Quantitative PCR and Histopathological Assessment of Cereal Infection by *Fusarium pseudograminearum*.

by

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A dissertation submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

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Certificate of Originality

I certify that the experimental work, results, analyses and conclusions reported in this dissertation are entirely my own effort, except where otherwise acknowledged. The text of this thesis contains no material which has been accepted for the award of any other degree or diploma in any University, unless stated. To the best of my knowledge, the text of this thesis is original and contains no material previously published or written by another person, except where due reference is made.

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Signature of Supervisor         Date
Acknowledgments

A PhD is a challenge, an adventure to be embarked upon. It is a test of mental strength, endurance and resolve. In a world of so much, a PhD encompasses relatively little, yet it becomes your life, your world. It represents your sacrifices, your enthusiasm, your highs and lows, your successes and failures, your strengths and weaknesses. A PhD represents work predominantly done alone, however it cannot be achieved alone. For this I must firstly thank my supervisor Professor Mark Sutherland, a source of not only funding, but of guidance, encouragement, wisdom and opportunity. I greatly appreciate the rigorous and thorough thought behind our discussions and the many opportunities I have been given to present my work and interact with other researchers from around the world.

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Abstract

The assessment of crown rot (F. pseudograminearum) infections in winter cereals was explored using a quantitative polymerase chain reaction (PCR) approach. A range of cereal genotypes of varying resistance to crown rot were monitored during seedling and adult growth stages. A histopathological investigation of F. pseudograminearum (Fp) growth during pathogenesis in seedling and adult growth stages was also performed across a range of cereal genotypes. This utilised a novel staining method developed during this project.

Visual assessment of crown rot symptoms in wheat seedlings is commonly conducted in order to rapidly identify resistant genotypes. Ratings rely heavily on discolouration of seedling tissues, predominantly the leaf sheaths. This study is the first to explore the relationship between seedling tissue discolouration and Fp DNA content in wheat genotypes of differing resistance. The partially resistant wheat 2-49 exhibited lower visual and qPCR values than the susceptible wheat Puseas. The rate of disease development and Fp growth in seedling leaf sheaths was slower in 2-49 than Puseas. The rates of symptom development in intermediate genotypes EGA Wylie and EGA Gregory were not significantly different from that recorded in 2-49. A comparison of visual ratings and qPCR values of Fp DNA indicated a strong correlation (r = 0.89, p < 0.01) between these characters at 14 days after inoculation across all genotypes. The correlation weakened over time. Furthermore, qPCR revealed differences between partially resistant and susceptible genotypes to a much greater extent than possible using visual discolouration.

Crown rot infections of adult cereal stems are typically rated at maturity by recording the amount of discolouration on individual internodes. A comparison was performed between visual ratings and Fp DNA content of four cereal genotypes (two bread wheats, one durum wheat and one barley) at anthesis (16 weeks after planting) and maturity (22 weeks after planting). At anthesis a strong correlation (r = 0.86, p < 0.01) was present between visual and qPCR values. At maturity this relationship was modest (r = 0.58, p < 0.01). Furthermore, differences between partially resistant and susceptible genotypes were greatest at anthesis.

The strong correlations between visual discolouration and fungal DNA content indicate that visual discolouration is a useful measure of fungal load in
seedling and adult cereal tissues. However, the degree to which these two parameters correlate varies with the time elapsed since tissue infection.

Fluorescence microscopy revealed no major differences between fungal growth patterns or structural characteristics in the host genotypes assessed. Leaf sheaths were most frequently penetrated via stomata, indicated by initial lesions forming at the guard cells. Leaf sheath tissues became extensively colonised in most cell types, except for the vascular bundles and abaxial silica cells. Colonisation of leaf sheaths resulted in the re-emergence of hyphae and occasionally conidiophores from stomata.

Colonisation of culm tissues frequently originated in the parenchymatous hypoderm, which became greatly discoloured, resulting in the visual discolouration used for disease rating. Early infection of pith parenchyma cells was also frequent. Infections typically spread from the culm base upwards through the tissues, typically only to internode three, with a much slower lateral spread of hyphae. Colonisation of sclerified cells occurred later in the infection process. Vascular tissues were frequently colonised by anthesis. This was more rapid in susceptible genotypes. Occlusion of large xylem vessels was rare during moderate infections.

The ability of quantitative PCR to accurately describe the extent of crown rot infection suggests that it could be utilised as a powerful technique for detecting new resistance sources or for identifying quantitative trait loci for resistance. This method, along with further microscopic and biochemical assessments of partially resistant and susceptible genotypes, may provide new information on the pathogenesis of crown rot and the nature of host resistance responses.
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List of Abbreviations

1°: primary
2°: secondary
Col: coleoptile
Cro: crown
Ct: threshold cycle
dai: days after inoculation
Fp: Fusarium pseudograminearum
hai: hours after inoculation
IN: internode
LB: leaf blade
LRC: Leslie Research Centre
LS: leaf sheath
LSb: base 2 cm of leaf sheath
LSt: top 2 cm of leaf sheath
NA: not applicable
PCR: polymerase chain reaction
PH: parenchymatous hypoderm
PP: pith parenchyma
qPCR: quantitative polymerase chain reaction
QTL: quantitative trait loci
SCI: sub-crown internode
SH: sclerenchymatous hypoderm
SNA: starch nitrate agar
VB: vascular bundle
Vis: visual discolouration
VP: vascular parenchyma
WAP: weeks after planting
Wh: wheat