Feeling The Heat: Investigating the dual assault of *Zymoseptoria tritici* & Heat Stress on What (*Triticum aestivum*)

Blyth, H.R.[1*], **Zarsav, A.**[1,2], Smith, D.[1], Kanyuka, K. [1,3], Hassall, K.L.[1], Rudd, J.J.[1] & **Haslam, R.P.**[1] *hannah.blyth@rothamsted.ac.uk

[1] Rothamsted Research, Harpenden, Herts, UK. [2] Swiss Federal Research Institute WSL, Zürcherstrasse 111 CH-8903 Birmensdorf, Switzerland. [3] National Institute for Agricultural Botany (NIAB), Cambridge, UK.

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1 INTRODUCTION

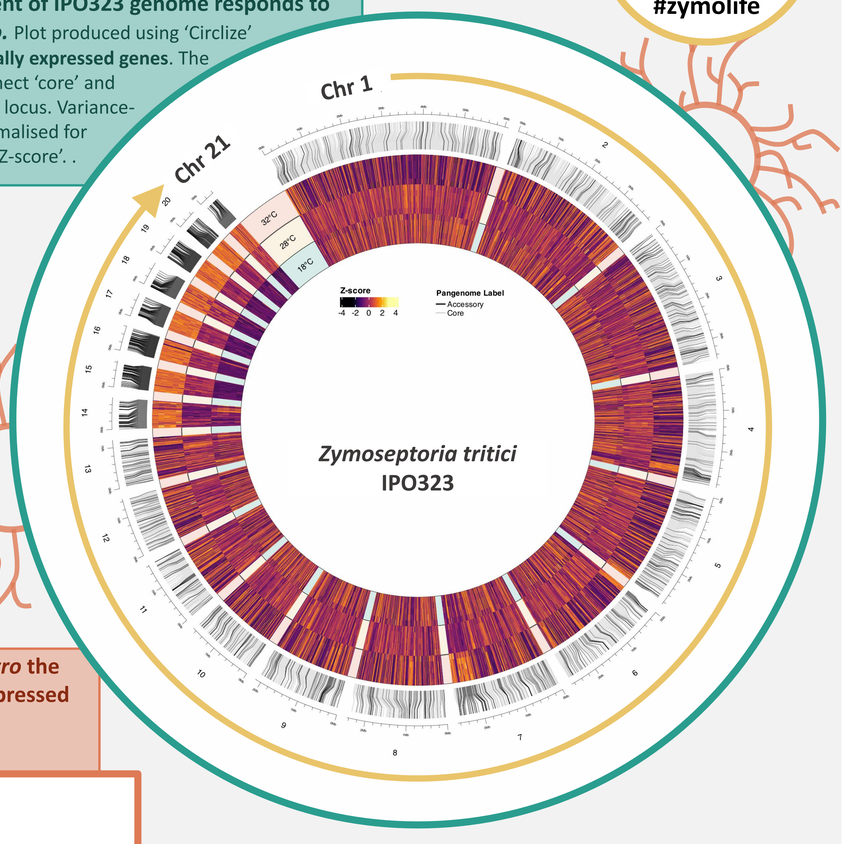
- **Combined abiotic and biotic stress** on plants is an understudied area and essential to understand in light of global climate change.
- Ascomycete *Zymoseptoria tritici* is a **foliar wheat pathogen** and a tractable model for phenotypic screening[1].
- **Lipid remodelling** is key in plant and fungal responses to heat stress[2].

Question: how does increased temperature impact the plant, the pathogen, their interaction and lipidome?

2 *IN VITRO* WORK

- Differential expression (DE) of **3489 genes** between three temperature treatments *in vitro*.
- Using a pangenome[3] to categorise genes, we identified that the flexible genome component was up-regulated at higher temperatures (**Figure 1, right**).
- The largest functional KOG group[4] was identified as 'Metabolism' (**Figure 2**). GO term enrichment analysis showed the top enriched group as oxidoreductase (GO:0016491).
- Principal component analysis (PCA) of the lipid profile indicates temperature-related differences (**Figure 3**).

Figure 1: "Accessory" component of IPO323 genome responds to increasing temperature *in vitro*. Plot produced using 'Circlize' in R showing **significantly differentially expressed genes**. The outer track grey and black lines connect 'core' and 'accessory' genes to their respective locus. Variance-stabilised counts are scaled and normalised for heatmap visualisation, producing a 'Z-score'. .



MATERIALS & METHODS

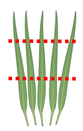
***In vitro* transcriptomics**

- YPD broth was inoculated with 1×10^5 IPO323 blastospores mL^{-1}.
- Three temperatures in a shaking incubator.
- Five replicates per condition.

***In planta* transcriptomics**

Day 21°C/Night 15°C **'Ambient'**

Day 28°C/Night 22°C **'High'**



Five leaves/sample & five replicates

Spray inoculated & negative control

- Wheat cv. Riband.
- Leaves harvested at three time points 9-, 14- and 21-days post inoculation (dpi).

Lipidome extraction

- ***In vitro***: five replica flasks of YPD-grown fungal cultures as above, then flash-frozen and freeze-dried.
- ***In planta***: three replica leaves per sample, harvested at 9-, 14- and 21- dpi and flash frozen. Total of five replica samples per time point.
- A modified Folch lipid extraction method[5] was utilised, followed by LC-MS analysis.

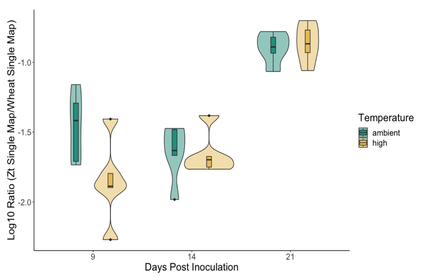
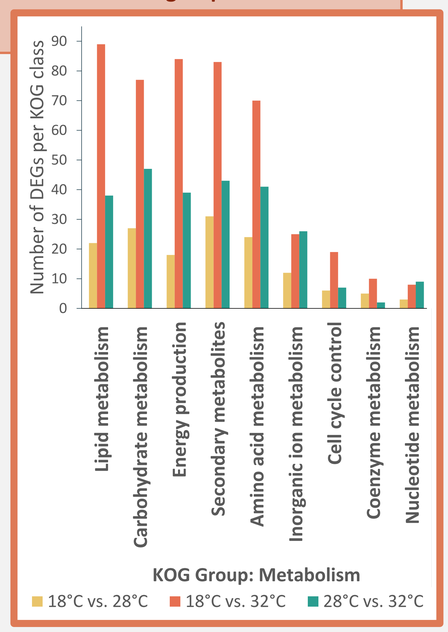


Figure 4: Is wheat 'shielding' *Z. tritici* (Zt) from impact of heat? A violin plot comparing the Log_{10} ratio of single mapped reads (Zt:Wheat) in infected wheat grown at two temperature conditions.

3 *IN PLANTA* FUNGAL

- Disease progression (measured by ratio of fungal to wheat reads) suggests **earlier** stages of fungal infection were **negatively impacted** at **higher temperatures** (**Figure 4, left**).
- DE of **376 fungal genes total** *in planta* contrasting ambient vs. high at 9 and 21 dpi (**Figure 5**).
- Over-represented GO terms exclusive to 'high' temperature infection contrasts predominantly concern **ribosome construction & assembly.**

Figure 2: Bar chart showing *in vitro* the total number of differentially expressed genes (DEGs) per class in the 'Metabolism' KOG group.



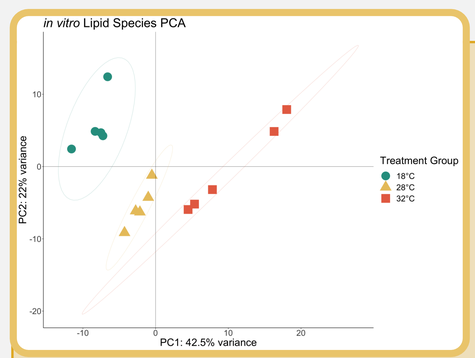


Figure 3: PCA indicates a temperature-related difference between lipid species identified in the fungal cultures. Represents the differences in 183 lipid species.

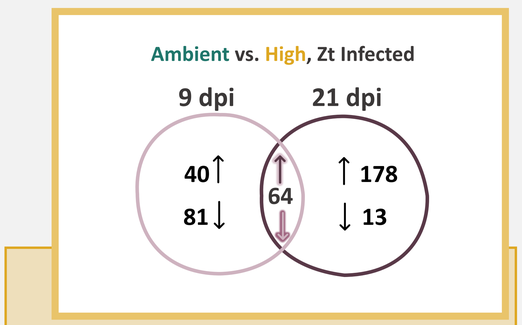


Figure 5: Venn diagram showing the numbers of DE fungal genes *in planta* between ambient vs. high temperature at 9 and 21 dpi.

There are even more DEGs *in planta* when we contrast by time!

4 *IN PLANTA* WHEAT

- **Lots** more work to do here!
- At 9 dpi contrasting ambient and high, there are 1,121 DE wheat genes without inoculation.
- One over-represented Biological Processing term in this list is Transmembrane Transport (GO:0090662).
- PCA of LC-MS analysis identified time and temperature-related differences between 171 lipid species (**Figure 6, right**).

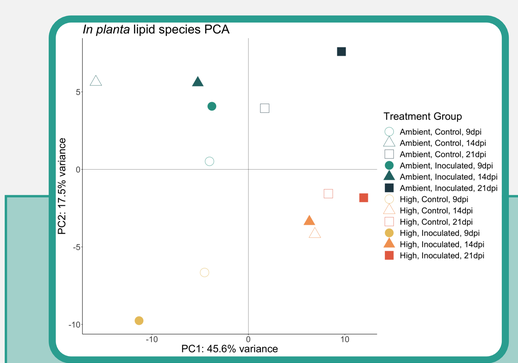


Figure 6: PCA of average group lipid group peak areas indicates temperature- and time-related differences between lipid species *in planta*. Infection with Zt does not appear to be a major component.

Manuscript in preparation!

In the meantime... want more? Scan here.

Want to see this poster again later? bit.ly/HRB_Fungi24

CITATIONS

[1]Blyth *et al,* (2023) Front. Plant Sci., 14
[2]Balogh *et al.,* (2013) FEBS Letters, 587(13)
[3]Chen *et al*., (2023) BMC Biology, 21:24
[4]Tatusov *et al.,* (2003) BMC Bioinform., 4:41.
[5]Folch *et al.,* (1957) J. biol. Chem., 226(1)

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