Project title:	Novel ways of managing tree crop fungal diseases: Using precision diagnostic technologies to tailor disease management strategies
Project number:	SF/TF 170
Project leader:	Thomas Heaven, University of Lincoln
Report:	Annual report, Oct 2020
Previous report:	Annual report, Oct 2019
Key staff:	Dr Helen Cockerton Prof Matthew Goddard
Location of project:	NIAB EMR
Industry Representative:	Megan McKertcher, Worldwide Fruit
Date project commenced:	01 October 2018

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

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GROWER SUMMARY

Headline

Collection, culture and DNA extraction methods for studying apple scab and powdery mildew genetics have been established and optimised. A high coverage genome assembly of apple powdery mildew has been generated – the first for a European isolate. Fungicide resistant strains of apple scab have been isolated.

Background

To better inform the disease management strategies of growers there is an immediate need for improved diagnostic testing for orchard diseases. Specifically, a test is needed that provides a quick diagnosis at a low cost to growers, ideally simultaneously, for a panel of diseases commonly found in orchards. This test also needs to be precise enough to identify key pathogen characteristics such as resistance to certain fungicides and virulence. One approach is a diagnosis by sequencing method which identifies genetic markers associated with resistance and virulence traits. Such a diagnostic could also be used to better track the effects of different planting decisions and agronomic practices, improving disease management over the longer term.

Commercial apple cultivation is hampered by its susceptibility to a number of fungal pathogens. The most serious among these is apple scab caused by the pathogen *Venturia inaequalis*, where production losses can be as high as 70% in heavily affected orchards (Biggs 1990; MacHardy 1996). Apart from scab the most common fungal disease found in UK apple cultivation is apple powdery mildew caused by *Podosphaera leucotricha* (Lesemann and Dunemann 2006).

Unfortunately, genomic resources for these two pathogens lag behind those available for the apple host or for other pathogenic fungi. The genetic basis for resistance to commonly used fungicides is not well understood in either scab or powdery mildew. Similarly, no virulence genes have been characterised in either species that facilitate the suppression of a host's natural immune response. Indeed, there were no genome assemblies available for apple powdery mildew prior to 2020. There has long been significant interest in developing new varieties of apple combining the fruit quality of commercial cultivars with the superior disease resistance of wild species. However, in the past the resistance of cultivars has quickly been broken in field conditions, to prevent this from happening in the future a better understanding of the pathogen is needed (Caffier et al. 2014, Caffier and Laurens 2015). The objective of this project is to generate some of the first genomic resources for the study of apple powdery

mildew. We aim to improve understanding of virulence in apple scab and powdery mildew by identifying putative virulence and avirulence genes that determine isolate host specificity as well as mutations leading to fungicide resistance. This will inform future management strategies and breeding efforts.

Summary

In the first two years of this PhD, techniques for collection, culture and DNA extraction from apple scab and powdery mildew have been refined. These methods have facilitated DNA and RNA sequencing of the apple powdery mildew genome as well as a number of other mildew species infecting Rosaceae crops. Comparison of these species genomes and transcriptomes will enable identification of key virulence and fungicide resistance factors. Additionally, strains of apple scab have been isolated for further study including fungicide resistant strains.

- Apple Scab was sampled from indicator trees known to carry resistance genes
- Apple Scab was sampled from problem orchards and confirmed to be fungicide resistant
- Fungicide resistant and susceptible strains of apple scab were crossed to generate a mapping population for the fungicide resistance trait
- Samples of powdery mildew were collected from susceptible apple, strawberry and raspberry plants
- DNA extraction and sequencing was performed on apple powdery mildew samples
- RNA extraction and sequencing was performed on strawberry powdery mildew samples
- A mapping population of apple trees were scored for susceptibility to apple powdery mildew

Financial Benefits

Apple growers are currently heavily dependent upon frequent fungicide applications to control disease in orchards. Without these products outbreaks inflict serious crop losses of up to 70%. There is increasing pressure to reduce to use of such crop protection products. This project lays the groundwork for diagnosis by sequencing of the two diseases apple powdery mildew and scab. Precision diagnosis this kind of rapid and precise diagnosis would allow the effects of management strategies to be tracked and modified, supporting the maximisation of yield whilst minimising application of fungicides.

Action Points

• There are no grower actions points at this stage of the project.

SCIENCE SECTION

Introduction

Apple is among the most important fruit crops globally, ranked third in terms of annual production at 84.6 million tonnes (FAOSTAT 2014). Apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) are key pathogens of apple cultivation. Currently, growers use a range of integrated pest management strategies to control this disease but remain heavily dependent upon fungicide use, applying pesticides throughout the season to limit damage to their crops (Roßberg 2003). It is thought that apple producers in the eastern United States spend as much as 18.6 million USD per year on scab control alone (Cox 2015). However, this practice may be unsustainable as fungicide resistant varieties have begun to emerge in both pathogen populations and increased restrictions on fungicide use are expected in the near future (Brent and Hollomon 1995; Lesemann et al. 2006).

It is thought that fungicide resistance can arise via a number of mechanisms; through alterations in the fungicide binding site, increased production of a targeted protein or an improved ability of fungi to remove toxins (Koenraadt et al. 1992; Hamamato et al. 2000; Deising et al. 2008). Resistance against some classes of fungicide have been associated with mutations in specific target regions, for example non-synonymous mutations in the CYP51A1 gene region have been associated with resistance to demethylase inhibitor fungicides (Villani et al. 2016). However, this does not fully explain resistance in apple scab. Detection of fungicide resistance often still relies upon microbiological methods for many fungicide classes.

Both apple scab and apple powdery mildew are known to be highly host specific. This host specificity is thought to arise from each pathogen's ability to suppress or avoid recognition by the hosts immune system. Fungi secrete toxins and proteins to aid infection. These are termed effectors. However, some of these effectors may be recognised by the host plant if it carries certain resistance genes. This results in effector triggered immunity. Fungi may gain or lose effectors to avoid recognition by the plant, allowing them to overcome resistance. The ability of an isolate to overcome known resistance genes in a host is used to define different races in a pathogen population. A race structure is well established in the apple scab pathosystem and there is also evidence for the existence of a race structure in the mildew population (Bowen et al. 2011). New patterns of virulence may occur due to the disruption of genes via the insertion of a transposon or as a result of mutation.

This project aims to identify key genetic markers in apple scab and apple powdery mildew, conferring traits such as host specificity and fungicide resistance. This lays the groundwork

for diagnosis by sequencing. We have collected samples of *P. leucotricha* as well as isolating *V. inaequalis* strains that display different virulence and fungicide resistance characteristics. Sequencing and analysis of these samples is now underway.

Project aims:

- To identify key virulence factors in resistance breaking strains of apple scab (V. *inaequalis*)
- To identify genetic markers for fungicide resistance in *V. inaequalis*
- To generate a first draft genome and transcriptome for a European isolate of apple powdery mildew *(P. leucotricha*)

Materials and methods

Powdery mildew sampling

Heavily infected leaves were removed and transported to the lab in paper envelopes taking care to retain as much mildew material as possible in transit. Leaves were then placed into a two-litre glass beaker containing one litre of water and stirred vigorously to wash off condia. Conidial suspensions were then sieved into 50 ml falcon tubes which were centrifuged at 5000 g for 5 mins. Following this the supernatant was removed via stripette leaving isolated condia. Washing of leaves was repeated into the same falcon tubes until all conidia had been dislodged and subsequent washes gave no increase in conidial yield. Any remaining water was the removed from the samples by pipette following a final centrifugation at 5000 g for 5 min. Samples were then freeze dried overnight and the fully dried conidial powder transferred to 1.5 ml Eppendorf tubes stored at -80 ºC.

V. inaequalis sampling and culture

Scab was collected from the field by using cork borers to excise single scab lesions from infected leaves, the resulting leaf discs were kept at -20ºC for long term storage. In order to bulk up individual isolates from these stocks the fungus was cultured from single spores grown on PDA plates containing 60 ppm rifamycin in 24 hour darkness at 17ºC; individual leaf discs collected from a given cultivar were defrosted and placed into 2 ml Eppendorf tubes with 300 µl of sterile water, discs were then vortexed for 1 min to wash off conidia. An optical microscope was used to confirm the presence of spores suspended in the water. 50 µl of spore suspension was then spread onto a plate. Another 300 µl of sterile water was then added to the remaining suspension and 50 µl of the more dilute spores spread onto a second plate. Germination was observed under a dissecting microscope and a scalpel was used to excise individual germinated spores onto fresh individual plates, these were sealed with

parafilm and left to develop for 6 weeks. For collection of mycelium the cultures were transferred to cellophane disc plates. These were prepared by pipetting 1 ml of sterile water onto standard PDA + rifamycin plates before spreading a cellophane disc flat across the agar surface. Plugs of mycelium were taken from uncontaminated plates mixed with 1 ml of sterile water, and spread over the top of the cellophane discs. These plates were then sealed with parafilm and the cultures grown until the disc was covered by mycelium, at this point sub culturing and mycelial harvest took place. Mycelium was harvested by scraping from the surface of a given cellophane disc and freeze drying in 1.5 ml Eppendorf tubes overnight. These stocks were then stored at -20ºC until use for either DNA extraction or for recovering growing cultures of contaminated isolates.

V. inaequalis crossing

Four fungicide resistant *V. inaequalis* isolates were crossed with seven susceptible isolates. An apple leaf decoction media was prepared; senescent apple leaves were simmered in distilled water for 15 mins, 25 g/L, the resulting decoction was filtered through muslin and used to prepare malt extract media (5 g/L malt extract,17 g/L agar). Crossing of two strains was achieved by subculturing mycelial plugs of each parent onto the same leaf decoction plate amended with 60 ppm rifamycin. Plugs were placed approximately 15 mm apart from each other and left for one month at room temperature to grow together. Once colonies from the two isolates had grown sufficiently to meet each other plates were moved to chilled 4ºC conditions for 6 months to facilitate sexual reproduction.

After 6 months the meeting point of two colonies was excised using a scalpel blade and placed on a microscope slide, sexual pseudothecial structures were separated from as much media as possible. Following this a few drops of sterile deionised water were added and samples were crushed, the release of ascospores was confirmed under via optical microscope. Spores were washed into Eppendorf tubes using sterile water. This spore suspension was then pipetted onto rifomycin amended PDA plates and allowed to dry before plates were sealed. 24-48 hours later plates were checked for germinating spores which were then isolated and removed to individual plates for culture as described previously.

Short read DNA extraction

DNA extraction was performed using a nucleospin plant II extraction kit following the manufacturer's instructions for fungal samples, with the modification that homogenization and lysis was performed using a geno/grinder with 2 ball bearings inserted into 2 ml Eppendorf tubes at 1500 rpm in 20 s bursts for a total of 2 mins chilling with liquid nitrogen between bursts. RNase A and proteinase K were used as described in step 2 of the manufacturer's instructions.

High molecular weight DNA extraction

High molecular weight DNA extractions were based upon the CTAB extraction protocol of Schwessinger (2016) which was progressively modified to obtain high quality DNA from mildew. Premade buffers were combined to form lysis buffer (Buffer A; 0.35 M sorbitol, 0.1 M TrisHCl, 5 mM EDTA pH 8 Buffer B; 0.2 M Tris-HCl, 50 mM EDTA pH 8, 2 M NaCl, 2% CTAB Buffer C; 5% Sarkosyl N-lauroylsarcosine sodium salt Buffer D; PVP40 10% Buffer E PVP10 10%) in the ratios 5:5:2:1:1, 10 µL (10kU) RNase A was added to this. Fungal samples were ground in a geno/grinder with 2 ball bearings inserted into 2 ml Eppendorf tubes at 1500 rpm in 20 s bursts for a total of 2 min chilling with liquid nitrogen between bursts. They were then incubated at room temperature for 30 min in 1.5 ml lysis buffer whilst being inverted, ball bearings were left in the tubes to increase mixing, 20 µL of proteinase K was then added and incubation continued for another 30 min. Following this, samples were cooled on ice for 5 min before 250 µl of 5 M potassium acetate was added and cooling continued for an additional 5 min. Samples were then centrifuged for 12 min at 5000 g and the supernatant removed to safe lock tubes. Washing was then carried out by the 1:1 addition of Phenol:Chloroform:Isoamyalcohol 100 mM Tris-EDTA pH 8 (P:C:I), samples were mixed by inversion for 2 hours and centrifuged for 10 min at 4000 g before transfer of the supernatant to a fresh tube. This wash was repeated three times per sample, followed by a fourth wash using Chloroform:Isoamylalcohol (C:I) in place of P:C:I. DNA precipitation was performed with 200 µl of Sodium Acetate (3 M pH 5.2) and 800 µl Isopropanol, added to approximately 1 ml of supernatant and mixed by inversion at room temperature for 10 min. DNA was pelleted by centrifugation at 8000 g for 30 min. The supernatant was discarded and the pellet washed three times by resuspension in 1.5 ml of 70% ethanol, centrifugation at 13000 g for 5 min and discarding of the supernatant. Following the final wash step remaining ethanol was allowed to evaporate for 30 min before the DNA pellet was dissolved in 100 µL of 10 mM Tris pH 8.5 at room temperature overnight. DNA was then re-precipitated using 20 µl Sodium Acetate (3 M pH 5.2) and 1.5 ml 100% ethanol overnight at - 20°C. Following this the previous washing steps were repeated; x3 P:C:I, x1 C:I, isopropanol precipitation, x3 70% ethanol and DNA was redissolved in 100 µL of 10 mM Tris pH 8.5 at room temperature overnight.

The quality of extracted DNA was assessed initially using a Nanodrop 1000 spectrophotometer (Thermo Scientific), if these results were in the target range then quality was further assessed using a Qubit 2.0 fluorometer (Invitrogen) and using these results 100 ng of DNA was run on a 1.5% agarose gel with GelRed to assess contamination with RNA and finally these results were compared to a qubit RNA reading.

RNA extraction

RNA Extraction was performed using 3% CTAB extraction buffer as described in Yu et al. 2012 with the following modifications; chloroform:isoamyl alcohol (24:1) washing was omitted, precipitation was performed at -20°C for four hours. Resulting RNA concentration and RNA integrity number (RIN) of samples was assessed using the Agilent RNA ScreenTape System with a 2,200 Tapestation (Agilent Technologies, Germany) according to the manufacturers protocols. DNA concentration was assessed via Qubit dsDNA HS assay kit with a Qubit 3.0 fluorometer (Life Technologies, Waltham, MA USA).

ITS sequencing

The ITS region of isolates was amplified using serial dilutions of DNA samples. PCR was performed using 5 µL Taq 5X master mix Mix (NEB), 16 µL water, 1 µL ITS-1 primer (TCCGTAGGTGAACCTGCGG), 1 µl ITS-4 primer (TCCTCCGCTTATTGATATGC) and 2 µl DNA dilutions. PCR was performed on a Veriti thermal cycler (Applied biosystems) using the following cycle: an initial 95°C for 3 min; 35 cycles of 95°C for 20 s, 60°C for 15 s and 68°C for 2 mins; then a final extension at 68°C for 2 mins. PCR products were visualised on a 1.5% agarose gel with GelRed (0.5 µl/L) before being purified using a monarch PCR & DNA clean-up kit (5 µg) following the manufacturer's instructions. Purified PCR products were submitted for sequencing by Eurofins genomics LIGHTrun tube service. The resulting sequences were aligned to a reference ITS region (Locus EU035437 for scab and JQ999954 for mildew) downloaded from NCBI and analysed using the program Geneious V10.0.2.

DNA/RNASeq Sequencing

DNA and RNA samples were submitted to the commercial sequencing company Novogene for total RNA depletion, purification, fragmentation, cDNA library construction, enrichment and sequencing. Sequencing was performed on an illumina HiSeq platform using PE150 chemistry.

Bioinformatic pipelines

Our genome assembly pipeline was as follows; raw sequencing reads were subjected to a quality control check using FastQC v0.11.9 (Andrews, 2010). Sequences were trimmed and adapters removed using Trimmomatic v0.39 (Bolger et al, 2014). Reads were then aligned to the apple genome (GDDH14_1-1, Daccord et al, 2017) and apple powdery mildew genome (JAATOF000000000, Gañán et al. 2020) using bowtie2 v2.4.2. Coverage was assessed using samtools v1.1 (Handsaker et al, 2009) ‘coverage’ function and the kmer analysis toolkit v2.4.2 (Mapleson et al, 2017) function ‘kat plot spectra-cn’. Those reads not aligning to the

apple genome were carried forward for de novo genome assembly using the programme SPAdes v3.14.1 (Bankevich et al, 2012). Quality of the resulting transcriptome was assessed by looking for benchmarking universal single-copy orthologs (BUSCO) with BUSCO v 4.0.6 (Simão et al. 2015).

The transcriptome assembly pipeline used was as follows: Sequence reads from RNA-seq were subjected to a quality control check using FastQC v0.11.9. Sequences were trimmed and adapters removed using Trimmomatic v0.39. Reads were then aligned to the apple genome using STAR v2.7.3 (Dobin et al. 2013). Those reads successfully aligned to apple were omitted from further analysis. *De novo* transcriptome assembly was performed using Trinity v2.11.0 (Grabherr et al. 2011) and assembled sequences with a minimum length of 201 bp recorded as contigs. Quality of the resulting transcriptome was assessed by BUSCO analysis. These results were compared with BUSCO results from eucalypt, cucurbit and pea powdery mildew (GHEF00000000, GEUO01000000, GHDE00000000; Fonseca et al. 2019, Corsia et al. 2016, Angelini et al. 2019).

Results

Apple scab

Scab isolates have been collected from two orchards; one problem orchard containing scab isolates displaying resistance to fungicides and one orchard which has never been sprayed and contains isolates presumed to be fungicide susceptible. Isolates taken from the problem orchard were first assessed for their ability to germinate in the presence of different chemicals. Successfully germinated spores displaying resistance to 4 chemicals (pyraclostrobin, cyprodinil, difenoconazole and pyrimethanil) were cultured, these represent strobilurin, demethylase inhibitor and anilinopyramidine class fungicides.

Resistant and susceptible isolates were then crossed to produce offspring segregating for the resistance trait. Around ⅓ of crosses went on to produce viable sexual spores. These spores were cultured to generate mapping populations. Approximately 800 progeny isolates have now been cultured, the majority of these fall into four mapping populations (Table 1). Each resistant parent has been tested for resistance to one chemical. These will be used to map the genetic basis of fungicide resistance.

Table 1. Scab fungicide resistance mapping populations: Results of four scab crosses, including the identity of the resistant and susceptible parent and the number of individual progeny in each population.

Resistant Parent	Susceptible parent	No. progeny
Cyprodinil (Anilinopyramidine) (7)	Cox 128	96
Pyrimethanil (Anilinopyramidine) (1)	Cox 101	213
Pyrimethanil (Anilinopyramidine) (1)	Cox 128	288
Difenoconazole (DMI) (17)	Gala 82	154

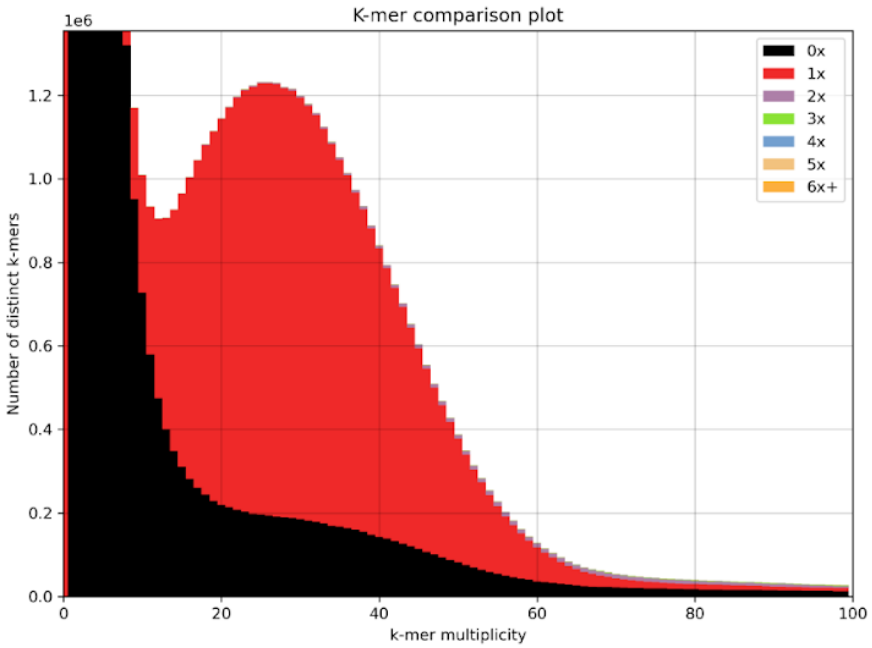
Additionally, race 7 scab isolates have been sampled from naturally occurring 2019 outbreaks of the disease on apple trees carrying the major resistance gene Rvi7. Cultures of these isolates have been established and their DNA extracted for future sequencing.

Powdery mildew

Mildew samples have been collected from field outbreaks of the pathogen *P. leucotricha* affecting apple trees and the pathogen *Podosphaera aphanis* infecting both strawberry and raspberry. DNA has been extracted for each of these three pathosystems and short read Illumina sequencing completed for *P. leucotricha.*

DNA sequencing of apple powdery mildew samples yielded 121,174,496 raw reads. Following quality control, we found that 13.67% of trimmed reads aligned to the apple genome. By contrast, 64.15% of reads aligned to the available *P. leucotricha* genome. Alignment to the available *P. leucotricha* genome provided an estimated a median sequencing coverage of x119. Some 22% of reads do not align to either the apple host or existing *P. leucotricha* genome assembly. The quality of reads was assessed using the programme KAT (kmer analysis toolkit), much of our read dataset represents sequences that appear as single copies in the available apple powdery mildew genome and a far lower proportion represent sequences that are found in the available apple genome (Figure 1). This shows that we have successfully extracted a relatively pure sample of apple mildew.

A)



B)

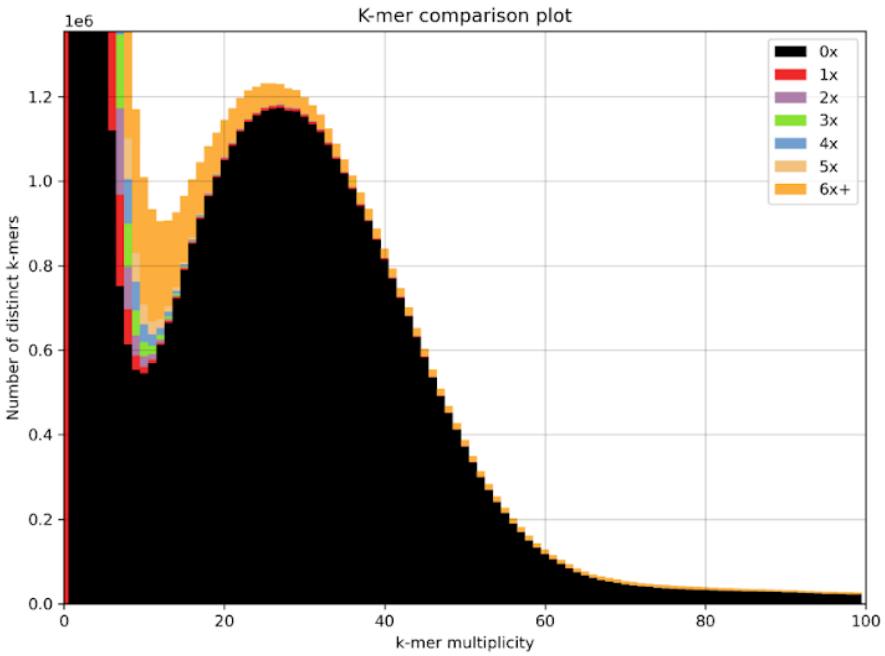


Figure 1. Read kmer comparison plots: Number of distinct kmers against their coverage in the trimmed sequencing read dataset; A) Coloured for the number of times a given kmer appears in the available apple powdery mildew genome B) Coloured for the number of times a given kmer appears in the available apple genome.

Genome assembly was performed using the programme ‘SPAdes’, the resulting assembly consisted of 7,860 contigs with an N50 of 19,411 and a genome size of 51.5 Mb. BUSCO analysis performed using databases of conserved genes for the kingdom: fungi, division:

ascomycota and order: erysiphales to which *P. leucotricha* belongs found that, respectively, 96.5%, 93.7% and 88.7% of conserved genes were represented in this genome assembly.

In addition to DNA sequencing for *P. leucotricha* RNA has been extracted from *P. aphanis* on a strawberry host and sequenced to generate a transcriptome assembly. RNA-seq yielded 1,988,067 raw reads. No genome assembly is publicly available for *P. aphanis* therefore the program 'Trinity' was used to generate a de novo transcriptome. This assembly consists of 104,167 transcripts. BUSCO analysis performed using databases of conserved genes for the kingdom: fungi, division: ascomycota and order: erysiphales to which *P. aphanis* belongs found that, respectively, 56.4%, 54.8% and 47.7% of conserved genes were represented in this transcriptome assembly. These results were compared to other de novo transcriptome assemblies of *Podosphaera* species in order to assess the quality our assembly (table 2; Fonseca et al. 2019, Corsia et al. 2016, Angelini et al. 2019).

Table 2. *Podosphaera* transcriptome: Basic quality measurements for different de novo transcriptome assemblies of *Podosphaera* species, including the species, origin of the transcriptome, N50 and BUSCO score versus an Ascomycota dataset.

Mildew Species	Assembly	Transcript no.	N50	Busco %
P. aphanis	T.Heaven	104,167	547	54.8
P. pannosa	N.Fonseca	12,106	1841	46.1
P. xanthii	D.Corsia	37,241	929	60.6
P. xanthii	R.Angelini	71.095	2251	90.5

Discussion

Apple Scab

We have now generated four scab populations large enough to conduct mapping of the fungicide resistance trait. It is now possible to proceed with phenotyping and genotyping experiments. Dose response experiments will be performed first on the cross parents, these results will then be used to determine a suitable discriminatory dose for investigation of the progeny populations. The progeny will be screened for variability in their resistance to DMIs using a single dose. We will then select a mapping population from within the ~800 progeny for DArTSeq and full dose response testing. Fungicide resistance quantitative trait loci (QTL) will then be mapped. Whilst resistant isolates have been selected based upon their ability to germinate in the presence of a single chemical, all isolates were sampled from the same problem orchard. It is expected that dose response testing of the parent isolates will reveal resistance to multiple chemicals. We will initially focus on resistance to the DMI class of

fungicides as this is most commonly used commercially for scab control, once genotypic data is available it may be possible to gather more phenotypic data for other chemicals.

For investigation of the scab Rvi7 virulence trait we will complement our 2019 samples with frozen isolates from previous years. Isolates are identified as race 7 by sampling from host trees carrying the Rvi7 gene, to ensure isolates taken from susceptible hosts are not race 7 frozen samples will be used that were collected before the emergence of race 7 in the UK. Genome assemblies from each population can then be created and genome comparisons can be made.

Powdery mildew

In our previous report sampling and DNA extraction methods had been optimized for apple powdery mildew, however we were unable to collect enough material in the first year of this project to conduct genomic sequencing. We have successfully overcome this hurdle in the second year of the project by pooling samples from numerous cultivars. Additionally, we have been able to apply the methods developed for apple powdery mildew to powdery mildew outbreaks affecting strawberry and raspberry.

Alignment of our sequencing reads to the available apple and apple powdery mildew genomes demonstrates that our sampling method is successfully isolating mildew material. Many more reads align to the apple powdery mildew genome than to the apple genome. KAT analysis further supports this as most reads aligning to the apple genome align multiple times indicating that they are repetitive sequences, whereas those reads aligning to the apple powdery mildew genome are unique, aligning only once. Around one fifth of reads did not align to either the apple or powdery mildew genome. Our KAT analysis indicates that these reads may be unrepresented in the currently available mildew assembly as there is an inflection in the distribution of unaligned reads corresponding to the peak in kmer coverage (Figure 1, A). Alternatively, these unaligned reads may originate from other contaminating organisms. Our draft *P. leucotricha* genome assembly appears relatively complete with a reported 1,598 complete BUSCOs of a total 1,706 core genes of the Ascomycota_odb10 data set. The quality of our assembly is good when compared to the only other available apple powdery mildew genome; 51.5Mb across 7,860 contigs with and N50 of 19,411 for our assembly compared to 43,8Mb across 8,921 contigs with and N50 of 8,371 (Ganan et al. 2020). We hope to further improve upon this draft assembly.

The results of our transcriptome assembly for *P. aphanis* are comparable to those for other *de novo* transcriptome assemblies generated from one sample. To include a greater proportion of BUSCO genes we plan to extract and submit multiple samples as was done in the case of *P. xanthii* by Angelini et al. 2019. Not all genes are expressed at all times and so only a subset may be captured by RNA extraction at a single point of time.

DNA extractions for *P. aphanis* on raspberry and strawberry are ready to be submitted for short read Illumina sequencing. We also plan to conduct RNA extractions for *P. leucotricha* and *P. aphanis* on raspberry. This data will be used to generate metagenome and transcriptome assemblies for each of these pathosystems, from which gene and effector predictions can be made. Whilst it is possible to generate genome assemblies using only Illumina sequence data, powdery mildew genomes are known to contain many repetitive sequences that are hard to resolve using only short read sequencing technologies (Frantzeskakis et al. 2018). In order to produce more contiguous genome assemblies we hope to use the nanopore sequencing platforms. Further DNA extractions for each pathosystem will be performed for Oxford Nanopore long-read sequencing.

Conclusions

- Different races of apple scab have been sampled and cultured
- Fungicide resistant and susceptible scab isolates have been sampled and crossed to generate a mapping population
- Powdery mildew samples have been collected from apple, raspberry and strawberry
- A first genome assembly has been constructed for apple powdery mildew

Knowledge and Technology Transfer

The student has presented this project at CTP (August) and AHDB (February) meetings, unfortunately COVID 19 has led to the cancellation of other conferences. Additionally, a case study of the project was created for worldwide fruit (February).

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