Needle disease strategy Research Programme

SCION forests · products · innovation

CLIENT REPORT (Confidential)

Validation of qPCR assays for *Cyclaneusma minus* 'simile' and 'verum' and assessment with *Pinus radiata* of varying susceptibility

Rebecca McDougal, Shannon Hunter & Charlie Low

Date: July 2016

Publication No: ND-T016

Milestone No: 1.5 Cyclaneusma screening

supported by forestgrowers commodity levy

FOREST OWNERS ASSOCIATION

REPORT INFORMATION SHEET

REPORT TITLE	VALIDATION OF QPCR ASSAYS FOR *CYCLANEUSMA MINUS* 'SIMILE' AND 'VERUM' AND ASSESSMENT WITH *PINUS RADIATA* OF VARYING SUSCEPTIBILITY
AUTHORS	REBECCA MCDOUGAL, SHANNON HUNTER & CHARLIE LOW
CLIENT	FOREST GROWERS LEVY TRUST
SIDNEY OUTPUT NUMBER	57894
SIGNED OFF BY	REBECCA GANLEY
DATE	JUNE 2016
CONFIDENTIALITY REQUIREMENT	CONFIDENTIAL (FOR CLIENT USE ONLY)
INTELLECTUAL PROPERTY	

Disclaimer

The information and opinions provided in the Report have been prepared for the Client and its specified purposes. Accordingly, any person other than the Client uses the information and opinions in this report entirely at its own risk. The Report has been provided in good faith and on the basis that reasonable endeavours have been made to be accurate and not misleading and to exercise reasonable care, skill and judgment in providing such information and opinions.

Neither Scion, nor any of its employees, officers, contractors, agents or other persons acting on its behalf or under its control accepts any responsibility or liability in respect of any information or opinions provided in this Report.

EXECUTIVE SUMMARY

Report Title: Validation of qPCR assays for *Cyclaneusma minus* 'simile' and 'verum' and assessment with *Pinus radiata* of varying susceptibility
Authors: Rebecca McDougal, Shannon Hunter & Charlie Low

The problem

Cyclaneusma needle cast (CNC) impacts *Pinus radiata* forests across New Zealand, especially in certain regions. Diagnostic evaluation of disease and screening for resistance is complicated by the fact that two different morphotypes ('simile' and 'verum') of the causal agent, *Cyclaneusma minus,* exist and it is not known which is responsible for disease. A quantitative real-time PCR (qPCR) assay was previously developed that allows distinction of the two morphotypes from needle samples, allowing rapid and quantitative identification from infected trees (McDougal & Hunter, 2015). This molecular assay required further validation with needle samples, as well as optimisation for use with a molecular assay for *P. radiata* to enable relative quantification.

This project

The objectives of this study were to:

1. Complete the development and validation of quantitative molecular assays for *Cyclaneusma minus* 'simile' and 'verum' and for *P. radiata*.
2. Perform a comparative assessment of the *Cyclaneusma* qPCR and the qualitative assessment method (needle retention).

Key Results

Objective 1 (qPCR):

The PCR conditions were optimised for *Cyclaneusma minus* 'simile' and 'verum' qPCR assays and standard curves for quantification were generated. The new PCR conditions were also applied to the previously tested *P. radiata* qPCR assay, which improved the amplification from some samples. Generation of a standard curve with *P. radiata* DNA yielded a narrow range of sensitivity for quantification, and needs further improvement.

Objective 2 (qPCR vs. needle retention assessments):

The qPCR assay was applied to samples collected from the Long Mile Road trial site (Forest Genetics, Scion and Radiata Pine Breeding Company (RPBC)) with a range of susceptibilities to *Cyclaneusma* infection. Both pine and *Cyclaneusma* DNA could be detected in all samples analysed. The majority of samples contained 'simile', with 'verum' only detected in three samples.

Comparison of *Cyclaneusma* quantification by qPCR to the needle retention assessments of this trial site were performed to determine if qPCR could be a potentially new and more efficient way of phenotyping susceptibility and tolerance in genetic trials. The common tolerant and susceptible genotypes from both 2014 and 2015 needle retention data were compared with the qPCR-based quantification of infection in these genotypes. This analysis showed a clear difference between the susceptible and tolerant strains, with the tolerant genotypes harbouring more *Cyclaneusma* than the susceptible genotypes. However, when comparing this data across all genotypes the results were not as clear.

Implications of Results for Client

The qPCR methods provide useful and efficient tools for diagnostics and potentially show promise for screening genetic material for disease. Ultimately, a qPCR-based method for screening genetic material would improve the time, cost and efficiency of the screening process. A quantitative method could also provide more confidence in assessment of genetic trials for disease tolerance.

Further Work

Further analysis of the current dataset, and with additional data from earlier in the infection cycle is needed to provide further confidence in using this testing method with material from genetic trials for disease resistance. This will also require an improved standard curve for more accurate quantification of pine DN