Determining the role of secondary metabolism in the pathology of Ramularia collo-cygni, the fungus responsible for Ramularia leaf spot disease of barley.

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Identification and characterisation of polyketide synthases in the barley pathogen *Ramularia collo-cygni*.

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Ramularia leaf spot

Ramularia collo-cygni (Rcc) is the causative agent of Ramularia leaf spot (RLS), a late season disease of barley occurring in temperate regions worldwide (Figure 1; Havis *et al.,* 2015). RLS can cause substantial yield losses estimated to be as high as 70% but typically around 5-20%. Development of RLS is thought to be linked with the production and release *in planta* of a set of anthraquinone-derived phytotoxic fungal secondary metabolites (SMs) called rubellins (Miethbauer *et al.*, 2003; Walters *et al.*, 2008). Rubellins are predicted to be synthesised through the polyketide synthase (PKS) pathway (Miethbauer *et al.*, 2006). This study aimed at identifying putative SM gene clusters located near Rcc PKS core genes and to assess the expression of PKSs and SM-related genes during RLS development.

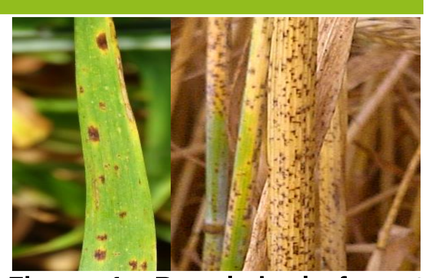

Figure 1: Ramularia leaf spot symptoms on barley leaf and stem

In silico identification of PKSs in *Ramularia collo-cygni*

I) Identification of putatively functional PKSs in Rcc

Protein BLAST (pBLAST) analysis using known fungal PKSs sequences as queries identified 30 gene models in the Rcc genome (McGrann *et al.*, 2016) as putative PKS candidates. Only 10 of these Rcc gene models encoded proteins containing the three domains KS-AT-ACP required for PKS function (Figure 2).

Figure 2: Domain organisation of *R. collo-cygni* putatively functional PKSs

SAT: starter unit: acyl carrier protein transacylase, KS: keto-synthase, AT: acyl-transferase, DH: dehydrogenase, PT: product template, MT: methyl-transferase, ER: enoyl-reductase, KR: keto-reductase, ACP: acyl carrier protein, TE: thiol-esterase, R: reductase

II) Identification of putative PKS-based SM gene clusters in Rcc

Using *in silico* genome walking, Rcc genes with a predicted function associated with secondary metabolism were identified near PKS core genes. Eight putative SM gene clusters containing at least one PKS were discovered in the genome of Rcc (Figure 3). Several clusters exhibited similarity to known SM biosynthetic clusters in other fungi.

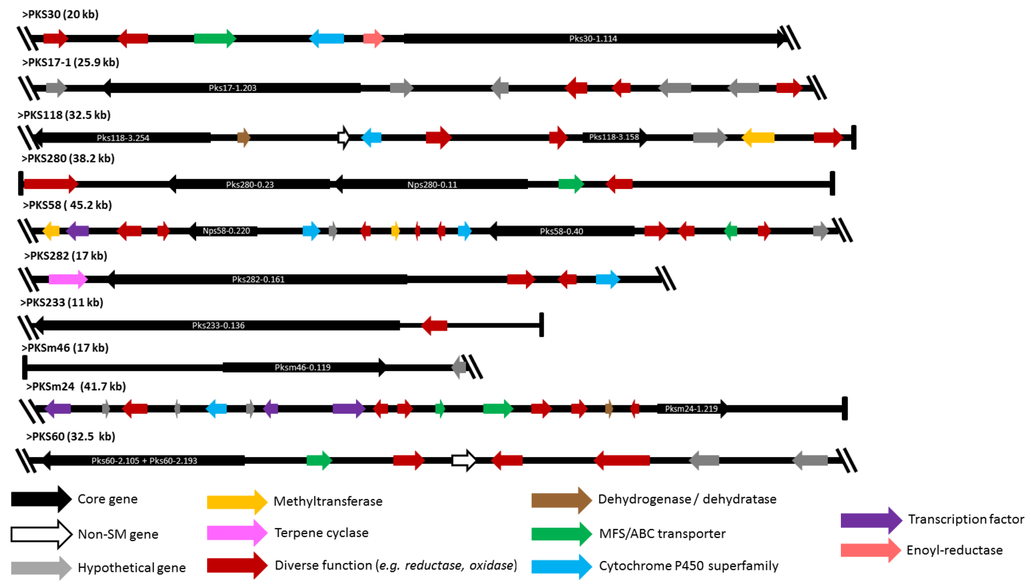

Figure 3: Putative SM gene clusters located near PKS genes identified in *R. collo-cygni*

In planta expression of PKSs in *Ramularia collo-cygni*

I) Expression of Rcc PKSs during RLS development

Five of the putative PKSs identified in the Rcc genome were most highly expressed during the early stages of disease development (Figure 5).

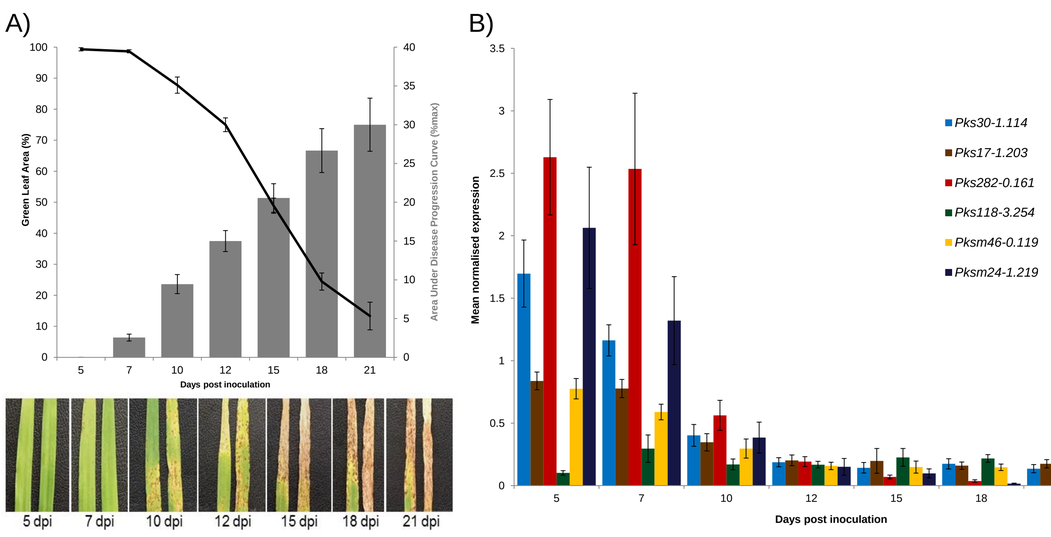

Figure 5: *R. collo-cygni* PKSs are preferentially expressed during the asymptomatic and early lesion development stages of RLS development.

A) RLS disease development over 21 day post inoculation time course. B) Transcript levels of Rcc PKSs during disease development.

II) Rcc PKSs and SM-related genes appear to be co-regulated

The putative homologs of *A. flavus AflR*, *AflJ* and *StcQ* identified in a putative SM gene cluster containing no core gene in Rcc appear to be co-regulated with several Rcc PKSs, including the *AfPksA* putative homologs *Pksm24 and Pksm46*. Transcript levels were highest during the asymptomatic and early symptoms formation stages of RLS development (Figure 6).

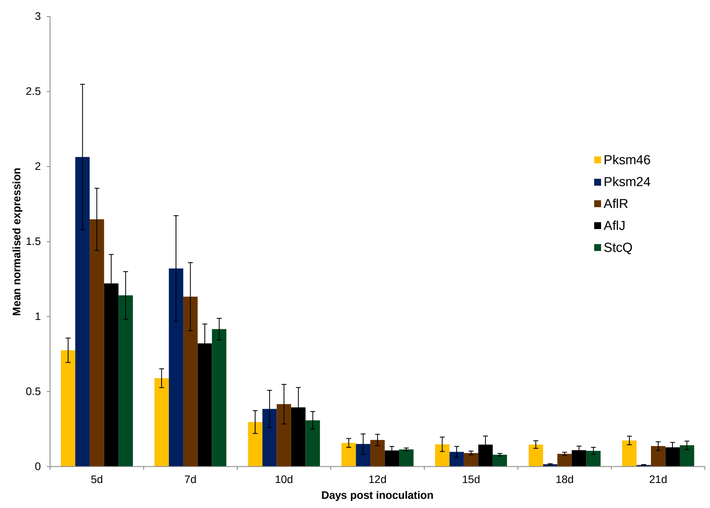

Figure 6: Co-regulation of *R. collo-cygni* PKSs with SM-related genes

Conclusions

- *Rcc* appears to have the genetic equipment to produce several polyketide-derived SMs.
- Most of Rcc PKSs are expressed early during RLS development questioning the role of these genes in disease development but suggesting a putative role in host manipulation.

Future work

- Identifying and disrupting candidate Rcc PKS genes to determine their role in rubellin biosynthesis to characterise the function of Rcc PKSs in RLS development.
- Characterisation of new SMs produced by Rcc may help relate their core gene expression to the biology of RLS.

References

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