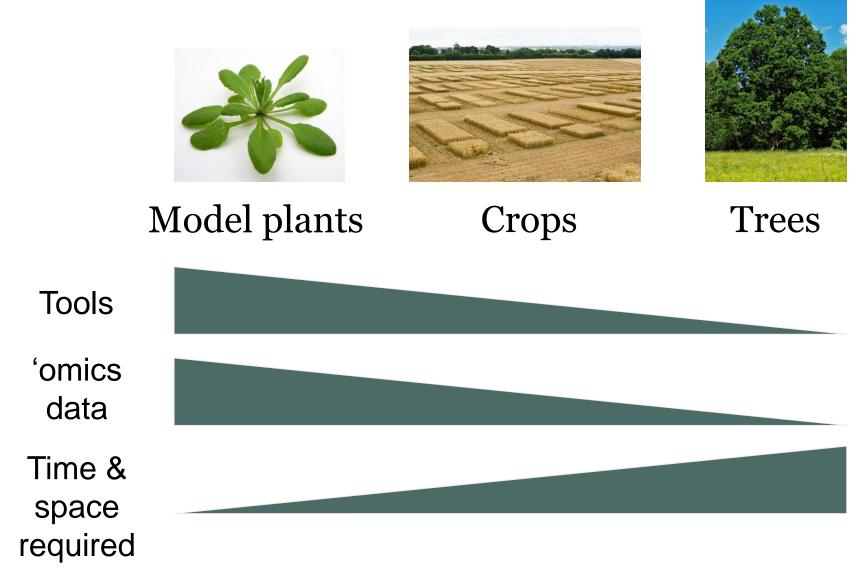
# Development of tools for dissection of oak tree diseases

Dr Graeme Kettles 3<sup>rd</sup> annual BIFoR Community Meeting 30<sup>th</sup> January 2019



# The challenge of trees (from the perspective

of a molecular biologist)



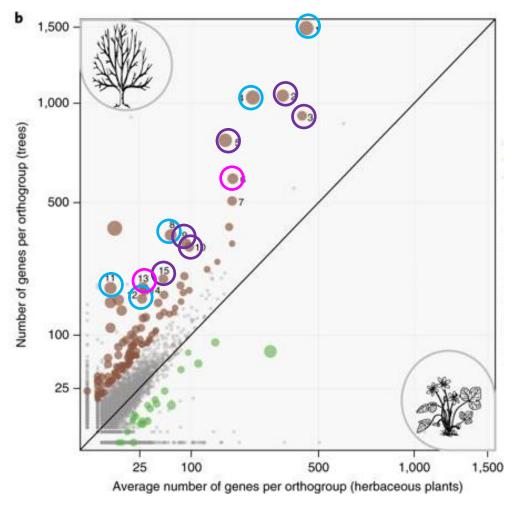
## What tools do we need?

□ Genomic data for tree species (leads!)

- □ A method for reverse genetics
- Markers of immune status

- □ Expression of transgenes
- □ Pathogen bioassays

## What do tree genomes tell us?



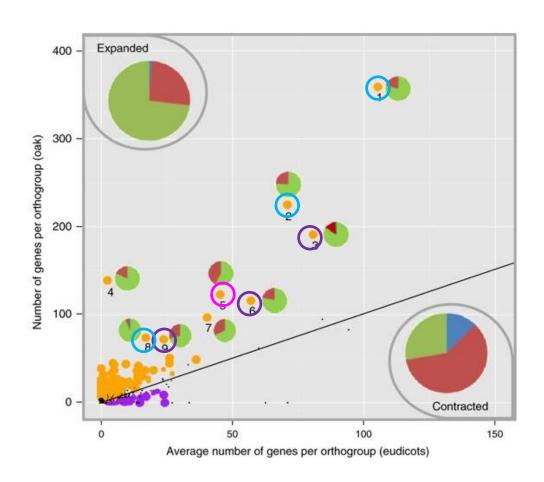
Nucleotide-binding and leucine-rich repeat (NLR) immune sensors

Receptor-like kinase (RLK) immune sensors

Receptor-like protein (RLP) immune sensors



## The Quercus robur genome



Nucleotide-binding and leucine-rich repeat (NLR) immune sensors

Receptor-like kinase (RLK) immune sensors

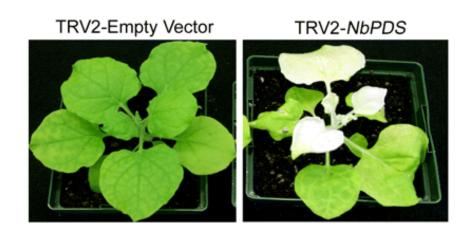
Receptor-like protein (RLP) immune sensors

#### How do you chase these leads?



## A method for reverse genetics

- Analysis of gene function often involves targeted mutagenesis
- Stable transformation is difficult in tree species
- Transient methods such as Virus-Induced Gene Silencing (VIGS) are a fast, high-throughput alternative

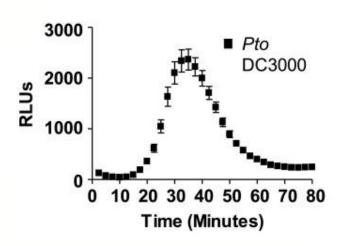


Silencing of the PDS gene induces photobleaching in the tobacco Nicotiana benthamiana

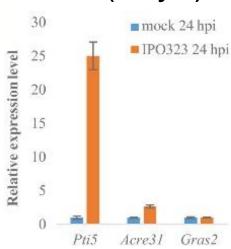
## Markers of immune status

- What markers are hallmarks of immune activation?
- How do they differ between resistant and susceptible individuals?

### Early (minutes)



#### Late (days)



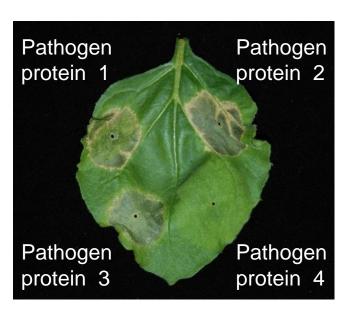
(Smith and Heese, 2014, *Plant Methods*) (Kettles et al., 2017, *New Phytologist*)

## Expression of transgenes

- Viral and bacterial systems allow expression of foreign proteins in plants
- □ Proteins can be of plant, animal or pathogen origin



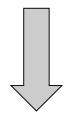
Expression of Green Fluorescent Protein (GFP) in the tobacco *Nicotiana*benthamiana



## Glasshouse bioassays

Field symptoms





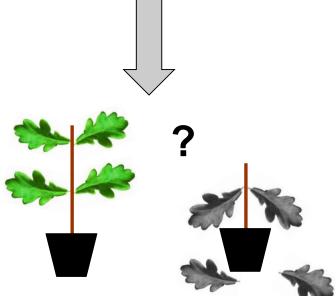
**R**esistant **S**usceptible

Bioassay











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