A model system using insects to vector *Fusarium tumidum* for biological control of gorse (*Ulex europaeus*)

A thesis submitted in partial fulfilment
of the requirements for the degree of

Doctor of Philosophy

at

Lincoln University

by

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2007

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy (Ph.D.)

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The overall objective of this study was to test the hypothesis that insects can vector *F. tumidum* conidia to infect gorse plants with the aim of developing an alternative approach to mycoherbicide delivery to control weeds. Four potential insect species (*Apion ulicis, Cydia ulicetana*, *Epiphyas postvittana* and *Sericothrips staphylinus*) were assessed for their ability to vector *F. tumidum* conidia. To achieve this, the external microflora (bacteria and fungi) and the size and location of fungal spores on the cuticle of these insect species were determined. In addition, the ability of the insects to pick up and deposit *F. tumidum* conidia on agar was studied. Based on the results from these experiments, *E. postvittana* was selected for more detailed experiments to determine transmission of *F. tumidum* to infect potted gorse plants. The factors promoting pathogenicity of *F. tumidum* against gorse and the pathogen loading required to infect and kill the weed were also determined.

The external microflora of the four insect species were recovered by washing and plating techniques and identified by morphology and polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) and sequencing of internally transcribed spacer (ITS) and 16S rDNA. A culture-independent technique (direct PCR) was also used to assess fungal diversity by direct amplification of ITS sequences from the washings of the insects. All insect species carried *Alternaria, Cladosporium*, *Nectria*, *Penicillium*, *Phoma, Pseudozyma* spp. and entomopathogens. Ninety four per cent of the 178 cloned amplicons had ITS sequences similarity to *Nectria mauritiicola. E. postvittana* carried the largest fungal spores (mean surface area of 125.9 μm^2) and the most fungal CFU/insect.

About 70% of the fungi isolated from the insects were also present on the host plant (gorse) and the understorey grass. The mean size of fungal spores recovered from the insect species correlated strongly with their body length (R^2 = 85%). *Methylobacterium aquaticum* and *Pseudomonas lutea* were common on all four insect species. *Pseudomonas fluorescens* was the most abundant bacterial species.

In the pathogenicity trials, the effectiveness of *F. tumidum* in reducing root and shoot biomass of 16 and 8 wk old gorse plants was significantly increased with wounding of the plants. Older plants (32 wk old) which were wounded and inoculated were significantly shorter, more infected and developed more tip dieback (80%) than plants which were not wounded (32%). This indicates that damage caused by phytophagous insect species present on gorse through feeding and oviposition may enhance infection by *F. tumidum*. Wounding may release nutrients (e.g. Mg and Zn) essential for conidia germination and germ tube elongation and also provide easier access for germ tube penetration. Conidial germination and germ tube length were increased by 50 and 877%, respectively when incubated in 0.2% of gorse extract solution for 24 h compared with incubation in water. Inoculum suspensions amended with 0.2% of gorse extract caused more infection and significantly reduced biomass production of 24 wk old gorse plants than suspensions without gorse extract. A minimum number of about 900 viable conidia/infection site of *F. tumidum* were required to infect gorse leaves. However, incorporation of amendments (which can injure the leaf cuticle) or provision of nutrients (i.e. gorse extract or glucose) in the formulation might decrease the number of conidia required for lesion formation. Scanning electron micrographs showed that germ tube penetration of gorse tissue was limited to open stomata which partly explain the large number of conidia required for infection. The flowers and leaves were more susceptible to *F. tumidum* infection than the spines, stems and pods. An experiment to determine the number of infection sites required to cause plant mortality showed that the entire plant needs to be inoculated in order for the pathogen to kill 10 wk old plants as *F. tumidum* is a non systemic pathogen. The number of infection sites correlated strongly with disease severity (R^2 = 99.3%). At least 50% of the plant was required to be inoculated to cause a significant reduction in shoot dry weight.

F. tumidum, applied as soil inoculant using inoculated wheat grains in three separate experiments, significantly suppressed gorse seedling emergence and biomass production.

In experiments to determine the loading capacity of the insect species, *E. postvittana*, the largest insect species studied, carried significantly more (68) and deposited significantly more (29) *F. tumidum* conidia than the other species. Each *E. postvittana*, loaded with 5,000 conidia of *F. tumidum*, transmitted approximately 310 conidia onto gorse plants but this did not cause any infection or affect plant growth as determined by shoot fresh weight and shoot height. *E. postvittana* on its own did not cause any significant damage to gorse and did not enhance *F. tumidum* infection. It also failed to spread the pathogen from infected plants to the healthy ones. There was no evidence of synergism between the two agents and damage caused by the combination of both *E. postvittana* and *F. tumidum* was equivalent to that caused by *F. tumidum* alone.

This study has shown that *E. postvittana* has the greatest capacity to vector *F. tumidum* since it naturally carried the largest and the most fungal spores (429 CFU/insect). Moreover, it naturally carried *Fusarium* spp. such as *F. lateritium*, *F. tricinctum* and *Gibberella pulicaris* (anamorph *Fusarium sambucinum*) and was capable of carrying and depositing most *F. tumidum* conidia on agar. Coupled with the availability of pheromone for attracting the male insects, *E. postvittana* may be a suitable insect vector for delivering *F. tumidum* conidia on gorse using this novel biocontrol strategy. Although it is a polyphagous insect, and may visit non-target plants, *F. tumidum* is a very specific pathogen of gorse, broom and a few closely related plant species. Hence, using this insect species to vector *F. tumidum* in a biological control programme, should not pose a significant threat to plants of economic importance. However, successful control of gorse using this "lure-load-infect" concept would depend, to a large extent on the virulence of the pathogen as insects, due to the large size of *F. tumidum* macroconidia, can carry only a small number of it.

Keywords: *Fusarium tumidum*, insect microflora, PCR-RFLP, ITS, 16S rDNA, mycoherbicide, gorse, biological control, wounding, insect vectors, lure-load-infect.

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ABBREVIATIONS AND SYMBOLS

A	adenine
ANOVA	analysis of variance
bp	base pair
C	cystosine
CFU	colony forming unit
DAI	days after inoculation
DAS	days after sowing
DM	dry matter
DNA	deoxyribose nucleic acid
dNTP	deoxy-ribonucleotide triphosphate
DSI	disease severity index
DSS	disease severity score
Fig.	figure
G	guanine
h	hour
Kb	kilobase
L	litre
LSD	least significant difference
mg	miligram
min	minute
mL	millilitre
mM	millimolar
NaOCl	sodium hypochlorite
nd	not determined
ng	nanogram
OMA	oatmeal agar
P	probability
PCR	polymerase chain reaction
PDA	potato dextrose agar
®	registered trademark
RFLP	restriction fragment length polymorphism
rpm	revolutions per minute
s	second
SEM	scanning electron microscope
sp.	species
spp.	species (plural)
T	thymine
TAE	tris acetate ethylenediaminetetra-acetic acid
™	trademark
U	units
USA	United States of America
WAI	week(s) after inoculation
wk	week(s)
µL	microlitre
x *g*	gravity, measured in metres per second

ACKNOWLEDGEMENTS

I gratefully acknowledge the assistance of the following:

Prof. Alison Stewart, Dr Eirian Jones, Dr Max Suckling, Dr Graeme Bourdôt and Dr Richard Weld who supervised this research and offered invaluable professional advice and comments. Dr Nick Waipara and Neil Andrews for their respective assistance in morphological identification of the fungal isolates and with regards to the scanning electron microscopy. Dr Richard Hill for providing information and materials about gorse and Hugh Goulay for teaching me the techniques of insect trapping and providing materials for catching the insects. Dr Monika Walter, Dr Alvin Hee and Kirsty Boyd-Wilson for their contribution in conducting the second experiment on insect microflora. Alison Lister for her advice on statistical analyses. Margaret, Candice and Brent for their technical support. To all Bio-Protection staff and postgraduate students, I say "me da mo ase" (I thank you) for your friendship.

The New Zealand Tertiary Education Commission for funding this research and Landcare Research, Auckland, for providing the *Fusarium tumidum* isolates used for this study.

To my parents (Christiana and Samuel Nyarko), my wife (Olivia) and kids (Samuel, Linda and Justina) I am grateful for your love and support which motivated me to complete this interesting study.