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Development of a pathogenicity testing
system for *Dothistroma pini* infection of
Pinus radiata.

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Abstract

Dothistroma pini is a fungal pathogen of pine species around the world and can be found in most parts of New Zealand. Infection by *D. pini* causes a disease commonly known as Dothistroma needle blight. Dothistroma needle blight has a significant financial impact on New Zealand's forestry industry. Although control of infection by *D. pini* is currently very successful there is a possibility that a new strain introduced from another country could be a lot more damaging and overcome current control measures. In recent years both the incidence and severity of the disease have increased in the northern hemisphere and other parts of the world.

A distinctive characteristic of *Dothistroma* needle blight is the production in the infected needle of a toxic red pigment called dothistromin. Dothistromin is produced as a secondary metabolite by *D. pini* and has known phytotoxic properties as well as clastogenic and mutagenic properties towards human cells. Purified dothistromin toxin injected into pine needles has been shown to reproduce symptoms similar to those observed during *D. pini* infection. Because of this production, dothistromin is thought to play an important role in the infection process. Mutants of *D. pini* that are deficient in dothistromin production have been made recently that will allow this role to be investigated.

The aim of this study was to develop a pathogenicity testing system under PC2 containment (required for dothistromin deficient mutant) and to develop microscopy methods required to monitor both epiphytic and endophytic growth of the fungus on the needle. *D. pini* requires high light intensity, continuous leaf moisture and a specific temperature range in order to infect pine needles. Progress was made towards developing a robust pathogenicity testing system.

This study has also developed several microscopy techniques for the visualisation of epiphytic growth including a fluorescent microscopy technique. Other bright field and fluorescent staining techniques were investigated with some success.

Staining techniques were not successful for the visualisation of endophytic *D. pini* growth but a green fluorescent protein (*sgfp*) reporter construct was obtained and two

gfp plasmid constructs were developed for the transformation of *D. pini* for use as biomarkers. Successful introduction of the *gfp* constructs into *D. pini* will allow *in situ* visualisation of endophytic and epiphytic *D. pini* growth.

The work done in this study will be useful for the further investigation into the role of dothistromin toxin, which may lead to new or more efficient methods of controlling *D. pini* as well as possibly providing information about other polyketide molecules of economic or medical significance.

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TABLE OF CONTENTS

Abstract.....	i
Acknowledgements.....	iii
Table of Contents.....	v
List of Figures.....	x
List of Tables.....	xiii
CHAPTER 1: GENERAL INTRODUCTION.....	1
1.1 Introduction to Dothistroma needle blight.....	1
1.1.1 General Introduction.....	1
1.1.2 Disease symptoms.....	2
1.1.3 Economic impact on New Zealand's Forestry Industry.....	3
1.1.4 Control.....	3
1.2 Introduction to <i>Pinus Radiata</i>	4
1.2.1 Classification.....	4
1.2.2 Dothistroma Resistance.....	5
1.3 Introduction to <i>Dothistroma pini</i>	6
1.3.1 Classification.....	6
1.3.2 Life Cycle of <i>D. pini</i>	7
1.3.3 Origin and Distribution.....	7
1.4 Effect of Environmental Condition on <i>D. pini</i> Growth and Infection of <i>Pinus Radiata</i>	8
1.4.1 Seasonal Effects on <i>D. pini</i> Infection Levels.....	8
1.4.2 Effect of Light Intensity.....	8
1.4.3 Effect of Humidity and Free Water.....	9
1.4.4 Effect of Temperature.....	9
1.5 Microscopy Methods to Visualise <i>D. pini</i> Infection.....	9
1.6 Dothistromin Toxin.....	11
1.6.1 Description.....	11
1.6.2 Evidence Suggesting a Biological Role.....	12
1.7 Generation of a Dothistromin Deficient Mutant (<i>ΔdotA</i>).....	15
1.8 Research Objectives.....	16
CHAPTER 2: GENERAL MATERIALS AND METHODS.....	17
2.1 Biological Strains.....	17
2.1.1 Bacterial Strains.....	17
2.1.2 Fungal Strains.....	17
2.1.3 Plant Material.....	18

2.2 Growth medium.....	18
2.2.1 Aspergillus minimal media.....	18
2.2.2 Dothistroma media (DM).....	18
2.2.3 Dothistroma Sporulation media (DSM).....	18
2.2.4 LB agar.....	18
2.2.5 LB broth.....	19
2.2.6 Potato Dextrose agar.....	19
2.3 Buffers and Solutions.....	19
2.3.1 CTAB buffer.....	19
2.3.2 Ethidium bromide staining solution.....	19
2.3.3 Gel loading dye.....	19
2.3.4 Fluorometer working solution.....	19
2.3.5 Hoechst dye stock solution.....	19
2.3.6 Fluorometer DNA standard.....	19
2.3.7 TBE buffer.....	20
2.3.8 TE buffer.....	20
2.3.9 TNE buffer.....	20
2.4 Culturing techniques.....	20
2.4.1 Maintenance of <i>D. pini</i> stocks.....	20
2.4.2 Obtaining and Quantifying <i>D. pini</i> spore suspensions.....	20
2.4.3 Inoculation of <i>P. radiata</i> seedlings with <i>D. pini</i> spore suspensions.....	21
2.5 Bacterial plasmid DNA preparation.....	21
2.5.1 Preparation of Electroporation Competent <i>E. coli</i> cells.....	21
2.5.2 Electroporation of <i>E. coli</i> XL-1 cells.....	22
2.5.3 Bacterial plasmid DNA extraction.....	22
2.6 Genomic DNA preparation.....	22
2.7 DNA quantification.....	23
2.7.1 Fluorometric assay.....	23
2.7.2 Gel electrophoresis assay.....	23
CHAPTER 3: METHODS TO VISUALISE INFECTION.....	25
3.1 Introduction.....	25
3.2 Materials and Methods.....	27
3.2.1 Plant Material and Inoculation Procedures.....	27
3.2.1.1 Pigment Clearing Trials.....	27
3.2.1.2 Cytoplasmic Stain Comparison.....	27
3.2.2 Fungal Strains and Inoculation.....	27
3.2.3 Microscopy Stains.....	28
3.2.3.1 Cytoplasmic Stains.....	28
3.2.3.2 Fluorescent Stains.....	29

3.2.4	Solutions.....	29
3.2.4.1	Preparation of Acetic acid.....	29
3.2.4.2	Formic acetic alcohol (FAA).....	29
3.2.4.3	Sodium hypchlorite Solutions.....	29
3.2.4.4	Shear's Mounting Fluid.....	29
3.2.5	Buffers.....	29
3.2.5.1	Phosphate Buffered Saline (PBS).....	29
3.2.5.2	Sucrose in Phosphate Buffered Saline (PBS).....	30
3.2.6	Clearing and Staining Procedures.....	30
3.2.6.1	FAA Pigment Clearing Method.....	30
3.2.6.2	Methanol Pigment Clearing Method.....	30
3.2.6.3	Sodium hypochlorite Pigment Clearing Method.....	30
3.2.6.4	Cytoplasmic Staining Comparison.....	31
3.2.6.5	Calcofluor white Staining Method.....	31
3.2.6.6	Gluteraldehyde Staining and Destaining Method.....	31
3.2.7	Microscopy.....	32
3.2.7.1	Bright Field Microscopy.....	32
3.2.7.2	Epi-illumintion Microscopy.....	32
3.2.7.3	Fluorescent Microscopy.....	32
3.2.7.4	Confocal Microscopy.....	32
3.3	Results.....	33
3.3.1	Clearing of Pigment from Pine Needles.....	33
3.3.1.1	Sodium hypochlorite with Combinations of Acetic acid and Heat.....	33
3.3.1.2	FAA Treatment.....	34
3.3.1.3	Methanol Treatment.....	34
3.3.2	Schiff Stained needles visualised by Epi-illumination.....	36
3.3.2.1	Background.....	36
3.3.2.2	Results.....	36
3.3.3	Cytoplasmic Staining and the Effect of Heat.....	38
3.3.3.1	Background.....	38
3.3.3.2	Results.....	38
3.3.4	Fluorescent Stains.....	41
3.3.4.1	Gluteraldehyde.....	41
3.3.4.2	Calcofluor white.....	42
3.4	Discussion.....	42

CHAPTER 4: PATHOGENICITY TEST OF *dotA* KNOCKOUT AND WILD TYPE *D. PINI*.....46

4.1	Introduction.....	46
4.2	Materials and Methods.....	49
4.2.1	Plant Material.....	49
4.2.2	<i>D. pini</i> Strains Used.....	49
4.2.3	Isolation of Fresh Wild Type <i>D. pini</i>	49
4.2.4	PCR amplification.....	50

4.2.5	DNA Sequencing.....	50
4.2.6	GMO Suite.....	50
4.2.7	Lighting.....	51
4.2.8	Light Intensity Measurements.....	51
4.2.9	Misting System.....	52
4.2.10	Watering System.....	52
4.2.11	Temperature Control.....	52
4.2.12	Growth Rack.....	52
4.2.13	Treatments and Tree Layout.....	53
4.2.14	Inoculation of <i>P. radiata</i> Trees.....	54
4.2.15	Sampling of Needles from Treatments.....	54
4.2.16	Microscopy.....	55
4.2.17	Spore viability on PDA and AMM.....	55
4.2.18	<i>D. pini</i> Spore Density Calculations on Inoculated <i>P. radiata</i> seedlings.....	55
4.3	Results.....	56
4.3.1	<i>DotA</i> Knockout Mutant and Wild Type <i>D. pini</i> Spore Viability.....	56
4.3.1.1	Background.....	56
4.3.1.2	Results.....	56
4.3.2	Development of Conditions Favourable for <i>D. pini</i> Infection of <i>P. radiata</i>	58
4.3.2.1	Background.....	58
4.3.2.2	Lighting.....	58
4.3.2.3	Leaf Wetness.....	60
4.3.2.4	Temperature.....	62
4.3.3	Confirmation of <i>D. pini</i> Strains Identity.....	62
4.3.3.1	Background.....	62
4.3.3.2	Results.....	62
4.3.4	<i>D. pini</i> Spore Density and Germination on Inoculated <i>P. radiata</i> seedlings.....	63
4.3.4.1	Background.....	63
4.3.4.2	Results.....	63
4.3.5	Monitoring of Epiphytic Growth by Fluorescent Microscopy.....	64
4.3.6	Visual observations of trees from different treatments.....	68
4.3.6.1	Background.....	68
4.3.6.2	Results.....	68
4.3.7	Percentage of Damaged Foliage.....	70
4.3.7.1	Background.....	70
4.3.7.2	Results.....	70
4.4	Discussion.....	77

CHAPTER 5: TRANSFORMATION OF *D. PINI* WITH GREEN FLUORESCENT PROTEIN (GFP)..... 81

5.1	Introduction.....	81
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5.2 Material and Methods.....	83
5.2.1 Plasmids.....	83
5.2.2 <i>D. pini</i> Strains.....	83
5.2.3 Media.....	83
5.2.3.1 Osmotically Stabilised Dothistroma media (DMSuc).....	83
5.2.3.2 Dothistroma media Top Agar.....	84
5.2.4 Buffers and Solutions.....	84
5.2.4.1 OM buffer.....	84
5.2.4.2 Polyethylene Glycol (PEG) 6000.....	84
5.2.4.3 ST buffer.....	84
5.2.4.4 STC buffer.....	84
5.2.5 Procedures.....	84
5.2.5.1 Restriction Digestion Protocol.....	84
5.2.5.2 Phosphatase Treatment.....	85
5.2.5.3 Phenol/Chloroform Extraction and Ethanol Precipitation.....	85
5.2.5.4 Ligation.....	85
5.2.5.5 Construction of <i>gfp</i> Vectors.....	86
5.2.5.6 Generation of Competent <i>D. pini</i> Protoplasts.....	87
5.2.5.7 Transformation of <i>D. pini</i> Protoplasts.....	88
5.3 Results.....	89
5.3.1 Generating GFP Constructs for Transformation of <i>D. pini</i> ... 5.3.1.1 Background..... 5.3.1.2 Results.....	89
5.3.2 Transformation of <i>D. pini</i> with GFP Constructs.....	91
5.4 Discussion.....	92
CHAPTER 6: GENERAL DISCUSSION.....	94
6.1 Introduction.....	94
6.2 Microscopy.....	95
6.3 Green Fluorescent Protein.....	96
6.4 Pathogenicity Test.....	97
6.5 Conclusions.....	98
REFERENCES.....	99
APPENDIX I: Visual observations of inoculated trees during pathogenicity test... 	109
APPENDIX II: Damage counts of needles collected from pathogenicity test.....	119
APPENDIX III: Statistical analysis.....	135

List of Figures

Figure 3.1	Whole pine needle section treated with water overnight Stained with Schiff's reagent.....	35
Figure 3.2	Whole pine needle section treated with sodium hypochlorite. Stained with Schiff's reagent.	35
Figure 3.3	Whole pine needle section treated with FAA overnight. Stained with Schiff's reagent.	35
Figure 3.4	Whole pine needle section treated with methanol overnight. Stained with Schiff's reagent.	35
Figure 3.5	Pine needle treated with methanol overnight. Stained with Schiff's reagent	37
Figure 3.6	Same frame as fig 3.5. Epi-illumination	37
Figure 3.7	Aniline blue stain followed by staining with calcofluor white. Bright field. Epi-illumination. Wide band UV excitation.....	39
Figure 3.8	Acid Fuschin stain followed by staining with calcofluor white. Bright field. Epi-illumination. Wide band UV excitation.	39
Figure 3.9	Coomassie blue stain followed by staining with calcofluor white. Bright field. Epi-illumination. Wide band UV excitation.	39
Figure 3.10	Lactophenol cotton blue stain followed by staining with calcofluor white. Bright field. Epi-illumination. Wide band UV excitation.....	39
Figure 3.11	Schiff's reagent stained followed by staining with calcofluor white. Bright field. Epi-illumination. Wide band UV excitation.....	40
Figure 3.12	Toluidine stain followed by staining with calcofluor white. Bright field. Epi-illumination. Wide band UV excitation.....	40
Figure 3.13	Trypan blue stain followed by staining with calcofluor white. Bright field. Epi-illumination. Wide band UV excitation.....	40
Figure 3.14	Trypan blue (heated 60°C) stain followed by staining with calcofluor white. Bright field. Epi-illumination. Wide band UV excitation.....	40
Figure 3.15	Pine needle stained with 4% gluteraldehyde PBS solution Wide band blue excitation. Confocal image	41
Figure 3.16	Pine needle stained with calcofluor white. Wide band UV excitation.....	42
Figure 4.1	Schematic representation of the growth rack used for pathogenicity testing.....	53
Figure 4.2	Treatment and tree layout of pathogenicity test	53
Figure 4.3	Spore germination on PDA media	57

Figure 4.4	Spore germination on AMM	57
Figure 4.5	Pine needle with mineral deposits on the tip.....	61
Figure 4.6	Pine needle with mineral deposits on edge.....	61
Figure 4.7	<i>D. pini</i> spore density within pathogenicity trial treatments.....	63
Figure 4.8	Mean spore germination within <i>D. pini</i> pathogenicity trial treatments.....	64
Figure 4.9	Needle taken from NZE7 treatment, 60/5 seedling, Day 0.	66
Figure 4.10	Needle taken from NZE7X treatment, 60/5 seedling, Day 0.....	66
Figure 4.11	Needle taken from 8A1 treatment, 13/11 seedling, Day 0.	66
Figure 4.12	Needle taken from NZE7 treatment, 60/5 seedling, Day 6.....	66
Figure 4.13	Needle taken from NZE7 treatment, 40/8 seedling, Day 12.....	66
Figure 4.14	Needle taken from 8A1 treatment, 40/8 seedling, Day 12.....	66
Figure 4.15	Needle taken from NZE7X treatment, 40/8 seedling, Day 21.....	67
Figure 4.16	Needle taken from 34C1 treatment, 13/11 seedling, Day 21.....	67
Figure 4.17	Needle taken from 34C1 treatment, 60/5 seedling, Day 26.....	67
Figure 4.18	Needle taken from 8A1 treatment, 13/11 seedling, Day 31.....	67
Figure 4.19	Needle taken from 34C1 treatment, 18/19 seedling, Day 48.....	67
Figure 4.20	Needle taken from 34C1 treatment, 18/19 seedling, Day 83.....	67
Figure 4.21	Small lesion on the base of a <i>P. radiata</i> fascicle taken from pathogenicity test.....	69
Figure 4.22	Large lesion on a <i>P. radiata</i> needle taken from pathogenicity test.....	69
Figure 4.23	Multiple lesions on a <i>P. radiata</i> needle taken from pathogenicity test.....	69
Figure 4.24	Lesions in the same position on different <i>P. radiata</i> needles within the same fascicle taken from pathogenicity test.....	69
Figure 4.25	Chlorosis in the same position on different <i>P. radiata</i> needles within the same fascicle needle taken from pathogenicity test.....	69
Figure 4.26	Tip browning of a <i>P. radiata</i> needle taken from pathogenicity test..	69
Figure 4.27	Mean percentage of damaged foliage (Lesions, chlorosis and or tip browning) in the different treatments of the pathogenicity test.....	71
Figure 4.28	Means percentage of damaged foliage (Lesions, chlorosis and or tip browning) in the different ramet groups of the pathogenicity test.....	71
Figure 4.29	Combinations and quantities of damage in treatment NZE7X.....	72
Figure 4.30	Combinations and quantities of damage to treatment NZE7.....	73

Figure 4.31	Combinations and quantities of damage to treatment 8A1.....	74
Figure 4.32	Combinations and quantities of damage to treatment 34C1.....	75
Figure 4.33	Combinations and quantities of damage to treatment Negative.....	76
Figure 5.1	Cloning strategy used to develop <i>gfp</i> constructs	87
Figure 5.2	Gel electrophoresis to check for presence of <i>gfp</i> insert in pBCH- <i>gfp</i>	89
Figure 5.3	Gel electrophoresis to check for presence of <i>gfp</i> insert in pBCH- <i>gfp</i> and pBCP- <i>gfp</i>	91

LIST OF TABLES

Table 2.1	Table of bacterial strains used in this study.....	17
Table 2.2	Table of fungal strains used in this study.....	17
Table 2.3	Ramet numbers of groups used in pathogenicity test and parent clone numbers.....	18
Table 3.1	Times needed to clear halved needles of pigment.....	34
Table 3.2	Comparison of different stains	38
Table 4.1	Photosynthetically active radiation (PAR) in growth rack on a sunny day.....	59
Table 4.2	PAR in growth rack on an overcast day.....	59
Table 4.3	Luminescence radiation (LR) in growth rack on a sunny day.....	59
Table 4.4	LR in growth rack on an overcast day.....	59
Table 5.1	Table of plasmids used in this study.....	83