Originally Published: Spring 2018
Washington State University - Viticulture and Enology Extension News
Michigan State University - Grapes Extension Blog

Detecting DMI Resistance in Powdery Mildew

By the Fungicide Resistance Assessment, Mitigation, and Extension (FRAME) Network

If you heard one the FRAME Network group talks about fungicide resistance in grape powdery mildew this past winter, this past winter. We hinted we would soon have a DMI-resistance (FRAC 3) screening test similar to the test we have for QoI fungicides (FRAC 11; QoI or strobilurin). You may be wondering: When will I be able to submit samples for potential DMI resistance screening? Is the test available yet?

The short answer: No; it is still a work in progress. While we can detect and quantify some of the mutations associated with DMI fungicide resistance, we are not sure what the testing results mean for field samples, and the potential of DMI control failures.

PCR is a rapid technique that can be used to look at the genetic information of an organism. In this case, we use it to look for genetic mutations.

The long answer: Kind of. We have a **PCR-based assay** that can detect and accurately quantify the **Y136F mutation** in the CYP51 gene that is associated with DMI resistance. The problem is that the association between the mutation and actual field-level resistance is not clear.

For those who are interested, the exact wording is the "Y136F allele", which is a specific mutation of the CYP51 gene. Genes can have many different types of alleles.

This is why the association is not clear: First, we have found some powdery mildew isolates with the Y136F mutation that have the same tolerance to DMI fungicides as sensitive isolates without any known mutation. The problem is that there are numerous copies of the CYP51 gene in a cell. These sensitive mutants have 1 to 2 copies of the mutant gene but also have many more copies of the normal gene. This is likely why they are still sensitive.

This result agrees with our observation that as the number of mutant copies of the gene increases in the cell, so does the tolerance of the cell to DMI fungicides tested (myclobutanil and tebuconazole).

Figure 1 - Managing grapevine powdery mildew was challenging in 2017 across the region. In addition to conducive weather for mildew outbreaks, as well as less-than-ideal spray practices, fungicide resistance development was also a potential player.

Easy, you might say--- just count the number of Y136F mutations present in a sample. Unfortunately, it is not that easy. Since this relationship is at the individual cell level, we have to determine how many cells were sampled in order to estimate how many mutant copies of the gene there are per cell. We have developed such a method, but the problem is interpreting the results for field samples which are mostly mixtures of multiple isolates.

Let us examine a hypothetical sample. We will realistically assume our sampling methods have picked up 4 isolates from a moderately infected leaf or berry. The hypothetical Y136F copy number that would be detected for each isolate if sampled individually and the result from the hypothetical field sample is presented in Table 1.

Assume that 20 mutant gene copies per cell are needed to cause a control failure (actual number is not known) and that each mildew isolate had the same number of cells in the hypothetical field sample (this is never really the case, but it keeps the math relatively simple). Our DMI test results for this hypothetical sample would indicate that there were eight Y136F mutant genes per cell; which could suggest a low risk DMI fungicide failure. But that is not true! In fact, 25% (1 of the 4) of your isolates (Isolate #4) are resistant, meaning they contain more mutant gene copies than the threshold for control failure. The subsequent use of DMI fungicides could allow this resistant isolate to rapidly increase; and if those DMI fungicides are used at a critical time (i.e., bloom to late fruit-set) a field control failure could be likely. We could be falsely thinking that we can use DMI fungicides when instead we should be implementing strict fungicide resistance mitigation practices.

Table 1- Potential results from PCR-tests for DMI fungicide resistance.

	# of mutant gene copies detected	Hypothetical risk of DMI fungicide failure
Isolate #1	0	Low
Isolate #2	1	Low
Isolate #3	6	Low
Isolate #4	25	High
Average	**8**	**Low**

The conservative approach to using this data would be to not use any DMI fungicides if a Y136F mutation is detected. But this conservative

DMI Fungicides, con't.

approach also has some drawbacks - if we stop using DMIs when only a single mutant gene is detected, we would be placing undue resistance development pressure on the remaining fungicide modes-of-action. It likely takes a lot of copies of the mutant gene in order to get to the point of field-level control failures, considering we have been living with DMI resistance since 1996.

To make things even more complicated, we also have found powdery mildew isolates without the Y136F mutation but resistant to the DMIs tested (myclobutanil and tebuconazole). This means that you could get a negative test result back (no Y136F detected), but still have DMI resistant powdery mildew isolates. This tells us that the Y136F mutation is likely not the only genetic trait associated with DMI fungicide resistance in the field.

As the FRAME Network group, we are expanding our sample testing in 2018 to improve our understanding of how to use these tools and interpret results. We are not confident that existing genetic tests can be used to make accurate field management decisions regarding the DMI fungicides because of our limited understanding of mutation frequency.

One last point. The fungicide resistance data to date is only representative of the samples we received. Since these samples were not randomly collected, they do not represent the state of the powdery mildew population in any region. We also do not know how many samples are needed to make an accurate management decision. This is ongoing research that is partially funded by the American Vineyard Foundation.

Grape FRAME Network

Fungicide Resistance Assessment, Mitigation and Extension Network

Team participants:

- **Walt Mahaffee (USDA-Agriculture Research Service):**
  walt.mahaffee@ars.usda.gov
- **Phil Brannen (University of Georgia):**
  pbrannen@uga.edu
- **Monica Cooper (University of California Cooperative Extension):**
  mlycooper@ucdavis.edu
- **Melanie Ivey (The Ohio State University):**
  ivey.14@osu.edu
- **Tim Miles (Michigan State University):**
  milesti2@msu.edu
- **Michelle Moyer (Washington State University):**
  michelle.moyer@wsu.edu
- **Rachel Naegele (USDA-Agriculture Research Service):**
  rachel.naegele@ars.usda.gov
- **Ioannis Stergiopoulos (University of California - Davis):**
  istergiopoulos@ucdavis.edu
- **Rob Stoll (University of Utah):**
  rstoll@eng.utah.edu

Project Funding

This project is funded by the United States Department of Agriculture – National Institute for Food and Agriculture – Specialty Crop Research Initiative Award No. 2018-03375 titled "FRAME: Fungicide Resistance Assessment, Mitigation and Extension Network for Wine, Table, and Raisin Grapes."Preliminary research supporting this project was funded by: the American Vineyard Foundation, the Oregon Wine Board, and the Washington State Grape and Wine Research Program.