

SUMMARY – Aneen Belgrove

Fusarium oxysporum Schlecht is a cosmopolitan fungus and causes Fusarium wilt of economically important crops. The species complex contains pathogenic and non-pathogenic strains that cannot be distinguished morphologically. The pathogenic *F. oxysporum* can penetrate the root, spread in the xylem vessels and block water transport, thereby causing a lethal vascular wilt. Most individuals belonging to *F. oxysporum* are non-pathogenic, saprophytic soil inhabitants. These non-pathogens are efficient colonisers of the plant rhizosphere and the root cortex but do not induce any symptoms in plants. Pathogenic strains of *F. oxysporum* can survive in the soil for long periods of time without a host, making it impossible to eradicate them from infested agricultural fields.

Fusarium oxysporum f.sp. *cubense* (*Foc*) is the Fusarium wilt pathogen of banana. Chemical and cultural control has been used with little success to control this disease. Since Fusarium wilt is significantly influenced by host genotype, the best means of controlling this disease is by using disease resistant planting material. Resistance breeding can be difficult when no dominant gene is known. In recent years, the use of biological control agents such as *Pseudomonas fluorescens* and non-pathogenic *F. oxysporum* has resulted in a reduction in Fusarium wilt incidence. Suppressive soils generally host potential biocontrol agents. In this thesis, the control of *Foc* using biocontrol agents such as non-pathogenic *F. oxysporum* isolated from suppressive soils and *P. fluorescens* WCS 417 was investigated. Non-pathogenic *F. oxysporum* isolates from disease suppressive soils were subjected to Restriction Fragment Length Polymorphisms analyses of the intergenic spacer region. Great diversity exists in the non-pathogenic strains, which might suggest that the genotypes are widely distributed, or that great movement of these genotypes occurred. The clonal nature and stability of *Foc* was confirmed when all the pathogenic isolates grouped into a single genotype. The selected non-pathogenic *F. oxysporum* isolates reduced Fusarium wilt of banana effectively in the greenhouse, but the field trial failed due to unfavourable environmental conditions.

In this study, it was demonstrated that non-pathogenic *F. oxysporum* and *P. fluorescens* WCS 417 induced disease resistance in banana roots both locally and systemically. *Pseudomonas fluorescens* WCS 417 induced significant higher levels of total phenolic content in the one half on the banana roots that were not wounded than in the roots other half of the same rootball of the banana plant that were wounded and inoculated. Non-pathogenic *F. oxysporum* stimulated banana roots to produce high levels of antimicrobial phenolic compounds that possibly diffused out of storage, became polymerised and

increased cell wall-bound phenolics. The cell wall-bound phenolics can be lignified in cell walls or could aid in the formation of tyloses, gums and papillae, blocking the pathogen from further invasion. The role of these changes in phenolic composition of banana roots, induced by non-pathogenic *F. oxysporum*, should be further demonstrated in histochemical studies.

A non-pathogenic *F. oxysporum* isolate was successfully transformed with the *GFP*- and *DsRed-Express* genes. Fluorescent microscopy showed that all the structures of the fungus fluoresced brightly, and successive transfers to non-selective media proved that the transformation was stable. The transformed isolates were then used for infection studies on the banana root in a non-invasive and non-destructive manner. In this study, the interaction between the putative biological control organisms and a GFP-transformed *Foc* isolate was investigated by using a confocal laser scanning microscope. Our results demonstrated that competition for infection sites is an unlikely mode of protection. When applied simultaneously and at equal concentrations, the non-pathogenic *F. oxysporum* isolate and *Foc* extensively colonised the banana root in the first few days. The density of the non-pathogenic *F. oxysporum* decreased from day 4. Whether timing and concentration of the non-pathogenic *F. oxysporum* is important to compete with *Foc* on banana roots is not clear. No inhibition of *Foc* by non-pathogenic *F. oxysporum* and *P. fluorescens* WCS 417 was observed *in vitro*, suggesting that antibiosis does not play a role in the reduction of Fusarium wilt disease.

A split-root technique was used to study whether induced systemic resistance may influence infection of banana roots by the GFP-transformed *Foc* isolate. The one side of the banana root system was inoculated with non-pathogenic *F. oxysporum* or *P. fluorescens* WCS 417 2 days before inoculation of the other side with *Foc*. Microscopic analyses suggested that the non-pathogenic *F. oxysporum* and *P. fluorescens* WCS 417 isolates did not induce a systemic response that prevented banana roots from becoming colonised by *Foc*. One can argue that a biochemical, rather than a structural response, prevented infection of distant banana roots from taking place, since induced resistance was suggested as a mode of action of non-pathogenic *F. oxysporum* and *P. fluorescens* WCS 417 in earlier greenhouse pathogenicity trials. The inability of *Foc* to infect non-wounded banana roots in control treatments, unfortunately, prevents this hypothesis from any further exploitation. None of the roots were wounded in this experiment, thus the results indicate that wounding might be essential for *Foc* penetration. The *Foc* isolate used in this study is known to attack Cavendish bananas under abiotic stress conditions only. Whether the non-pathogenic *F. oxysporum* and *P. fluorescens* WCS 417 isolates used would still protect bananas by means of induced resistance after wounding and following abiotic stressful conditions also needs to be further investigated.

