

Sporulation of *Stagonospora nodorum*

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This thesis is presented for the degree of
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Declaration

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

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Abstract

Stagonospora nodorum is a necrotrophic fungal pathogen that is the causal agent of leaf and glume blotch on wheat. Very little is currently known about the molecular mechanisms required for pathogenicity of *S. nodorum*, despite its major impact on Australian agriculture. *S. nodorum* is a polycyclic pathogen. Rain-splashed pycnidiospores attach to and colonise wheat tissue and subsequently sporulate within 2-3 weeks. Several cycles of infection are needed to build up inoculum for the damaging infection of flag leaves and heads, sporulation is therefore a critical component of the infection cycle of *S. nodorum*; our aim is to determine the genetic and biochemical requirements for sporulation for development of control of the pathogen. Disease progression of *S. nodorum* on wheat cv. Amery was monitored by light microscopy to determine the time point when pycnidia development began. Early pycnidia development was evident 12 days post-infection. This information was used to guide a genomics and a metabolomics based approach to determine the requirements for sporulation in *S. nodorum*. The genomics approach utilised two cDNA libraries created from sporulating and non-sporulating cultures. EST frequency was used to determine highly expressed genes under the two developmental states. Gene expression from the most highly represented genes during sporulation were confirmed using quantitative PCR. A gene encoding an arabitol 4-dehydrogenase (*Abd1*), was mutagenised, in its absence sporulation was reduced by approximately 20%. The metabolomics approach isolated metabolites from both *in planta* infection and *in vitro* growth. Rapid changes in the abundance of metabolites were detected during the onset of sporulation. Key fungal metabolites identified include mannitol and trehalose. The concentration of both mannitol and trehalose increased dramatically in concert with pycnidia formation. Both

mannitol and trehalose have also been linked to pathogenicity in filamentous fungi. Creation of deletion mutants of the gene encoding trehalose 6-phosphate synthase showed the synthesis of trehalose is required for full sporulation of *S. nodorum* *in planta* and *in vitro*.

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Table of contents

CHAPTER 1 INTRODUCTION.....	1
1.1 WHEAT PRODUCTION AND FUNGAL DISEASE.....	2
1.2 STAGONOSPORA NODORUM.....	2
1.3 NECROTROPHIC FUNGAL PATHOGENS.....	7
1.4 STAGONOSPORA NODORUM AND WHEAT LIFE CYCLE.....	7
1.5 WORLDWIDE DISTRIBUTION.....	12
1.6 CONTROL OF LEAF AND GLUME BLOTCH.....	14
1.7 MOLECULAR GENETICS IN <i>STAGONOSPORA NODORUM</i>	15
1.8 MOLECULAR BIOLOGY OF PATHOGENICITY OF <i>STAGONOSPORA NODORUM</i>	17
1.9 SPORULATION IN THE FILAMENTOUS FUNGI.....	20
1.10 SUMMARY.....	24
CHAPTER 2 GENERAL MATERIALS AND METHODS.....	25
2.1 GENERAL REAGENTS.....	26
2.2 MEDIA.....	26
2.2.1 <i>Luria-Bertani medium</i>	26
2.2.2 <i>SOC medium</i>	26
2.2.3 <i>CZV8CS solid medium</i>	26
2.2.4 <i>CZV8CS broth</i>	26
2.2.5 <i>Minimal medium</i>	27
2.2.6 <i>Benzimidazole agar</i>	27
2.2.7 <i>CZV8 protoplast medium</i>	27
2.2.8 <i>CZV8 protoplast top agar</i>	27
2.3 MEDIA ADDITIONS.....	27
2.3.1 <i>Ampicillin</i>	27
2.3.2 <i>Chloramphenicol</i>	27
2.3.3 <i>Kanamycin</i>	28
2.3.4 <i>Tetracycline</i>	28
2.3.5 <i>Phleomycin</i>	28
2.3.6 <i>Hygromycin</i>	28
2.3.7 <i>IPTG</i>	28
2.3.8 <i>X-Gal</i>	28
2.3.9 <i>Complete supplement (CS)</i>	29
2.3.10 <i>100 x trace solution</i>	29
2.3.11 <i>Centrifuged V8 juice</i>	29
2.4 <i>S. NODORUM</i> STRAIN.....	29
2.5 WHEAT CULTIVAR AND GROWTH CONDITIONS.....	29
2.6 <i>S. NODORUM</i> CULTURE GROWTH CONDITIONS:.....	30
2.7 TRANSFORMATION OF <i>S. NODORUM</i>	30
2.7.1 <i>Solutions</i>	30
2.7.1.1 <i>Protoplast wash solution</i>	30
2.7.1.2 <i>Glucanex digestion solution</i>	30
2.7.1.3 <i>1 M Sorbitol solution</i>	30
2.7.1.4 <i>Protoplast overlay solution</i>	30
2.7.1.5 <i>STC solution</i>	31
2.7.1.6 <i>PEG solution</i>	31
2.7.2 <i>Procedures</i>	31
2.7.3 <i>PCR screening of <i>S. nodorum</i> transformants</i>	32
2.8 SOUTHERN BLOT.....	32
2.8.1 <i>Digestion and separation of genomic DNA for Southern blot</i>	32
2.8.2 <i>Transfer of nucleic acids to nylon membrane</i>	33
2.8.3 <i>Probe labeling</i>	33
2.8.4 <i>Hybridisation of probe to transferred nucleic acids</i>	33
2.8.5 <i>Detection of probe hybridised to transferred nucleic acids</i>	33
2.9 PLASMIDS.....	34
2.10 POLYMERASE CHAIN REACTION (PCR).....	35
2.10.1 <i>PCR reagents</i>	35
2.10.2 <i>DNA polymerase</i>	35

2.10.3	<i>dNTP solution</i>	35
2.10.4	<i>10 x PCR buffer</i>	35
2.10.5	<i>Primer stocks</i>	35
2.10.6	<i>Standard PCR reaction components</i>	35
2.10.7	<i>High-fidelity PCR reaction components</i>	35
2.10.8	<i>Thermal cycler settings</i>	36
2.11	PCR PRIMERS	37
2.12	RESTRICTION ENZYME DIGESTION OF DNA.....	40
2.13	DNA LIGATION	40
2.14	ESCHERICHIA COLI STRAINS	40
2.15	BACTERIAL TRANSFORMATION	41
2.15.1	<i>Blue/white selection</i>	41
2.15.2	<i>E. coli competent cells preparation</i>	41
2.15.3	<i>Plasmid DNA isolation</i>	42
2.15.4	<i>Genomic DNA isolation</i>	42
2.16	AGAROSE GEL ELECTROPHORESIS.....	42
2.16.1	<i>Agarose gel electrophoresis method</i>	42
2.16.2	<i>DNA cleanup</i>	43
2.16.2.1	<i>Gel extraction</i>	43
2.16.2.2	<i>PCR product clean up</i>	43
2.16.3	<i>Automated sequencing of DNA</i>	43
2.17	<i>S. NODORUM</i> SPORE HARVEST.....	43
2.18	<i>S. NODORUM</i> PATHOGENICITY ASSAYS.....	44
2.18.1	<i>Growth of wheat cv. Amery</i>	44
2.18.2	<i>Detached leaf assay (DLA)</i>	44
2.18.3	<i>Whole plant spray (WPA)</i>	44
2.18.4	<i>Whole plant spray disease progression scoring system</i>	45
2.18.5	<i>Latent period assay (LPA)</i>	45
2.18.6	<i>Pycnidium growth stages</i>	45
2.19	STATISTICAL TESTS.....	46
2.19.1	<i>The t-test</i>	46
2.19.2	<i>Tukey-Kramer significance test</i>	46
2.20	<i>S. NODORUM</i> GENOME SEQUENCE ACCESS.....	47
2.20.1	<i>Genomic sequence</i>	47
2.20.2	<i>Expressed sequence tags (ESTs)</i>	47
CHAPTER 3 CYTOLOGY OF SPORULATION IN <i>STAGONOSPORA NODORUM</i>		48
3.1	INTRODUCTION.....	49
3.2	MATERIALS AND METHODS.....	51
3.2.1	<i>Trypan blue staining</i>	51
3.2.1.1	<i>Trypan blue staining solution</i>	51
3.2.1.2	<i>Trypan blue staining procedure</i>	51
3.2.2	<i>Sectioning of infected leaves</i>	51
3.3	RESULTS.....	52
3.3.1	<i>Infection progress from penetration to sporulation</i>	52
3.3.2	<i>Specific stages of pycnidial development</i>	57
3.4	DISCUSSION.....	61
3.4.1	<i>Sampling for transcript profiling and metabolite analysis</i>	61
3.4.2	<i>The infection cycle of <i>S. nodorum</i> in planta</i>	61
3.4.3	<i>Asexual development of <i>S. nodorum</i> in planta</i>	62
CHAPTER 4 TRANSCRIPT ANALYSIS OF <i>S. NODORUM</i> GROWTH IN PLANTA AND IN VITRO.		64
4.1	INTRODUCTION.....	65
4.1.1	<i>Transcriptomics</i>	65
4.1.1.1	<i>DNA microarray</i>	65
4.1.1.2	<i>SAGE</i>	66
4.1.1.3	<i>EST analysis</i>	66
4.1.2	<i>EST studies of filamentous fungi</i>	67
4.2	MATERIALS AND METHODS.....	72
4.2.1	<i>RNA isolation for in planta cDNA library construction</i>	72

4.2.1.1	DEPC-treated H ₂ O	72
4.2.2	<i>DNase treatment of in planta RNA</i>	72
4.2.3	<i>In planta cDNA library construction and manipulation</i>	72
4.2.4	<i>In vitro oleate-induced cDNA library</i>	73
4.2.4.1	Growth conditions for <i>in vitro</i> oleate-induced cultures	73
4.2.4.2	cDNA library construction	73
4.2.5	<i>Bioinformatics</i>	74
4.2.5.1	Blast searches	74
4.2.5.2	EST trimming	74
4.2.5.3	EST clustering	74
4.2.5.4	EST annotation	75
4.2.6	<i>Growth of SN15 in vitro for sporulating and non-sporulating cultures</i>	75
4.2.7	<i>Reverse transcription of RNA for quantitative PCR</i>	76
4.2.8	<i>Quantitative PCR</i>	76
4.2.9	<i>Overlap PCR for <i>Abd1</i> knockout construct</i>	77
4.2.9.1	DNA template	77
4.2.9.2	Reaction components	77
4.2.9.3	Thermocycler settings	78
4.3	RESULTS	79
4.3.1	<i>RNA isolation for cDNA library</i>	79
4.3.2	<i>cDNA library construction</i>	79
4.3.3	<i>Analysis of the pilot study of the in planta library</i>	80
4.3.4	<i>Trimming and sequence quality control</i>	81
4.3.5	<i>Clustering of ESTs</i>	83
4.3.6	<i>Gene annotation</i>	83
4.3.7	<i>Gene expression analysis by EST abundance</i>	86
4.3.8	<i>Gene ontology analysis</i>	86
4.3.9	<i>Candidate selection</i>	100
4.3.10	<i>Gene expression of candidate genes during sporulation in vitro and in planta</i>	100
4.3.11	<i>Annotation of SNOG_14274</i>	106
4.3.12	<i>Deletion of the gene <i>Abd1</i>, encoding arabitol 4-dehydrogenase</i>	107
4.3.13	<i>Pathogenicity of <i>abd1</i> strains in a detached leaf assay</i>	109
4.3.13.1	Pycnidia development	109
4.3.13.2	Lesion development	112
4.4	DISCUSSION	112
4.4.1	<i>Analysis of the two cDNA libraries</i>	114
4.4.2	<i>Analysis of genes up-regulated in each library</i>	115
4.4.2.1	GO annotation of EST libraries	115
4.4.2.2	Genes up regulated in <i>planta</i>	115
4.4.3	<i>Genes up regulated in vitro</i>	117
4.4.4	<i>Quantitative expression analysis of genes upregulated in planta</i>	118
4.4.5	<i>Sporulation of <i>abd1</i> mutants</i>	119
CHAPTER 5 METABOLOMICS OF IN PLANTA SPORULATION		122
5.1	INTRODUCTION	123
5.1.1	<i>Metabolomics</i>	123
5.1.1.1	Metabolomics and metabonomics	124
5.1.2	<i>Technologies</i>	124
5.1.2.1	Nuclear magnetic resonance spectroscopy	125
5.1.2.2	Gas chromatography-mass spectrometry	126
5.1.2.3	Liquid chromatography-mass spectrometry	128
5.1.2.4	Minor platforms for metabolomics	128
5.1.3	<i>Metabolomics studies of fungi</i>	130
5.1.4	<i>Metabolomics of plant pathogen interactions</i>	130
5.1.5	<i>This study</i>	132
5.2	MATERIALS AND METHODS	134
5.2.1	<i>Infected wheat leaves for GC-MS analysis</i>	134
5.2.2	<i>Extraction of metabolites for GC-MS analysis</i>	134
5.2.3	<i>Derivatisation of metabolites for GC-MS analysis</i>	134
5.2.4	<i>GC-MS analysis</i>	135
5.2.4.1	Gas chromatography temperature settings	135
5.2.4.2	Chromatogram deconvolution	136

5.2.5	<i>Metabolite identification</i>	136
5.2.5.1	GC-MS retention-time and mass-spectral libraries.....	137
5.2.5.2	Retention time comparison between experimental and the “Pol_fa TMS” library.....	138
5.2.6	<i>Metabolite abundance calculation</i>	139
5.2.6.1	Data processing.....	139
5.2.7	<i>Hierarchical cluster analysis</i>	139
5.2.8	<i>Principal components analysis</i>	140
5.3	RESULTS.....	141
5.3.1	<i>GC-MS analysis of polar metabolites from S. nodorum infected wheat</i>	141
5.3.2	<i>Metabolite distribution at time points</i>	151
5.3.3	<i>Hierarchical clustering of in planta metabolomics data</i>	156
5.3.4	<i>Hierarchical clustering of individual metabolite profiles</i>	156
5.3.5	<i>Principle components analysis of in planta metabolite profiles during sporulation</i>	161
5.3.6	<i>t-Test statistical analysis of metabolite abundances</i>	167
5.3.7	<i>Tukey-Kramer statistical analysis of metabolite abundances</i>	169
5.4	DISCUSSION.....	182
5.4.1	<i>Aims</i>	182
5.4.2	<i>Method development</i>	182
5.4.3	<i>GC-MS analysis of S. nodorum infected wheat</i>	184
5.4.4	<i>Sporulation associated metabolites</i>	185
5.4.4.1	The biological significance of mannitol and trehalose.....	187
5.4.5	<i>Metabolite distribution at time points</i>	189
5.4.6	<i>HCA of in planta metabolite changes</i>	190
5.4.7	<i>PCA of in planta metabolomic changes</i>	193
CHAPTER 6 METABOLOMICS OF <i>IN VITRO</i> SPORULATION.....		195
6.1	INTRODUCTION.....	196
6.2	MATERIALS AND METHODS.....	197
6.2.1	<i>Growth of SN15 in vitro cultures for GC-MS analysis</i>	197
6.2.2	<i>Extraction of metabolites for GC-MS analysis</i>	197
6.2.3	<i>GC-MS analysis</i>	197
6.2.3.1	Retention-time locking.....	197
6.2.3.2	Analysis of saturated metabolite extracts.....	198
6.2.4	<i>Principal components analysis</i>	198
6.2.4.1	Varimax rotation of components.....	198
6.2.5	<i>Metabolic pathway data mapping</i>	198
6.3	RESULTS.....	199
6.3.1	<i>A method to capture the entire metabolome range by GC-MS</i>	199
6.3.2	<i>The metabolome of S. nodorum during sporulation in vitro</i>	201
6.3.3	<i>Hierarchical cluster analysis of metabolite profiles</i>	213
6.3.4	<i>Principle components analysis of in vitro metabolite profiles during sporulation</i>	220
6.3.5	<i>Statistical analysis of in vitro metabolite abundances</i>	227
6.3.6	<i>Metabolite composition of S. nodorum pycnidiospores</i>	238
6.4	DISCUSSION.....	241
6.4.1	<i>Method development - diluted samples</i>	241
6.4.2	<i>Method development - RT locking</i>	241
6.4.3	<i>GC-MS analysis of S. nodorum SN15 grown in vitro</i>	242
6.4.4	<i>Distribution of metabolites across time-points</i>	243
6.4.5	<i>Sporulation associated metabolites</i>	245
6.4.6	<i>The metabolome of the S. nodorum pycnidiospore</i>	246
6.4.7	<i>Lipid metabolism and the asexual life cycle of S. nodorum</i>	247
6.4.8	<i>Glycerol biosynthesis and early growth of S. nodorum</i>	250
6.4.9	<i>Summary</i>	256
CHAPTER 7 TREHALOSE BIOSYNTHESIS AND SPORULATION IN <i>STAGONOSPORA NODORUM</i> 257		
7.1	INTRODUCTION.....	258
7.1.1	<i>Trehalose structure</i>	258
7.1.2	<i>Trehalose biosynthesis and degradation</i>	258
7.1.3	<i>The OtsA/B pathway</i>	261
7.1.3.1	The TreY/Z and TreS pathways.....	262

7.1.4	<i>Roles of trehalose with the cell</i>	262
7.1.4.1	Trehalose biosynthesis as a control point for glycolysis.....	263
7.1.4.2	Desiccation stress.....	263
7.1.4.3	Heat stress.....	264
7.1.4.4	Oxidative stress.....	265
7.1.4.5	Osmotic stress.....	265
7.1.4.6	Carbohydrate store.....	266
7.1.4.7	Pathogenicity.....	266
7.1.4.8	Sporulation.....	267
7.1.5	<i>This study</i>	268
7.2	MATERIALS AND METHODS.....	269
7.2.1	<i>Liquid culture growth assay</i>	269
7.2.2	<i>Oxidative stress assay</i>	269
7.2.3	<i>Heat stress assay</i>	269
7.2.4	<i>Germination assay</i>	269
7.2.4.1	Thin agarose slides.....	269
7.2.4.2	Germination procedure.....	270
7.2.5	<i>SIM mode GC-MS</i>	270
7.2.6	<i>Bioinformatics</i>	270
7.2.6.1	Sequence similarity searches.....	270
7.2.6.2	Multiple sequence alignment.....	271
7.2.6.3	Phylogenetic tree building.....	271
7.2.6.4	<i>S. nodorum</i> SN15 genome sequence.....	271
7.2.6.5	Contig building.....	271
7.3	RESULTS.....	272
7.3.1	<i>Identification of the gene encoding trehalose 6-phosphate synthase</i>	272
7.3.2	<i>Disruption of the gene encoding trehalose 6-phosphate synthase</i>	278
7.3.3	<i>The ability of tps1 mutants to synthesise trehalose</i>	283
7.3.4	<i>Ability of tps1 strains to utilise various nutrient sources</i>	290
7.3.5	<i>The ability of tps1 strains to cause disease</i>	293
7.3.6	<i>The ability of tps1 strains to sporulate</i>	297
7.3.6.1	Pycnidia formation during latent period assay.....	297
7.3.6.2	Pycnidia formation during a detached leaf assay.....	297
7.3.6.3	Sporulation during <i>in vitro</i> growth.....	301
7.3.7	<i>Pycnidia morphology during growth in planta</i>	301
7.3.8	<i>Stress response in the tps1 strains</i>	303
7.3.9	<i>The effect of oxidative stress on tps1 strains</i>	304
7.3.10	<i>Nitrate utilisation by tps1 strains</i>	308
7.4	DISCUSSION.....	311
7.4.1	<i>Identification of a gene potentially encoding a trehalose 6-phosphate synthase</i>	311
7.4.2	<i>Deletion of Tps1 in S. nodorum</i>	312
7.4.3	<i>Strains lacking the Tps1 locus do not accumulate trehalose to wild-type levels</i>	313
7.4.4	<i>tps1 strains have markedly reduced sporulation</i>	314
7.4.5	<i>Tps1 is dispensable for lesion formation by S. nodorum</i>	317
7.4.6	<i>Strains lacking Tps1 are more sensitive to external stresses</i>	317
7.4.7	<i>Growth on rapidly fermentable carbon sources is unaffected in tps1 mutants</i>	319
7.4.8	<i>Summary</i>	320
	CHAPTER 8 FINAL DISCUSSION	321
8.1	<i>STAGONOSPORA NODORUM AS A MODEL NECROTROPH</i>	322
8.2	OVERVIEW OF KEY FINDINGS.....	323
8.3	TRANSCRIPTOMICS VERSUS METABOLOMICS.....	324
8.4	FUTURE DIRECTIONS.....	326
8.5	CONCLUSION.....	328
	CHAPTER 9 BIBLIOGRAPHY	329
	CHAPTER 10 APPENDICES	344
10.1	PLASMID AND CONSTRUCT MAPS.....	345
10.2	METABOLOMICS RETENTION-TIME LIBRARIES.....	350
10.2.1	<i>The MPIMP “Pol_fa TMS” library</i>	350
10.2.2	<i>ACNFP TMS library</i>	355

List of figures

Figure	Title	page
Figure 1.1	Wheat growing regions of the world.	3
Figure 1.2	Distribution of Australian wheat growing areas and losses due to <i>Stagonospora nodorum</i> .	6
Figure 1.3	Structure of wheat	9
Figure 1.4	Asexual sporulation of <i>S. nodorum</i> on wheat, under controlled conditions.	10
Figure 1.5	Leaf and glume blotch of wheat, field conditions.	12
Figure 1.6	Regulation of sporulation in <i>Aspergillus nidulans</i> .	38
Figure 3.1	Hyphae grow toward stomata on the leaf surface.	54
Figure 3.2	The early infection period.	56
Figure 3.3	The late infection period.	57
Figure 3.4	Early developmental stages of a pycnidium.	59
Figure 3.5	Late developmental stages of a pycnidium.	60
Figure 4.1	Size distribution of clustered ESTs from the <i>in planta</i> library and the <i>in vitro</i> library.	82
Figure 4.2	Distribution of uni-genes and ESTs among the two libraries.	85
Figure 4.3	Quantitative PCR analysis of gene expression from potential sporulation associated genes.	103
Figure 4.4	Gene knockout construct for <i>Abd1</i> .	108
Figure 4.5	Southern blot of <i>abd1</i> strains.	111
Figure 4.6	Pathogenicity of <i>abd1</i> strains on wheat <i>cv.</i> Amery.	113
Figure 5.1	Metabolomics publications written each year.	131
Figure 5.2	Infection progress for <i>in planta</i> metabolomics samples.	142
Figure 5.3	Typical examples of GC-MS traces from the <i>in planta</i> infection dataset.	143
Figure 5.4	Metabolites detected during the infection of wheat <i>cv.</i> Amery with <i>S. nodorum</i> SN15.	146
Figure 5.5	Venn diagram of metabolite distribution amongst the three SN15-infected time-points sampled.	153
Figure 5.6	Venn diagram of metabolite distribution amongst the three mock-infected time-points sampled.	154
Figure 5.7	Venn diagram of metabolite distribution amongst the two infection states, SN15 and Mock infected.	155
Figure 5.8	Hierarchical cluster analysis of <i>in planta</i> samples during sporulation.	157
Figure 5.9	Hierarchical cluster analysis of <i>in planta</i> metabolites during sporulation.	159
Figure 5.10	Principle components analysis of metabolite profiles by treatment.	162
Figure 5.11	Principle components analysis of metabolite profiles; Factor loadings of each component.	164

Figure	Title	page
Figure 5.12	Principle components analysis of <i>in planta</i> metabolites; relative metabolite responses.	166
Figure 5.13	Interesting metabolites based on all analyses completed.	180
Figure 6.1	Column saturation results in aberrant chromatography and underestimation of metabolite abundance.	200
Figure 6.2	Growth morphology for <i>in vitro</i> metabolomics samples.	202
Figure 6.3	Representative examples from gas chromatography-mass spectrometry (GC-MS) analysis of metabolites from <i>in vitro</i> grown SN15.	203
Figure 6.4	Metabolites detected during the growth <i>in vitro</i> of <i>S. nodorum</i> SN15.	207
Figure 6.5	Relative peak areas for metabolites detected during <i>in vitro</i> growth of <i>S. nodorum</i> SN15.	212
Figure 6.6	Venn diagram of metabolite distribution amongst the three SN15-infected time-points sampled.	215
Figure 6.7	Hierarchical cluster analysis of <i>in vitro</i> samples during sporulation.	216
Figure 6.8	Hierarchical cluster analysis of metabolites during growth <i>in vitro</i> .	218
Figure 6.9	Principle components analysis of metabolite profiles by treatment.	221
Figure 6.10	Factor loadings of metabolites within principle components one and two.	223
Figure 6.11	Principle components analysis of <i>in vitro</i> metabolites; relative metabolite responses.	226
Figure 6.12	Metabolites that show significant changes in the Tukey-kramer test.	234
Figure 6.13	Composition of <i>S. nodorum</i> pycnidiospores.	239
Figure 6.14	Venn diagram of metabolite distribution amongst the two growth scenarios, <i>in planta</i> and <i>in vitro</i> .	244
Figure 6.15	Primary metabolism of <i>S. nodorum</i> during sporulation.	248
Figure 7.1	Disaccharides.	259
Figure 7.2	Overview of trehalose biosynthesis and breakdown.	260
Figure 7.3	Identification of conserved sequence domains in SNOG_9603.1 and the manually annotated sequence using the CD database at the NCBI.	274
Figure 7.4	Sequencing annotation of <i>Tps1</i> .	275
Figure 7.5	Phylogenetic tree of enzymes involved in trehalose biosynthesis and breakdown in fungi.	277
Figure 7.6	Multiple sequence alignment of <i>Tps1</i> sequences.	279
Figure 7.7	Diagram of <i>Tps1</i> knockout construct.	282
Figure 7.8	PCR Screening of <i>Tps1</i> transformants.	284
Figure 7.9	Southern blot of <i>tps1</i> strains.	285
Figure 7.10	Key metabolite levels of <i>tps1</i> strains during growth <i>in vitro</i> .	286
Figure 7.11	Relative trehalose levels of <i>tps1</i> strains during growth <i>in</i>	288

Figure	Title	page
	<i>planta.</i>	
Figure 7.12	Absolute trehalose concentrations in the spores of <i>tps1</i> strains.	289
Figure 7.13	Growth in liquid culture of <i>tps1</i> strains in various defined media.	291
Figure 7.14	Growth behavior of <i>tps1</i> strains on solid media.	292
Figure 7.15	Growth rate of <i>tps1</i> strains on solid media.	294
Figure 7.16	Pathogenicity of <i>tps1</i> strains in a whole plant spray assay.	295
Figure 7.17	Pathogenicity of <i>tps1</i> strains in a detached leaf assay.	296
Figure 7.18	Pycnidia development by <i>tps1</i> strains <i>in planta</i> , in a latent period assay	298
Figure 7.19	Pycnidiospore production by <i>tps1</i> strains <i>in planta</i> .	299
Figure 7.20	Pycnidia development by <i>tps1</i> strains during the detached leaf assay.	300
Figure 7.21	Sporulation of <i>tps1</i> strains <i>in vitro</i> .	302
Figure 7.22	Microscopic morphology of <i>tps1</i> strains during sporulation <i>in planta</i> .	305
Figure 7.23	Germination rates of <i>tps1</i> strains under heat stress.	306
Figure 7.24	Growth rates of <i>tps1</i> strains under heat stress.	307
Figure 7.25	Growth rates of <i>tps1</i> strains under oxidative stress.	309
Figure 7.26	Nitrate utilisation in the <i>tps1</i> strains	310

List of tables

<i>Table</i>	<i>Title</i>	<i>page</i>
Table 1.1	Top ten wheat producers - 2003, 2004	4
Table 1.2	Australian wheat production statistics	5
Table 2.1	Plasmid vectors used in this study	34
Table 2.2	PCR primers used in this study	37
Table 4.1	Selected EST studies on filamentous fungi	69
Table 4.2	Clustering of ESTs	84
Table 4.3	The top 100 genes overrepresented in the <i>in planta</i> library	87
Table 4.4	The top 100 genes overrepresented in the <i>in vitro</i> library	91
Table 4.5	Gene ontology (GO) annotation of the EST set.	95
Table 4.6	Biological processes up-regulated in the <i>in planta</i> library or the <i>in vitro</i> library.	97
Table 4.7	Cellular components up-regulated in the <i>in planta</i> library or the <i>in vitro</i> library.	98
Table 4.8	Molecular functions up-regulated in the <i>in planta</i> library or the <i>in vitro</i> library.	99
Table 4.9	Candidate genes selected as potential sporulation associated genes.	102
Table 5.1	Metabolites detected during the infection of wheat cv. Amery with <i>S. nodorum</i> SN15.	144
Table 5.2	Metabolite classes detected during the infection of wheat cv. Amery with <i>S. nodorum</i> SN15.	152
Table 5.3	Correlated metabolites in the <i>in planta</i> samples	168
Table 5.4	Comparison of metabolite abundances by t-test, between infected and uninfected samples.	170
Table 5.5	Comparison of metabolite abundances by t-test, between time-points.	174
Table 5.6	Tukey-Kramer significance test of SN15 infection across time-points.	178
Table 6.1	Metabolites detected during the growth <i>in vitro</i> of <i>S. nodorum</i> SN15.	204
Table 6.2	Classes of metabolites detected during the growth of <i>S. nodorum</i> SN15.	214
Table 6.3	Comparison of <i>in vitro</i> metabolite abundances by t-test, between time-points.	228
Table 6.4	Tukey-Kramer comparison of metabolite abundances between time points.	232

List of abbreviations

1-P	1-phosphate
3-P	3-phosphate
6-P	6-phosphate
A	absorbance
Abd1	arabitol 4-dehydrogenase 1
acetyl-CoA	acetyl-coenzyme A
ALA	delta-aminolaevulinic acid
amp	ampicillin
AUD	Australian dollars
ave	average
bp	nucleotide base pair(s)
cDNA	complementary deoxyribonucleic acid
CE-MS	capillary electrophoresis-mass spectrometry
cv	cultivar
CZV8CS	Czapek Dox V8 juice complete supplement
d	day
DEPC	diethylpyrocarbonate
DIG	digoxigenin
DLA	detached leaf assay
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
dpi	days post inoculation
E	exponential
EST	expressed sequence tag
FT-ICR	Fourier transform-ion cyclotron resonance
GABA	gamma-aminobutyric acid
GC-MS	gas chromatography-mass spectrometry
gDNA	genomic deoxyribonucleic acid
GO	gene ontology
HCA	hierarchical cluster analysis
hyg	hygromycin
IPTG	isopropyl-beta-D-thiogalactopyranoside
kb	kilobase pairs
LB	Luria Bertani
LC-MS	liquid chromatography-mass spectrometry
LPA	latent period assay
malonyl-ACP	malonyl-acyl carrier protein
Mb	megabase pairs
MCS	multiple cloning site
MEOX	methoxylamine
MM	minimal medium
mRNA	messenger ribonucleic acid
MST	mass-spectral tag

n	number
NMR	nuclear magnetic resonance
P Value	probability value
PC	principal component
PCA	principal component analysis
PCR	polymerase chain reaction
pers. comm.	personal communication
phleo	phleomycin
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
RNase	ribonuclease
RT	retention time
SAGE	serial analysis of gene expression
SDS	sodium dodecyl sulphate
stdev	standard deviation
T6P	trehalose 6-phosphate
TCA cycle	tricarboxylic acid cycle
TMS	trimethylsilyl
TOF	time of flight
Tps1	trehalose 6-phosphate synthase 1
v/v	volume per volume
w/v	weight per volume
WPA	whole plant spray
X-GAL	5-bromo-4-chloro-3-indolyl-beta-D-galactoside