

# Natural variation in oomycete infection of *Marchantia polymorpha*



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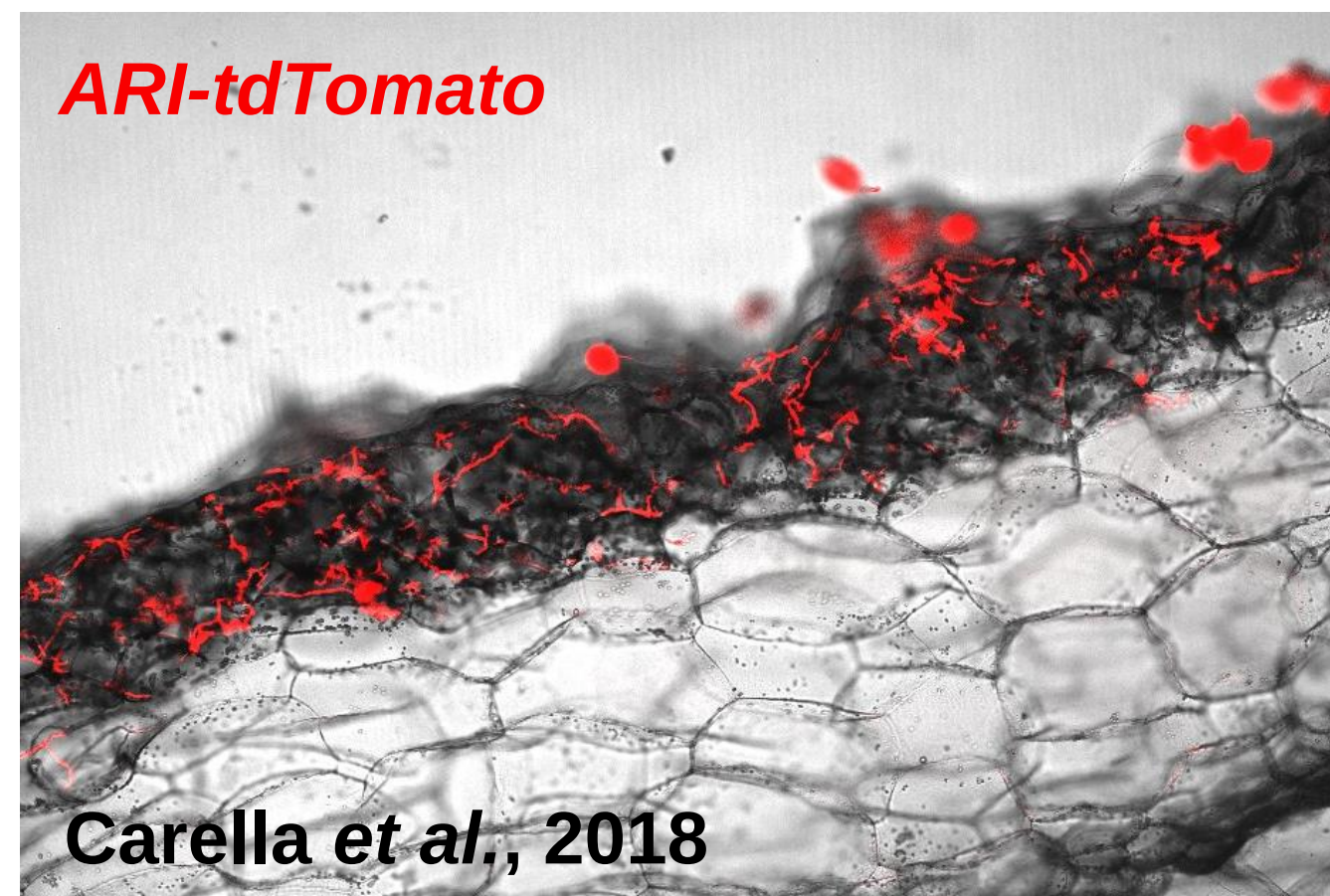
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## 1. Introduction

- Liverwort and angiosperm lineages diverged around 450 million years ago.
- Infection of *Marchantia polymorpha* has been established as a model pathosystem for macroevolutionary studies in oomycetes, fungus, and bacteria.
- Phytophthora palmivora* is a hemi-biotrophic broad-host, and infects through air chambers and colonises the upper photosynthetic layer.
- Most studies of *Marchantia* are on a small number of accessions, and the genomic resources which are available for other more established model organisms are not currently available.
- Our **aim** is to establish these genetic resources, and our **working hypothesis** is that variation in the infection of natural accessions of *Marchantia* is caused by variable genetic elements.



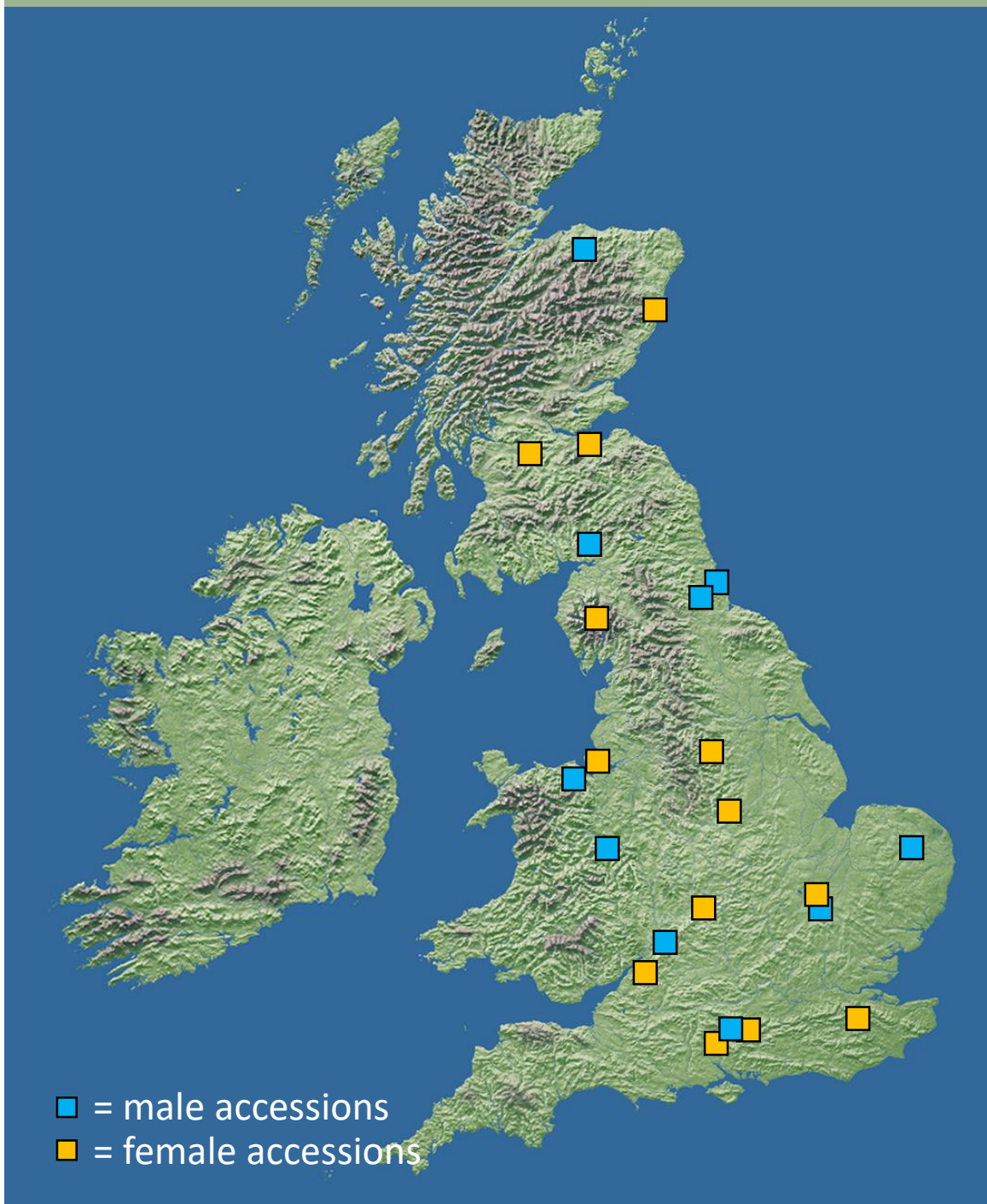
Infected *Marchantia polymorpha* (5dpi)



## 2. Collecting wild-type accessions of *Marchantia* (and *Lunularia*).



The Great British Liverwort Hunt Map



Scan here to watch how we processed samples submitted by the public



We have so far collected 92 accessions of *Marchantia polymorpha*: some through our citizen science project, and some by us and collaborators on field trips or holidays! Male and female accessions were determined by amplification of PCR markers.

## 3. WT accessions of *Marchantia* display many morphological differences. These traits have potential to be used in genome wide association studies.

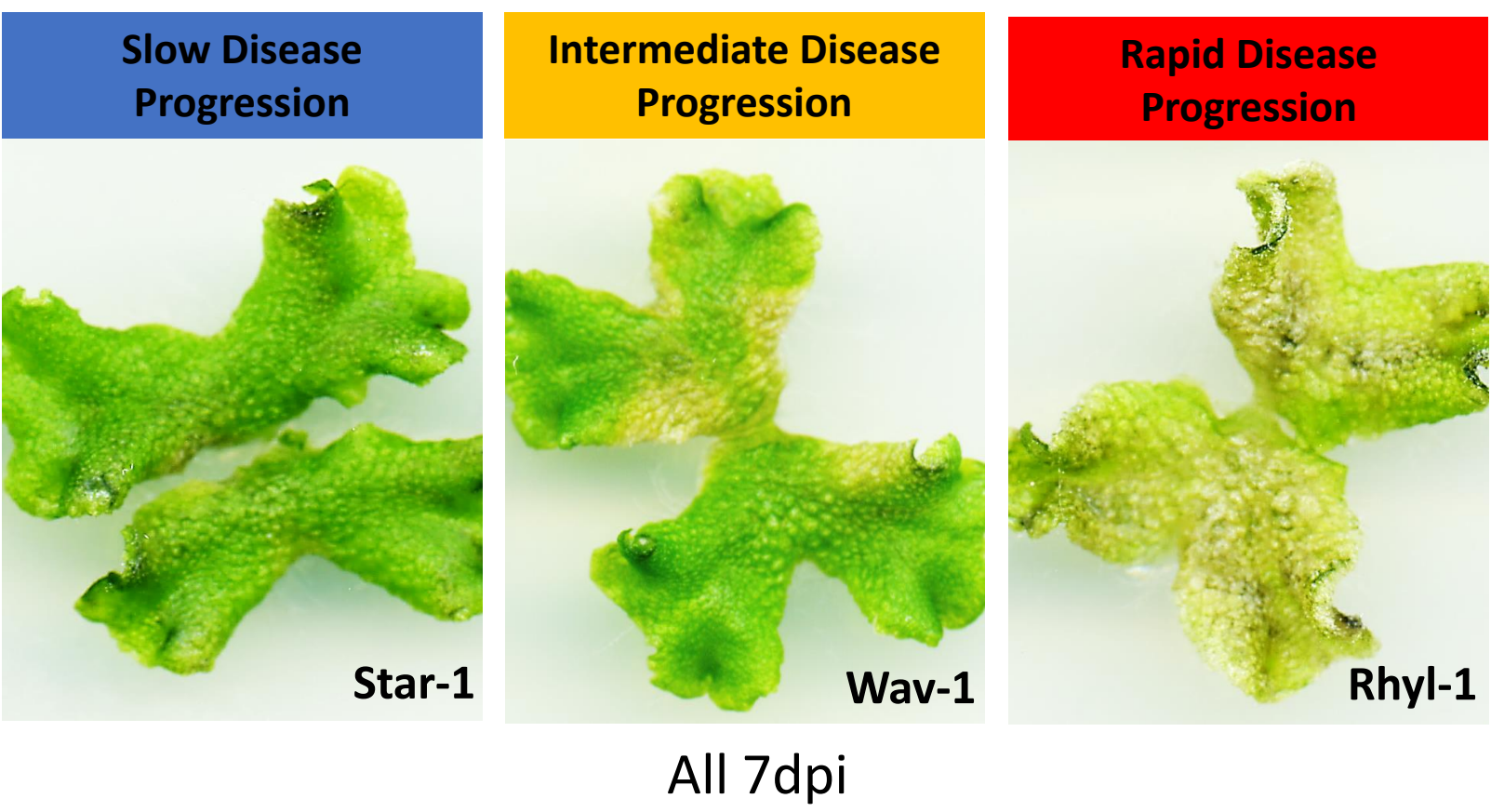


A selection of the accessions obtained from the Great British Liverwort Hunt grown for 3 weeks on 1/2 MS B5 (pH5.5) media under 24h light. Morphological traits which show natural variation include: number of bifurcations, planar growth, and number of gemma cups.

## 4. Initial survey of the Liverwort Hunt accessions using a subjective rating system of symptoms.

Three major categories of disease progression were observed in the Liverwort Hunt accessions

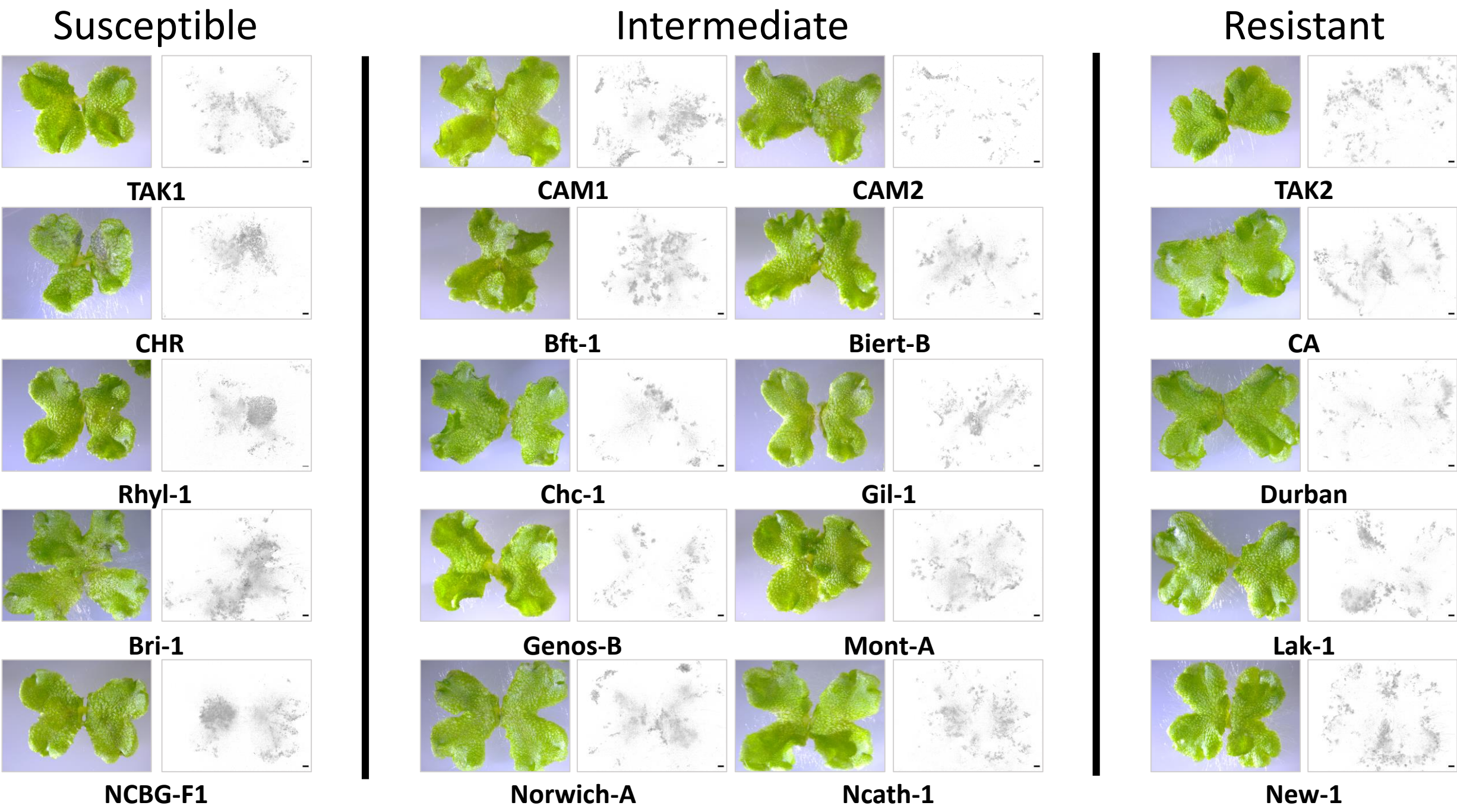
- Of all accessions tested, none were completely resistant to infection.
- However, the rates of disease severity varied between the different accessions.
- The majority displayed intermediate disease progression, while some accessions either had slower or more rapid disease progression phenotypes (see below).



| Accession Name | Sex | 1dpi | 3dpi | 5dpi | 7dpi |
|----------------|-----|------|------|------|------|
| Gor-1          | F   | 1    | 1    | 1    | 1    |
| Star-1         | F   | 1    | 1    | 1    | 1    |
| Jim-1          | M   | 1    | 1    | 1    | 1    |
| TAK2           | F   | 1    | 1    | 1    | 1    |
| Aber-1         | F   | 1    | 1    | 1    | 2    |
| Fie-1          | F   | 1    | 1    | 1    | 2    |
| Fie-2          | F   | 1    | 1    | 1    | 2    |
| Glo-1          | M   | 1    | 1    | 1    | 2    |
| Lak-1          | F   | 1    | 1    | 1    | 2    |
| Mtw-1          | M   | 1    | 1    | 1    | 2    |
| New-1          | F   | 1    | 1    | 1    | 2    |
| Hope-1         | M   | 1    | 1    | 1    | 2    |
| Pot-1          | M   | 1    | 1    | 1    | 2    |
| War-1          | F   | 1    | 1    | 1    | 2    |
| Gil-1          | M   | 1    | 1    | 2    | 2    |
| Maud-1         | M   | 1    | 1    | 2    | 2    |
| Rin-1          | F   | 1    | 1    | 2    | 2    |
| Tony-1         | F   | 1    | 1    | 2    | 2    |
| Bft-1          | F   | 1    | 1    | 2    | 3    |
| Bla-1          | F   | 1    | 1    | 2    | 3    |
| Bri-1          | F   | 1    | 1    | 2    | 3    |
| Gra-1          | F   | 1    | 1    | 2    | 3    |
| Lib-1          | F   | 1    | 1    | 2    | 3    |
| Ncath-1        | M   | 1    | 1    | 2    | 3    |
| Sev-1          | F   | 1    | 1    | 2    | 3    |
| Wav-1          | F   | 1    | 1    | 2    | 3    |
| Whit-1         | M   | 1    | 1    | 2    | 3    |
| Wies-1         | F   | 1    | 1    | 2    | 3    |
| Chc-1          | F   | 1    | 1    | 2    | 3    |
| Lh-1           | M   | 1    | 2    | 2    | 3    |
| TAK1           | M   | 1    | 2    | 3    | 3    |
| Crow-1         | F   | 1    | 2    | 3    | 3    |
| Rhyl-1         | M   | 1    | 1    | 3    | 4    |

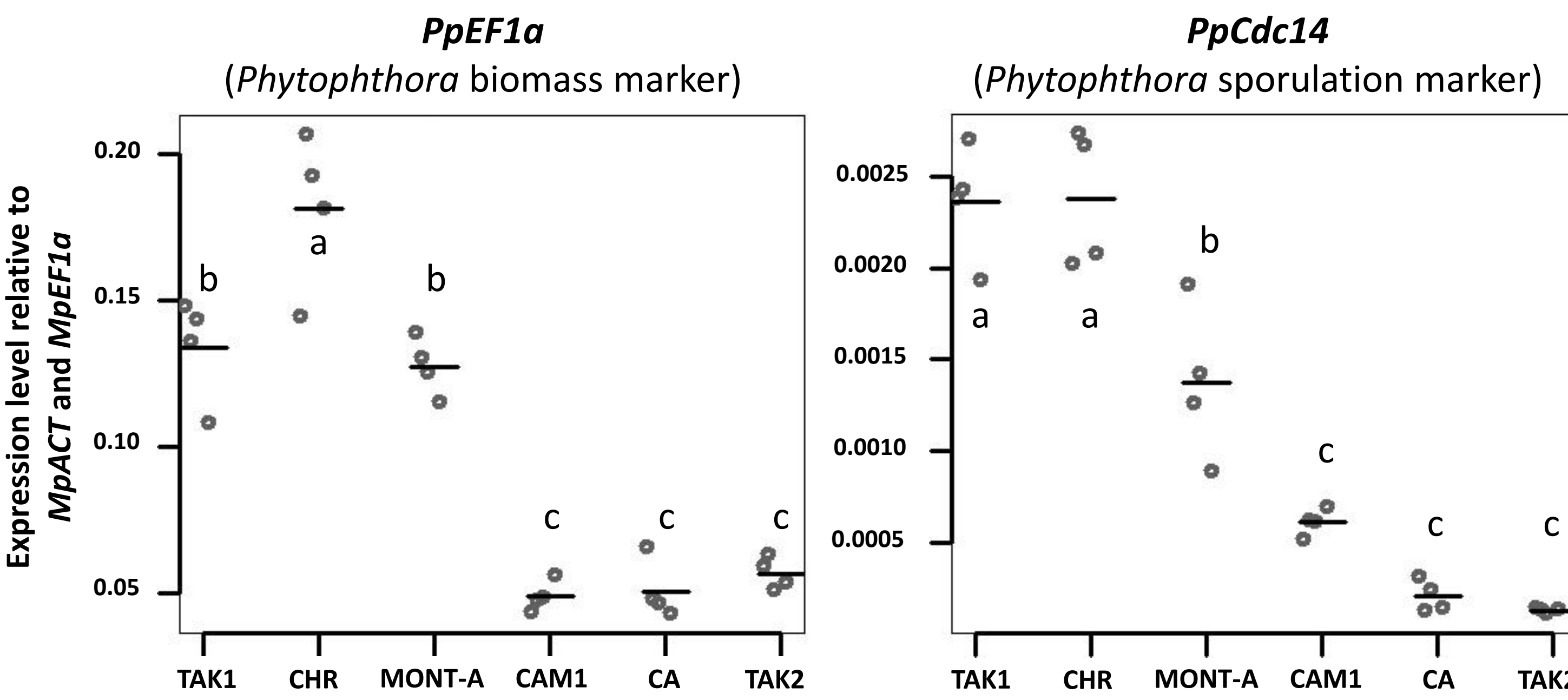
1 = little to no symptoms 2 = intermediate symptoms 3 = clear symptoms 4 = severe symptoms

## 5. Scaling up: for quantitative analysis, infection of additional accessions at a late biotrophy time point (3 days post infection).



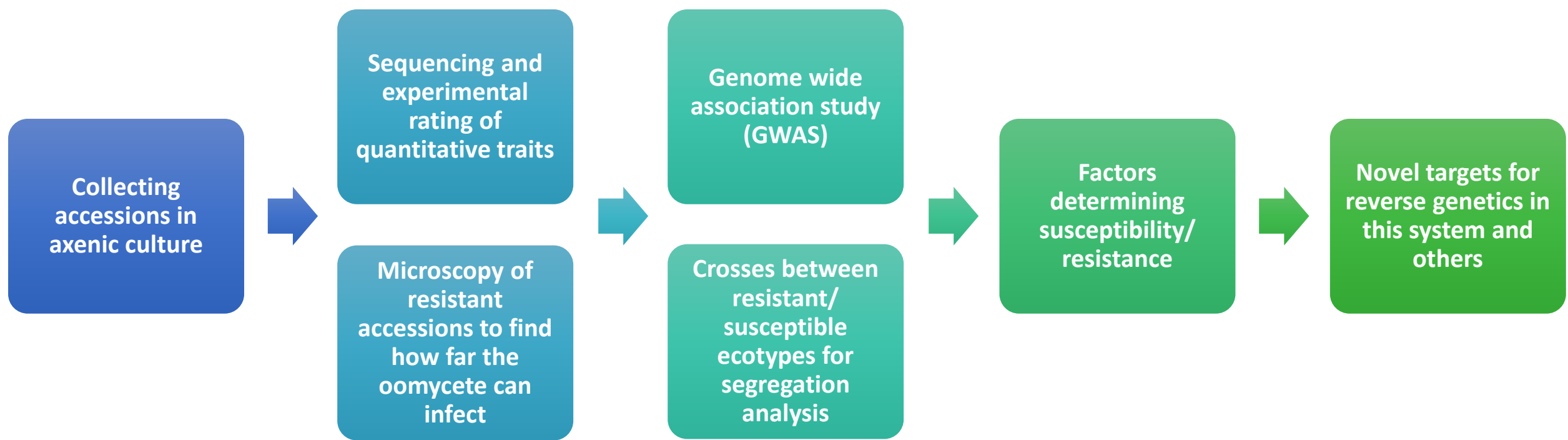
A subset of plants infected at 3dpi in our most recent experiments for qPCR experiments. For each ecotype, left image is bright-field; right image is DsRed pathogen epifluorescence. Scale = 1mm. At 3dpi, susceptible accessions are beginning to move into necrosis. Pathogen growth in resistant accessions is mainly surface growth or growth on the media at this time point.

## 6. qPCR quantification of *Phytophthora* infection across accessions is in progress, with a goal of a linear assembly of this trait.



Initial proof-of-concept experiment. qPCR data showing 2 susceptible, 2 intermediate, and 2 resistant accessions collected at a single time point. N=4, each data point performed in triplicate. Each sample contains 3 individual plants. Statistical groupings from Tukey's HSD post-hoc analysis. In meta-analysis of separate infection experiments, we will use TAK1 (susceptible) and TAK2 (resistant) as baselines for pathogen biomass.

## 7. Future steps: genome wide association studies will reveal novel factors of resistance/susceptibility in this model pathosystem.



### Summary & Conclusions:

- Marchantia polymorpha* and *Phytophthora palmivora* are a model pathosystem for macroevolutionary studies.
- However, as a relatively new model system the genetic resources which are available for other systems are not yet established.
- We are working to establish these resources for the community by collecting and sequencing accessions.
- Most accessions display intermediate symptoms, though some are very resistant and some are very susceptible.
- None of the accessions tested so far are completely resistant to the pathogen.
- We plan to use the *Marchantia-Phytophthora* pathosystem as a quantitative trait for future genome-wide association studies.

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