



**University of  
Nottingham**

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**Large scale  
phenotyping  
looking at  
temperature stress  
on reproduction in  
Brassica**

**Alison Tidy**

**16<sup>th</sup> October 2023**



Female fertility

Gene regulatory networks



Male fertility



Seed vigour



WARWICK



# BRAVO

Brassica Rapeseed And Vegetable Optimisation

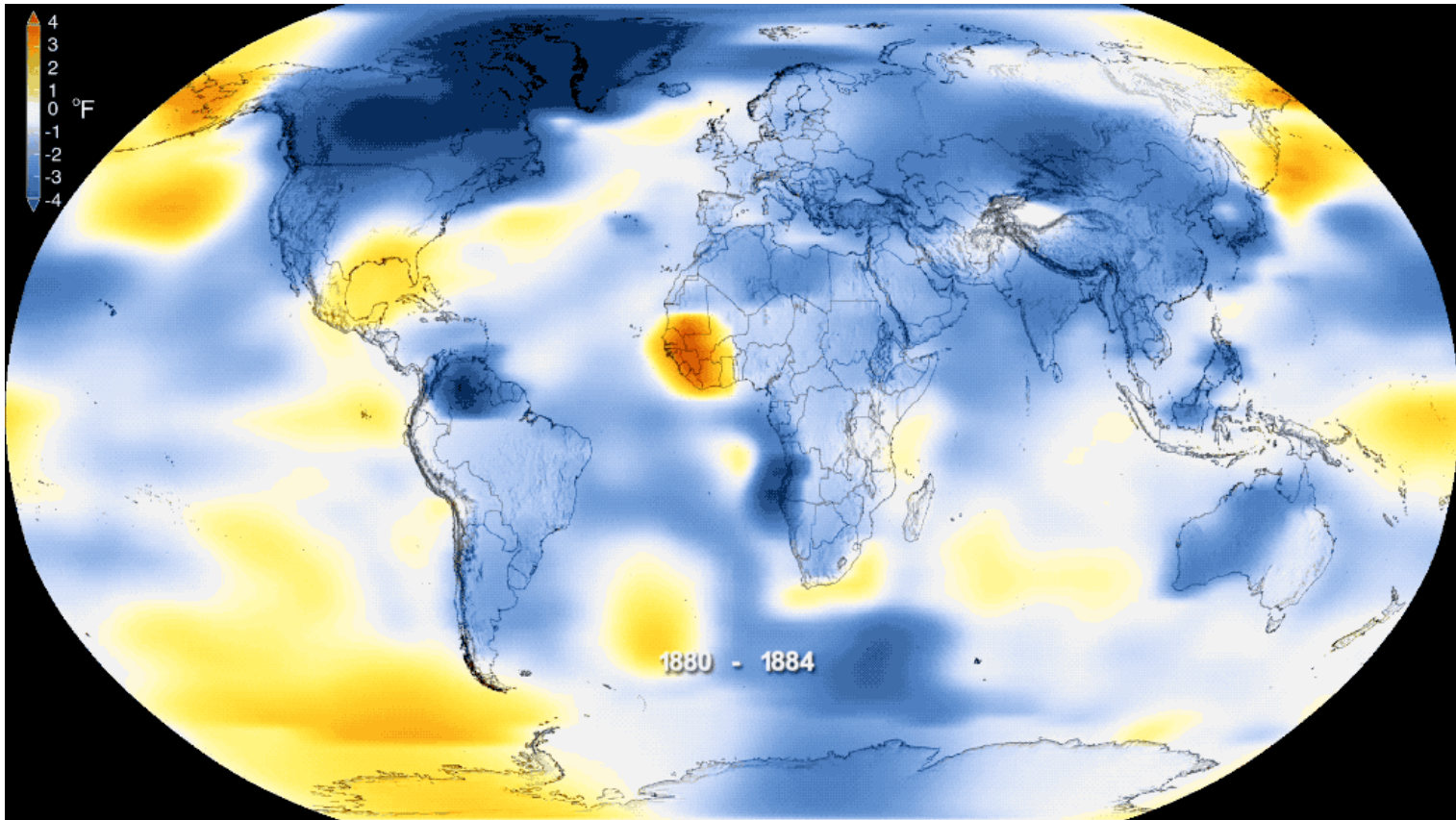
Floral transitions



Seed size, number and yield



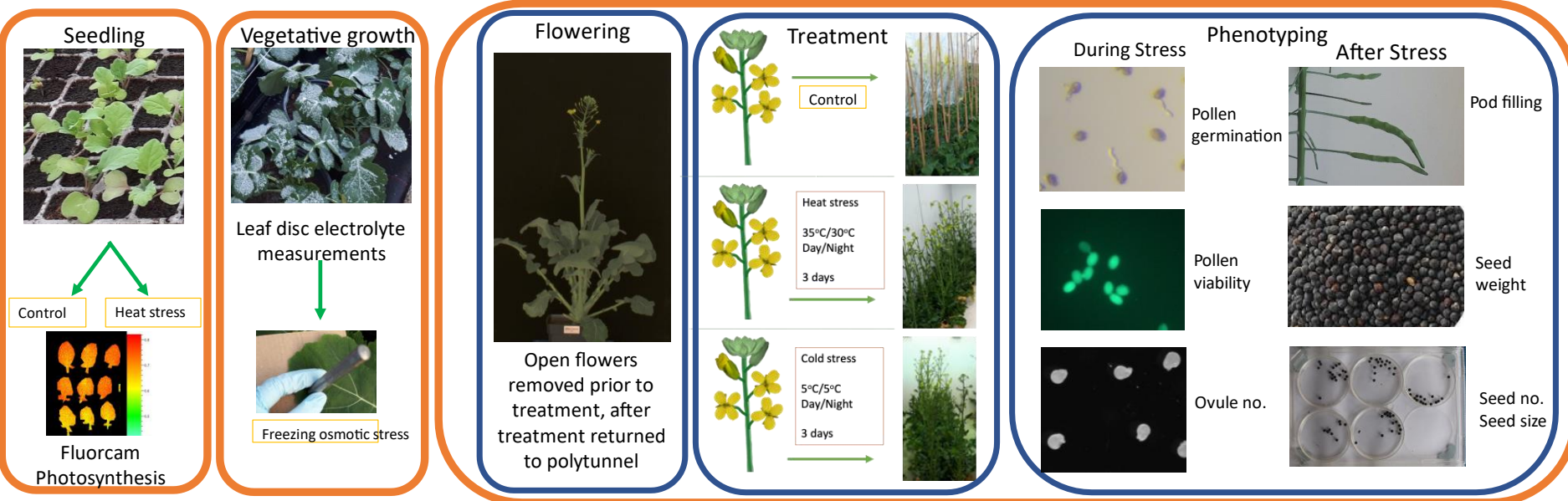
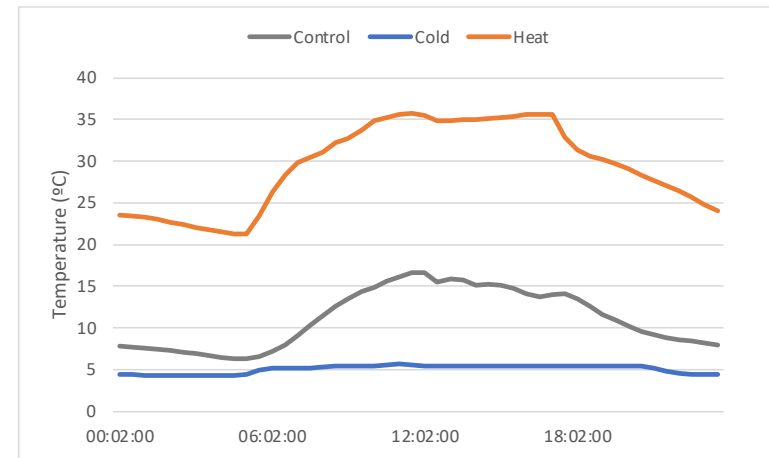




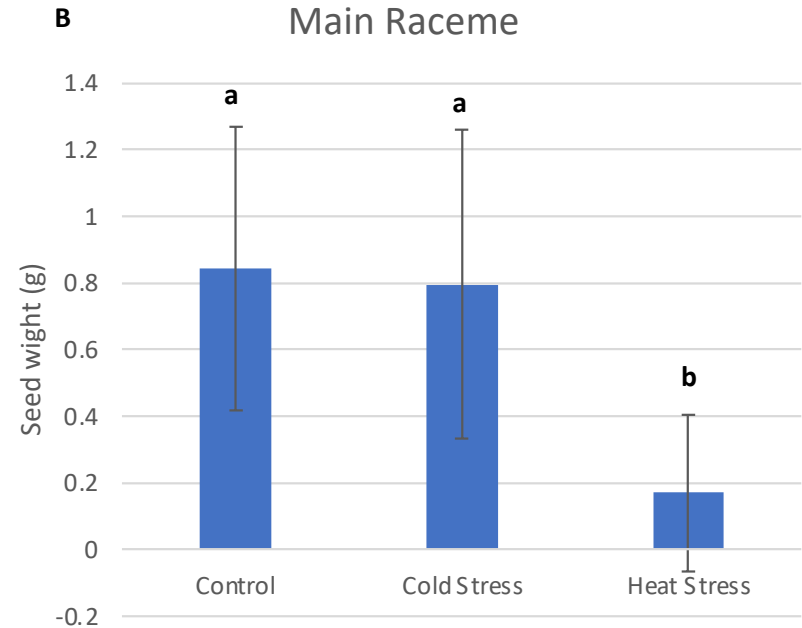
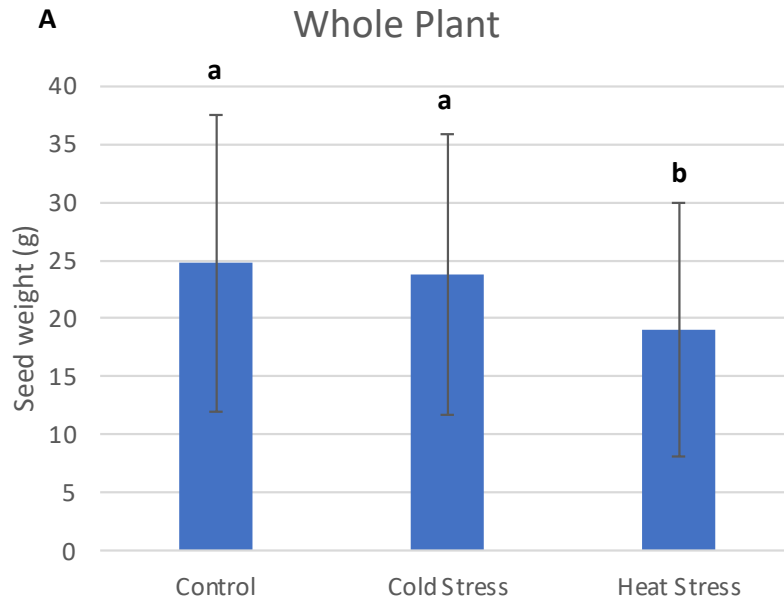
Climate change is resulting in increased global temperature, changes in temperature can effect when plants flower resulting in both extremes of temperature during flowering.

## *B. napus* BnaDFFS panel

- 99 lines
- 3 treatments
- 5 bio replicates per treatment
- 36 phenotypic features collected



# Seed yield for all accessions

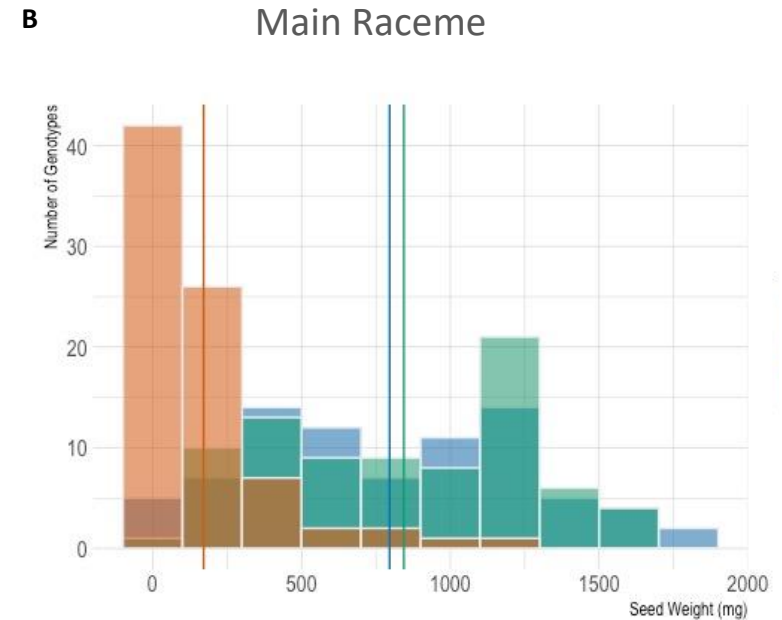
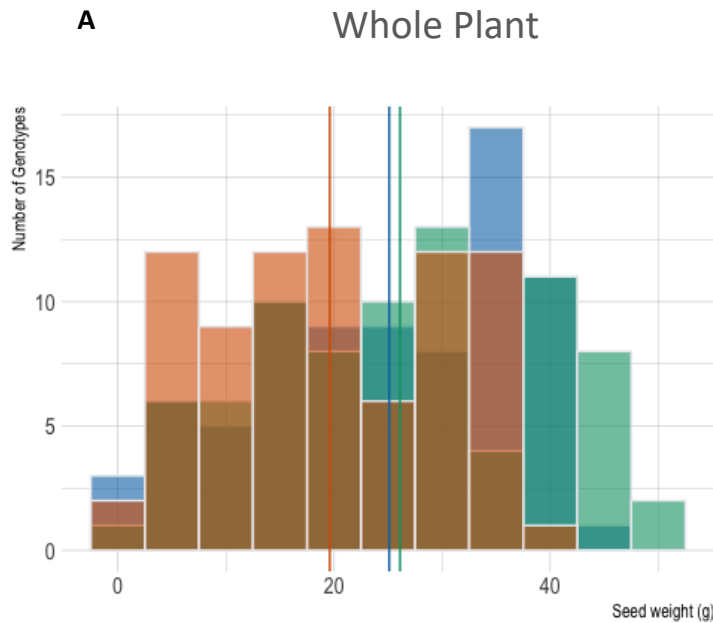


## Yield reduction

- 4% in cold stress (not sig)
- 28% in heat stress ( $P > 0.05$ )

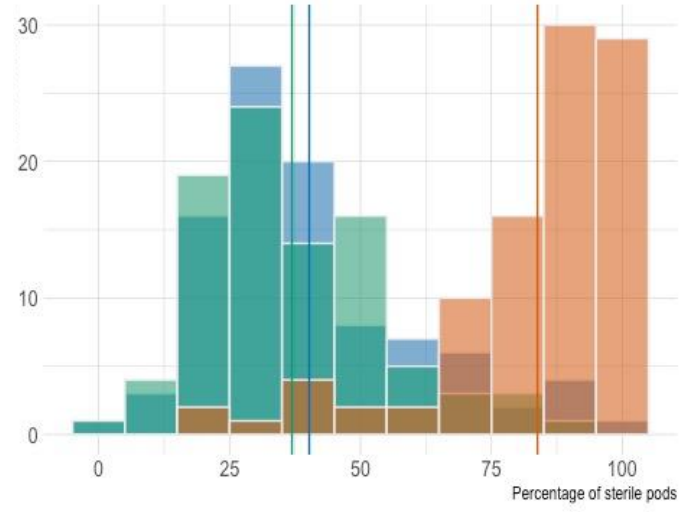
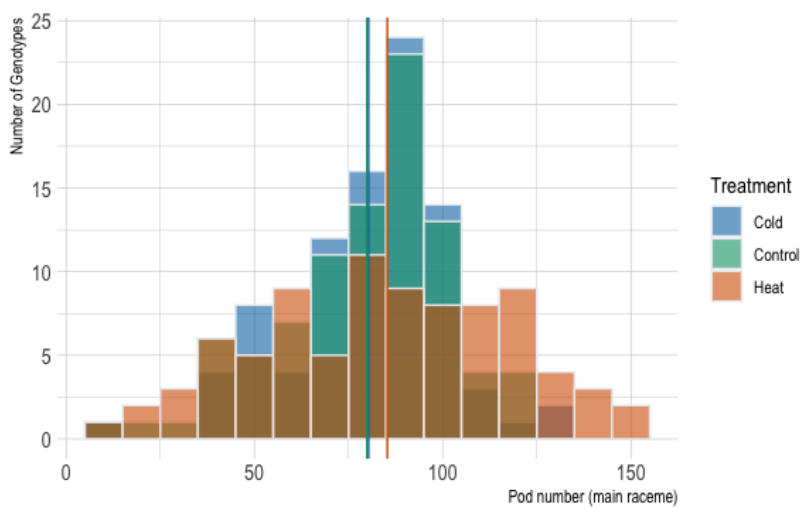
## Yield reduction

- 4% in cold stress (not sig)
- 72% in heat stress ( $P > 0.05$ )



High variability in seed yield between accessions in control and their response to heat stress

- Allow identification of those lines with increased tolerance to heat stress



Shift to more diversity in pod number, suggesting two mechanisms to stress;

- Giving up on main raceme and focusing on branching
- Producing more pods per raceme
  - High percentage of the pods on main raceme are sterile in heat stress (sterility is well known to increase flowering time and number of pods)





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# Causes of sterility - Pollen



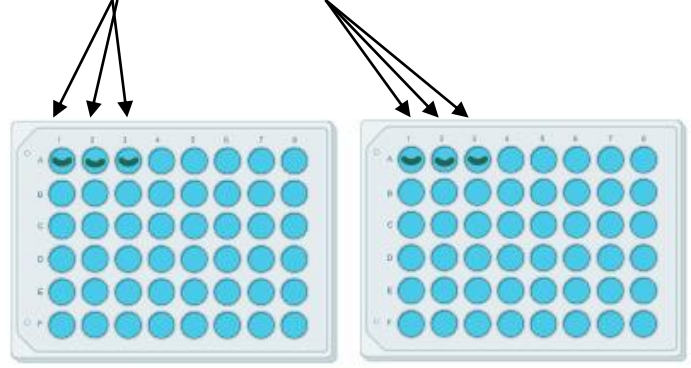
# Pollen Germination – 24hr after moved to stress



Youngest open flower collected into an Eppendorf tube



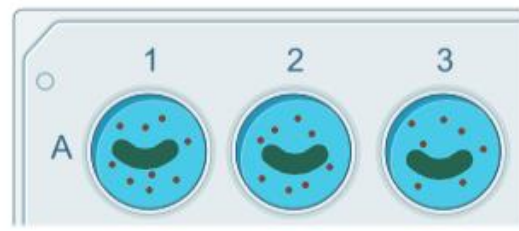
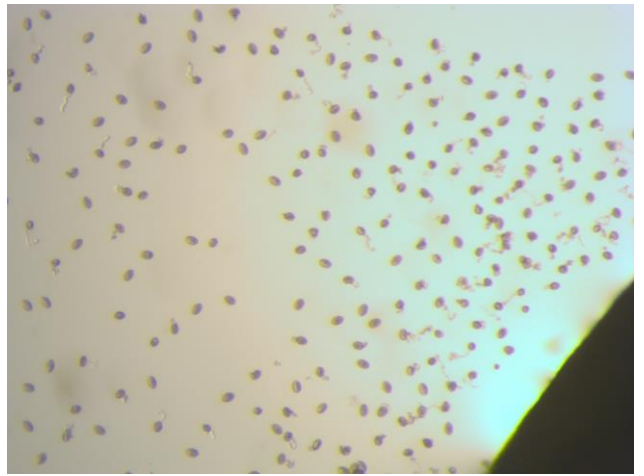
Individual anthers removed from a single flower and used to inoculate liquid pollen germination media



Control  
21oC

Grow overnight in the dark, and then take pictures

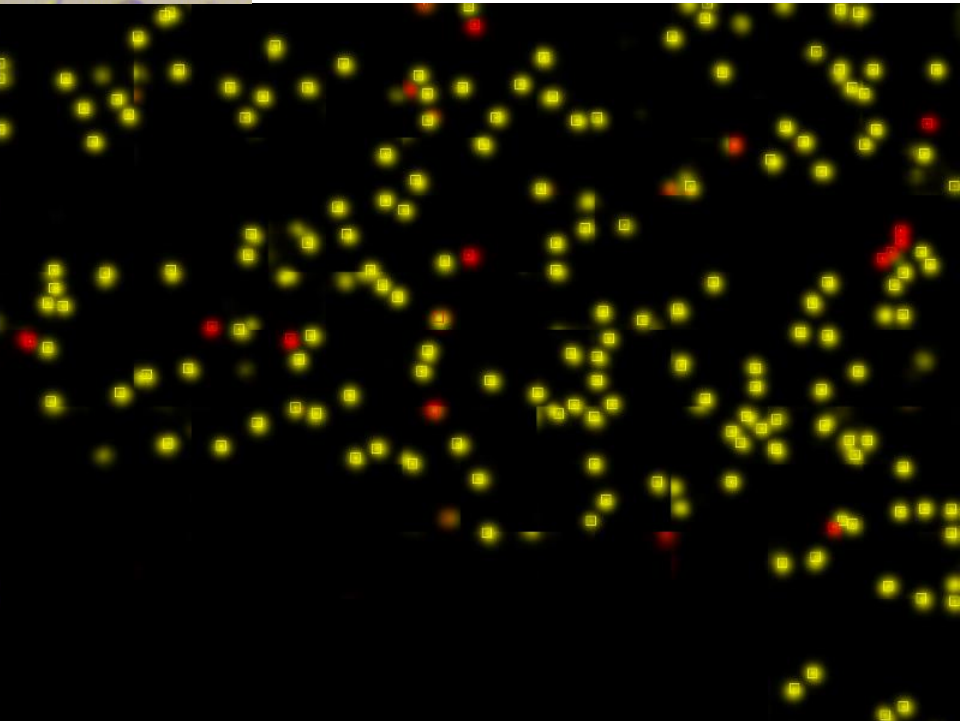
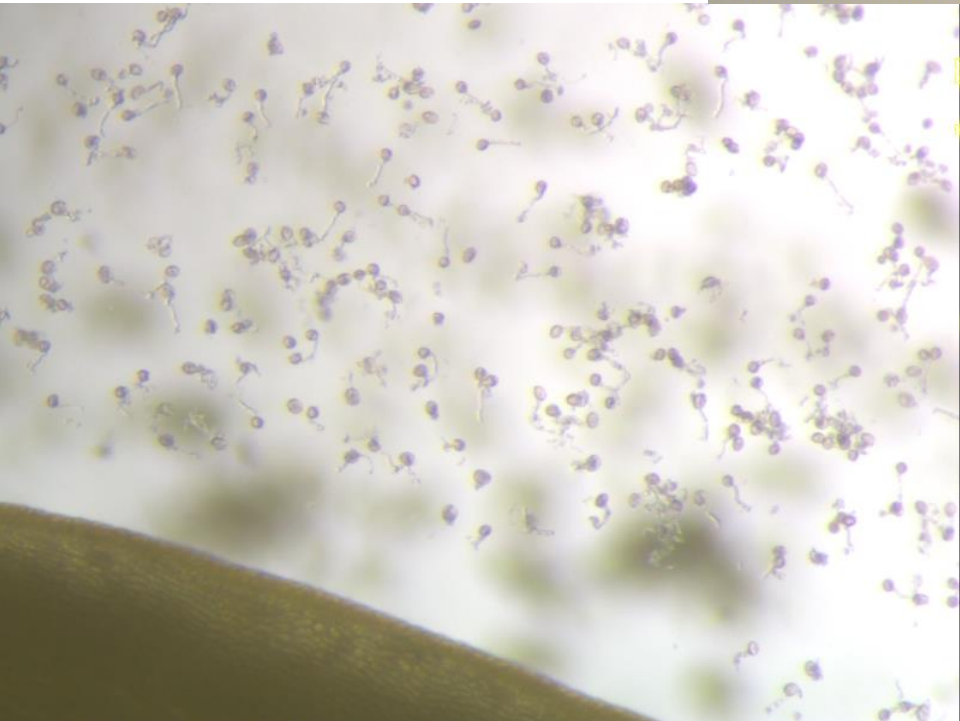
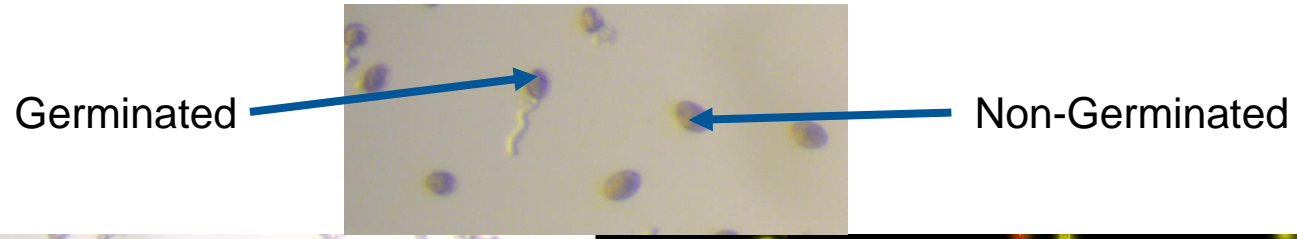
Treatment  
Incubation at  
35 or 4oC





Simon Castle-Green

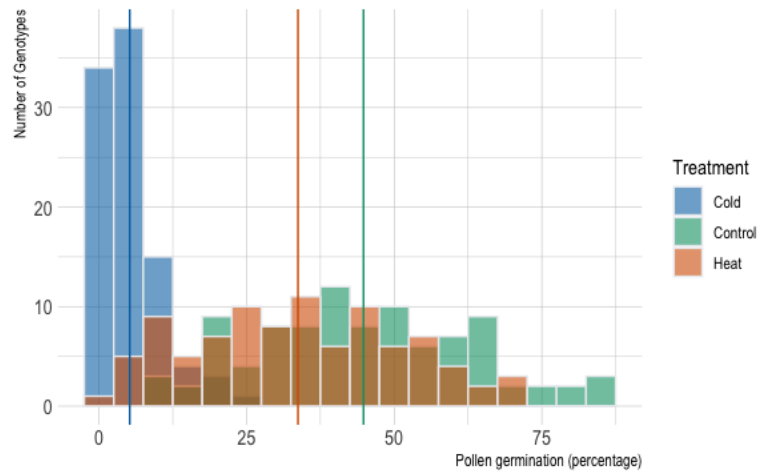
Computer training using 100 annotated images of germinated or non-germinated pollen



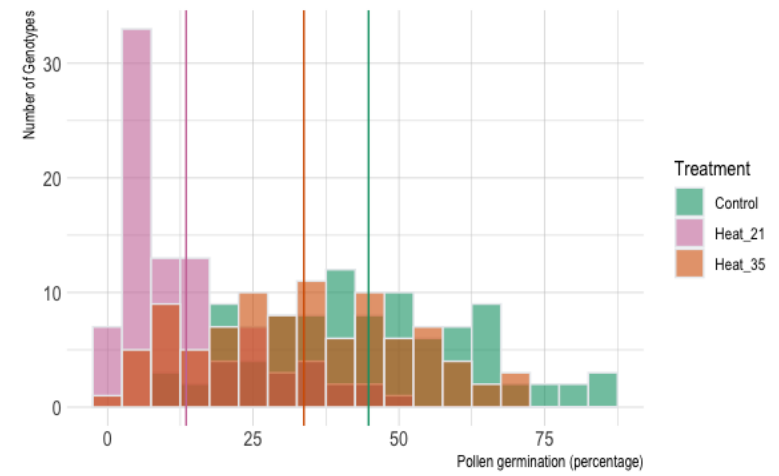
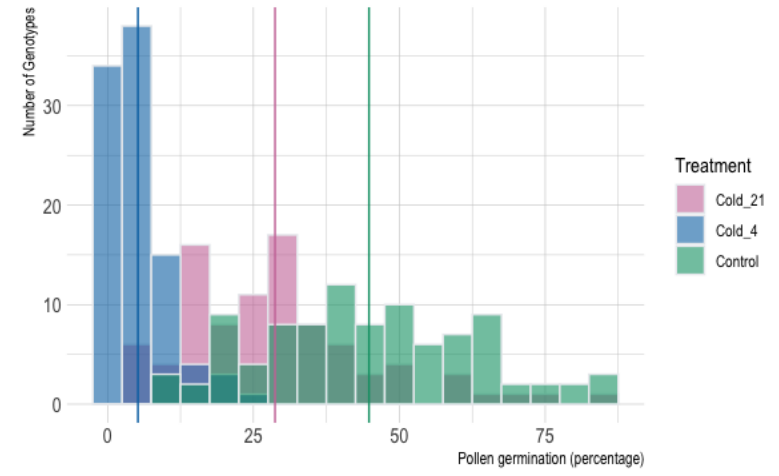
Trained program good at identifying pollen and labelling correctly  
Yellow = Germinated  
Red = Non-Germinated



Pollen germination is significantly reduced in cold. Rescued by returning to non-stress conditions

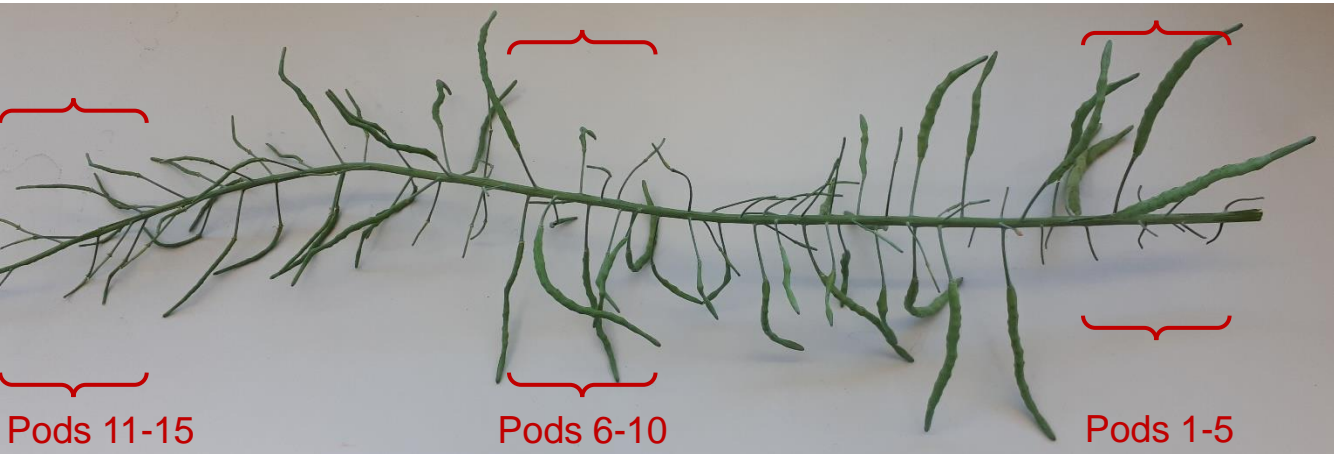


Pollen germination is reduced in heat but drops further once returned to non-stress conditions – suggests a longer negative effect

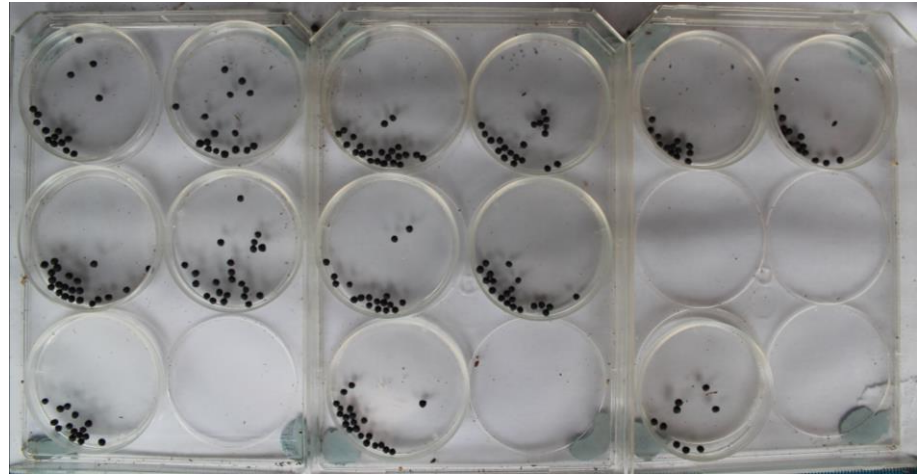




# Seed pods



5 oldest pods, 5 middle pods and 5 youngest pods taken from dried OSR plants

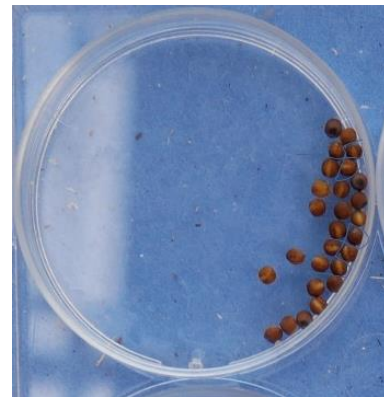
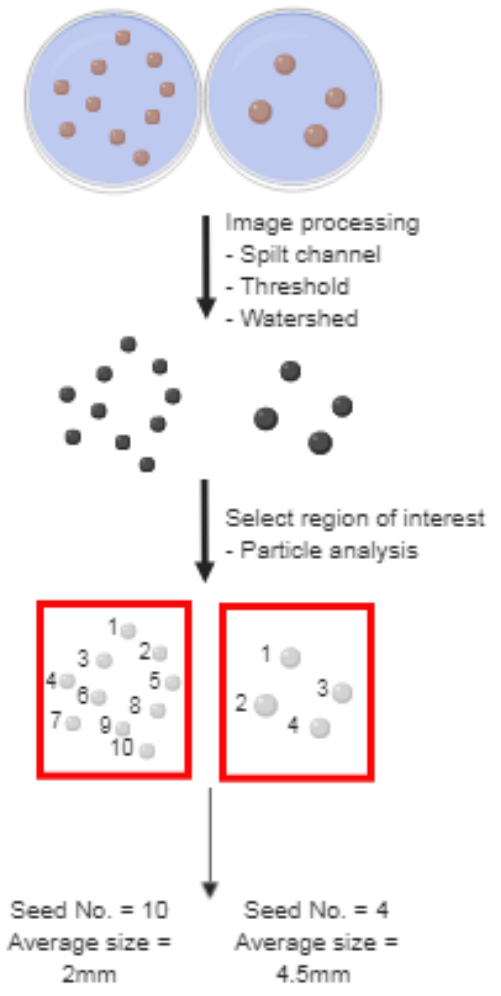


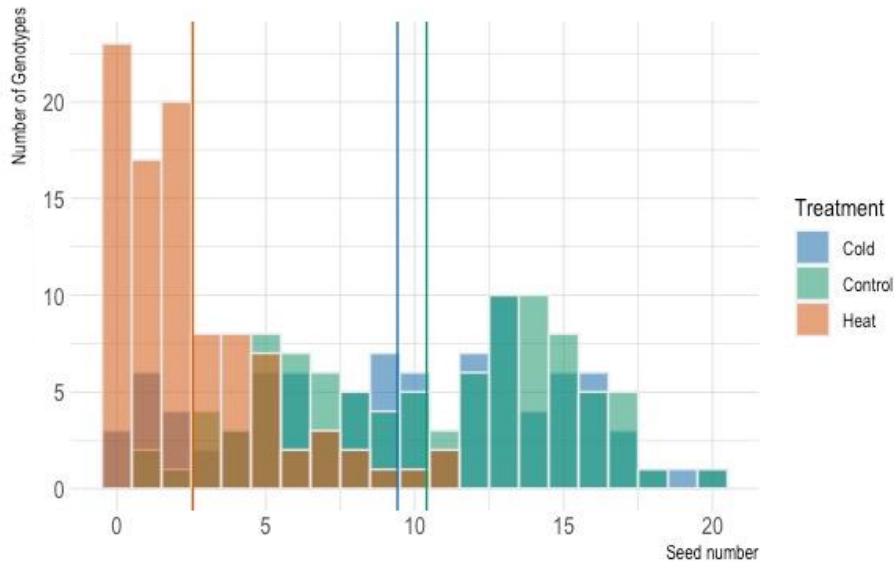
Pods 1-5                      Pods 6-10                      Pods 11-15



Laura Siles-Suarez

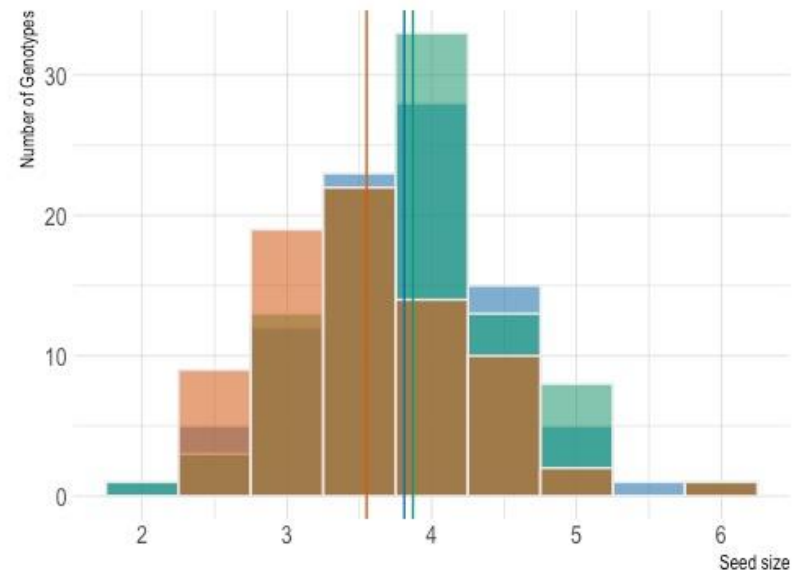
## Image J Macro written by Alison for automatic processing of seeds



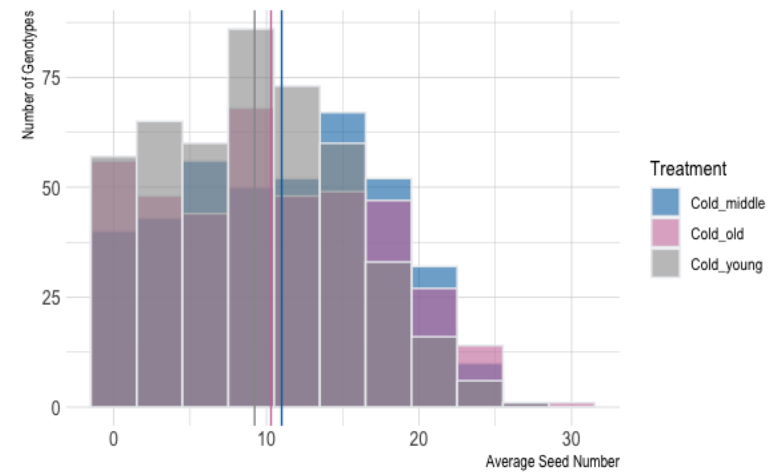
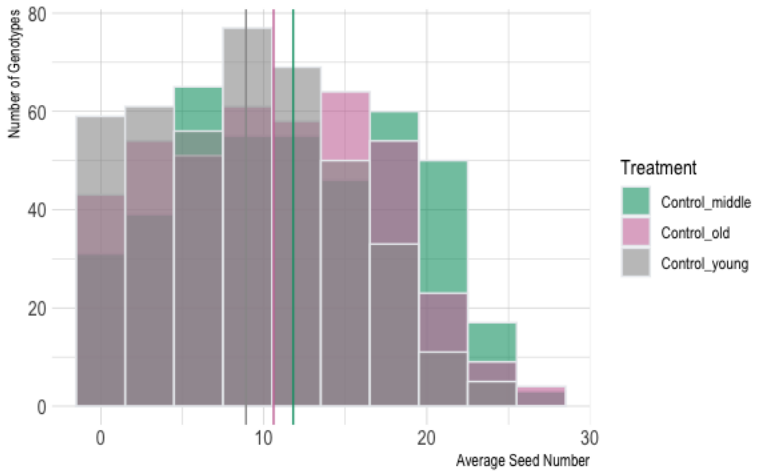


Seed number is heavily affected by heat, with less seeds being produced.  
Little variability between cold/control

Slight decrease in seed size in heat.  
Little variability between cold/control

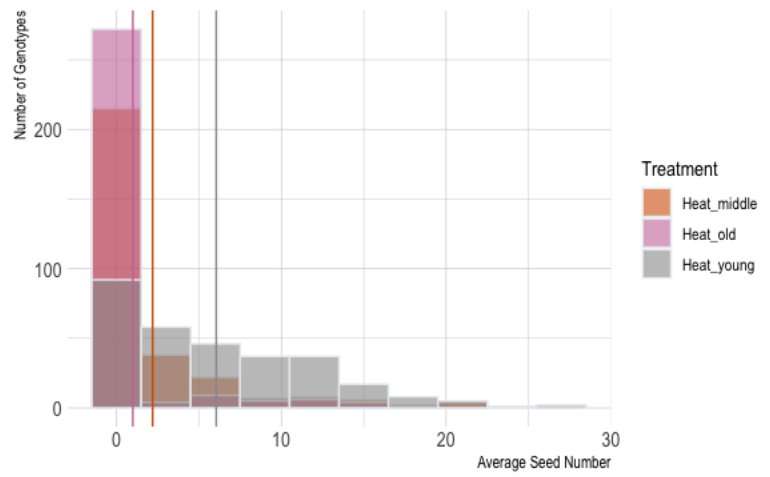






Rescue in seed number in the youngest pods (undeveloped during heat stress)

Only slight rescue in seed pods from the middle of the plant, suggesting whole developing buds are damaged by heat





# Summary

Short 3 day stress at the start of flowering has a significant effect on numerous plant developmental processes including

- Male fertility
- Female fertility

Heat stress has a significant effect in final yield in comparison to cold stress

Reducing yield not only during the 3 day heat stress but a longer negative development affecting pollen or ovule development, germination or fertilisation preventing seed set causing a significant reduction over the whole plant yield



Unlocking Nature's Diversity



WARWICK



University of Nottingham  
Zoe Wilson  
Rory Hayden  
Simon Castle-Green – Computer training

Rothamsted Research  
Smita Kurup  
Laura Siles-Suarez – Female analysis of stressed plants