Molecular biological studies of the Fusarium wilt pathogen of banana in South Africa.

BY

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Declaration

I, the undersigned, declare that the work contained in this thesis is my own and original work and that it has not previously in its entirety or part submitted for a degree to any other university.

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PREFACE

Fusarium wilts, induced by special forms of the soilborne fungus *Fusarium oxysporum,* are considered to be among the most severe plant diseases in the world. Possibly the most devastating of these wilts is Fusarium wilt of banana, caused by *F. oxysporum* f. sp. *cubense* (*Foc*). Historically this disease became notorious for destroying many thousands of hectares of prime banana fields in Central America, but it is still considered a major constraint to banana production and expansion in many countries including South Africa.

Effective management of Fusarium wilt of banana requires a good knowledge of *Foc* in order to design the necessary control measures. The occurrence of variants of the pathogen has inspired scientists to extensively study this fungus in the last 20 years. These studies used both phenotypic and genotypic techniques such as VCGs and DNA fingerprinting techniques, and have resulted in useful information on pathogen diversity, origin, distribution, phylogeny and dispersal. Studies in this thesis were aimed at making deductions on the phylogeny, reproductive potential, host pathogen interactions and understanding the genetic diversity at the loci level. It shed light on pathogen diversity using several different molecular techniques. Each chapter in this thesis is presented as an independent entity with redundancy between chapters being unavoidable.

Chapter 1 presents the history of Fusarium wilt of banana, and provides the reader with a general review on the taxonomy, origin and spread of *Foc* in the world. The chapter gives a broad overview of techniques currently used to study the population diversity of the pathogen. The application of molecular techniques such as sequencing of the different gene genealogies used to subdivide *Foc* populations and understand evolutionary patterns of origin and distribution are discussed. The review concludes by emphasizing shortcomings in current research on this pathogen.

Knowing the reproductive capability of *Foc* is important in order to predict the ability of the fungus to diversify and overcome disease management strategies. Knowledge of the population structure of the pathogen in South Africa could further provide an indication as to its diversity. In **Chapter 2** the presence of mating type genes and *Foc*'s ability to reproduce sexually have been investigated. The *MAT*-2 gene region was sequenced and the phylogenetic relationships of the South African isolates compared to those of representative genotypes from different geographical regions.

Phylogenetic analysis of DNA characters from two loci was used to help resolve the genetic relationships among *Foc* isolates from diverse geographic origins in **Chapter 3**. These two regions represent a nuclear region and a highly polymorphic region of mitochondrial DNA respectively. The results strongly indicate the presence of clonal lineages among isolates of *Foc*.

Little is known about the interaction between banana plants and *Foc*. With the new knowledge available on the diversity in pathogenic populations of the fungus, studies on host-pathogen interactions are becoming increasingly more important. In **Chapter 4** the stable transformation of *Foc* with the green fluorescent protein (GFP) protein is described. GFP provides a powerful tool to study early stages of fungal infection and to quantify resistance responses in bananas.

A study of the population structure of *Foc* using both VCGs and molecular markers was needed to provide insights into the diversity of the pathogen in South Africa. This required the development of molecular markers that are highly reproducible and codominant. Microsatellite assays have rapidly become established as a powerful tool for the analysis of population structure, reproductive mode and genetic isolation of fungal populations. **Chapters 5** and **6**

deal with the development, testing and the use of these polymorphic microsatellite markers on a worldwide collection of *Foc* isolates. Developing microsatellite markers would be essential for solutions to the current questions still pertaining to diversity in *Foc*. The abilities of pathogen populations to overcome control measures such as resistant cultivars and chemical control can be deducted from such studies.