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Investigation of dothistroma needle blight development on *Pinus radiata*

A thesis presented in the partial fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in Microbiology and Genetics at Massey University, Manawatu, New Zealand

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2014
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

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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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### Abbreviation

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