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

List of Figures

LIST OF TABLES