Needle disease strategy Research Programme

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# CLIENT REPORT (Confidential)

## Development of quantitative molecular assays for *Cyclaneusma minus* "simile" and "verum"

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Report: ND-T008

## REPORT INFORMATION SHEET

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| ***REPORT TITLE*** | DEVELOPMENT OF QUANTITATIVE MOLECULAR ASSAYS FOR *CYCLANEUSMA MINUS* "SIMILE" AND "VERUM" |
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| ***CLIENT*** | FOREST GROWERS LEVY TRUST |
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| ***INTELLECTUAL PROPERTY*** |  |

# EXECUTIVE SUMMARY

**Report Title:** Development of quantitative molecular assays for *Cyclaneusma minus* "simile" and "verum".
**Authors:** Rebecca McDougal & Shannon Hunter

## The problem

*Cyclaneusma minus* exists as two morphotypes "simile" and "verum" which were recently proposed to be different species. In New Zealand we do not know if one or both of these types are responsible for cyclaneusma needle cast (CNC). This makes both diagnostic evaluation of disease and screening for resistance complicated. A rapid molecular assay could potentially resolve some of these issues.

## This project

The objectives of this study were (i) to develop quantitative real-time PCR (qPCR) assays for detection and relative quantification of *Cyclaneusma minus* from pine needles, and (ii) to obtain cultures for further characterisation of the genetic diversity of *Cyclaneusma* morphotypes in New Zealand.

## Key Results

*Objective 1 (qPCR):*
Molecular assays specific to each morphotype were designed and tested for specificity to previously characterised *Cyclaneusma* isolates, without cross-reaction to the opposite morphotype or to selected fungi or oomycetes known to be associated with *Pinus radiata*. Two fungal isolates did cross-amplify but not consistently and very late in the PCR–cycling this; these were therefore regarded as an equivocal result, but were not considered to be of significance to the utility of the qPCR assays.

Standard curves of known concentrations of *Cyclaneusma* DNA were run and the sensitivity was determined. The qPCR assay for simile had a detection limit of 1pg/µL whereas the verum qPCR had a detection limit of 10 pg/µL indicating that the simile qPCR assay is more sensitive than the verum qPCR assay.

DNA extractions were performed from both symptomatic (CNC) and asymptomatic pine needles. Detection of the *Cyclaneusma* morphotypes was observed very late in the PCR cycling, and did not cross the threshold for fluorescence detection, giving a "no amplification" result. These data suggest that the DNA extraction procedure needs modification to ensure better lysis of pine tissues and more efficient DNA extraction.

A molecular assay previously established for relative quantification of *Dothistroma septosporum* in *Pinus radiata* did not perform well in the assay conditions required for the *Cyclaneusma* detection. A new assay will need to be investigated.

*Objective 2 (New Zealand distribution of Cyclaneusma* morphotypes*):*
A further 63 isolates were recovered from a secondary collection of *Cyclaneusma* isolates at Scion. New isolates were obtained from locations not previously tested such as Central Otago, North Canterbury, Rangitikei, Sounds (Marlborough) and Wanganui. The collection also contained more cultures from other locations previously tested, providing more confidence in the dataset. Overall, the most prevalent morphotype was simile, which was found in 85% of samples from the North Island and 60% of samples from the South Island.

## Implications of Results for Client

The new molecular assay shows potential as a diagnostic tool and for quantification of *Cyclaneusma* infection, but requires further validation.

## Further Work

Further work is required to complete the validation and testing of the molecular assays to provide confidence in the detection of the two morphotypes. In addition, a new *P. radiata* assay needs to be investigated to allow relative quantification of the biomass ratio (*Cyclaneusma*:*P. radiata*). This will provide a quantitative measure for infection levels between samples. Finally further optimisation of the DNA extraction procedure for pine needle tissue will enable more efficient testing of pine samples.