Metabolism and Infection in the *Stagonospora nodorum*-Wheat Pathosystem

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I declare that this thesis is my own account of my own work and contains as its main content work which has not been previously submitted for any degree at any tertiary institution.

……

Ormonde Dominick Creagh Waters.

Ode to an Ectopic Fungal Mutant (Pmk1-61)

Thy hyphae fair didst bloom upon my plate
Of medium minimal, yet enough to grow.
And with selective fungicides to ensure
Lest non-transformants would contaminate.
In Stygian darkness, but near-UV also
I nourished you and waited you to spoor.
A picture portrait I did make of you,
Your handsome colours did my eye delight
And I did hope that you might be the one!
An homologous recombinant mutant – Oh so true
On you an Honours chapter I would write
And you a thesis cover would become.
Alas! By PCR you proved ectopic
And now you moulder in a bin necrotic.

Ormonde Waters 2007

ABSTRACT

Stagonospora nodorum is a necrotrophic fungal pathogen, and the causal agent of stagonospora nodorum blotch of wheat. Despite the economic importance of this disease, the molecular basis of the pathosystem is poorly understood. The aim of this study was to investigate the interaction between metabolism and infection in this pathosystem, with particular reference to the metabolism of mannitol.

In common with many fungi, the main metabolite produced by *S. nodorum* is the acyclic hexitol mannitol. Among the previously suggested roles for this compound is a role in pathogenicity. The metabolism of mannitol has been hypothesised as occurring in a cycle involving the enzymes mannitol 2-dehydrogenase (*Mdh1*) and mannitol 1-phosphate 5-dehydrogenase (*Mpd1*). A strain was created harbouring disruption constructs for both of these genes. The double mutant was unable to synthesise or catabolise mannitol, and was unable to sporulate. Addition of exogenous mannitol completely restored *in vitro* sporulation, and partially restored *in planta* sporulation. This demonstrated an essential role for mannitol in asexual sporulation. This is the first demonstrated role for this compound.

A ^{13}C NMR study of the wild type strain, the *mdh1* and *mpd1* single mutants, and *mpd1mdh1* double mutant was undertaken to investigate carbon utilisation and cycling. Disruption of *Mpd1* significantly altered the metabolite profile with the *mpd1* mutants producing trehalose and glycerol in place of mannitol. Labelling patterns in the double mutant showed that scrambling of label can be explained by the

triosephosphate isomerase triangle and pentose phosphate pathway. This suggests the contribution of mannitol to label scrambling has been overstated in previous studies.

The evidence did not support the metabolism of mannitol in *S. nodorum* as occurring in a cycle, but rather as two separate pathways.

A GC-MS analysis of diseased and non-diseased tissue from infected leaves, compared to non-infected and mock-inoculated leaves, could not detect any metabolites associated with a systemic host reaction to pathogen attack.

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ABBREVIATIONS

1-P	1-phosphate
6-P	6-phosphate
ACNFP	Australian Centre for Necrotrophic Fungal Pathogens
b	base(s)
BCA	bicinchoninic acid
BLAST	Basic Local Alignment Search Tool
bp	nucleotide base pair(s)
BSA	bovine serum albumin
cDNA	complementary DNA
CFE	cell-free extract
cm	centimetre(s)
cv.	cultivar
CzV8CS	Czapek Dox V8 juice complete supplement
DLA	detached leaf assay
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate(s)
dpi	days post inoculation/days post infection
EC	Enzyme Commission
EDTA	ethylenediaminetetra-acetic acid, disodium salt pH 8.0
EST	expressed sequence tag
f. sp.	*forma(e) species*
FT	Fourier transform
g	gram(s)

g	gravity
GC-MS	Gas Chromatography-Mass Spectrometry
gDNA	genomic DNA
GFP	Green Fluorescent Protein
GPS™-M	Genome Priming System - Mutagenesis
h	hour(s)
HSD	honestly significant difference
HST	host-specific toxin
kb	kilobase(s)
kPa	kiloPascal(s)
kV	kilovolt(s)
L	litre(s)
µg	microgram(s)
µL	microlitre(s)
µM	microMolar
µm	micron(s)
M	Molar
MAP kinase	mitogen-activated protein kinase
Mb	Megabase(s)
Mdh1/Mdh1	mannitol 2-dehydrogenase (gene/protein)
mg	milligram(s)
min	minute(s)
mL	millilitre(s)
MM	minimal medium
MM-C	minimal medium minus carbon

mM	milliMolar
mm	millimetre(s)
mol	mole(s)
MPa	Mega Pascal(s)
Mpd1/Mpd1	mannitol-1-phosphate 5-dehydrogenase (gene/protein)
NA	natural abundance
NAD^+	nicotinamide adenine dinucleotide (oxidised)
NADH	nicotinamide adenine dinucleotide (reduced)
$NADP^+$	nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
ng	nanogram(s)
nm	nanometre(s)
NMR	nuclear magnetic resonance
PCA	Principal Components Analysis
PCR	polymerase chain reaction
PEG	polyethylene glycol
PEP	phosphoenolpyruvate
pers. comm.	personal communication
pH	potential of hydrogen
P_i	inorganic phosphate
pl.	plural
ppm	parts per million
QTL	quantitative trait locus/loci
qPCR	quantitative polymerase chain reaction
rcf	relative centrifugal force

RNA	ribonucleic acid
RNAase	ribonuclease
ROS	reactive oxygen species
rpm	revolutions per minute
SDBS	Spectral Database for Organic Compounds
SDS	sodium dodecylsulphate
SE	standard error
sec	second(s)
sing.	singular
SNB	stagonospora nodorum blotch
sp.	species (sing.)
spp.	species (pl.)
subsp.	subspecies
syn.	synonym
TCA	tricarboxylic acid
TMS	trimethylsilyl
Tween 20	polyoxyethylenesorbitan monolaurate
U	unit(s)
UV	ultraviolet
V	volt(s)
v/v	volume per volume
WT	wild type
w/v	weight per volume

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PAPERS PUBLISHED FROM THIS STUDY

Solomon, P.S., Waters, O.D.C. and Oliver, R.P. (2007) Decoding the mannitol enigma in filamentous fungi. *Trends in Microbiology* **15 (6)**: 257-262.

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*As equal first author. My contribution to this paper included the creation and characterisation of the *mdh1* mutant and the *mpd1mdh1* double mutant and the discovery that the double mutant was unable to undergo asexual sporulation without the addition of exogenous mannitol.

A paper reporting the results of the ^{13}C-NMR study conducted here is in preparation.