AmpSeq: use of a new genotyping tool to address practical questions in pathogen biology, population studies, and fungicide resistance

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Introduction

On many high value specialty crops, such as grapes, hops, and strawberries, powdery mildews cause economically important diseases and are primarily managed with fungicides, including sterol demethylase inhibitors (DMIs), and quinone outside inhibitors (QoIs) among others. DMIs target fungal membrane function, but single nucleotide polymorphisms (SNPs), increased copy number, and increased expression of the CYP51 gene can confer resistance to this class of fungicide. QoIs disrupt mitochondrial respiration by inhibiting the cytochrome bc1 complex, but there are three known SNPs in the CYTb gene that can lead to resistance (Miles, 2014).

This study uses a highly multiplexed, inexpensive AmpSeq technology to identify known SNPs and copy number variants in field-collected samples that confer fungicide resistance. The high multiplexing of AmpSeq enables simultaneous data collection for mating type and other molecular markers relevant to pathogen biology.
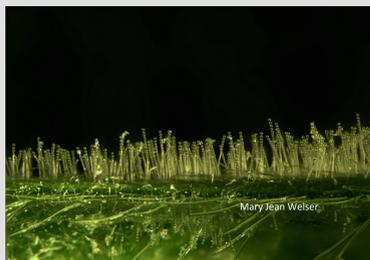
Fig. 1. *Podopshaera macularis* (hop powdery mildew) conidia atop conidiophores shown under a microscope at approximately 250X magnification.

Fig. 2. *Erysiphe necator* (grape powdery mildew) infection on grape berries in a vineyard.

Methods

Sample Collection & Preparation:
Conidia and mycelia were collected using a 1-cm piece of tape or Microtube Tough-Spots from single, sporulating colonies on in vitro or field-sampled leaves. Microfuge tubes containing the pathogen samples were then stored in an -80°C freezer overnight. Either CTAB or Chelex DNA extraction protocols were used. For a subset of samples DNA quality using absorbance at 260/280nm and 260/230nm, quantity was estimated by Qubit fluorometry, and PCR competence was tested using ITS primers. For *Podosphaera aphanis* samples, inconsistent PCR amplification was remedied by using a 5-fold dilution of DNA template.

AmpSeq & Data Analysis:
The Cornell University Genomics Facility performed highly multiplexed PCR, with up to 200 primers per PCR and with Nextera dual barcoding to multiplex up to 4608 samples per Illumina sequencing lane. Sequencing data was converted to genotype calls using two semi-automated computational pipelines incorporating de novo and reference-based strategies.

For more information on AmpSeq, read:
Yang S, et al. 2016. A next-generation marker genotyping platform (AmpSeq) in heterozygous crops: a case study for marker assisted selection in grapevine. Horticulture Research 3: 16002.

Results
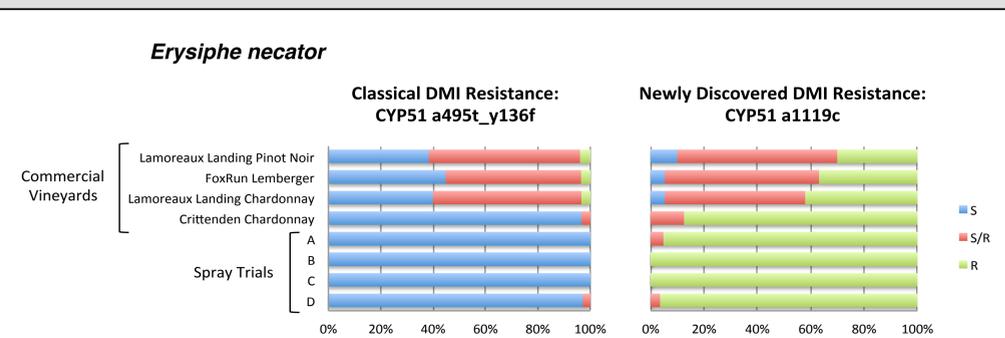
Fig. 3. Frequency of fungicide sensitive (S) and resistant (R) Alleles found across 246 *Erysiphe necator* samples collected from fungicide spray trial vineyards as well as commercial vineyards in NY

The classical DMI fungicide resistance allele was found in about 60% of samples collected from commercial vineyards but was rarely found in any of the spray trials. The recently discovered DMI fungicide resistance allele was found in almost all samples collected in commercial vineyards as well as spray trial vineyards. Commercial vineyards display heterozygosity in ~50% samples collected for both CYP51 loci.

Across all 570 *Erysiphe necator* samples studied, 26.0% had the classical DMI resistance allele and 79.2% had the new newly discovered DMI resistance allele.
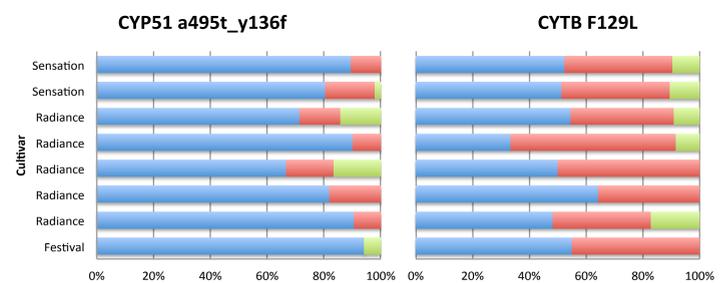
Results Cont'd

Frequency of Fungicide Sensitivity and Resistance Alleles in 134 *Podosphaera macularis* and 198 *Podosphaera aphanis* Samples:
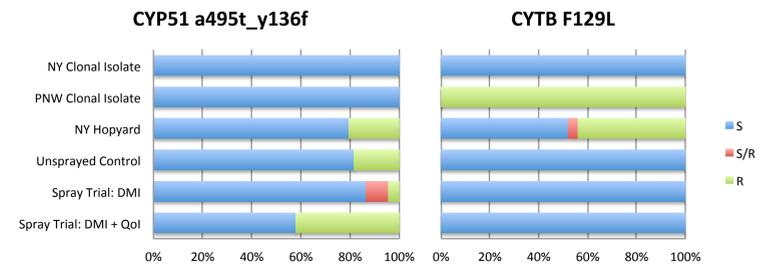
Fig. 4. The frequency of fungicide sensitive (S) and resistant (R) alleles across 134 *Podosphaera macularis* samples collected from different fungicide treatments. These results suggest that hop powdery mildew exposed to fungicide treatments contain a higher percentage of resistance than those unexposed. The clonal isolate collected from the Pacific Northwest demonstrates QoI resistance while the NY isolate that had not been grown under conditions with fungicide is 100% sensitive for both alleles.

Fig. 5. The frequency of fungicide sensitive (S) and resistant (R) alleles across 198 *Podosphaera aphanis* samples collected from 3 different cultivars sourced from 8 different nurseries. These results suggest that ~20% of samples are DMI resistant and ~50% of the, are QoI resistant.

Discussion

Erysiphe necator
- Commercial vineyards display heterozygosity in ~50% *Erysiphe necator* samples for both CYP51 loci despite it being a haploid fungus due to gene duplication. Increased copy numbers can increase CYP51 gene expression and confer resistance to DMI fungicides (Jones, 2014).

Podosphaera macularis
- As expected, the unsprayed control and wild NY isolate samples did not contain high proportions of fungicide resistant alleles. In spray trial 2, a DMI was used in conjunction with a QoI, which could have resulted in less intense selection for DMI resistance in the pathogen population.

Podosphaera aphanis
- The *Podosphaera aphanis* results show that among several cultivars sampled, ~20% of samples contain the DMI resistance allele and ~50% have the QoI resistant allele.

Future Directions:
We are performing data analyses to track mating type frequency and distribution among these powdery mildew pathosystems. We also hope to elucidate more information on the copy number variants we are finding in commercial vineyards and their DMI fungicide resistance.

References:
Jones L, et al. 2014. Adaptive genomic structural variation in the grape powdery mildew pathogen, Erysiphe necator. BMC genomics 15(1): 1081
Miles T. D, et al. 2014. Screening and characterization of resistance to succinate dehydrogenase inhibitors in Alternaria solani. Plant Pathology 63(1): 155-164