SUMMARY – Susan Groenewald

Fusarium oxysporum Schlecht. causes vascular wilt diseases to many economically important agricultural crops. The fungus is taxonomically complex, and consists of many pathogenic and non-pathogenic forms that are morphologically similar. One of the most important pathogenic forms is *F. oxysporum* f.sp. *cubense* (EF Smith) Snyd. and Hans (*Foc*), the causal agent of Fusarium wilt of banana. *Foc* has a worldwide distribution and consists of three races (1, 2 and 4) and 21 vegetative compatibility groups (VCGs). Race 4 is divided into 'tropical' and 'subtropical' isolates, dependant on their ability to cause disease to Cavendish bananas in the tropics and subtropics, respectively. In *Foc*, the same race includes several DNA fingerprinting groups and represents a number of VCGs. In this thesis, the diversity in *Foc* has been investigated by means of phenotypic and genotypic analysis. An attempt was made to develop a molecular marker for VCG 0120, the most widely distributed VCG of the pathogen in the world, and to understand virulence in the fungus.

An important finding in this study was that the genotypically uniform population of *Foc* 'subtropical' race 4 (VCG 0120) is phenotypically diverse. This study provided the first evidence that certain genetically uniform isolates of *Foc* caused more severe disease to Cavendish bananas than others under controlled environmental conditions. Variation in fungal virulence to Cavendish bananas does not appear to be a function of growth tempo and/or sporulation, but could rather be due to the influence of other factors such as the production of toxins and suppression of plant defence responses. The finding that *Foc* 'subtropical' race 4 isolates showed optimal growth at 25ºC, supports previous views that the increased disease incidence on Cavendish bananas in the subtropics is primarily a function of the banana plant being more vulnerable to infection under winter temperatures, rather than the pathogen becoming more competitive. *Foc* grew better on nitrate medium than on ammonium medium *in vitro*, which does not reflected Fusarium wilt development in the field. The more pronounced disease development in soils fertilized with NH_4-nitrogen, compared to NO_2-nitrogen, is because nitrate causes an increase in pH and ammonium a decrease. Fusarium wilt is associated with acidic soils rather than with alkaline soil.

Previous studies gave a good indication of the diversity of the worldwide population of *Foc*, but did not always agree in terms of genetic relationships among clonal lineages of this pathogen. Amplified fragment length polymorphism (AFLP) analysis of *Foc* isolates supported previous findings that divided a worldwide population of *Foc* into two major clades, but gave higher resolution within clades. It further suggests that the current race

designation cannot be considered accurate, since race 1 and race 4 isolates grouped together. These isolates are currently assigned to different races based on their pathogenicity to Cavendish bananas under field conditions. Another important finding was that VCG 0121, previously considered to belong to 'subtropical' race 4, grouped closer to isolates of VCGs 01213 and 01216 that belong to 'tropical' race 4 than other 'subtropical' race 4 isolates. This suggests that the Cavendish banana variety 'GCTCV 218' that proofed to be tolerant to *Foc* VCG 0121 in Taiwan, could also be tolerant to VCGs 01213 and 01216, the 'tropical' race 4 currently causing devastating losses to Cavendish bananas in Malaysia, Indonesia and northern Australia.

AFLP analysis provided sufficient polymophisms among VCGs of *Foc* for conversion to simple single locus markers. In an effort to develop a VCG 0120-specific marker, a DNA fragment was isolated and analysed for single nucleotide polymorphisms (SNPs) that could potentially be developed into sequence characterised amplified region (SCAR) or cleaved amplified polymorphic site (CAPS) markers. Due to problems arising during the cloning of the excised AFLP fragment, the original polymorphism was lost. The conversion from AFLP marker to a sequence-specific PCR or PCR-RFLP marker could, therefore, not be achieved in this study. SNPs were found in the flanking regions of the AFLP fragment that could potentially distinguish between *Foc* VCG 0120 and other VCGs of *Foc*.

Three virulence-associated genes, *fmk1*, *pg1* and *xyl3* were found in *Foc*, but were not unique to the banana pathogen. Sequence analysis of *fmk1* and *pg1* from a diverse range of *F. oxysporum* isolates proved that these genes were useful in phylogenetic analysis of a worldwide *Foc* population. They can also be applied for comparative analysis of *formae speciale*s within *F. oxysporum*. From the high similarity in amino acid sequences among *fmk1*, *pg1* and *xyl3* genes in *F. oxysporum* pathogenic and non-pathogenic to banana, it can be concluded that these genes play a limited role in host specificity.