DETECTING and MONITORING DMI RESISTANCE in GRAPE POWDERY MILDEW

If you heard one of the recent talks about fungicide resistance from the Grape FRAME Network, we hinted we would soon be able to screen for DMI-resistance (FRAC 3), similar to how we assess samples for QoI (strobilurin) fungicide resistance (FRAC 11).

You may be wondering: Is the test available yet and when will I be able to submit samples?

The short answer—No and not yet; it is still a work in progress. While we can detect and quantify some of the genetic mutations associated with DMI fungicide resistance, we are not sure what the results mean, particularly with regards to predicting potential DMI control failures in the field.

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.

This is why: First, some powdery mildew isolates with the Y136F mutation have the same tolerance to DMI fungicides as sensitive isolates without any known mutation. This is because 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, meaning that DMIs remain effective against these populations.

This result agrees with our observation: 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). Easy, you might say—just count the number of Y136F mutations present in a sample to determine whether you have a sensitive or resistant population. Unfortunately, it's more complicated: since this relationship is at the individual cell level, we first must determine how many cells were sampled to be able to estimate the number of mutant copies of the gene present per cell. We have developed such a method, but have had difficulty interpreting the results for field samples that primarily contain a mixture of multiple isolates.

Let's 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 results are presented in Table 1.

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

	Isolate#1	Isolate #2	Isolate #3	Isolate #4	Field Sample (average of all isolates)
Number of mutant gene copies detected	0	1	6	25	8
Hypothetical risk of DMI fungicide failure	Low	Low	Low	High	Low

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 average results for this hypothetical sample would indicate that there were eight Y136F mutant genes per cell; which could suggest a low risk of DMI fungicide failure. But that is not true! In fact, 25% (1 of the 4; Isolate #4) of your isolates 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. An alternate, conservative approach would be to not use any DMI fungicides if a Y136F mutation is detected. However, this also has some drawbacks - if we stop using DMIs when the risk is low (samples 1-3), we would place 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. For now, 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 generated to date is only representative of the samples we received. Since these samples were not randomly collected, they do not represent the state of fungicide resistance in 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.

For more information on fungicide resistance testing and management contact a member of the Grape FRAME (Fungicide Resistance, Assessment, Mitigation and Extension) Network:

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