Image analysis methods for studying infection of barley by *Rhynchosporium commune*

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Introduction

Rhynchosporium commune

- Fungal pathogen of barley
- Causes barley leaf scald
- Annual losses in the UK ≈ £7.2M
- Long asymptomatic phase
  - Important stage in epidemic development

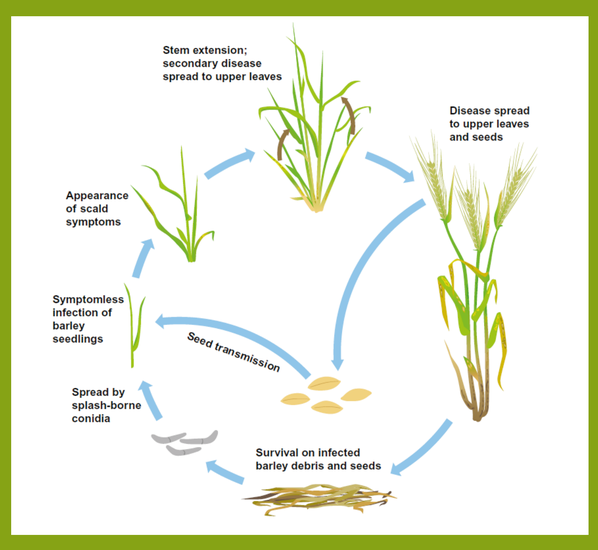

Figure 1: *R. commune* epidemic development during the barley growing season [1].

GFP expressing *R. commune* isolates and confocal microscopy have been used to investigate early host pathogen interactions during the asymptomatic phase [2].

However, such experiments have not looked at detailed differences in growth morphology, and experiments using modest numbers of lines can generate large numbers of images.

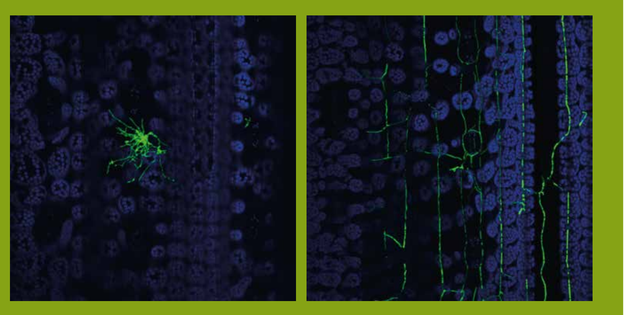

Figure 2: Confocal micrographs showing *R. commune* growth on the leaf surface of a susceptible line (Saffron; left) and a resistant line (Retriever; right). GFP fluorescence is shown in green, and chlorophyll auto-fluorescence in blue.

The aim of this work was to develop automated analysis tools to study growth morphology of *R. commune* hyphae on the barley leaf surface, and identify quantitative traits associated with resistance.

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Technology Strategy Board

The Scottish Government

Methods

5 barley cultivars, with varying resistance ratings, were inoculated with a GFP expressing *R. commune* isolate and imaged at 2, 3, 8 & 9 days post inoculation (dpi).

Image Segmentation

Automatic thresholds were applied to 2D projections of confocal image stacks to separate hyphae from background. Small (non-hyphal) particles were identified and removed. Binary images were converted into image skeletons in order to provide simplified representations of hyphal growth.

Error Correction & Image Analysis

- From image skeletons, neighbour relationships between foreground pixels were stored as an adjacency matrix and converted to a graph (vertices corresponding to foreground pixels and edges reflecting adjacencies).
- Gaps in hyphal branches (caused by variation in levels of GFP expression or thresholding errors) were corrected by interpolation across gaps.
- Breadth first search algorithm used to identify hyphal branch paths within the graph.
- Lists of branches were stored for each image and used to derive a number of quantitative descriptions of growth morphology.

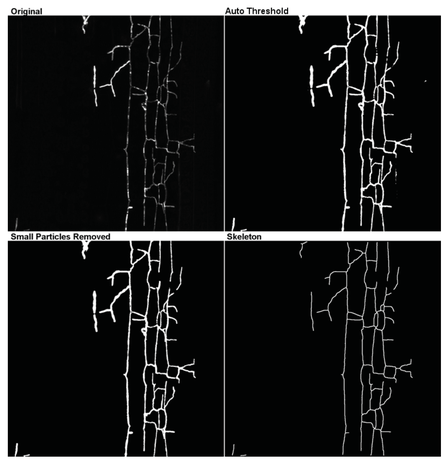

Figure 3: Steps in image segmentation. Top left: Original image (GFP channel); Top Right: Threshold set; Bottom Left: Small particles removed; Bottom Right: skeleton image.

Statistical Analysis

- 14 traits associated with growth morphology were scored.
- Principal component analysis used to examine relationships between traits.
- Spore germination rates have previously been associated with resistance, and these were scored at 2 dpi.

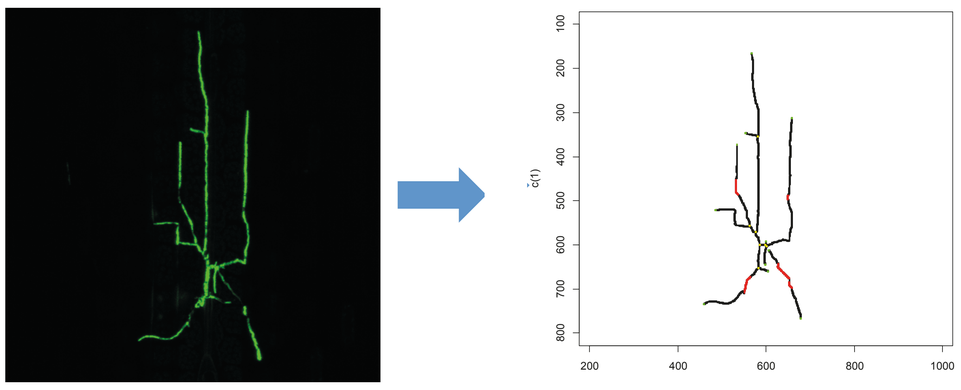

Figure 4a: Representative image showing GFP fluorescence from *R. commune* hyphae on a barley leaf.

Figure 4b: The same image following processing. Interpolations across gaps are shown in red.

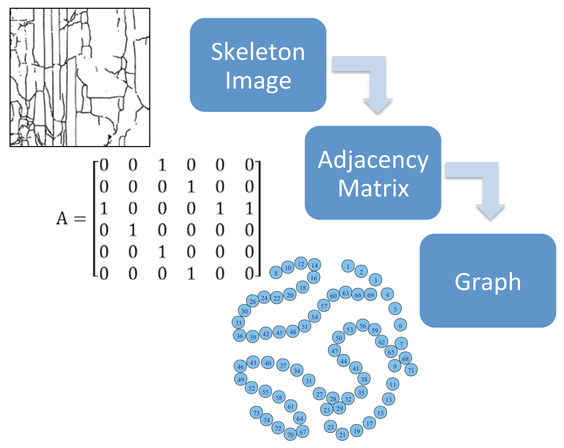

Figure 5: Steps in the conversion of a binary skeleton image to a graph.

Results

- Most variation in growth morphology due to two sets of highly correlated traits:
  - Total length of fungal hyphae (total branch length)
  - Average distance between branching points (mean branch length)
- Strong cultivar effects were identified for both of these traits and spore germination rates
- Patterns of cultivar variation differed between traits, suggesting differences in the effects of specific resistance genes.

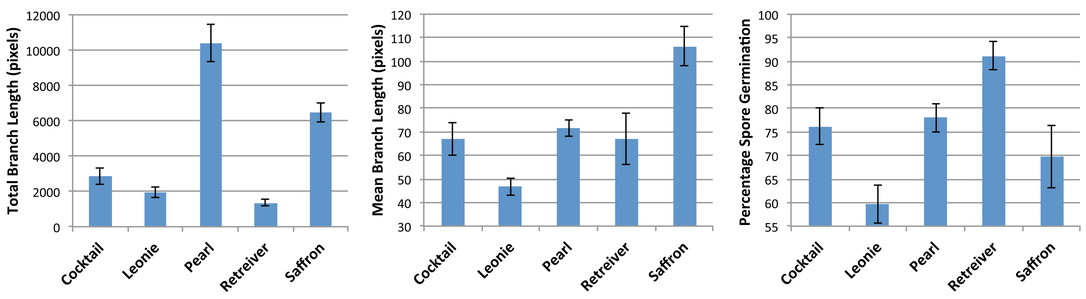

Figure 6: Cultivar means for the two morphological traits identified (at 8 dpi) and spore germination rates at 2 dpi.

Software

- **FIJI**: Image segmentation.
- **R**: Image processing, trait scoring and statistical analysis.
- **igraph** (R package): graph production and path searching.

igraph 0.6.5

References

Avrova A, Knogge W (2012) *Rhynchosporium commune*: a persistent threat to barley cultivation. Molecular Plant Pathology 13 (9): 986-997
Thirugnanasambandam A, Wright KM, Atkins SD, Whisson SC, Newton AC (2011) Infection of Rrs1 barley by an incompatible race of the fungus *Rhynchosporium secalis* expressing the green fluorescent protein. Plant Pathology 60 (3):513-521

Summary

- Methods developed to automatically process and analyse confocal micrographs
- Variation in growth morphology affected by cultivar
- Cultivars show response in specific resistance traits

Future work

- Test effects of specific resistances on growth morphology in common genetic background
- Relate information on *R. commune* effector recognition to growth morphology