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

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.

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.

**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.

**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.

**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.

**Software**

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

**References**


**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