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Investigation of dothistroma needle blight development on *Pinus radiata*

A thesis presented in the partial fulfilment of the
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Abstract

Dothistroma needle blight (DNB), caused by the fungi *Dothistroma septosporum* and *Dothistroma pini*, is an important foliar disease of pine species throughout the world and predictions of the future spread of this disease have been made using climate models. Although DNB infection is prevalent in many forests, attempts to achieve infection under controlled laboratory or glasshouse conditions are notoriously difficult. However, artificial infection is a very important tool for studying different aspects of plant-microbe interactions, such as pathogen life style and roles of virulence factors. *D. septosporum* was thought to have a hemi-biotrophic life style but this was not formally investigated *in planta*. The non-host selective toxin dothistromin produced by this fungus was shown not to be essential for pathogenicity but its role in pathogen virulence was unknown.

The aims of this study were to improve the DNB pathogenicity assay and to use this system to test the hypotheses that *D. septosporum* is a hemi-biotrophic pathogen and that dothistromin plays a role in virulence.

A new sporulation medium (pine needle medium with glucose) was used to obtain sufficient viable *D. septosporum* spores. The critical microclimatic component of leaf wetness was optimised to have a short (4-7 d) high wetness period followed by 'medium' wetness (continual misting), and using these conditions >80% needle infection was routinely achieved on *Pinus radiata* seedlings.

A combination of microscopy, biochemical and molecular studies over a time-course of infection of *P. radiata* by *D. septosporum* confirmed its hemi-biotrophic life style. Restricted mesophyll colonisation, shorter lesions and fewer spores from *P. radiata* needles infected with dothistromin-deficient mutants, compared to those with

wild type *D. septosporum*, suggested that dothistromin has a role in virulence. Interestingly ‘green islands’ in which chlorophyll levels were maintained at higher levels than adjacent chlorotic and necrotic regions, surrounded early-appearing lesions caused by both wild-type and mutant isolates. At a later developmental stage of the lesion the green islands were still present in the mutant but appeared to be masked by the extended dothistromin-containing lesions in the wild type, which lead to the hypothesis that chloroplasts could be a site of action of dothistromin.

The discovery that dothistromin is a virulence factor opens up new insights into the *Dothistroma*-pine interaction. This fundamental finding will be useful for management strategies for this important disease in the future.

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Table of contents

Abstract	i
Acknowledgements	iii
Table of contents	v
List of tables	ix
List of figures	x
Abbreviation	xiv
Chapter 1: Introduction	1
1.1. Dothistroma needle blight disease	1
1.1.1. Incidence of the disease	1
1.1.2. Dothistroma needle blight symptoms	4
1.1.3. Dothistroma needle blight and its hosts	4
1.1.4. Management of dothistroma needle blight	5
1.2. Dothistroma pathogens cause dothistroma needle blight	6
1.3. Parasitic phases of plant-fungal interactions	7
1.3.1. Different life styles in plant-microbe interactions	7
1.3.2. Molecular aspects of biotrophic plant-fungal interactions	8
1.3.3. Molecular aspects of necrotrophic plant fungal interactions	10
1.3.4. Molecular aspects of hemi-biotrophic plant fungal interactions	11
1.3.5. Fungi having a latent phase <i>in planta</i> and their quantification	13
1.3.6. Identification of life styles of plant-microbe interactions	14
1.3.7. Use of green fluorescence protein in fungal biology	14
1.4. Microbial toxins and plant disease	15
1.4.1. Introduction and classification of toxins	15
1.4.2. Host-selective toxins and their roles	16
1.4.3. Non-host selective toxins and their roles	17
1.5. Dothistromin toxin	17
1.5.1. Biosynthesis of dothistromin <i>in vitro</i> and <i>in planta</i>	18
1.5.2. Genetics of dothistromin biosynthesis	22
1.5.3. Mode of action of dothistromin	26
1.5.4. Role of dothistromin in dothistroma needle blight pathogenesis	27
1.6. Hypotheses, aims and objectives	29

Chapter 2: Materials and Methods.....	31
2.1. Biological materials	31
2.2.1. Fungal isolates	31
2.2.1.1. Isolation and confirmation of <i>Dothistroma septosporum</i>	31
2.1.2.2. Maintenance of fungal isolates	32
2.2. Plant material	32
2.3. Pathogenicity assay	33
2.3.1. Sporulation of <i>Dothistroma septosporum</i> (<i>in vitro</i> and <i>in planta</i>)	33
2.3.2. Spore germination, surface growth and penetration	34
2.3.3. Pathogenicity assay chamber	35
2.3.4. Plant inoculation techniques	36
2.3.5. Adhesion tests	36
2.3.6. Microclimate optimisation	37
2.3.7. <i>Dothistroma</i> needle blight infection and scoring	37
2.3.8. Pathogenicity assays for role of dothistromin	38
2.4. Microscopy.....	39
2.4.1. Light microscopy	39
2.4.2. Confocal microscopy	39
2.4.3. Transmission electron microscopy	40
2.4.4. Scanning electron microscopy	41
2.5. Histo-chemical studies	41
2.5.1. Cell viability.....	41
2.5.2. Reactive oxygen species (ROS) staining	42
2.5.3. Lignification test	42
2.6. Molecular and biochemical studies.....	43
2.6.1. PCR Diagnostics	43
2.6.1.1. DNA extraction	43
2.6.1.2. <i>Dothistroma</i> needle blight confirmation by PCR.....	43
2.6.2. <i>Dothistroma septosporum</i> biomass and dothistromin toxin quantification ..	44
2.6.2.1. Needle sampling.....	44
2.6.1.2. Quantification of <i>Dothistroma septosporum</i> biomass	45
2.6.2.3. Dothistromin toxin quantification	46
2.6.3. Semi-quantitative gene expression	48
2.6.4. Gene expression studies	49

2.6.5. Chlorophyll quantification	50
2.7. Statistical analysis	51
2.7.1. ANOVA	51
2.7.2. Scattered plot analysis.....	51
2.7.3. Student t-test	51
Chapter 3: Pathogenicity assay optimisation.....	53
3.1. Introduction	53
3.2. Results.....	55
3.2.1. Sporulation	55
3.2.2. Inoculation	56
3.2.3. Needle Wetness.....	57
3.2.4. Host Genotype.....	60
3.3. Discussion	63
Chapter 4: Life style of <i>Dothistroma septosporum</i> in planta	69
4.1. Introduction	69
4.2. Results.....	72
4.2.1. Microscopy, molecular and biochemical studies of <i>Dothistroma septosporum</i> life style.....	72
4.2.1.1. Overview of <i>Dothistroma septosporum</i> life cycle in planta.....	72
4.2.1.2. Host cell disintegration during stages 3 and 4 of dothistroma needle blight	80
4.2.1.3. Histo-chemical analysis of cell death and host response during dothistroma needle blight.....	86
4.2.1.4. Chlorophyll loss during dothistroma needle blight infection.....	87
4.2.1.5. Growth and toxin production by <i>Dothistroma septosporum</i> during dothistroma needle blight infection.....	89
4.2.2. Gene expression studies and the life style of <i>Dothistroma septosporum</i>	96
4.2.2.1. Expression of dothistromin genes in planta.....	96
4.2.2.2. Expression of other genes associated with life style.....	101
4.3. Discussion	103

Chapter 5: Role of dothistromin during dothistroma needle blight.....	113
5.1. Introduction.....	113
5.2. Results.....	115
5.2.1. Spore germination and surface growth of dothistromin-deficient mutants at stage 1	115
5.2.2. Needle penetration of dothistromin-deficient mutants at stage 2	117
5.2.3. Mesophyll colonisation of dothistromin-deficient mutant at stages 3 and 4	118
5.2.4. Effect of dothistromin on lesion development at stages 3 and 4	119
5.2.5. Cell damage by dothistromin-deficient mutants	122
5.2.6. Fruiting body formation and sporulation by dothistromin-deficient mutants	125
5.2.7. Chlorophyll quantification in needles with dothistroma needle blight lesions	126
5.3. Discussion.....	128
Chapter 6: Conclusions and future directions.....	135
Appendices	141
Appendix 1: Media, buffers, primers and probes.....	141
Appendix 2: Calculations of <i>Dothistroma septosporum</i> biomass and dothistromin toxin	144
Appendix 3: Pathogenicity assay optimisation	149
Appendix 4: Life style of <i>Dothistroma septosporum</i>	153
Appendix 5: Role of dothistromin	161
Appendix 6: Publication and conference presentation.....	166
References	167

List of tables

Table 1.1. Biological functions of some secondary metabolite and proteinaceous host selective toxins from <i>Dothideomycete</i> spp.....	11
Table 1.2. Characteristics of 'core' dothistromin genes of <i>Dothistroma septosporum</i>	23
Table 2.1. <i>Dothistroma septosporum</i> isolates used in experiments	31
Table 2.2. Summary of experiments conducted to determine the role of dothistromin <i>in planta</i>	38
Table 2.3. Summary of needle sampling, sampling time and other related information for <i>Dothistroma septosporum</i> biomass and dothistromin toxin quantification during dothistroma needle blight	45
Table 3.1. Sporulation of <i>Dothistroma septosporum</i> on different media	56
Table 3.2. Effect of inoculation techniques on dothistroma needle blight.....	56
Table 3.3. Effect of adhesives on dothistroma needle blight incidence and red bands...	57
Table 3.4. Effect of needle wetness on dothistroma needle blight (DNB) infection	58
Table 3.5. Effect of initial high wetness on spore germination, penetration and dothistroma needle blight symptoms	59
Table 3.6. Dothistroma needle blight events on <i>Pinus radiata</i> clones.....	61
Table 4.1. Macroscopic and microscopic overview of the <i>Dothistroma septosporum</i> life cycle	73
Table 5.1. Spore germination, surface growth and penetration of dothistromin-deficient mutants ($\Delta PksA:gfp$ and $\Delta HexA$) and wild type (WT) <i>Dothistroma septosporum</i>	115
Table 5.2. Comparison of fruiting bodies and spores per lesion between needles infected with dothistromin-deficient mutants and wild type (WT) <i>Dothistroma septosporum</i>	125

Appendix tables

Table A1.1. Primers and probes used for <i>Dothistroma septosporum</i> identification and biomass quantification	143
Table A2.1. Calculation of unknown DNA from pine needle	145
Table A2.2. Calculation of unknown dothistromin from needle	148

Table A3.1. Spore germination of <i>Dothistroma septosporum</i> on PMMG at 7, 12 and 17 day	149
Table A3.2. Percent adhered <i>Dothistroma septosporum</i> spores (<i>in vitro</i> glass surface) after wash, and spore germination using adhesives	149
Table A3.3. Dothistroma needle blight infection with optimised wetness conditions .	150
Table A3.4. Dothistroma needle blight events on <i>Pinus radiata</i> clones.....	152
Table A3.5. Comparison of variance between seedlings and clones	152
Table A4.1. Genes associated with fungal life style	158
Table A4.2. Summary results of <i>Dothistroma septosporum</i> biomass and dothistromin toxin quantification along with environmental parameters.....	159
Table A4.3. Interim transcriptome read counts for the time-course of dothistroma needle blight	160
Table A5.1. Spore germination, surface growth and penetration of dothistromin-deficient mutant ($\Delta PksA:gfp$) and wild type (WT) <i>Dothistroma septosporum</i> .	161
Table A5.2. Spore germination, fungal surface growth and penetration of dothistromin-deficient mutant ($\Delta HexA$) and wild type (WT) <i>Dothistroma septosporum</i>	161
Table A5.3. Comparison of fruiting body and spores per lesion between dothistromin-deficient mutants and wild type <i>Dothistroma septosporum</i> infected needle lesions	164

List of figures

Fig. 1.1. Location of <i>Dothistroma</i> spp. on pines throughout the world.....	1
Fig. 1.2. Distribution and prediction of dothistroma needle blight severity in New Zealand.....	3
Fig. 1.3. Symptoms of dothistroma needle blight	4
Fig. 1.4. Zigzag model of plant immune system.....	9
Fig. 1.5. Plant-microbe interaction of <i>Colletotrichum</i> fungi along with gene expression	12
Fig. 1.6. Similarity of chemical structures of dothistromin and aflatoxin precursors versicolorin A and versicolorin B	18
Fig. 1.7. Growth and dothistromin biosynthesis in liquid media by <i>Dothistroma septosporum</i>	20
Fig. 1.8. <i>gfp</i> expression of <i>Dothistroma septosporum</i>	21

Fig. 1.9. Dothistromin (DOTH) fragmented gene cluster and proposed dothistromin biosynthetic pathway.....	24
Fig. 2.1. Pathogenicity assay chamber.....	35
Fig. 3.1. Dothistroma needle blight (DNB) progression.....	60
Fig. 3.2. Dothistroma needle blight (DNB) symptoms on pine clones.....	61
Fig. 3.3. Scatter plots showing percent of needles showing dothistroma needle blight symptoms in <i>Pinus radiata</i> clones or seedlings.....	62
Fig. 4.1. Time course of <i>Mycosphaerella graminicola</i> (septoria leaf blotch) on wheat.....	70
Fig. 4.2. Macroscopic overview of <i>Dothistroma septosporum</i> life cycle.....	73
Fig. 4.3. Microscopic (SEM) overview of the <i>Dothistroma septosporum</i> life cycle.....	74
Fig. 4.4. Stages 1 and 2 of <i>Dothistroma septosporum</i> life cycle.....	76
Fig. 4.5. Stage 3 (mesophyll colonisation) of <i>Dothistroma septosporum</i> life cycle.....	78
Fig. 4.6. Stage 4 (maturation of fruiting bodies) of <i>Dothistroma septosporum</i> life cycle.....	79
Fig. 4.7. Light microscopic observations on pine needle cross sections during dothistroma needle blight progression.....	83
Fig. 4.8. Ultra-structure of dothistroma needle blight infection on <i>Pinus radiata</i> needle at stage 2 and 4.....	85
Fig. 4.9. Neutral red stain indicates loss of mesophyll cell viability in stage 3 of dothistroma needle blight.....	86
Fig. 4.10. Mesophyll colonisation and chlorophyll auto-florescence in <i>Pinus radiata</i> needles by <i>gfp</i> -labelled <i>Dothistroma septosporum</i>	88
Fig. 4.11. Quantification of <i>Dothistroma septosporum</i> biomass and dothistromin toxin from whole needles.....	91
Fig. 4.12. Quantification of <i>Dothistroma septosporum</i> biomass and dothistromin toxin from dothistroma needle blight lesions.....	93
Fig. 4.13. Quantification of <i>Dothistroma septosporum</i> biomass and dothistromin toxin from lesions of susceptible clonal plants.....	95
Fig. 4.14. Semi-quantitative <i>PDotA:gfp</i> gene expression during dothistroma needle blight.....	97
Fig. 4.15. Dothistromin regulated gene <i>PDotA:gfp</i> expression during dothistroma needle blight.....	99
Fig. 4.16. Dothistromin gene expression <i>in planta</i>	100
Fig. 4.17. Expression of genes associated with life style <i>in planta</i>	102

Fig. 5.1. Spore germination, surface growth and penetration of dothistromin-deficient mutant ($\Delta PksA:gfp$) and wild type (WT) <i>Dothistroma septosporum</i> at stages 1 and 2.....	116
Fig. 5.2. Comparison of mesophyll colonisation between dothistromin-deficient mutant ($\Delta PksA:gfp$) and wild type (WT: gfp) <i>Dothistroma septosporum</i> infected needles	118
Fig. 5.3. Comparison of dothistroma needle blight (DNB) events caused by dothistromin-deficient mutants ($\Delta PksA:gfp$ and $\Delta HexA$) and wild type (WT) <i>Dothistroma septosporum</i> at stages 3 and 4	120
Fig. 5.4. Comparison of dothistroma needle blight (DNB) lesions on needles infected with dothistromin-deficient mutants ($\Delta PksA:gfp$, $\Delta HexA$) and wild type (WT) <i>Dothistroma septosporum</i>	121
Fig. 5.5. Cross sections through dothistroma needle blight lesions infected with dothistromin-deficient mutant $\Delta HexA$ and wild type (WT) <i>Dothistroma septosporum</i> at stages 3 and 4.....	123
Fig. 5.6. Scanning electron microscopy of transverse sections of late-stage dothistroma needle blight lesions from dothistromin-deficient mutant ($\Delta HexA$) and wild type (WT) infected needles.....	124
Fig. 5.7. Comparison of fruiting body eruptions from needles infected with dothistromin-deficient mutants and wild type <i>Dothistroma septosporum</i>	126
Fig 5.8. Comparison of chlorophyll content between distinct regions of needles infected with dothistromin mutant and wild type (WT) <i>Dothistroma septosporum</i>	127

Appendix figures

Fig. A2.1. Amplifications and standard curves of <i>PksA</i> and <i>CAD</i> gene.....	144
Fig. A2.2. Standard curve of dothistromin.....	147
Fig. A3.1. Types of <i>Dothistroma septosporum</i> spores seen during sporulation tests...	149
Fig. A3.2. Dothistroma needle blight incidence between taller and smaller seedlings	150
Fig. A3.3. Optimised dothistroma needle blight.....	151
Fig. A3.4. Dothistroma needle blight progression on pine clones.....	151
Fig. A4.1. Spore germination and fungal growth on pine needle surface.....	153
Fig. A4.2. Fruiting body of <i>Dothistroma septosporum</i>	154
Fig. A4.3. Young pine needle	154

Fig. A4.4. Light microscopic view of lignification on pine needle during dothistroma needle blight.....	155
Fig. A4.5. Reactive oxygen species staining during dothistroma needle blight	156
Fig. A4.6. Absolute biomass quantification.....	157
Fig. A5.1. Comparison of dothistroma needle blight (DNB) events caused by dothistromin-deficient mutants and wild type (WT) <i>Dothistroma septosporum</i>	162
Fig. A5.2. Comparison of dothistroma needle blight needle (DNB) lesions between dothistromin-deficient mutant and wild type (WT) <i>Dothistroma septosporum</i> at stages 3 and 4	163
Fig. A5.3. PCR diagnostics of DNB lesions caused by dothistromin-deficient <i>Dothistroma septosporum</i>	165

Contents of CD

1. Raw data of Figs. 4.11, 4.12 and 4.13 (growth and toxin production of *Dothistroma septosporum* in experiment 1 to 5 of section 4.2.1.5).
2. Raw data of Figs 4.16 and 4.17 (quantitative gene expression in section 4.2.2.1.2).
3. Digital version of thesis

Abbreviation

Abbreviation	Meaning
µg	micro gram
µl	micro litre
µM	micro molar
ai	after inoculation
bp	base pair
d	day
DNB	dothistroma needle blight
h	hour
g	gram
GFP	green fluorescent protein
kb	kilo base pair
L	litre
M	molar
min	minute
ml	milli litre
mm	milli metre
mM	milli molar
MQ	Milli Q water
nm	nano metre
°C	degree celsius
PCR	polymerase chain reaction
rpm	revolutions per minute
UV	ultra violet
V	volt
w/v	weight per volume
wpi	weeks post inoculation
WT	wild type