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How does *Austropuccinia psidii* infect mānuka to cause myrtle rust?

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Introduction

Generally, rust fungi are specialised plant pathogens with highly specific host ranges. However, the pandemic biotype of *Austropuccinia psidii* (the causative agent of myrtle rust disease) has a current host range of almost 500 known Myrtaceae species. This host range is exceptional, and potentially unique. *A. psidii* infects *Leptospermum scoparium* (mānuka) as well as many other New Zealand native plants. Effectors and CAZymes are known to be key components during rust infection processes (Figure 1). The expression levels of effectors and CAZymes were measured over time following inoculation of mānuka plants to understand how *A. psidii* causes myrtle rust.

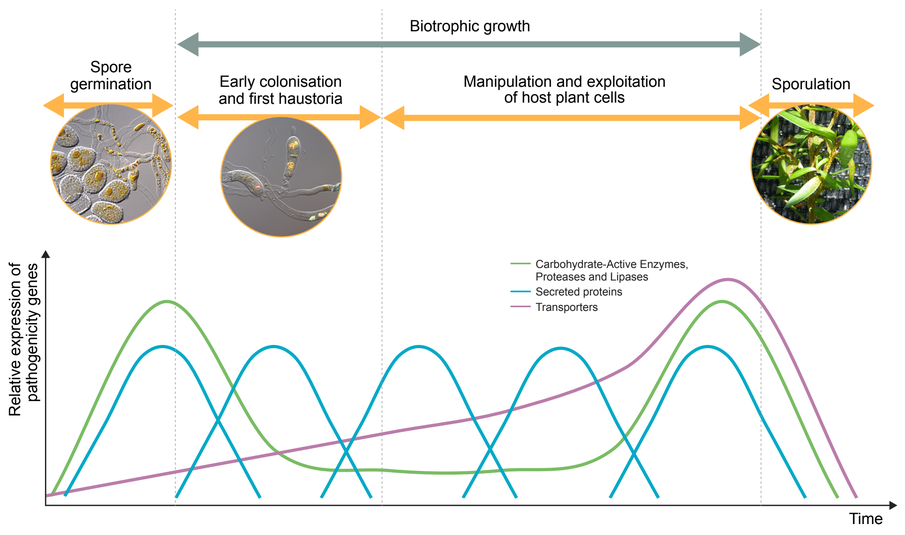

Figure 1: Generalised model of rust fungi expression of pathogenicity genes (adapted from Lorrain et al. 2019).

Methods

Mānuka plants from sibling families were selected based on pre-determined resistance phenotypes. All plants in the experiments were stem infection resistant (S1) but the leaf resistance scores varied:

- L1 immune resistant
- L2 hypersensitive response resistant
- L5 susceptible

Plants with fresh growth were inoculated with fungal spores and samples taken 0*, 6, 12, 24, 36 hours post inoculation (hpi). Environmental conditions optimal for spore germination and infection were maintained for the duration of the experiment.

RNA was extracted from the samples and sequenced. The RNAseq data were mapped to reference genomes and differential gene expression profiles identified.

Reference

1. Lorrain C, et al. 2019. New Phytol, 222: 1190-1206. https://doi.org/10.1111/nph.15641

Acknowledgements

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Results

Fungal reads were detected in 0* samples (taken immediately after inoculation); no fungal reads were detected in the uninoculated plant samples.

A. psidii gene expression was similar on all plant phenotypes (L1, L2, L5) but changed significantly 6 hpi (Figure 2). Further changes were seen from 6 through to 36 hours.

Different *A. psidii* effectors showed different expressions patterns over the course of the infection. Two examples are shown in Figure 3. CAZyme gene expression levels were lower at 36 hours than immediately after inoculation (Table 1).

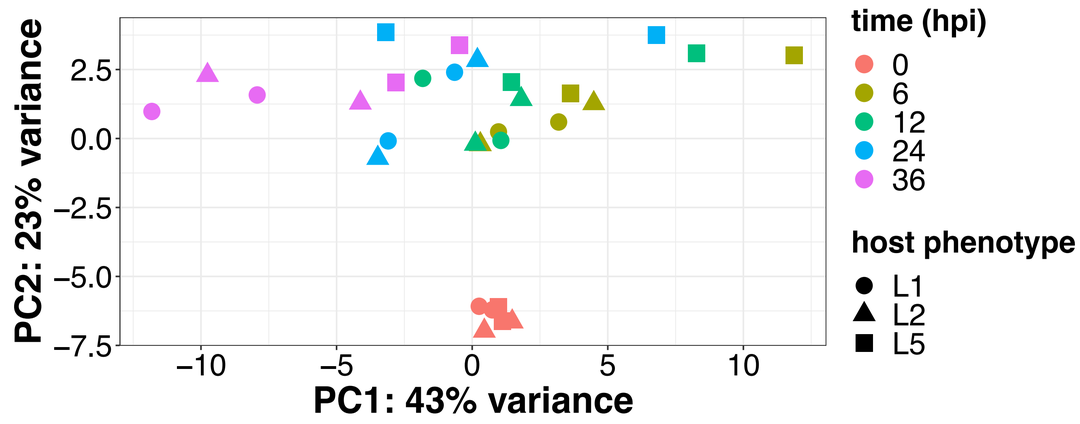

Figure 2: Principal component analysis based on differential gene expression data from the 50 *Austropuccinia psidii* genes with the smallest adjusted *p*-value. The sample time (hours post inoculation) accounts for most of the variation. The mānuka host phenotype does not appear to contribute much to the variation.

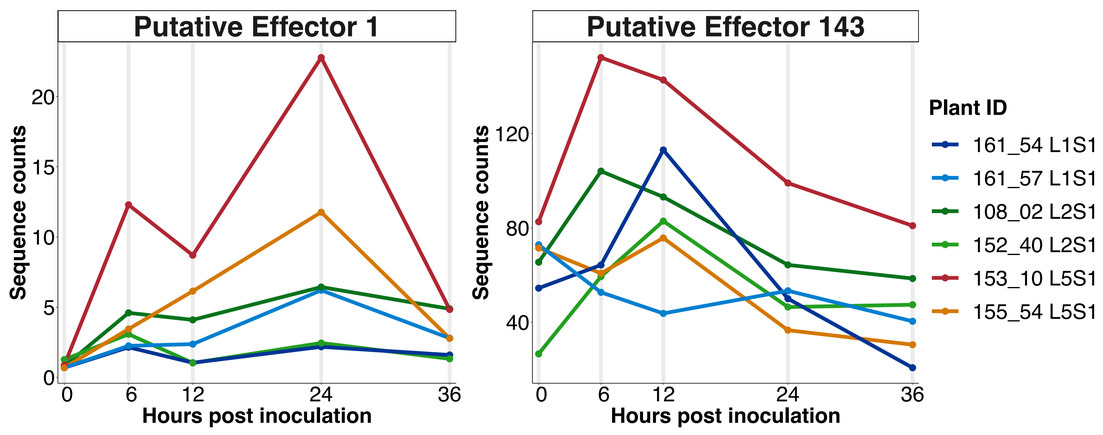

Figure 3: *Austropuccinia psidii* effectors are differentially expressed during the first 36 hours in the infection process on mānuka. Example expression profiles are shown for two putative effectors.

Table 1: *Austropuccinia psidii* CAZyme expression levels decrease in expression level over the infection process on mānuka.

CDS	Putative function	Log_2 Fold Change*	Adjusted *p*-value
APSI_P007.14261.t1	Beta-1,3-galactosyltransferase pvg3	-2.53	2.25E-04
APSI_P019.8073.t1	1,3-beta-glucan synthase component FKS1	-2.52	3.39E-05
APSI_P004.3209.t1	Uncharacterized beta-glucan synthesis-associated protein C23H3.11c	-2.36	1.06E-06
APSI_P005.10511.t1	Chitin synthase 8	-2.22	4.31E-04
APSI_P014.1335.t1	Uncharacterized protein YMR196W	-2.20	1.33E-03
APSI_P020.4849.t1	Inositol phosphoceramide mannosyltransferase 3	-2.00	1.08E-04
APSI_P007.13768.t1	Cell wall alpha-1,3-glucan synthase mok13	-1.86	3.78E-04
APSI_P005.10717.t1	Chitin synthase 3	-1.79	2.52E-04
APSI_P010.11958.t1	Probable glucan 1,3-beta-glucosidase D	-1.78	1.34E-02
APSI_P023.8558.t1	Chitin synthase 4	-1.77	7.20E-03

**Change in gene expression level at 36 hpi compared with immediately after inoculation*

Conclusions

- *A. psidii* gene expression over the first 36 hpi does not appear to change in response to the mānuka resistance phenotype.
- Effectors and CAZymes are expressed early in the infection process, suggesting that they have important roles in establishing the fungal disease cycle.
- The results from this analysis will be used to design gene targets for different control options to try to prevent myrtle rust.