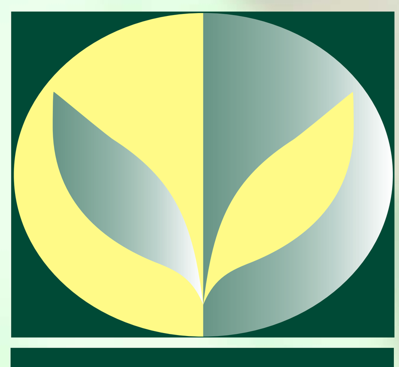

Exploring appressorial formation in the biotrophic cereal fungus powdery mildew

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Introduction

Powdery mildew caused by *Blumeria graminis,* affects a wide range of grass hosts, in particular cereals. *Blumeria graminis* f.sp *hordei* (*Bgh*) and *Blumeria graminis* f.sp *tritici* (*Bgt*) are the infective agents on barley and wheat, respectively. As biotrophs, these pathogens live on, and obtain nutrients from a living host. This can lead to a significant reduction in yield, which in turn has serious financial implications for farmers.

The fungus spreads through the production of asexual spores known as conidia. Once a conidium lands on a leaf, a programmed sequence of events takes place that leads to the formation of an appressorium ~8h post inoculation (h.p.i.) This structure is able, via a penetration peg, to puncture the surface of the plant. Within 24h a specialised structure involved in nutrient uptake (haustorium) develops in the penetrated cell. This supports the development of white, powdery conidial colonies on the surface of the leaf that are visible within 5-7 days post inoculation. A model of the signal transduction pathway that governs appressorial development has been predicted (figure 2).

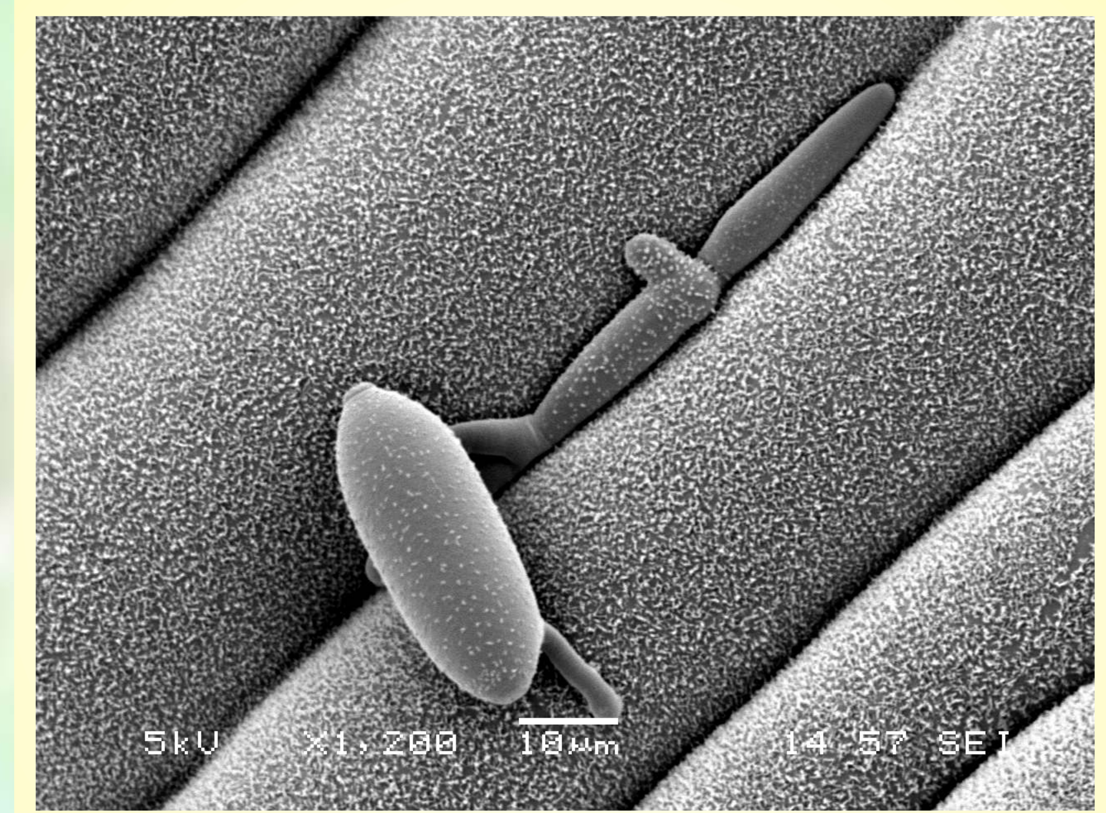


Figure 1: Conidia (24h.p.i.) showing Primary Germ Tube (PGT) and hooked appressoria.

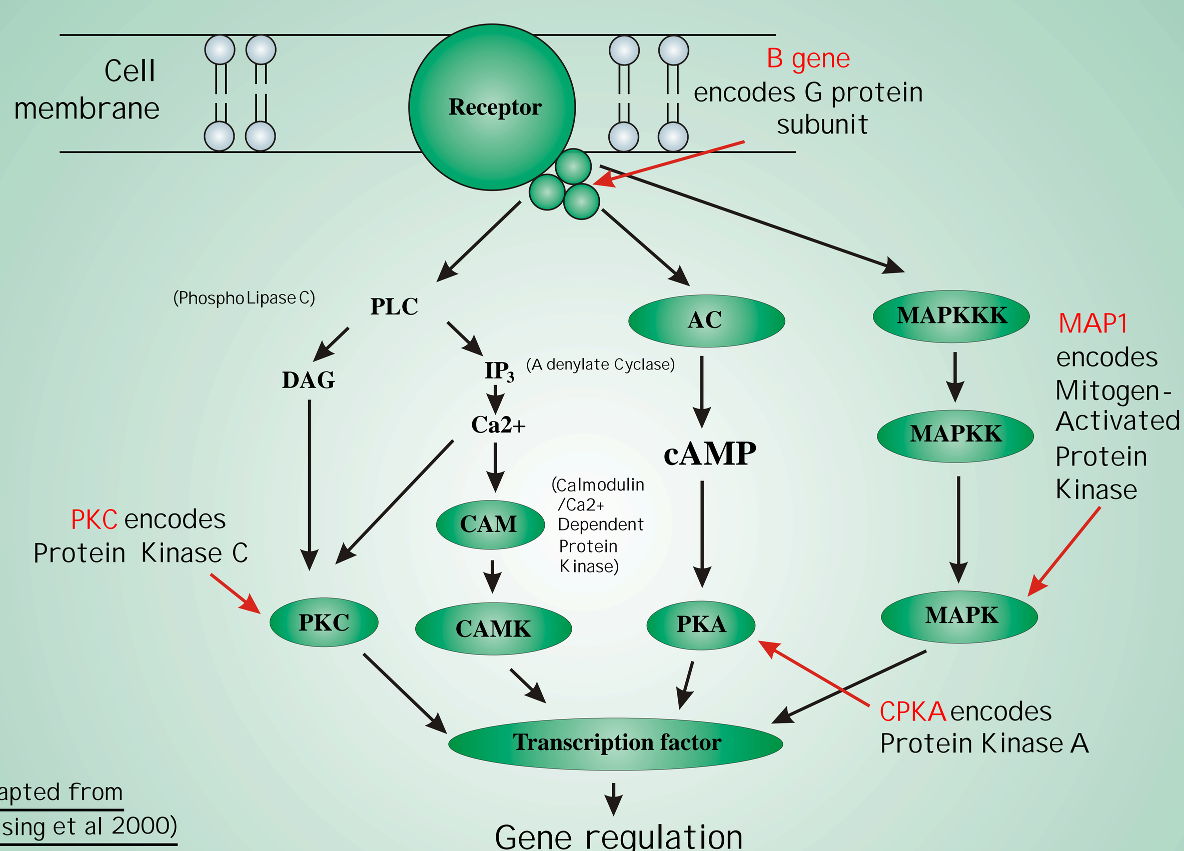


Figure 2: Model of signal transduction pathway that governs appressorial development. Deising *et al*, (2000) Fragments of genes encoding elements of the different pathways have been cloned (shown in red) and their expression analysed using real-time RT-PCR.

Aims

This project investigates the genes and signalling pathways involved in the development of the appressorium (figure1).

Methods

Leaf material was heavily inoculated with conidia and RNA extracted from infected leaf samples at time points post inoculation. Transcript levels for the genes of interest were then assessed using real-time RT-PCR. All work to date has been carried out on wild-type *Bgh* isolate 23D5.

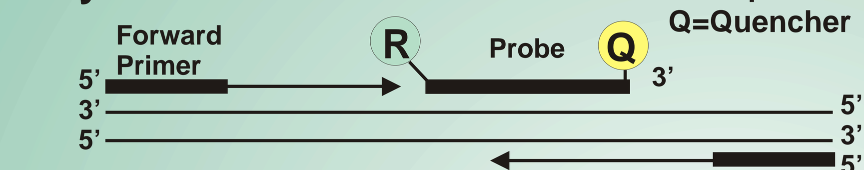
Development of Gene Expression Studies

Fragments of genes from both *Bgh* and *Bgt* encoding components of this pathway have been cloned and sequenced. From the resulting sequences, Taqman® probes have been designed in areas of homology, between *Bgh* and *Bgt*, for use in gene expression assays. Relative transcript quantities were calculated using the 2- [△][△]Ct method (Muller *et al,* 2002) which measures the level of gene expression, relative to a control/calibrator, in a treated sample when normalised to an appropriate housekeeping gene, in this case β-tubulin.

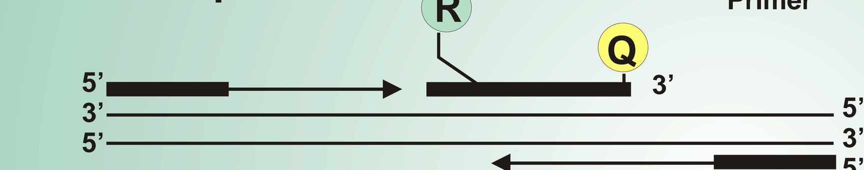
Results

Polymerization

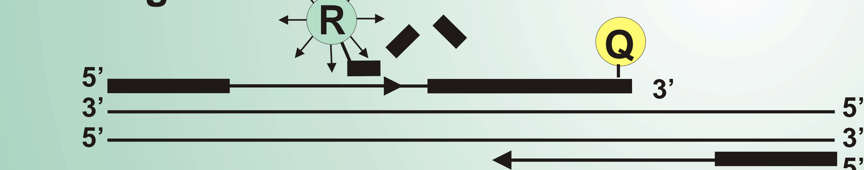
R=Reporter
Q=Quencher



Strand displacement



Cleavage



Polymerization completed

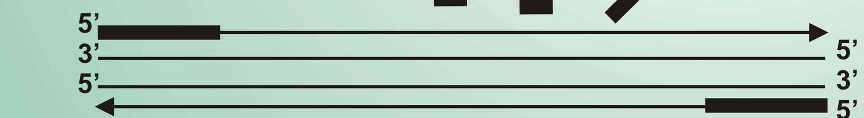


Figure 3: The general principle of real-time RT-PCR: During amplification each probe will bind specifically to its target DNA strand. DNA polymerase cleaves the reporter dye from the attached probe. This results in an increase in fluorescence due to the reporter dye and quencher becoming separated.

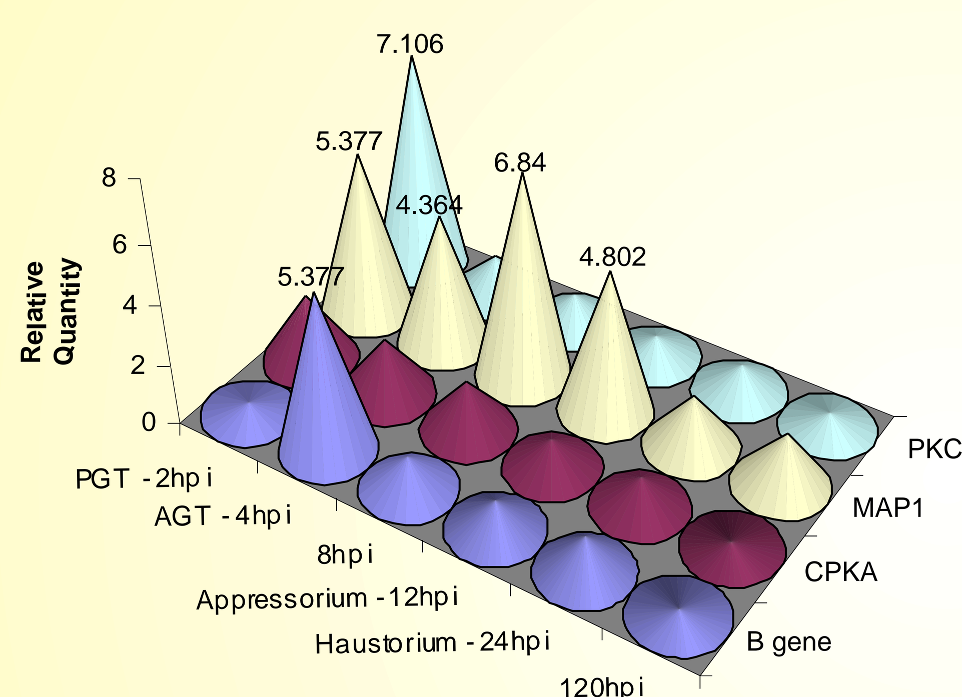


Figure 4: Relative expression of genes of interest for isolate 23D5 (untreated) at morphologically significant time points within conidial development. Expression level values are indicated on the graph where the highest relative increases were observed.

Transcript abundance for 23D5 in untreated conditions (Figure 4) shows:

- PKC transcription levels are elevated 2hpi around Primary Germ Tube (PGT) emergence
- B gene levels peak at 4hpi and hence likely to contribute to Appressorial Germ Tube (AGT) emergence
- MAP1 expression is high between 2hpi and 12hpi. This increase is in conjunction with AGT and appressorium differentiation
- CPKA transcription is low at 12hpi, resulting in inactivated cAMP pathway and triggering successful differentiation

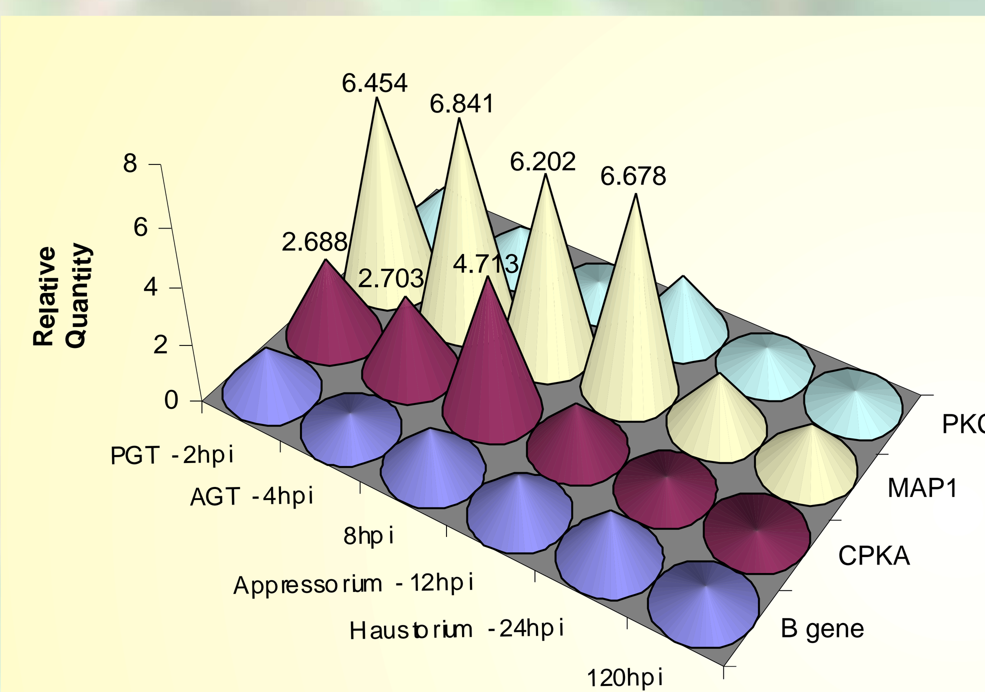


Figure 5: Relative quantity of genes of interest for isolate 23D5 on Quinoxyfen-treated leaves at morphologically significant time points within conidial development. Expression level values are indicated on the graph where the highest relative increases were observed.

Transcript profiles after treatment with Quinoxyfen (Figure 5) show:

- PKC and B gene expression at 2hp and 4hpi respectively, are considerably lower than in untreated samples
- MAP1 expression between 2hpi and 12hpi remains high
- CPKA level is higher at 8hpi, but remains low at 12hpi when appressorium differentiation normally occurs.

Discussion and Future Work

Expression profiles for the genes of interest correlate with previous work carried out (Zhang *et al.*, 2001). Future studies will look at the transcript abundance of other genes implicated in appressorial formation in a variety of isolates in the presence of various inhibitors and fungicides.This should give an indication of the gene(s) and/or pathways that are targeted by fungicides that inhibit appressorial formation.

Reference

Muller P.Y., Janovjak H., Miserez A.R and Dobbie Z (2002) *Biotechniques* **32,** 1372-1379
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Acknowledgements

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