# Revealing host metabolome reprogramming by the economical important fungal plant pathogens *Botrytis cinerea* and *Magnaporthe grisea*.

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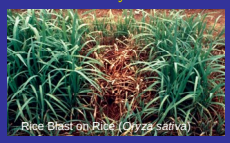
## BOX 1: INTRODUCTION

**Fungal plant pathogens impose economic penalties through reductions in crop yield and/or quality.**

**Many fungal pathogenic mechanisms aim to re-programme the host metabolome to suppress or avoid defences and mobilise nutrients.**

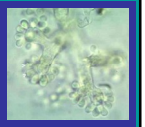
**This makes plant-pathogen interactions appropriate targets for metabolomic analyses**




### The hosts:

- **The model *Arabidopsis thaliana* has well-characterised mutants altered in either ethylene perception or signal transduction.**
- ***etr1-1* is an ethylene-insensitive mutant which has a dominant mutation in the gene that codes for the ethylene receptor.**
- ***ctr1-1* has a mutation in the gene encoding the CTR1-1 MAP kinase which is involved in the signal transduction pathway resulting in a constitutive ethylene response.**
- **The model grass species *Brachypodium distachyon*.**
- **Accessions have been shown to exhibit single gene-mediated resistance to *Magnaporthe grisea* –the causal agent of Rice Blast.**

### The pathogens:

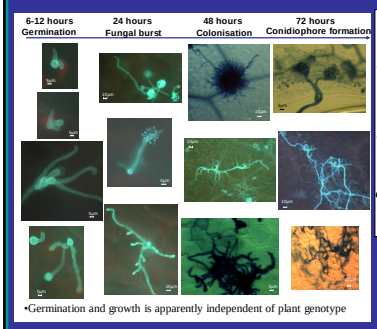


- ***Botrytis cinerea* is the casual agent of grey mould.**
- **Causes serious pre- and post-harvest diseases in a vast host range.**
- **As a necrotrophic pathogen it kills plant cells in advance of its own growth.**
- **Can result in losses of up to 50% in susceptible grape varieties.**

- ***Magnaporthe grisea* has a wide host range**
- **The causal agent of rice blast disease.**
- **Can result in losses of up to 30% of the rice crop**

## BOX 2: METABOLOMIC ANALYSIS OF *BOTYRTIS CINERA – ARABIDOPSIS* INTERACTION

**Fig 1. Microscopic analysis of *Botrytis cinerea* development using Aniline Blue (+UV light) and vital staining using Trypan / Evans Blue**




•Germination and growth is apparently independent of plant genotype

**Fig 2. *etr1-1* shows greater lesion development than wild-type. In contrast, *ctr1-1* shows little lesion formation.**

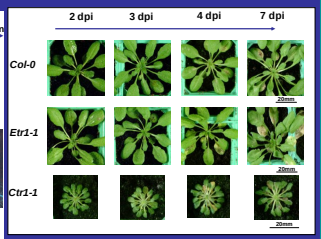



**Fig 3. Timing of necrosis**

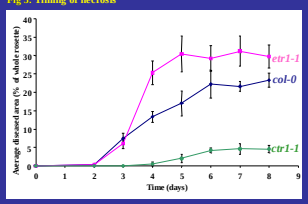



**Fig 4. Multivariate statistical analysis of metabolite fingerprinting (Fourier-transform IR data) FT-IR data shows major differences six hours post infection.**

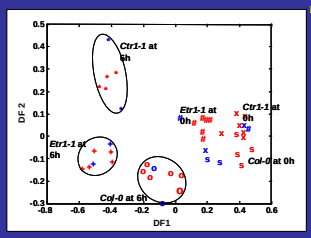
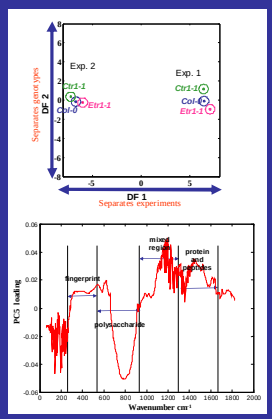



**Table 1. the MANOVA table using 5 PCs (>95% variance) derived from the FT-IR data, where df is the degrees of freedom and P is the probability**

| | df | ~F | [df1, df2] | P |
|---|---|---|---|---|
| Experiment (E) | 1 | 815.09 | [5, 68] | 0.0000 |
| Genotype (G) | 2 | 6.38 | [10, 136] | 0.0000 |
| GxE | 2 | 3.35 | [10, 136] | 0.0006 |
| Time (T) | 3 | 3.42 | [15, 188] | 0.0000 |
| ExT | 3 | 0.92 | [15, 188] | 0.5413 |
| GxT | 6 | 1.48 | [30, 274] | 0.0550 |
| GxExT | 6 | 0.81 | [30, 274] | 0.7546 |
| Total within plant | 72 | 0.98 | [360, 464] | 0.5889 |
| Residual | 96 | | | |

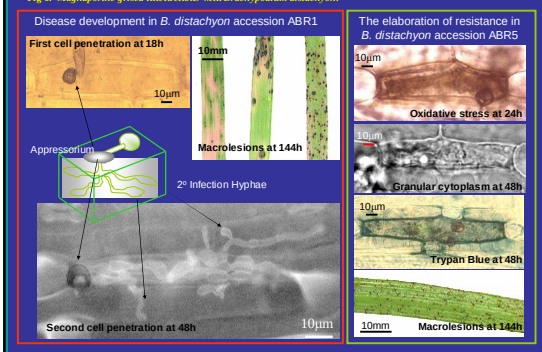
**Fig 5a. Discriminant function analysis (DFA) plot of the experiment x genotype interaction (ExG) . DF2 separates experiment 1 which is highly positively loaded on PC5 (0.8298)**

**Fig 5b. PC5 loading plot showing important metabolite regions**




## BOX 3: METABOLOMIC ANALYSIS OF *MAGNAPORTHE GRISEA – BRACHYPODIUM DISTACHYION* INTERACTION

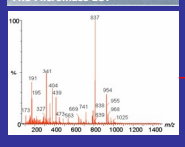
**Fig 6. *Magnaporthe grisea* interactions with *Brachypodium distachyon*.**



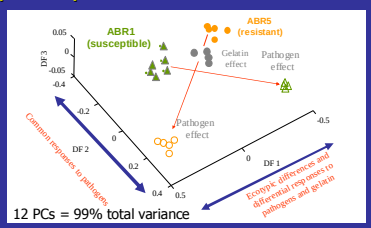

**FT-IR**

(This validates Experimental design; see Box 2)

**Metabolite Profiling**

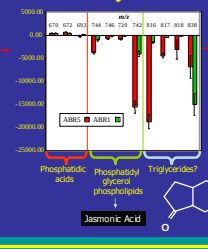
**Principal Component Discriminant Function Models**




**Data Mining**

Loading Plots
Subtraction Spectra

**Discriminatory metabolites**




**Confirmation By Tandem MS**

Defence Gene Expression

## BOX 4 . CONCLUSION AND FURTHER WORK

### Conclusion

- Metabolic changes can be detected in response to pathogenic challenge before visible localised symptoms
- Robust and reproducible mathematical models can be derived irrespective of sources of variation
- By applying variable selection techniques regions of spectra that best describe genotypic differences can be revealed
- Metabolite targets from fingerprint spectra and profiles can be identified in metabolomic reprogramming experiments

### Future work

- Biochemical confirmation of metabolite targets
- Based on chemometric models, mutants will allow the elucidation of signalling pathways involved in resistance

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bbsrc biotechnology and biological sciences research council