Bridging the gap between powdery mildew genomics and valuable culturing methods of *Erysiphe necator* and *Podosphaera aphanis*

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1 Introduction

Powdery mildew fungi are obligate, biotrophic pathogens in the phylum Ascomycota (Heffer et al., 2006). These highly specialized phytopathogens affect over 10,000 monocotyledonous and dicotyledonous plant species and consist of over 873 species of pathogenic fungi (Bradshaw and Tobin, 2020). Powdery mildew species cause economically important fungal diseases of both agricultural and horticultural crops, including wheat, barley, grapevine, fruit, and vegetable species (Glawe, 2008). The introduction of molecular methods has provided insight into the phylogenetic structure of *Erysiphales* and led to significant discoveries revolving around genomic structure and variation (Braun and Cook, 2012). Technical progress in the last decades in electron microscopy and molecular biology have strongly influenced further development of the taxonomy of the *Erysiphales* and its phylogenetic interpretation (Braun and Cook, 2012). But genomic resources and information on powdery mildews are relatively scarce. Further analysis of haustorial interfaces, weapons of pathogenesis (i.e., interacting proteins and effector proteins) and genomic sequences are necessary to determine/identify what makes powdery mildew specific to their host.

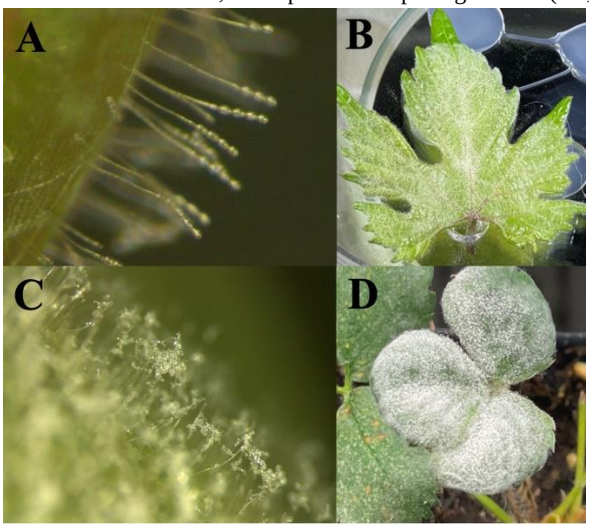

Figure 1: Infection of *Erysiphe necator* (grapevine powdery mildew) and *Podosphaera aphanis* (strawberry powdery mildew) grown in growth chambers. (A) Conidiophores formed via microcyclic conidiogenesis on a single isolate, i.e., germinating conidia produce new conidiophores direct (B) Visual hyphae and mycelium growth of *E. necator* on 'Chardonnay' grape leaves. (C) Non-uniform growth of conidiophores and conidia visible on an isolate of *P. aphanis.* (D) Visual hyphae and mycelial growth covering the leaf surface of a strawberry plant.

The taxonomy of powdery mildew fungi (order *Erysiphales*) recently underwent extensive revision based on DNA sequence data. This revision produced further classification based on a corresponding narrow host range (Heffer *et al*., 2006). In the past, identification was solely based on the teleomorph stage (sexual) and morphology of the chasmothecium and its appendages (Heffer *et al*., 2006). Identification of powdery mildews now requires attributes of the anamorph (asexual), or conidial producing stage (Fig. 1). Characteristics separating powdery mildew species and/or genera include the number of asci in an ascoma (one or several); number of ascospores in an ascus (eight or fewer); morphology of appendages; conidiogenesis, conidia maturing in chains, catenescent, or maturing one at a time; and mycelium displaying ectotrophic or endotrophic growth patterns (Braun and Cook, 2012). Multiple scientific speculations on phylogenetic relationships and evolution of powdery mildews based on the morphology and host-parasite interaction have been investigated (Takamatsu, 2013). The initiation of molecular biology has facilitated a more precise dissection of the relationships among taxa at the species level (Kiss *et al*., 2020). The most useful methods that have facilitated vast support in the identification and classification of genera include the use of DNA barcodes, particularly of the nrDNA 18S and 28S region and sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) at the species level (Braun *et al*., 2001; Takamatsu, 2004; Takamatsu, 2004, 2013; Takamatsu *et al*., 2015; Cabrera *et al*., 2018; Marmolejo *et al*., 2018).

Analysis of the *Erysiphe necator* (grapevine powdery mildew) genome reveals highly repetitive regions with frequent structural variations, playing a crucial role in the adaptive responses of isolates to fungicide stress (Zaccaron *et al*., 2021). Large amounts of copy number variations (CNVs) have been linked to the structure of the *E. necator* genome pointing to unbalanced changes and an excess of complex traits. In a study looking at the large and highly repetitive genome of *E. necator*, researchers completed a shotgun approach to sequence and assemble the genome along with RNA-seq and comparative genomics approach to predict and annotate protein-coding genes (Jones *et al*., 2014). The genome of *E. necator* showed repetition and suggested that transposable elements may be responsible for genome expansion (Jones *et al*., 2014). The results of the study demonstrate the effectiveness of using genomes to dissect

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complex traits with limited molecular information and suggest broader implications for understanding genomic dynamics in pathogenic systems with similar genome architectures (Jones *et al*., 2014).

The strict host specification of powdery mildew species, of which have significantly larger and difficult to interpret genomes, yields challenges to our current understanding of plant-pathogen interactions at the molecular level (Spanu and Panstruga, 2012). Powdery mildews cannot be cultured *in vitro* due to their obligate nature. Therefore, a reliable transformation protocol has not yet been established, resulting in serious challenges for use in experimental systems (Spanu & Panstruga, 2012). Due to the similar nature in control of *E. necator* and the main causal agent of strawberry powdery mildew, *Podosphaera aphanis*, we have established a viable method of culturing both *E. necator* and *P. aphanis* for further investigation.

2 Culturing *Erysiphe necator* (grapevine powdery mildew)

In this work, a method for culturing *E. necator* from isolated leaves of *Vitis vinifera* 'Chardonnay' was evaluated. Growth chambers of 'Chardonnay' grape plants in hydroponics systems were supplemented with nutrients using Hoagland's (1x) solution. Specific isolates we inoculated of *E. necator* from the field were placed in incubators to induce sporulation (22ºC at 60-100% RH). Young grape leaves collected from the 3rd and 4th node of each 'Chardonnay' plant were taken from the growth chamber and inoculated by single conidial chain transfer and incubated until conidial growth was visible (7-10d). Double petri-dish plates with sterile water in the lower region and a hole for the petiole were used to keep each 'Chardonnay' leaf alive (Quinn & Powell, 1982). New leaves were collected from the growth chamber and disinfested with 0.8% sodium hypochlorate for 30 sec then twice rinsed for 30 sec with sterile water. After drying in a laminar hood, each leaf was inoculated with a single isolate of *E. necator* by brushing conidia from an infected leaf onto the young, susceptible leaf. Leaves were then incubated at 22-25ºC and a RH of 60-100%. Sporulation of *E. necator* is visible within 7-10d and additional transferring is necessary for further analysis.

Figure 2: Growth chamber of strawberry plants inoculated and infected with *Podosphaera aphanis* (strawberry powdery mildew).

3 Culturing and DNA extraction of *Podosphaera aphanis* (strawberry powdery mildew)

A method for culturing *P. aphanis* from isolated leaves of strawberry was evaluated. A single isolate of *P. aphanis* was isolated from the field and transferred into an incubator shown in Fig. 2 with proper temperature and humidity to induce sporulation (22ºC at 70% RH). Non-inoculated strawberry leaves were rinsed in three separate tubs with a 1:20 dilution of bleach and sterile water, and two sterile water tubs for 30 sec each. A single conidial chain was transferred onto susceptible strawberry leaf tissue and stored in an incubator with temperature and RH conditions stated previously. An entire growth chamber was filled with non-inoculated strawberry plants and conditions ranged from 20-

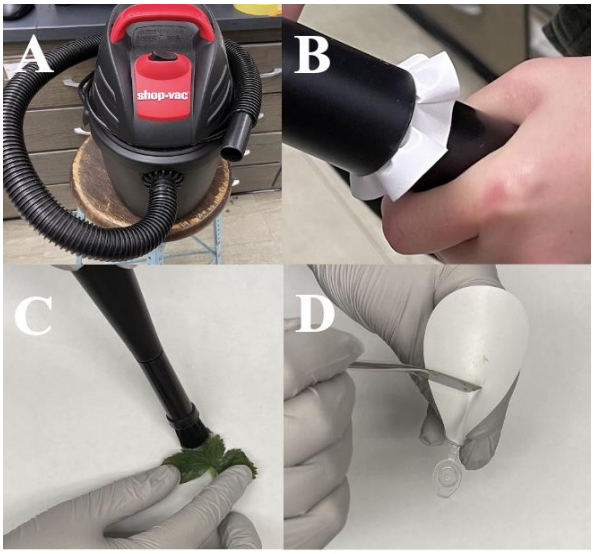

Figure 3: Collection of *Podosphaera aphanis* conidia. (A) Vacuum and hose wand attachment used for extracting spores from strawberry leaves. (B) Placing Whatman #1 filter paper between hose and wand attachment. (C) Removal of conidia by vacuuming from the leaf surface. (D) Use of spatula to transfer conidia from filter paper into a 2mL conical tube.

23ºC with a RH of 60-80%. When conidia were visible on the inoculated strawberries, leaves were brought into the incubator and strawberry plants were inoculated by touching the infected leaf to the non-infected leaf tissue. Within 7-10 days, strawberries were covered in a powdery dusting on the leaf tissue shown in Fig. 2. Roughly 200 infected Strawberry leaves were collected, and spores were vacuumed using the Shop Vac Micro-cleaning kit (Fig 3A). Whatman #1 filter paper (size 4.25 cm) was placed between the host and wand attachment (Fig 3B). *P. aphanis* conidia was then vacuumed from the leaf surface and collected onto the filter paper (Fig 3C). After collecting conidia from leaves, the wand was carefully separated from the hose and conidia gathered on the filter paper was removed and placed into a 2mL conical tube (Fig. 3D). The conical tube containing 150 mg of *P. aphanis* conidia and mycelium was then stored at -20ºC for further analysis. Using a Qiagen Genomic Tip DNA (Qiagen Scientific, Germantown, Maryland, USA) extraction method, high molecular weight (MW) DNA of *P. aphanis* rendered

1.8 µg of DNA with an average read length of 5Kb or less, however, it was highly fragmented (Fig 4).

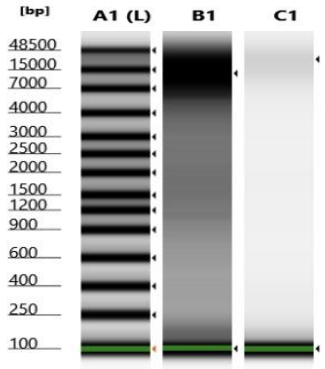

Figure 4: Highly fragmented PCR amplification rendering 1.8 ug of DNA with an average read length of 5Kb or less.

and unable to be sequenced using Nanopore. Our results suggested that we were unable to render high quality DNA for PacBio or Nanopore analysis. Illumina sequencing of high MW DNA from the isolate of *P. aphanis* will be used to obtain a detailed assembly of the transcriptome. High-quality-paired-end reads obtained will be assembled into contigs corresponding to putative transcripts that will be functionally annotated. Assembled transcripts will undergo a BLASTx search to compare sequences related to transposable elements.

4 Conclusions

Large-scale "omics" techniques including genomics, transcriptomics, proteomics, and metabolomics are useful tools used to generate complex data sets and dissect difficult biological systems such as powdery mildews (Bindschedler *et al*., 2016). Sequencing of *Erysiphe necator* (grapevine powdery mildew) and *Blumeria graminis* f.sp. *tritici* (wheat powdery mildew) has rendered significantly large genomes. The expansion in genome size is accompanied by a reduction in the number of protein-coding genes and caused by the accumulation of repetitive DNA, which is the result of retro-transposon activity occurring throughout the evolution of powdery mildew fungi (Spanu *et al*., 2010; Wicker *et al*., 2013; Jones *et al*., 2014; Amselem *et al*., 2015; Bindschedler *et al*., 2016). Comparative analyses of *P. aphanis*, *E. necator*, and *B. gramminis* sequence structure is required to fill the gaps in understanding the complexity of genes regulating these complex obligate biotrophic fungi. Investigation of the multifaceted host-pathogen interactions, specification, and identification of genes present during the infection process is necessary at the molecular level to better understand these complex pathogenic fungi.

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