Identifying candidate resistance genes for acute oak decline and oak powdery mildew using transcriptome sequencing

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Figure 1: Oak powdery Mildew

Introduction

Resistance (R) genes in plants play a key role in recognising pathogen-derived molecules and triggering downstream immune responses. Typically, R-genes fall into several gene families, including receptor-like kinases (RLKs) and genes with nucleotide binding site – leucine rich repeat domains (NLRs). Following sequencing of the *Quercus robur* genome, it was discovered that these gene families are massively expanded in oak, leading to strong speculation that they may be important for oaks long life-span (Plomion et al. 2018). Despite their seemingly important role, no R-gene has yet been functionally characterized in oak, an important knowledge gap given the threat oaks currently face from invasive diseases. Two of the most threatening of these diseases are oak powdery mildew (OPM) (figure 1) and Acute oak decline (AOD) (figure 2).

OPM is a foliar disease caused by the biotrophic fungus *Erysiphe alphitoides.* Severe infections of mature trees can impact their health by limiting photosynthesis and shortening leaf life-span. Severe infections of young trees and seedlings can be lethal. AOD is caused by a system of at least three necrotizing bacteria, including *Brenneria goodwinii, Gibbsiella quercinecans,* and *Rahnella victoriana.* Colonization of the inner bark and sapwood by these bacteria causes stem bleeds from lesions in the trees trunk.

Our aim is to identify and understand R-genes that contribute to successful immune response against OPM and AOD, this can be accomplished by genetic comparisons between highly resistant and susceptible individuals. We have therefore established and executed bioassays to identify such individuals.

Figure 2: Acute Oak Decline

OPM Methods and Results

A detached-leaf bioassay was established for OPM by Bartholome *et al.* (2019), who demonstrated that resistance to OPM exists in the European oak population. In this bioassay, rate of mycelial growth is used as an analogue for degree of quantitative resistance to OPM. We adapted this assay (Fig.3), to inoculate *E. alphitoides* spores onto leaves excised from individual plants.

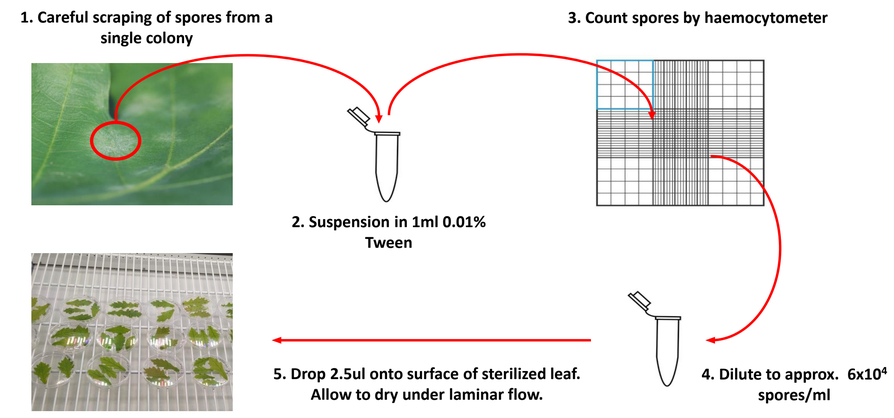

Figure 3: Steps followed in the inoculation of excised leaves. Following inoculation, leaves were maintained in controlled light/dark and temperature conditions in a growth chamber.

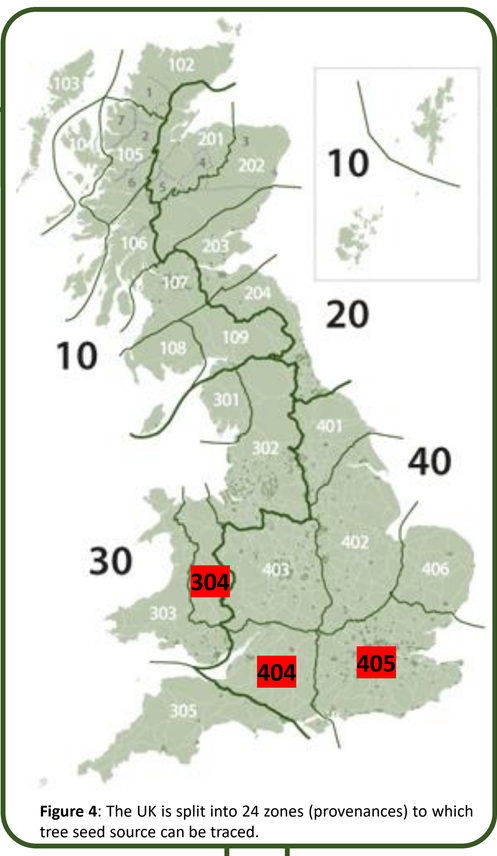

Figure 4: The UK is split into 24 zones (provenances) to which tree seed source can be traced.

The UK is divided into 24 zones (provenances) for seed source tracking (Fig.4). Distribution of resistance in the UK oak population is unknown. In our bioassay we screened individual seedlings sourced from several provenances for resistance to OPM. Mycelial growth was scored 14 days post inoculation (Fig.5).

Figure 5: Seedlings of all levels of resistance were observed, from highly resistant (left) to highly susceptible (right)

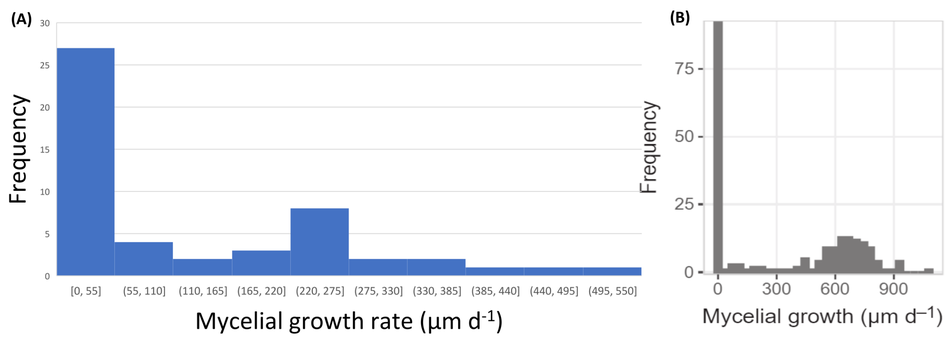

Figure 6: distribution of levels of mycelial growth observed in our bioassay on UK oaks (A) compared to that observed in Bartholome et al's (2019) bioassay on European oaks (B).

The distribution of levels of resistance we have observed (Fig.4a) is similar to that which was previously observed by Bartholome et al. (2019) (Fig.4b), when using the same bioassay to quantify resistance in the European oak population.

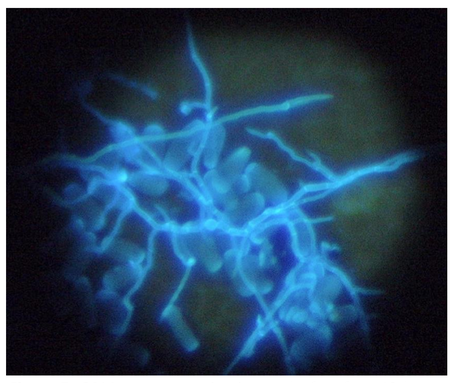

Figure 7: OPM spores germinating on leaf surface 48 hours post inoculation.

RNA will be extracted from sites of inoculation at 48 hours post inoculation (Fig.7) and sequenced. Sequenced RNA will be compared among resistant and susceptible individuals to identify genetic differences, and among pathogen and control inoculated samples to identify gene expression differences induced by OPM infection.

Discussion and Future directions

- Our aim is to identify R-genes in the oak genome that play an important role in successful immune response to OPM and AOD.
- We have so far in our research shown that OPM resistance appears to be as common in the UK oak population as it is in the European population. A similar range of resistances appears to exist for AOD. We have also identified trees highly resistant and susceptible to OPM and AOD.
- Given the major expansion of gene families associated with disease resistance in the oak tree genome, we hypothesise that these genes contribute to immune response against OPM and AOD. Within these gene families, genetic variants that are consistently present in resistant trees and absent in susceptible trees (or visa versa) will likely point to important R-genes.
- We are therefore in the process of sequencing the transcriptomes of resistant and susceptible trees, and genotyping them as illustrated in the sequence data analysis section.

AOD Methods and Results

To screen for disease resistance or susceptibility to bacteria associated with AOD, we sourced acorns produced from three provenances with different levels of reported AOD severity (405, 304, and 404) (Fig.4). Approximately 150 seedlings were inoculated with a mixture of the three AOD causing bacteria at six points on the stem (Fig.8). Three of these pieces of the stem were then sampled on the day of inoculation, and the other three were sampled 50 days later.

Figure 8: (left) 149 oak seedlings aged 200 days awaiting inoculation, (right) three (of six) points on the stem into which a mix of AOD causing bacteria were deposited and taken up by capillary action.

Our objective was to identify individual seedlings that were either highly susceptible or highly resistant to bacterial colonisation. At 50dpi, stem samples were homogenised and bacteria quantified by plate counting. A wide range of colony counts were observed (Fig.9), ranging from plants which had cleared infection, to those which harboured high bacterial loads (refer to figure annotation).

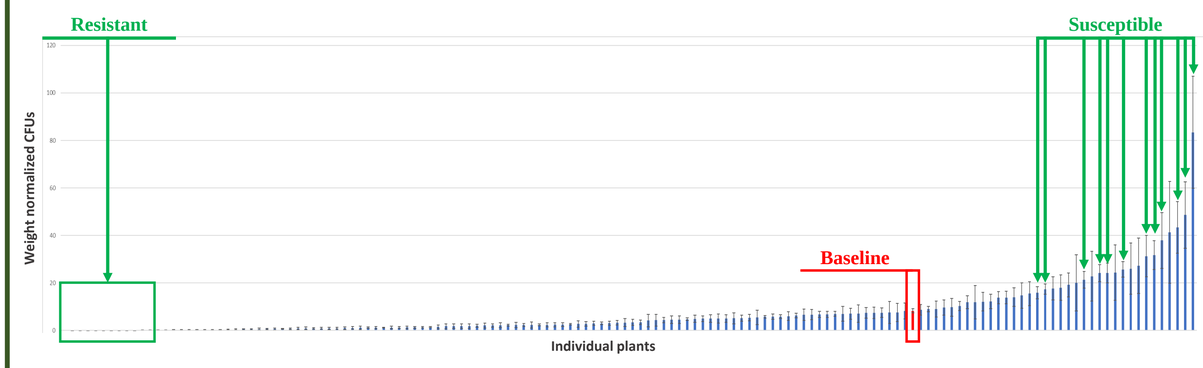

Figure 9: Quantities of bacteria isolated from each individual tree in the AOD bioassay. Bar highlighted in red denotes approximate quantity of bacteria in each tree at 0dpi. Green arrows point to highly resistant and susceptible trees chosen for future experiments.

The goal of this bioassay was to identify highly resistant and susceptible individuals for genetic comparison. Twelve resistant and 12 susceptible individuals (green arrows) were identified as those whose colony counts were most statistically different from this baseline level (red box), taking into account variation between sample replicates.

Sequence data analysis

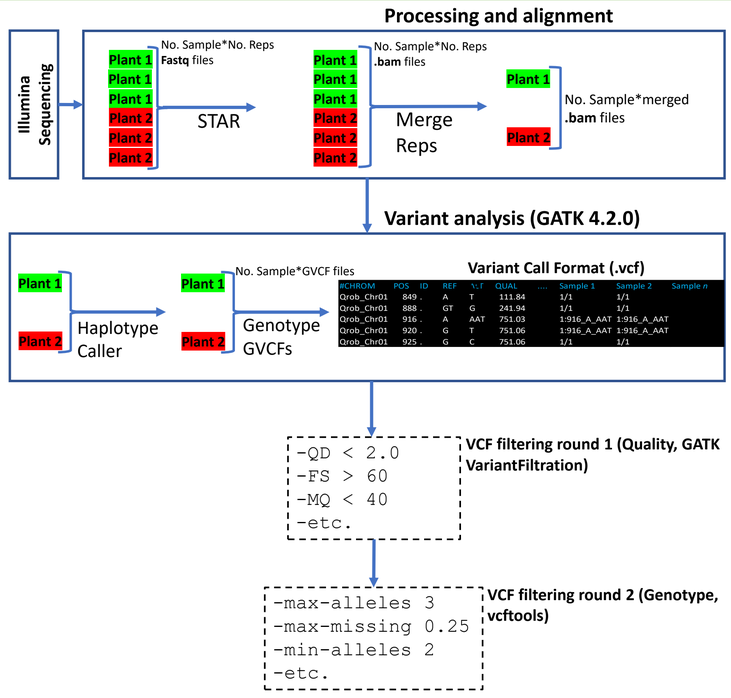

Figure 10: Brief overview of software pipeline for identification of genetic variants.

- Align RNA sequence (.fastq) files to reference genome using STAR splice aware aligner. Outputs binary alignment map (.bam) file.
- Use gatk –HaplotypeCaller to call SNPs and Indels, outputs genomic variant call format file (GVCF).
- Use gatk –GenotypeGVCFs for joint genotyping of all samples in a combined GVCF file. Output is a final standard .vcf file on which variant filtering can be conducted.
- Filter variant calls by quality metrics, e.g. mapping quality (MQ), quality by depth (QD), etc.
- Filter variants by genotype, e.g. maximum and minimum number of alleles.

References

- Bartholomé, J., Brachi, B., Marçais, B., Mougou-Hamdane, A., Bodénès, C., Plomion, C., Robin, C. and Desprez-Loustau, M., 2019. The genetics of exapted resistance to two exotic pathogens in pedunculate oak. *New Phytologist*, 226(4), pp.1088-1103.
- Plomion, C., … H., Kremer, A. and Salse, J., 2018. oak genome reveals facets of long lifespan. *Nature Plants*, 4(7), pp.440-452.

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