Investigating the effect of phenolic acids present in wheat cell walls on growth of the pathogenic fungi *Zymoseptoria tritici* and *Fusarium graminearum*

My project at Rothamsted Research Centre was based on investigating the effects of phenolic acids present in the cell walls of grass species, on the growth of 2 species of pathogenic fungi – *Zymoseptoria tritici* (causal agent of Septoria leaf blotch) and *Fusarium graminearum* (causal agent of Fusarium head blight) in wheat. Phenolic acids, primarily ferulic acid and *p*-coumaric acid, have been shown to play a role in the strengthening of cell walls, especially ferulic acid which can form cross-links between arabinoxylan chains, and also the arabinoxylan and lignin molecules. This has been proposed to play a role in plant defence against both pests and pathogens.

We therefore set out to answer three main questions:
1. Do genes involved in phenolic acid formation have altered expression in wheat tissues when they are infected with Fusarium or Septoria?
2. Does the content of bound *p*-coumaric and ferulic acid change in wheat leaves during Septoria or Fusarium infection?
3. Do cell wall phenolics (as free or bound compounds) have an anti-fungal inhibitory effect on either Septoria or Fusarium?
This research combined two of the departments at Rothamsted – the Wheat Pathogenomics group and the Cell Wall group, meaning luckily I had a large team to help me!

So answering whether the genes involved show altered expression when the plant is infected with either disease compared with when it is uninfected was relatively straight forward, as we found that the Wheat Expression Browser (http://www.wheat-expression.com/) had information on gene expression levels from uninfected and Fusarium infected spikes, and uninfected and Septoria infected seedlings. This meant that once I had a list of all of the candidate genes, I could check them against this database and found that yes, several members of each gene family had altered expression when infected with either Septoria or Fusarium. This means that we now have potential target genes which can be focused on to observe what they encode and what that gene product does.

The second question I tackled by extracting the phenolics from Septoria-infected and uninfected (control) leaves, and also from Fusarium-infected and uninfected (control) wheat ears, and running them on a HPLC to quantify the phenolic content in each sample set. We unfortunately found that there was no difference between the infected tissues and their corresponding controls in this study. There is also current ongoing research to determine if wheat varieties with naturally higher resistance to these pathogens have higher levels of phenolic compounds.

The third question of whether these compounds had an inhibitory effect was tackled with plate assays of serial dilutions of pure phenolic acid compounds – p-coumaric acid, ferulic acid and a third diferulate, poacic acid, which has recently been identified as an anti-fungal compound, grown with both species of fungi. I found that all of the pure compounds exhibited an anti-fungal effect albeit at quite high concentrations. In these plates I also ran serial dilutions of the phenolics I extracted from the plants themselves, as this would obviously give more biologically relevant results. I encountered a few problems with dissolving them, but in my last plate found some indication that these phenolic mixtures potentially inhibit Septoria and Fusarium slightly, suggesting that even at low levels these phenolics have an effect.

This work was actually part of the background work for a much larger project, which aims to look at whether plants with higher phenolic acid content display increased tolerance to pathogens. I enjoyed the placement and learnt so many new skills that will inevitably help with future careers, and it helped to determine that research is definitely the career path I want to go down.

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