CHARACTERISATION OF A GLOBAL COLLECTION OF *DOTHISTROMA PINI* ISOLATES.

A thesis presented in partial fulfilment of the requirements
for the degree of Master of Science in Molecular Biology
at Massey University, Palmerston North, New Zealand.

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Errata (October 2000)

Page 1: Authorities for the subsequent species are as follows,
Pinus radiata D. Don
Mycosphaerella pini E. Rostrup apund Munk
Scirrhia pini Funk & Parker

Page 4:
'*Ophiostroma*' should be *Ophiostoma*

Section 2.1.1: Description of NEB10
NEB10 was received from Ned Klopfenstein, National Agroforestry Center, U.S.A. Records show that NEB10 was isolated in 1975 in Nebraska, USA from *P. nigara*. It is not know whether the NEB10 isolate received was the original strain (incorrectly assumed to be *D. pini*) or a cultured contaminant.

Page 28, Section 2.11.1: Description of 10C12
Monoclonal antibody 10C12 is a murine monoclonal subclass IgG1. It was produced by immunising inbred BalbC female mice with dothistromin conjugated, through the hydrogen atoms adjacent to aromatic hydroxyls on the anthroquinone skeleton, to Bovine serum albumin using the Mannich reaction. Mab 10C12 was tested against dothistromin analogues and it was shown that the bifuran ring of dothistromin was an important structure recognised by this antibody.

Page 50: Replace Table 4.2 with the following Table

Table 4.2 Effects of medium on dothistromin production by ALP3 (with shaking & light)

	Dothistromin		Mycelium
Culturing Conditions	($\mu g\ ml^{-1}$)	($\mu g\ mg^{-1}$ mycelium)	($mg\ ml^{-1}$)
'low' DB	1.00±0.20	1.19±0.11	0.88±0.23
AMM + 2% glucose	0.72±0.07	0.80±0.04	0.91±0.12

Results are mean±S.E.M. ($n = 3$)

Page 74:
The 10 d time point was chosen over 7 d as previous extractions of cultures incubated for 7 d had not tested positive for aflatoxin (see page 65).

Pages 55 & 74:
It was assumed that the 10 d isolates were in stationary phase of the growth cycle. However, growth curves (two replicates) completed by Monahan (1998) showed that the 10 days growth point marked the transition from exponential to stationary phase. More thorough studies would be required to verify this and to define the stages of development. In view of this, the 10 d growth point phase should be re-classified from stationary to late exponential.

ABSTRACT

Dothistroma pini is a filamentous fungus which infects *Pinus radiata*, New Zealand's predominant forest species. *Dothistroma* blight causes premature defoliation, a reduction in the rate of growth and, in extreme cases, death of the trees. This forest pathogen produces a toxin, dothistromin, which is implicated in the development of the disease symptoms. Only one strain of *D. pini* is thought to be present in New Zealand. However, world-wide there is a diverse range including the sexual form. A collection of *D. pini* strains from eight countries was collated in the UK. To prevent further introductions of 'foreign' *D. pini* to New Zealand and to assist in the identification and appropriate containment, should a new outbreak of needle blight occur, the *D. pini* isolates in this collection were characterised at both the species and individual strain level.

Sequence analysis of the ribosomal internal transcribed spacer (ITS) and the production of dothistromin by the isolates in the collection confirmed all were *D. pini*. Quantification of the levels of dothistromin produced by the isolates, in culture, showed a large variation between the strains. Isolates MIN11, NEB8, GUA1 and, in particular, ALP3 produced significantly more dothistromin than NZE1. Changes in culture environment and media types were shown to affect the levels of dothistromin produced by the *D. pini* isolates. However, these changes were not sufficient to support the production of aflatoxin.

To analyse the genetic diversity among the overseas *D. pini* isolates, a robust microsatellite-based DNA fingerprinting system was developed. Microsatellite loci were isolated. Primers designed to flank the microsatellite repeats were used for PCR amplification in the 'core' twelve *D. pini* strains. The unique fingerprint patterns obtained from these loci were used to distinguish the isolates to the individual strain level. This system of identification provides an effective tool for screening and prognosis of infected pine forest sites.

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