

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

by

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SUMMARY

Fusarium subglutinans sensu lato is a complex of fungi, which are the causal agents of important diseases on a wide variety of plants. Two important diseases caused by *F. subglutinans sensu lato* are pitch canker and mango malformation. *F. subglutinans sensu lato* isolates causing pitch canker on pine trees have been described as a separate species, *F. circinatum*, whereas *F. subglutinans sensu lato* isolates associated with mango malformation have not been formally described. The objective of study was to clarify the taxonomy and population genetics of the pitch canker and mango malformation fungi residing in the *Gibberella fujikuroi* complex.

The introductory chapter of this thesis provides a review of the taxonomic classifications used for *Fusarium* spp. in the *G. fujikuroi* complex. In addition, the current knowledge pertaining to the population structure of the pitch canker and mango malformation fungi is discussed.

In the second chapter the occurrence of *F. circinatum* was investigated in Mexico. *Fusarium* isolates were collected from pine trees in Mexico and identified as *F. circinatum*. Morphology, sexual compatibility studies, pathogenicity tests and histone H3-RFLPs were used to identify and characterize this fungus.

The pitch canker fungus, *F. circinatum* and its teleomorph, *G. circinata* has been recently described. However, the name *G. circinata* is invalid, because insufficient information was provided to characterize the type specimen in the description. Additional information and a selection of *F. circinatum* isolates were, therefore, obtained and studies were undertaken in order to validate the description of *G. circinata*. The teleomorph *G. circinata* was validated and morphological criteria were shown to clearly distinguish *F. circinatum* from other *F. subglutinans sensu lato* isolates.

Chapter four presents a study aimed at better understanding relationships between populations of *F. circinatum* from different geographical areas. For this study co-dominant molecular markers were developed. These were then used to determine the genetic diversity, genetic distance and migration between different *F. circinatum*

populations. Analysis revealed a high diversity in the Florida population and a low diversity in the South African population. Genetic analysis also indicated that the South African *F. circinatum* population originated in Mexico.

In chapter five, the influence of sexual reproduction on the *F. circinatum* populations sampled over ten years in South Africa were studied. Sexual compatibility, vegetative compatibility and allelic diversity that were determined using polymorphic markers, developed in chapter four, were used. These results suggested that sexual reproduction is occurring more frequently in the more recently collected populations than in the initial population.

Mango malformation is an important disease in mango growing areas. The study presented in chapter six indicated that this disease is associated with two distinct *Fusarium* spp. in the section *Liseola*. The two new *Fusarium* spp. are thus described as *F. mangiferae* and *F. sterilihyphosum* using morphological criteria.

In chapter seven, the distribution and vegetative compatibility of both *F. mangiferae* and *F. sterilihyphosum* was determined for the South Africa populations. Results revealed that each of these species differ in their distribution in South Africa. Vegetative compatibility tests also suggest that both species represent single genets in South Africa.

Fusarium subglutinans sensu lato isolates associated with pine and mango are economically important fungi. The focus of the studies presented in this thesis has been on the taxonomy and population genetics of these fungi, with special reference to their occurrence in South Africa. Each of the chapters will contribute towards a better understanding of the taxonomy, population genetics and biology of these fungi.

OPSOMMING

Fusarium subglutinans sensu lato is 'n kompleks van fungi wat belangrike siektes op verskeie plante veroorsaak. Twee van die belangrike siektes wat *F. subglutinans sensu lato* veroorsaak is 'pitch canker' en 'mango malformation'. *F. subglutinans sensu lato* isolate wat 'pitch canker' veroorsaak op pine bome is beskryf as a aparte spesie, *F. circinatum* en *F. subglutinans sensu lato* isolate wat met 'mango malformation' geassosieer word is nog nie formeel beskryf nie. Die doel van die studie was om die taksonomie en populasie genetica van die 'pitch canker', en 'mango malformation' fungi, wat in die *Gibberella fujikuroi* kompleks behoort, te verklaar.

'n Oorsig van die taksonomiese klassifikasies wat gebruik is vir *Fusarium* spp. in die *G. fujikuroi* kompleks is voorgelê in die inleidende hoofstuk van die tesis. Verder is die huidige kennis wat betrekking het tot die populasie strukture van die 'pitch canker' en 'mango malformation' fungi ook bespreek.

In die tweede hoofstuk is die voorkoms van *F. circinatum* in Mexiko ondersoek. *Fusarium* isolate was versamel in Mexiko en geïdentifiseer as *F. circinatum*. Morfologie, seksuele verwantskap studies, patogenisiteit toetse en histone H3-RFLPs was gebruik om die fungus te identifiseer en te karakteriseer.

Die 'pitch canker' fungus, *F. circinatum* en sy teleomorf, *G. circinata* was onlangs beskryf. Die naam *G. circinata* is ongeldig, omdat nie genoegsame informasie weer gegee is nie in die beskrywing om die tipe spesiment te karakteriseer. Addisionele informasie en 'n verskeidenheid van *F. circinatum* isolate is bestudeer om die *G. circinata* beskrywing geldig te verklaar. Die teleomorf *G. circinata* is geldig gemaak en morfologiese kriteria kon duidelik onderskei tussen *F. circinatum* en ander *F. subglutinans sensu lato* isolate.

Hoofstuk vier stel 'n studie bekend wat gemik is om verwantskappe tussen *F. circinatum* populasies van verskeie geografiese areas beter te verstaan. Ko-dominante molekulêre merkers in ontwikkel in die studie. Hierdie merkers is gebruik om die genetiese diversiteit,

genetiese afstand en migrasie tussen *F. circinatum* populasies te bepaal. Analiese het gewys dat 'n hoë diversiteit in die Florida populasie en 'n lae diversiteit in die Suid-Afrikaanse populasie teenwoordig is. Genetiese analiese het ook aangedui dat die Suid-Afrikaanse *F. circiantum* populasie vanaf Mexiko afkomstig is.

In hoofstuk vyf is die invloed van seksuele reproduksie op die *F. circinatum* populasies wat oor tien jaar versamel is bepaal. Seksuele kruisbaarheid, vegetatiewe kruisbaarheid en alleliese diversiteit, wat bepaal is met die polimorfiese merkers wat in hoofstuk vier ontwikkel is, is gebruik. Hierdie resultate het getoon dat seksuele reproduksie meer voorkom in meer onlangs versamel populasies as in die oorspronklike populasie.

'Mango malformation' is 'n belangrike siekte in areas waar mangoes gekweek word. Die studie in hoofstuk ses bewys dat die siekte met twee verskillende *Fusarium* spp. in die seksie *Liseola* geassosieer word. Die twee nuwe *Fusarium* spp. is beskryf as *F. mangiferae* en *F. sterilihyphosum* met gebruik van morfologiese kriteria.

In hoofstuk sewe is die verspreiding en vegetatiewe kruisbaarheid van beide die *F. mangiferae* en *F. sterilihyphosum* populasies in Suid-Afrika bepaal. Resultate bewys dat elkeen van die spesies verskil in hul verspreiding in Suid-Afrika. Vegetatiewe verwantskaps toetse het voorgestel dat beide spesies gelyksoortig in Suid-Afrika.

Fusarium subglutinans sensu lato isolate wat met pine en mango assosieer is ekonomiese belangrike fungi. Die fokus van die studies, voorgelê in die tesis, was op die taksonomie en populasie genetica van die fungi met spesiale verwysing na hul voorkoms in Suid-Afrika. Elke hoofstuk het bygedra tot 'n beter begrip van die taksonomie, populasie genetica en biologie van die fungi.

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PREFACE

Fusarium subglutinans (section *Liseola*) has been described as a cosmopolitan fungus residing in three mating populations of the *Gibberella fujikuroi* complex. Morphological characters did not differentiate *F. subglutinans* strains from different hosts (= *F. subglutinans sensu lato*). Pathogenicity tests and sexual compatibility were, therefore, used to differentiate among *F. subglutinans sensu lato*. More recently, many *F. subglutinans sensu lato* isolates residing in mating populations in the *G. fujikuroi* complex have been elevated to species that were justified by molecular phylogeny and supported by associated morphological characters.

As a result of recent taxonomic studies, the pitch canker fungus, previously known as *F. subglutinans* f. sp. *pini*, has been described as *F. circinatum* (teleomorph *G. circinata*). However, the taxonomy of the *F. subglutinans sensu lato* strains causing mango malformation remains undefined. The major goal of studies in this dissertation has been to characterize *F. subglutinans sensu lato* isolates from mango and pine based on molecular techniques, morphological characteristics and sexual compatibility. Furthermore, the genetic diversity of South African populations of these two pathogens has also been investigated using various techniques. Each of the chapters in this thesis thus deals with different approaches to investigate the taxonomy and genetics of the pitch canker and mango malformation fungi.

The first chapter provides an overview of the literature published on the morphology, biological species and phylogenetic studies of *Fusarium* spp. in the *G. fujikuroi* complex. Studies leading to the most recent taxonomic system, where the morphological classification supports the mating populations, pathogenicity and phylogenetic species classification are treated. Emphasis in this chapter is placed on the taxonomy and population genetics of *F. subglutinans sensu lato* strains causing pitch canker and mango malformation.

Fusarium circinatum, the causal agent of pitch canker, has a world-wide distribution. However, very little is known about the occurrence of this fungus in Mexico. In chapter two, the identity of *Fusarium* isolates collected in Mexico was determined. Techniques used included morphological characteristics such as conidial morphology, origin of conidiophore from substrate, type of conidiophore branching and the presence of sterile coiled hyphae. Pathogenicity tests, sexual compatibility studies and histone *H3*-RFLPs were also used to characterize these isolates.

The name of the teleomorph of *F. circinatum*, *G. circinata* has been declared invalid, because insufficient information was provided to characterize the type specimen in the description. The aim of the study presented in chapter three was to provide information regarding the holotype specimen, names of collectors of isolates, date of collection and designation of the holotype specimen. Morphological criteria proposed in the recent classification were critically examined in order to determine whether *F. circinatum* could be differentiated from other *F. subglutinans sensu lato* isolates based on these characteristics.

Fusarium circinatum causes significant losses to the forestry industry and various strategies are being implemented to reduce them. An understanding of the population biology of the pathogen will lead to durable disease control. Population genetic analysis requires the identification of markers that are unambiguous and polymorphic. In chapter four, molecular markers that are co-dominant and polymorphic were developed. These markers were then used to analyze the genetic relationships among populations of *F. circinatum* from California, Florida, Mexico and South Africa.

Population genetics of *F. circinatum* is influenced by asexual and sexual reproduction. Clonal propagation is the result of the asexual cycle, while new genotypes are produced by the sexual cycle. The contribution of the sexual and asexual cycle to the *F. circinatum* population structure was investigated by comparing isolates from the initial *F. circinatum* outbreak, with isolates from recent outbreaks. Techniques used in this chapter included sexual compatibility, vegetative compatibility tests and molecular markers.

Two distinct phylogenetic species associated with mango malformation were previously recognized based on DNA sequences of histone *H3* and β -tubulin genes. In chapter six, these two new species have been formally described and differentiated from other species based on morphological characteristics.

Chapter seven reports on the distribution of the two *Fusarium* spp. described in chapter 6 from mango malformation symptoms in South Africa. A rapid molecular technique was used to differentiate these two species from one another. The genotypic diversity of both species was also determined using vegetative compatibility tests.

Each chapter this dissertation has been treated as an independent entity. Thus, redundancy between chapters could not be avoided. It is my hope that the results of these studies on two of the most important pathogens of tree crops in South Africa will contribute to a better understanding of the diseases that they cause. Also that these studies will form the basis of detailed and future investigations.