Kaemmer, D., Kahl, G., Fischer, D. & Weising, K. (1999) Polymorphic markers for the banana pathogen *Mycosphaerella fijiensis*. *Molecular Ecology* **8:** 523-526.

Nirenberg, H. I. & O'Donnell, K. (1998) New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* **90:** 434-458.

Noriega-Cantú, D. H., Téliz, D., Mora-Aguilera, G., Rodríquez-Alcazar, J., Zavaleta-Mejía, E., Otero-Colinas, G. & Lee Cambell, C. (1999) Epidemiology of mango malformation in Guerrero, Mexico with traditional and integrated management. *Plant Disease* **83:** 223-228.

O'Donnell, K. (1992) Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Current Genetics* **22:** 213-220.

O'Donnell, K. & Cigelnik, E. (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* **7:** 103-116.

O'Donnell, K., Cigelnik, E. & Nirenberg, H. I. (1998) Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia* **90:** 465-493.

O'Donnell, K., Nirenberg, H. I., Aoki, T. & Cigelnik, E. (2000) A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. *Mycoscience* **41:** 61-78.

Old, J. M., Moran, G. F. & Bell, J. C. (1984) Isozyme variability among isolates of *Phytophthora cinnamomi* from Australia and Papua New Guinea. *Canadian Journal of Botany* **62:** 2016-2022.

Ostrander, E. A., Jong, P. M., Rine, J. & Duyk, G. (1992) Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences. *Proceedings of the National Academic of Science, U. S. A.* **89:** 3419-3423.

Ploetz, R. C. (1994a) Part III. Mango. In *Compendium of Tropical Fruit Diseases* (R. C. Ploetz, G. A. Zentmyer, W. T. Nishijima, K. G. Rohrbach & H. D. Ohr, eds): 33-44. St. Paul, MN, American Pathological Society, USA.

Ploetz, R. C. (1994b) Distribution and prevalence of *Fusarium subglutinans* in mango trees affected by malformation. *Canadian Journal of Botany* **72:** 7-9.

Ploetz, R. C. & Gregory, N. F. (1993) Mango malformation in Florida: distribution of *Fusarium subglutinans* in affected trees, and relationships among strains within and among different orchards. *Acta Horticulturae* **341:** 388-394.

Prasad, A., Singh, H. & Shukla, T. N. (1965) Present status of mango malformation disease. *Indian Journal of Horticulture* **22:** 254-265.

Puhalla, J. E. (1985) Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Canadian Journal of Botany* **63:** 179-183.

Rafalski, J. A., Vogel, J. M., Morgante, M., Powell, W., Andre, C. & Tingey, S. V. (1996) Generating and using DNA markers in plants. In *Analysis of non-mammalian*

genomic analysis - a practical guide (B. Birren & E. Lai, eds): 75-134. Academic Press Inc., USA.

Rohrbach, K. G. & Pfeiffer, J. B. (1976) Susceptibility of pineapple cultivars to fruit diseases incited by *Penicillium funiculosum* and *Fusarium moniliforme*. *Phytopathology* **66:** 1386-1390.

Rowan, S. J. (1982) Tip die-back in southern pine nurseries. *Plant Disease* **66:** 258-259.

Runion, G. B. & Bruck, R. I. (1988) Effects of thiabendazole-DMSO treatment of longleaf pine seed contaminated with *Fusarium subglutinans* on germination and seedlings survival. *Plant Disease* **72:** 872-874.

Santos, J. J. G. & Tovar, D. B. (1991) Algunos aspectos sobre el cancro resinoso de los pinos. In *VI Simposio Nacional Sobre Parasitologia Forestal,* October 1991. Unidad de Congresos del Colegio de Postgraduados Montecillos, Edo, Mexico.

Sattar, A. (1946) Diseases of mango in the Punjab. *Punjab Fruit Journal* **10:** 56-58.

Schmidt, R. A., Wilkinson, R. C., Moses, C. S. & Broerman, F. S. (1976) Drought and weevils associated with severe incidence of pitch canker in Volusia County, Florida. *University of Florida Institute Food Agricultural Science Program Report* **76-2**.

Singh, L. B., Singh, S. M. & Nirvan, R. S. (1961) Studies on mango malformation: review, symptoms, extent, intensity and cause. *Horticultural Advance* **5:** 197-207.

Smith, D. N. & Devey, M. E. (1994) Occurrence and inheritance of microsatellites in *Pinus radiata*. *Genome* **37:** 977-983.

Snyder, W. C., Toole, E. R. & Hepting, G. H. (1949) Fusaria associated with mimosa wilt, sumac wilt, and pine pitch canker of southern pines. *Journal of Agricultural Research* **78:** 365-382.

Steenkamp, E. T. (2000). *Gibberella fujikuroi* mating population E associated with maize and teosinte species. In *Molecualr taxonomic studies of selected species in the Gibberella fujikuroi complex,* (Ph.D thesis): 103-119. University of Pretoria, Pretoria, South Africa.

Steenkamp, E. T., Britz, H., Coutinho, T. A., Wingfield, B. D., Marasas, W. F. O. & Wingfield, M. J. (2000a) Molecular characterization of *Fusarium subglutinans* associated with mango malformation. *Molecular Plant Pathology* **1:** 187-193.

Steenkamp, E. T., Wingfield, B. D., Coutinho, T. A., Wingfield, M. J. & Marasas, W. F. O. (1999) Differentiation of *Fusarium subglutinans* f. sp. *pini* by histone gene sequence data. *Applied and Environmental Microbiology* **65:** 3401-3406.

Steenkamp, E. T, Wingfield, B. D., Coutinho, T. A., Zeller, K. A., Wingfield, M. J., Marasas, W. F. O. & Leslie, J. F. (2000b) PCR-based identification of *MAT*-1 and *MAT*-2 in the *Gibberella fujikuroi* species complex. *Applied and Environmental Microbiology* **66:** 4378-4382.

Storer, A. J., Gordon, T. R. & Clark, S. L. (1998) Association of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini,* with Monterey pine seeds and seedlings in California. *Plant Pathology* **47:** 649-656.

Storer, A. J., Gordon, T. R., Dallara, P. L. & Wood, D. L. (1994) Pitch canker kills pines, spreads to new species and regions. *California Agriculture* **48:** 9-13.

Storer, A. J., Gordon, T. R., Wood, D. L. & Bonello, P. (1997) Pitch canker disease of pines, current and future impacts. *Journal of Forestry* **95:** 21-26.

Storer, A. J. & Wood, D. L. (1998) Association between a native spittlebug (Homoptera: *Cercopidae*) on Monterey pine and an introduced tree pathogen which causes pitch canker disease. *Canadian Entomologist* **130:** 783-792.

Summanwar, A. S. & Raychaudhuri, S. P. (1968) The role of eriophyid mite (*Aceria mangiferae*) in the causation of mango malformation. *Indian Phytopathology* **21:** 463.

Summanwar, A. S., Raychaudhuri, S. P. & Phatak, S. C. (1966) Association of the fungus *Fusarium moniliforme* Sheld with the malformation in mango (*Mangifera indica* L.). *Indian Phytopathology* **19:** 227-228.

Sun, S-K. & Snyder, W. C. (1981) The bakane disease of the rice plant. In *Fusarium: disease, biology and taxonomy* (P. E. Nelson, T. A. Toussoun & R. J. Cook, eds): 104-113. University Park, PA. The Pennsylvania State University Press, USA.

Tautz, D. (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* **17:** 6463-6471.

Tautz, D. & Renz, M. (1984) Simple sequences are ubiquitous repetitive components of eukaryote genomes. *Nucleic Acids Research* **12:** 4127-4138.

Tenzer, I., Degli Ivanissevich, S., Morgante, M. & Gessler, C. (1999) Identification of microsatellite markers and their application to population genetics of *Venturia inaequalis*. *Phytopathology* **89:** 748-753.

van der Nest, M. A., Steenkamp, E. T., Wingfield, B. D. & Wingfield, M. J. (2000) Development of simple sequence repeat (SSR) markers in *Eucalyptus* from amplified inter-simple sequence repeats (ISSR). *Plant Breeding* **119:** 433-436.

Varma, A. (1983) Mango malformation. In *Exotic plant quarantine pests and procedures for introduction of plant materials* (K. G. Singh, ed): 173-188. Asean plant quarantine centre and training institute, Malaysia.

Varma, A., Lele, V. C., Raychaudhuri, S. P., Ram, A. & Sang, A. (1974) Mango malformation: a fungal disease. *Phytopathologische Zeitschrift* **79:** 254-257.

Varma, A., Raychaudhuri, S. P., Lele, V. C. & Ram, A. (1971) Preliminary investigations on epidemiology and control of mango malformation. *Proceedings of Indian National Science Academy* **37B:** 291-300.

Ventura, J. A., Zambolim, L. & Gilbertson, R. L. (1993) Pathogenicity of *Fusarium subglutinans* to pineapples. *Fitopathologia Brasileira Suplemento* **18:** 280.

Viljoen, A., Marasas, W. F. O., Wingfield, M. J. & Viljoen, C. D. (1997a) Characterization of *Fusarium subglutinans* f. sp. *pini* causing root disease of *Pinus patula* seedlings in South Africa. *Mycological Research* **101:** 437-445.

Viljoen, A., Wingfield, M. J., Gordon, T. R. & Marasas, W. F. O. (1997b) Genotypic diversity in a South African population of the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. *Plant Pathology* **46:** 590-593.

Viljoen, A., Wingfield, M. J., Kemp, G. H. J. & Marasas, W. F. O. (1995a) Susceptibility of pines in South Africa to the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. *Plant Pathology* **44:** 877-882.

Viljoen, A., Wingfield, M. J. & Marasas, W. F. O. (1994) First report of *Fusarium subglutinans* f. sp. *pini* on pine seedlings in South Africa. *Plant Disease* **78:** 309-312.

Viljoen, A., Wingfield, M. J., Marasas, W. F. O. & Coutinho, T. A. (1995b) Pitch canker of pines - a contemporary review. *South African Journal of Science* **93:** 411-413.

Voigt, K., Schleier, S. & Brückner, B. (1995) Genetic variability in *Gibberella fujikuroi* and some related species of the genus *Fusarium* based on random amplification of polymorphic DNA (RAPD). *Current Genetics* **27:** 528-535.

Waalwijk, C., de Koning, J. R. A., Baayen, R. P. & Gams, W. (1996) Discordant groupings of *Fusarium* spp. from sections *Elegans, Liseola* and *Dlaminia* based on ribosomal ITS1 and ITS2 sequences. *Mycologia* **88:** 361-368.

Wahba, M. L., El-Enany, M. A. M. & Farrag, A. M. I. (1986) Five mango varieties as affected by malformation phenomenon and bud mite infestation in Egypt. *Agricultural Research Review* **61:** 193-201.

Wallace, M. M. & Covert, S. F. (2000) Molecular mating type assay for *Fusarium circinatum*. *Applied and Environmental Microbiology* **66:** 5506-5508.

Weber, J. L. & May, P. E. (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics* **44:** 388-396.

Welsh, J. & McClelland, M. (1990) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* **18:** 7213-7218.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: a guide to methods and applications*, (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315-322. Academic Press, New York, USA.

Wikler, K. & Gordon, T. R. (1999) Population dynamics of *Fusarium circinatum*. In *Current and potential impacts of pitch canker in radiata pine* (M. E. Devey, A. C. Matheson & T. R. Gordon, eds): 33-34. Proceedings of the IMPACT Monterey Workshop.

Wikler, K., Gordon, T. R., Clark, S. L., Wingfield, M. J. & Britz, H. (2000) Potential for outcrossing in an apparently asexual population of *Fusarium circinatum*, the causal agent of pitch canker disease. *Mycologia* **92:** 1085-1090.

Wikler, K. & Gordon, T. R. (2000) An initial assessment of genetic relationships among populations of *Fusarium circinatum* in different parts of the world. *Canadian Journal of Botany* **78:** 709-717.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18:** 6531-6535.

Wingfield, M. J., Wingfield, B. D., Coutinho, T. A., Viljoen, A., Britz, H. & Steenkamp, E. T. (1999) Pitch canker: a South African perspective. In *Current and potential impacts of pitch canker in radiata pine,* (M. E. Devey, A. C. Matheson, & T. R. Gordon, eds): 62-69. Proceedings of the IMPACT Monterey Workshop, California, USA.

Wollenweber H. W. & Reinking, O. A. (1925) Aliquot Fusaria tropicalla nova vel revisa. *Phytopathology* **15:** 155-169.

Wollenweber H. W. & Reinking, O. A. (1935) Die Fusarien, ihre Beschreibung, Schadwirkung und Bekämpfung. Berlin, Paul Parey. 355 pp.

Wright, S. (1931) Evolution in Mendelian populations. *Genetics* **16:** 97-159.

Xu, J-R., Yan, K., Dickman, M. B. & Leslie, J. F. (1995) Electrophoretic karyotypes distinguish the biological species of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). *Molecular Plant-Microbe Interaction* **8:** 74-84.

Yoder, O. C., Valent, B. & Chumley, F. (1986) Genetic nomenclature and practice for plant pathogenic fungi. *Phytopathology* **76:** 383-385.

Zheng, Q. & Ploetz, R. (2001) Genetic diversity in, and development of a PCR assay for identifying, the mango malformation pathogen. *Plant Pathology* (in press).

Table 1. A representation of various classifications that have been used for *Fusarium subglutinans*.

Wollenweber & Reinking (1935)	Bilai (1955)	Booth (1971)	Gerlach & Nirenberg (1982)	Nelson *et al.* 1983	Nirenberg & O'Donnell (1998)
F. neoceras			*F. neoceras*		
			F. sacchari var. *sacchari*		*F. sacchari*
				F. subglutinans f. sp. *pini*	*F. circinatum*
	F. moniliforme var. *subglutinans*			*F. subglutinans* f. sp. *ananas*	*F. guttiforme*
			F. sacchari var. *subglutinans*	*F. subglutinans*	*F. subglutinans*
F. moniliforme var. *subglutinans*		*F. moniliforme* var. *subglutinans*			
			F. sacchari var. *elongatum*		*F. bulbicola*
	F. moniliforme var. *anthophilum*		*F. anthophilum*	*F. anthophilum*	*F. anthophilum*

Table 2. Mating populations in the *Gibberella fujikuroi* complex

MP	*Fusarium* species	Synonym	Host	References
A	*F. verticillioides*	*F. moniliforme*	Maize	Booth, 1971
		G. fujikuroi var. *moniliformis*		Kuhlman, 1982
B	*F. sacchari*	*F. moniliforme* var.	Sugarcane	Booth, 1971
		subglutinans		Gerlach & Nirenberg, 1982
		F. sacchari var. *sacchari*		Kuhlman, 1982
		G. fujikuroi var. *subglutinans*		Nelson *et al.*, 1983
		F. subglutinans		
C	*F. fujikuroi*	*G. fujikuroi* var. *fujikuroi*	Rice	Booth, 1971
		F. proliferatum		Gerlach & Nirenberg, 1982
				Kuhlman, 1982
				Nelson *et al.*, 1983
D	*F. proliferatum*	*G. fujikuroi* var. *intermedium*	Asparagus	Booth, 1971
				Gerlach & Nirenberg, 1982
				Kuhlman, 1982
				Nelson *et al.*, 1983

MP	*Fusarium* species	Synonym	Host	References
E	*F. subglutinans*	*F. moniliforme* var. *subglutinans*	Maize	Booth, 1971 Gerlach & Nirenberg, 1982
		F. sacchari var. *subglutinans*		Kuhlman, 1982
F	*F. thapsinum*	*F. moniliforme*	Sorghum	Booth, 1971 Nelson *et al.*, 1983 Klittich *et al.*, 1997
G	*F. nygamai*	-	Sorghum	Burgess & Trimboli, 1986
H	*F. circinatum*	*F. moniliforme* var. *subglutinans*	Pine	Booth, 1971 Kuhlman, 1982
		F. subglutinans f. sp. *pini*		Nelson *et al.*, 1983 Correll *et al.*, 1991 Nirenberg & O'Donnell, 1998 Britz *et al.*, 1999

CHAPTER 2

CHARACTERIZATION OF THE PITCH CANKER FUNGUS, *FUSARIUM CIRCINATUM* FROM MEXICO

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ABSTRACT

Fusarium circinatum (=*F. subglutinans* f. sp. *pini*) is the causal agent of pitch canker of pines. This fungus occurs in the United States, Japan, Mexico and South Africa and it can be introduced into new areas on seed and infected plant material. Its presence in cones from symptomless trees is of concern, particularly with respect to seed transmission. In this study, isolates of *Fusarium* spp. were collected from *Pinus patula, P. greggii, P. teocote* and *P. leiophylla* trees in Mexico, showing typical symptoms of pitch canker, as well as from cones from apparently healthy trees. Morphological characteristics of the pitch canker fungus and isolates of *F. subglutinans* from other hosts are very similar. Therefore, pathogenicity tests, sexual compatibility studies and histone *H3*-RFLPs were used to characterize isolates. Isolates collected from *Pinus* spp. from Mexico were identified as *F. circinatum.* In this study we have thus confirmed that *F. circinatum* occurs on pines in Mexico and that the affected trees can be asymptomatic.

INTRODUCTION

Pitch canker, caused by *Fusarium circinatum* Nirenberg & O'Donnell (=*F. subglutinans* (Wollenweber & Reinking) Nelson *et al.* f. sp. *pini* Correll *et al.*), was first reported in the southeastern United States (Hepting & Roth, 1946). *F. circinatum* is now found throughout this region where it has caused significant losses on a wide variety of pine species. This led to the suggestion that pitch canker is endemic to the area (Dwinell *et al.*, 1985). More recently, pitch canker was identified and reported in California (McCain *et al.*, 1987), predominately on *Pinus radiata* planted in landscape settings (Correll *et al.,* 1991). Since 1992, it has been recognized as a threat to native *P. radiata* stands in California (Storer *et al.,* 1994; Storer *et al.*, 1998). *F. circinatum* is also found in Japan (Muramoto *et al.,* 1988; Kobayashi & Kawabe, 1992) and South Africa (Viljoen *et al.*, 1994; Viljoen *et al.*, 1997).

In South Africa, the fungus was reported from forestry nurseries where it has resulted in serious losses of *P. patula* (Viljoen *et al.,* 1994), *P. elliottii, P. greggii* and *P. radiata* seedlings. Stem cankers on larger trees such as those found in the United States (Hepting & Roth, 1946; Dwinell *et al.,* 1977) have not been seen in South

Africa (Wingfield *et al.*, 1999). Pitch canker has been reported in Mexico on a variety of native pine species (Santos & Tovar, 1991; Guerra-Santos, 1999) and this is thought to be the origin of the pathogen, for areas such as South Africa where Mexican pines are commonly propagated.

Fusarium circinatum has been isolated from stems and branches of trees (Hepting & Roth, 1946; Dwinell *et al.*, 1977), root collars of pine seedlings (Barnard & Blakeslee, 1980; Viljoen *et al.*, 1994), female strobili, mature cones and seeds (Dwinell *et al.*, 1977; Miller & Bramlett, 1978; Dwinell *et al.*, 1985; Barrows-Broaddus, 1986; 1987; Storer *et al.*, 1998; Dwinell, 1999). Recently, Storer *et al.* (1998) isolated *F. circinatum* from pine seedlings originating from seeds collected from cones on diseased as well as asymptomatic branches. Those authors hypothesized that *F. circinatum* can be carried inside seeds and may remain dormant until germination, which increases the possibility of seedling infections. The implication of seed transmission is serious, since current treatments may be ineffective in eliminating the pathogen. This would increase the possibility of introducing the pathogen into uninfested areas (Barrows-Broaddus & Dwinell, 1985; Storer *et al.*, 1998).

Fusarium subglutinans sensu lato includes species occurring on a wide variety of hosts, including pineapple, maize, mango, pine and sugarcane (Booth, 1971). Correll *et al.* (1991) distinguished pine and non-pine *F. subglutinans* isolates based on pathogenicity to pines. Those authors proposed that *F. subglutinans* from pine should be designated as *F. subglutinans* f. sp. *pini* based on its exclusive pathogenicity to pine trees (Correll *et al.*, 1991). Restriction fragment patterns of the mtDNA and random amplified polymorphic DNA (RAPD) also indicated that pine isolates differed from non-pine isolates (Correll *et al.*, 1992; Viljoen *et al.*, 1997).

Sexual compatibility among isolates causing pitch canker on pines confirmed that this group corresponded to a distinct biological species (Viljoen *et al.*, 1997). O'Donnell *et al.* (1998) showed pine isolates to be phylogenetically distinct and Nirenberg & O'Donnell (1998) thus proposed the name, *F. circinatum,* for it. Steenkamp *et al.*

(1999) could, furthermore, distinguish *F. circinatum* from other species in *F. subglutinans sensu lato* using histone *H3* gene sequences.

Until recently, the most reliable technique to distinguish *F. circinatum* from closely related *Fusarium* spp. has been sexual compatibility. A molecular technique based on RFLP profiles of the histone *H3* gene, reliably and rapidly distinguishes *F. circinatum* from other similar *Fusarium* spp. in the *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura complex (Steenkamp *et al.*, 1999). Sexual compatibility as well as histone *H3*-RFLPs can, therefore, be used to separate the eight different mating populations (biological species), designated by the letters A to H, in this complex. Heterothallic *F. circinatum* isolates reside in mating population H of the *G. fujikuroi* complex and tester strains representing opposite mating types have been selected and designated (Coutinho *et al.* 1995; Britz *et al.*, 1998; 1999). Sexual compatibility of field isolates with tester strains of mating population H (MRC 6213 and MRC 7488) provides a firm basis for the identification of field isolates as *F. circinatum.*

In this study, isolations from pine trees in Mexico showing typical canker symptoms were made. The possible association of *F. circinatum* with asymptomatic cones was also investigated. The identity of these isolates was verified using morphology (Nelson *et al.*, 1983; Nirenberg & O'Donnell, 1998) as well as pathogenicity, sexual compatibility and histone *H3*-RFLP comparisons.

MATERIALS AND METHODS

Isolates, cultural and morphological characteristics

Fusarium circinatum strains (MRC 7568-7587) were collected in Laguna Atezca and Hidalgo, Mexico, from cankers occurring on branches of native stands of *P. patula* and *P. greggii* (Table 1). Strains MRC 7568-7569 were isolated from apparently healthy cones collected from asymptomatic *P. patula* trees and strains MRC 7570-7587 were obtained from branches showing pitch canker symptoms. Strains MRC 7570–7572, MRC 7573-7576, 7577-7579, 7580-7582 and 7583-7585 were isolated from five trees. Strains MRC 7588-7601 were isolated from cankers on branches of

P. teocote and *P. leiophylla* in northern Michoacan, Mexico (Table 1). Single conidial isolates of all cultures were prepared and are maintained in 15% glycerol at –70°C in the *Fusarium* collection of the Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. Isolates have also been deposited in the culture collection of the Medical Research Council (MRC), P. O. Box 19070, Tygerberg, South Africa.

For isolations, pine cones were immersed in 70% ethanol for 2 min. Small pieces (approximately 5 mm^2) of the asymptomatic cone scale tissue and wood pieces (2 mm^2) from the infected tree branches were removed and plated on 2% malt extract agar (MEA). Fungi were allowed to grow for seven days at room temperature. Small agar pieces (approximately 5 mm^2) from the edges of the colonies were transferred to 90 mm plastic Petri dishes containing carnation leaf agar (CLA) (Fisher *et al.*, 1982). Cultures were incubated at 23°C under near-ultraviolet and cool-white light with a 12 h photoperiod to stimulate culture and conidium development. Isolates were identified and morphological features were compared with *F. circinatum* tester strains (MRC 6213 and MRC 7488) after 10 to 14 days, using a light microscope. Diagnostic characteristics used were those specified by Nirenberg & O'Donnell (1998) and Nelson *et al.* (1983).

All isolates obtained from Mexico (Table 1), as well as, *F. circinatum* tester strains (MRC 6213 and MRC 7488) from South Africa were used in sexual compatibility tests and for comparison based on histone *H3*-RFLPs. *F. sacchari* (Butler) W. Gams (= *F. subglutinans*) and *F. subglutinans* standard tester strains of the B (MRC 6524 and MRC 6525) and E (MRC 6483 and MRC 6512) mating populations of the *G. fujikuroi* complex were also included. Tester strains of the B mating population are progeny from a fertile cross between two isolates from sugarcane (*Saccharum officinarum*) from Hsingying, Taiwan and tester strains of the E mating population were isolated from maize (*Zea mays*) in St. Elmo, Illinois, USA (Leslie, 1991). *F. subglutinans* isolates from maize (MRC 1077) and mango (*Mangifera indica*) (MRC

7035) were included in the histone *H3*-RFLP comparisons as representatives of host specific isolates of *F. subglutinans* (Table 1).

RFLPs of Histone H3 gene

DNA was extracted from cultures (Table 1) grown for 10 days in complete medium broth (CM) (Correll *et al.*, 1987) using the protocol described by Steenkamp *et al.* (1999). PCR reactions were performed using primers (H3-1a and H3-1b) for amplification of the Histone *H3* gene PCR product as described by Glass & Donaldson (1995). The same conditions as those described by Steenkamp *et al.* (1999) were used except Boehringer Mannheim polymerase and reaction buffers (Boehringer Mannheim South Africa Pty. Ltd.) were used. The restriction enzyme, *Dde* I, which distinguishes *F. circinatum* isolates from other similar *Fusarium* spp. (Steenkamp *et al.* 1999), was used in this study. Digests were performed using this restriction enzyme in a total reaction volume of 20 µl containing 5 U of the enzyme. Sodium chloride was added to the reactions with the enzyme *Dde* I to a final concentration of 100 mM. All digestion reactions were incubated at 37°C for 7 h.

Restriction fragments were separated using agarose gels (Promega Corporation, Madison, Wisconsin, USA) in the presence of ethidium bromide (0.1 µg/ml). RFLP fragments were electrophoresed on 3% (w/v) agarose gels and visualized using an ultraviolet transilluminator (Ultra-Violet Product). The visualized RFLP fragments were photographed using a gel documentation system (Microsoft Corporation) and evaluated using the methods described by Steenkamp *et al.* (1999).

Sexual compatibility

Crosses to determine sexual compatibility were made on carrot agar as described by Klittich & Leslie (1988), except that 300 g fresh carrots were used rather than the recommended 400 g. All crosses were also done on V8-agar (325 ml canned V8-juice per liter and 2% agar, pH 5.8-6.2). Hermaphrodite tester strains of the B, E and H mating populations of *G. fujikuroi* were crossed with each another (MRC 6524 x MRC 6525; MRC 6483 x MRC 6512 and MRC 6213 x MRC 7488). Isolates from Mexico were crossed with standard tester strains from the B (MRC 6524 and MRC

6525), E (MRC 6483 and MRC 6512) and H (MRC 6213 and MRC 7488) mating populations of the *G. fujikuroi* complex. All field isolates of *F. circinatum* from Mexico were crossed with each other in all possible combinations. The isolates in this study were also crossed against themselves as a negative control, i.e. where no mature perithecia should be produced. Reciprocal crosses, where the isolates corresponded to the male and female parents were reversed, were done for all crosses.

All the crosses recorded as positive were repeated at least once. Crosses were examined weekly and scored as positive when ascospores were observed, either by their exudation from perithecia or after crushing these structures. The viability of the ascospores was determined by streaking a portion of the ascospore cirrhus onto the surface of 2% water agar plates and estimating the percentage germination after 24 h.

Pathogenicity tests

Due to quarantine constraints in South Africa, pathogenicity tests of *F. circinatum* isolates from Mexico were conducted in greenhouse facilities of the Department of Plant Pathology, University of California. Tests to confirm pathogenicity were performed at approximately 25°C during the day and 18°C at night with a 12 h day/night cycle. Tests were performed on *P. radiata* seedlings, 3-4 years of age using 19 *F. circinatum* isolates collected in Mexico (Table 1), as well as, two isolates of *F. circinatum* (FSP 24 and FSP 34), known to be pathogenic to pines in California. All *F. circinatum* isolates were grown on potato dextrose agar (PDA) at 25°C for 7–10 days. Inoculations were performed by making a small wound in the seedling stems and placing a spore suspension (approximately 500 spores in distilled water) into each wound. Each isolate was inoculated into two *P. radiata* seedlings. The lesion lengths under the bark of the inoculated *P. radiata* plants were measured 41 days after inoculation.

RESULTS

Isolates, cultural and morphological characteristics

Isolates obtained from diseased pine branches, cankers and asymptomatic cones were identified as *F. subglutinans* based on morphological characteristics described by Nelson *et al.* (1983). These isolates could also be identified as *F. circinatum* based on the characteristics proposed by Nirenberg & O'Donnell (1998). Branched and proliferating conidiophores were observed and the polyphialides had 2-5 conidiogenous openings (Fig. 1c). Sterile coiled hyphae (Fig. 1d) and lunate macroconidia (Fig. 1e), reported by Nirenberg & O'Donnell (1998) to distinguish *F. circinatum* from similar *Fusarium* spp. in *Liseola* and related sections, were observed.

RFLPs of Histone H3 gene

The PCR products obtained using the primers H3-1a and H3-1b were approximately 500 base pairs (bp) in size. None of the PCR products from isolates belonging to the E mating population (MRC 6483 and MRC 6512) of the *G. fujikuroi* complex and the *F. subglutinans* isolate from maize (MRC 1077) were cut by *Dde* I, whereas the *F. subglutinans* isolate from mango had three RFLP fragments of approximately 110, 170 and 220 bp. All the isolates identified as *F. circinatum* based on morphology had the same banding pattern as the *F. circinatum* tester strains (MRC 6213 and MRC 7488), where two fragments of approximately 230 and 270 bp were evident (Fig. 2). Two histone *H3*-RFLP fragments of approximately 190 and 310 bp could be visualized for isolates in the B mating population (Fig. 2).

Sexual compatibility

Using sexual compatibility tests, we were able to verify that isolates from pine in Mexico belong to mating population H and, therefore, *F. circinatum* (Fig. 1). Perithecia with exuding ascospores (Fig. 1a, b) were produced four weeks after fertilization of the control crosses between tester strains of the B, E and H mating populations (MRC 6524 x MRC 6525; MRC 6483 x MRC 6512 and MRC 6213 x MRC 7488). The isolates from Mexico did not produce perithecia when crossed with

tester strains of either the B (MRC 6524 and MRC 6525) or the E (MRC 6483 and MRC 6512) mating populations. Mexican isolates MRC 7568, 7569, 7572, 7591, 7592, 7593, 7595, 7597, 7599 and 7600 produced perithecia with viable ascospores when crossed with the mating population H tester strain, MRC 6213. These isolates only produced fertile crosses when MRC 6213 was used as the female parent and are thus female-sterile. No mature perithecia with viable ascospores resulted from crosses amongst *F. circinatum* isolates from Mexico.

All the fertile crosses recorded in this study were repeated and identical results were obtained in at least two different tests. The same results were also obtained on both carrot and V8-agar. The percentage germination of ascospores in this study varied between 85-98%. None of the isolates in this study produced perithecia when crossed with themselves as negative controls.

Pathogenicity tests

The 21 selected *F. circinatum* isolates gave lesions after 41 days that varied in length between 10 to 90 mm. These isolates were, thus, pathogenic to *P. radiata* seedlings in the glasshouse. The pathogenicity of the *F. circinatum* isolates from California (FSP 24 and FSP 34) was consistent with that reported previously (Correll *et al.*, 1991; Gordon *et al.*, 1996).

DISCUSSION

In this study, we were able to identify *F. circinatum* from branches, cankers and asymptomatic cones from Mexico, based on a wide range of criteria. The association of the pitch canker fungus with asymptomatic cones demonstrates the possibility of spread on apparently healthy seeds such as reported by Storer *et al.* (1998). *F. circinatum* isolates considered in this study were collected from *P. patula, P. greggii, P. teocote* and *P. leiophylla* in Mexico. *P. patula, P. elliottii,* and *P. radiata* are the most important, commercially planted species in South Africa (Hinze, 1993). More recently, *P. greggii* has also become important to the forestry industry in South Africa (Malan, 1994). The isolation of *F. circinatum* from *P. patula* and *P. greggii* in native stands in Mexico, indicates that the pitch canker fungus could have been introduced

into South Africa from Mexico. This would most likely have occurred through seed importation. These findings emphasize the importance of screening seed for *F. circinatum* infection before it is exported. Seed treatment with fungicides would also reduce the chances of new introductions occurring, although it might not effectively eliminate internal infections (Storer *et al.*, 1998; 1999). Small seed lots, where the risk can be reduced through propagation under controlled conditions, may be an acceptable practice. The importation of large collections of seed for commercial planting cannot be managed effectively, and should be avoided.

In this study, only a small number (less than 28%) of *F. circinatum* isolates from Mexico were able to cross with the single *F. circinatum* tester (MRC 6213), from South Africa. It is possible that the isolates from Mexico that did not cross with MRC 6213 all belonged to the same mating type as this South African tester. Alternatively, low fertility or sterility (Perkins, 1994) could explain why none of these isolates cross with the tester strain of the opposite mating type. Female-sterility (Leslie & Klein, 1996) could also have contributed to the lack of sexual compatibility seen between Mexican *F. circinatum* isolates. The low level of sexual compatibility might also suggest that the population of *F. circinatum* in Mexico is evolving towards an asexual life history (Leslie & Klein, 1996). This is indicated by the high percentage of female-sterile isolates found in the sexual compatibility study. Despite the low level of sexual compatibility, sexual crosses confirmed that some of the Mexican isolates belonged to mating population H of *G. fujikuroi*.

Steenkamp *et al.* (1999) showed that *F. subglutinans* isolates from various plant hosts can be distinguished from one another with histone *H3*-RFLPs. Those authors concluded that this technique could be used for routine identification of *F. circinatum*. In this study, the histone *H3*-RFLP technique was critical for positive identification of *F. circinatum*. This was particularly due to the sometimes inconclusive and time-consuming pathogenicity tests and the low fertility among the *F. circinatum* isolates from Mexico.

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REFERENCES

Barnard, E. L. & Blakeslee, G. M. (1980) Pitch canker of slash pine seedlings: a new disease in forest tree nurseries. *Plant Disease* **64:** 695-696.

Barrows-Broaddus, J. B. & Dwinell, L. D. (1985) Branch dieback and cone and seed infection caused by *Fusarium moniliforme* var. *subglutinans* in a loblolly pine seed orchard in South Carolina. *Phytopathology* **75:** 1104-1108.

Barrows-Broaddus, J. B. (1986) A cone and seed disease of southern pines caused by the pitch canker fungus. In *Current Topics in Forest Research: Emphasis on Contributions by Women Scientists*: 150-154. Proceedings of a National Symposium, Gainesville, Florida, USA. General Technical Report SE-46.

Barrows-Broaddus, J. B. (1987) Pitch canker. In *Cone and Seed Disease of North America Conifers* (J. R. Sutherland, T. Miller & R. S. Quinard, eds): *North American Forestry Commission Publication* **1:** 42-49. Victoria, British Columbia, Canada.

Booth, C. (1971) The genus *Fusarium.* Commonwealth Mycological Institute. Kew, Surrey, U. K.

Britz, H., Coutinho, T. A., Wingfield, M. J., Marasas, W. F. O., Gordon, T. R. & Leslie, J. F. (1999) *Fusarium subglutinans* f. sp. *pini* represents a distinct mating population in the *Gibberella fujikuroi* species complex. *Applied and Environmental Microbiology* **65:** 1198-1201.

Britz, H., Wingfield, M. J., Coutinho, T. A., Marasas, W. F. O. & Leslie, J. F. (1998) Female fertility and mating type distribution in a South African population of *Fusarium subglutinans* f. sp. *pini*. *Applied and Environmental Microbiology* **64:** 2094-2095.

Correll, J. C., Gordon, T. R. & McCain, A. H. (1992) Genetic diversity in California and Florida populations of the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. *Phytopathology* **82:** 415-420.

Correll, J. C., Gordon, T. R., McCain, A. H., Fox, J. W., Koehler, C. S., Wood, D. L. & Schultz, M. E. (1991) Pitch Canker Disease in California: pathogenicity, distribution, and canker development on Monterey pine (*Pinus radiata*). *Plant Disease* **75:** 676-682.

Correll, J. C., Klittich, C. J. R. & Leslie, J. F. (1987) Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* **77:** 1640-1646.

Coutinho, T. A., Viljoen, A., Wingfield, M. J. & Marasas, W. F. O. (1995) Selection of sexually fertile parents of *Fusarium subglutinans* f. sp. *pini*. *South African Journal of Science* **91:** 17.

Dwinell, L. D. (1999) Association of the pitch canker fungus with cones and seeds of pines. In *Current and potential impacts of pitch canker in radiata pine* (M. E. Devey, A. C. Matheson & T. R. Gordon, eds): 35-39. Proceedings of the IMPACT Monterey Workshop, California.

Dwinell, L. D., Barrows-Broaddus, J. B. & Kuhlman, E. G. (1985) Pitch canker: a disease complex of southern pines. *Plant Disease* **69:** 270-276.

Dwinell, L. D., Ryan, P. L. & Kuhlman, E. G. (1977) Pitch canker of loblolly pine in seed orchards. In *Proceedings of the 14th Southern Forest Tree Improvement Conference*: 130-137. Gainesville, Florida, USA.

Fisher, N. L., Burgess, L. W., Toussoun, T. A. & Nelson, P. E. (1982) Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* **72:** 151-153.

Glass, N. L. & Donaldson, G. C. (1995) Development of primer sets designed for use with the PCR to amplify filamentous ascomycetes. *Applied and Environmental Microbiology* **61:** 1323-1330.

Gordon, T. R., Storer, A. J. & Okamoto, D. (1996) Population structure of the pitch canker pathogen, *Fusarium subglutinans* f. sp. *pini,* in California. *Mycological Research* **100:** 850-854.

Guerra-Santos, J. J. (1999) Pitch canker on Monterey Pine in Mexico. In *Current and potential impacts of pitch canker in radiata pine* (M. E. Devey, A. C. Matheson & T. R. Gordon, eds): 58-61. Proceedings of the IMPACT Monterey Workshop, California, USA.

Hepting, G. H. & Roth, E. R. (1946) Pitch canker, a new disease of some southern pines. *Journal of Forestry* **44:** 742-744.

Hinze, W. H. F. (1993) Silviculture of pines. In *Forestry Handbook* (H. A. van der Sijde, ed): 38-48. Southern African Institute of Forestry, Pretoria, South Africa.

Klittich, C. J. R. & Leslie, J. F. (1988) Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). *Genetics* **118:** 417-423.

Kobayashi, T. & Kawabe, Y. (1992) Tree disease and their causal fungi in Miyako Island. *Japanese Journal of Tropical Agriculture* **36:** 195-206.

Leslie, J. F. (1991) Mating populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). *Phytopathology* **81:** 1058-1060.

Leslie, J. F. & Klein, K. K. (1996) Female fertility and mating type effects on effective population size and evolution in filamentous fungi. *Genetics* **144:** 557-567.

Malan, F. S. (1994) The wood properties and qualities of *Pinus pringlei* and *P. greggii* compared with that of *P. patula* and *P. elliottii* grown in South Africa. *South African Forestry Journal* **171:** 43-52.

McCain, A. H., Koehler, C. S. & Tjosvold, S. A. (1987) Pitch canker threatens California pines. *California Agriculture* **41:** 22-23.

Miller, T. & Bramlett, D. L. (1978) Damage to reproductive structures of slash pine by two seed-borne pathogens: *Diplodia gossypina* and *Fusarium moniliforme* var. *subglutinans*. In *Proceedings of Flowering and Seed Development in Trees* (F. Bonner, ed): 347-355. USDA Forest Service, Southern Forest Experimental Station, New Orleans, USA.

Muramoto, M., Minamihashi, H., Mitsuishi, K. & Kobayashi, T. (1988) Occurrence of resiniferous damage on *Pinus luchuensis* Mayr - Symptom and analysis of the damage. In *Trans 99th annual meeting of Japan Forestry Society*: 513-514.

Nelson, P. E., Toussoun, T. A. & Marasas, W. F. O. (1983) *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park, Pennsylvania.

Nirenberg, H. I. & O'Donnell, K. (1998) New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* **90:** 434-458.

O'Donnell, K., Cigelnik, E. & Nirenberg, H. I. (1998) Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia* **90:** 465-493.

Perkins, D. D. (1994) How should the interfertility of interspecies crosses be designated? *Mycologia* **86:** 758-761.

Santos, J. J. G. & Tovar, D. B. (1991) Algunos aspectos sobre el cancro resinoso de los pinos. In *VI Simposio Nacional Sobre Parasitologia Forestal,* October 1991. Unidad de Congresos del Colegio de Postgraduados Montecillos, Edo, Mexico.

Steenkamp, E. T., Wingfield, B. D., Coutinho, T. A., Wingfield, M. J. & Marasas, W. F. O. (1999) Differentiation of *Fusarium subglutinans* f. sp. *pini* by histone gene sequences data. *Applied and Environmental Microbiology* **65:** 3401-3406.

Storer, A. J., Gordon, T. R. & Clark, S. L. (1999) The association of the pitch canker pathogen with Monterey pine seeds in California. In *Current and potential impacts of pitch canker in radiata pine* (M. E. Devey, A. C. Matheson & T. R. Gordon, eds): 40-44. Proceedings of the IMPACT Monterey Workshop, California, USA.

Storer, A. J., Gordon, T. R. & Clark, S. L. (1998) Association of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini,* with Monterey pine seeds and seedlings in California. *Plant Pathology* **47:** 649-656.

Storer, A. J., Gordon, T. R., Dallara, P. L. & Wood, D. L. (1994) Pitch canker kills pines, spreads to new species and regions. *California Agriculture* **48:** 9-13.

Viljoen, A., Marasas, W. F. O., Wingfield, M. J. & Viljoen, C. D. (1997) Characterization of *Fusarium subglutinans* f. sp. *pini* causing root disease of *Pinus patula* seedlings in South Africa. *Mycological Research* **101:** 437-445.

Viljoen, A., Wingfield, M. J. & Marasas, W. F. O. (1994) First report of *Fusarium subglutinans* f. sp. *pini* on pine seedlings in South Africa. *Plant Disease* **78:** 309-312.

Wingfield, M. J., Wingfield, B. D., Coutinho, T. A., Viljoen, A., Britz, H. & Steenkamp, E. T. (1999) Pitch canker: a South African perspective. In *Current and potential impacts of pitch canker in radiata pine* (M. E. Devey, A. C. Matheson & T. R. Gordon, eds): 62-69. Proceedings of the IMPACT Monterey Workshop, California, USA.

Table 1. List of *Fusarium* cultures used in this study.

Fusarium species	Host, substrate and origin	Isolates[a]
F. circinatum	*P. patula* cones, Hidalgo, Mexico	MRC 7568*, 7569*
F. circinatum	*P. patula* branches, Hidalgo, Mexico	MRC 7570*, 7571*, 7572*
F. circinatum	*P. greggii* branches, Laguna Atezca, Mexico	MRC 7573*, 7574*, 7575*, 7576*, 7577*, 7578*, 7579*, 7580*, 7581*, 7582*, 7583*, 7584*, 7585*, 7586*, 7587
F. circinatum	*P. teocote*, Northeastern Michoacan, Mexico	MRC 7588, 7589, 7590, 7591, 7592, 7593, 7594, 7595, 7596
F. circinatum	*P. leiophylla,* North-central Michoacan, Mexico	MRC 7597, 7598, 7599, 7600, 7601
F. circinatum	*P. radiata,* California	FSP 24*, 34*
F. circinatum	*P. patula* seedling, Ngodwana, South Africa	MRC 6213, 7488
F. sacchari	*Saccharum officinarum,* Taiwan	MRC 6524, 6525
F. subglutinans	*Zea mays*, South Africa	MRC 1077
F. subglutinans	*Z. mays,* Illinois, USA	MRC 6483, 6512
Fusarium sp.	*Mangifera indica*, Florida, USA	MRC 7035

[a] MRC refers to the culture collection of the Medical Research Council, P.O. Box 19070, Tygerberg, South Africa and FSP = *F. circinatum,* Department of Plant Pathology, University of California, Davis, California 95616. * Isolates marked are those used in pathogenicity tests.

Fig. 1. Morphological characteristics of *Fusarium circinatum*. (a) Mature perithecia (bar = 100µm). (b) Single septate ascospores (bar = 10µm). (c) Branched conidiophores bearing polyphialides with conidiogenous openings indicated by arrows (bar = 10µm). (d) Coiled sterile hyphae (bar = 10µm). (e) Lunate macroconidia (bar = 10µm).

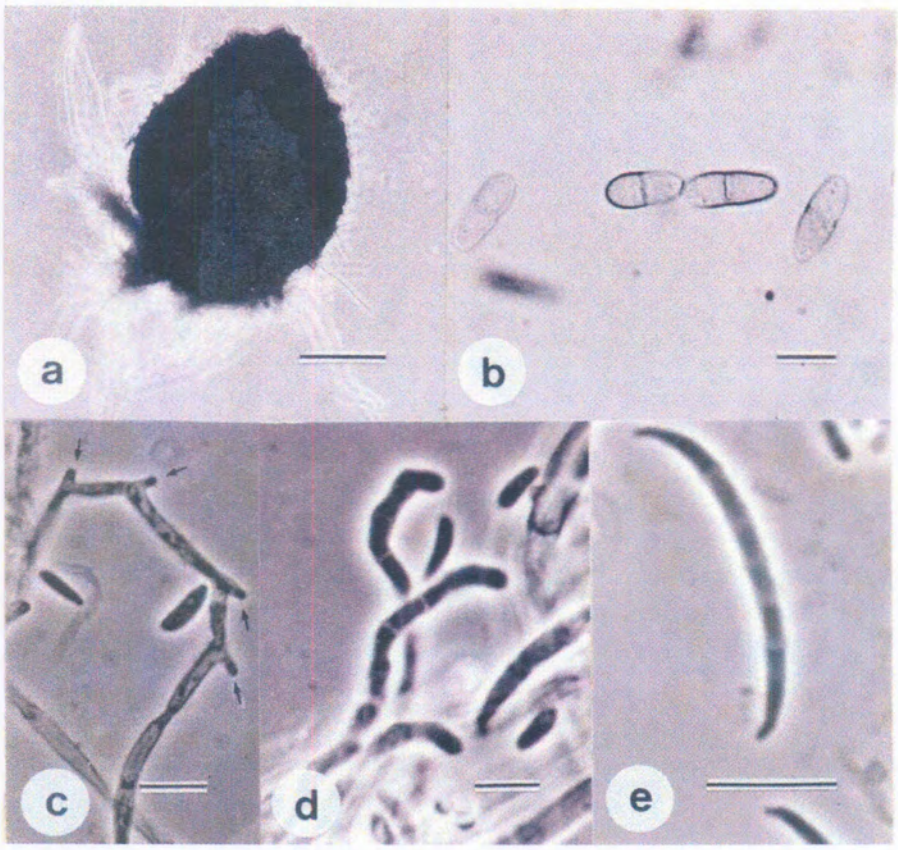

Fig. 2. *Dde* I RFLP profiles of the digestion of the histone *H3* gene amplification products on a 3% agarose gel. Lane marked as M = 100 bp ladder (1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100 bp). Lane 1 = *F. subglutinans* (MRC 6512) from the E-mating population. Lane 2 = *F. sacchari* (MRC 6525) from the B-mating population. Lane 3 = *F. subglutinans* isolate (MCR 7035) from mango. Lane 4-6 = digestion products of *F. circinatum* (MRC 7568, 7573, 7587, 7589, 7596 and 7598) from Mexico. Lane 7 = digestion product of *F. circinatum* tester strain (MRC 6213) from mating population H.