Investigation of dothistroma needle blight development on *Pinus radiata*

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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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The Author

Table of contents

List of tables

Appendix tables

List of figures

Appendix figures

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
^{o}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