Virginia Wine Board

Annual Progress Report (final report) for July 1, 2007–June 30, 2008

Survey of grape powdery and downy mildew sensitivity to commonly used fungicides, 2007-08

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Objectives

1. Evaluate Virginia grape powdery (PM) and downy mildew (DM) populations for resistance to fungicides, with emphasis on the ergosterol biosynthesis inhibiting fungicides (PM) and the QoI fungicides or strobilurins (PM and DM).
2. Develop a test for routine assay of fungicide resistance.

Progress, objective 1

Powdery (154 isolates from 31 vineyards) and downy mildew (153 isolates from 26 vineyards) isolates were obtained in 2006 and 2007 from locations in Maryland, North Carolina, Pennsylvania, and Virginia and bio-assayed for fungicide sensitivity.
Most downy mildew isolates that are not needed for immediate testing are now in ultra-cold storage at -80C. Powdery mildew isolates, however, are being transferred to fresh leaf disks every 2-3 weeks to maintain them until testing is completed, because attempts at live, frozen storage have not been successful.

Sensitivity testing, downy mildew

QoI (strobilurin) fungicides (Abound, Sovran, Flint, Pristine)

Over 90% of all downy mildew isolates tested, from all geographic areas (Figure 1), were highly resistant to strobilurin fungicides. In most vineyards, all samples tested were resistant. Application to single leaves or small plants at field concentrations (maximum label rate assuming a spray volume of 100 gallons per acre) followed by inoculation provided no (azoxystrobin) or little (pyraclostrobin) control of selected isolates of resistant grape downy mildew. EC50 values (concentration providing 50% inhibition) in leaf disk bioassay of resistant isolates were over 100 mg azoxystrobin per liter. Most sensitive isolates came from vineyards that had not been treated with strobilurin fungicides. A few commercial vineyards had a mixture of sensitive and resistant isolates, and for others, the number of isolates recovered was too small to tell. In most vineyards where strobilurins have been used regularly, they are unlikely to be effective against downy mildew anymore.

PCR testing for the G143A mutation was completed for all available downy mildew isolates. Almost all isolates that tested resistant by bioassay were positive for the G143A mutation for QoI resistance. All bioassay-sensitive isolates had <1% 143A. One isolate was resistant by bioassay but had only low levels of 143A; it may have had a different mutation but this was not determined.

In the vineyards for which spray records were available, the number of strobilurin applications per year averaged between 2 and 3.4. This indicates that following the resistance management recommendations on the labels of these fungicides did not prevent buildup of resistant populations of grape downy mildew. Former label recommendations were to apply fewer than four sprays per season. The latest labels have changed this to no more than one-third of all fungicide sprays, with a maximum of four per season.

Mefenoxam or metalaxyl (Ridomil)

None of the 153 isolates tested has shown evidence of resistance to mefenoxam. Since mefenoxam or metalaxyl has been available for use on grapes for many years, this indicates that resistance management strategies, including limiting use to four applications per season, selling only prepacks with multi-site fungicide, and the fact that this fungicide is fairly expensive, have been successful in discouraging overuse and in maintaining downy mildew sensitivity.

Sensitivity testing, powdery mildew.

QoI (strobilurin) fungicides (Abound, Sovran, Flint, Pristine)

Over 80% of the powdery mildew isolates tested, from all geographic areas (Figure 1), were resistant to strobilurin fungicides. In some cases, it appeared that the same vineyard can have resistant downy and sensitive powdery mildew populations (although it is possible that these occurred in different blocks).EC50 values for azoxystrobin were lower than those for downy mildew (Figure 2). However, when grape seedlings were treated with maximum label

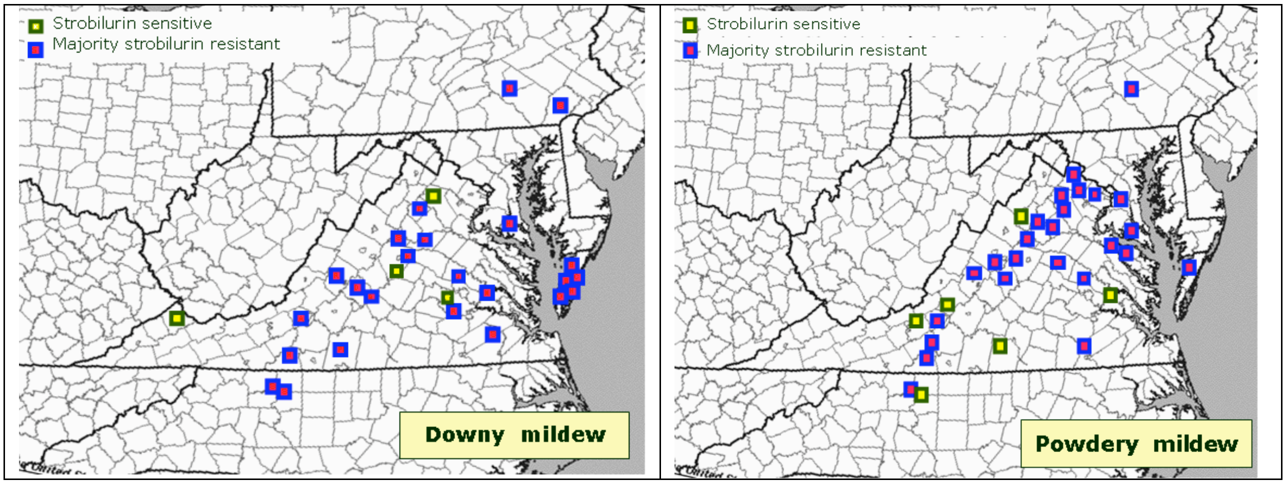

Figure 1. Map of the Mid-Atlantic USA region showing the locations where grape downy and powdery mildew isolates were collected and the reaction of the isolates to azoxystrobin. Note that for some of the "sensitive" locations, only one or two isolates were available

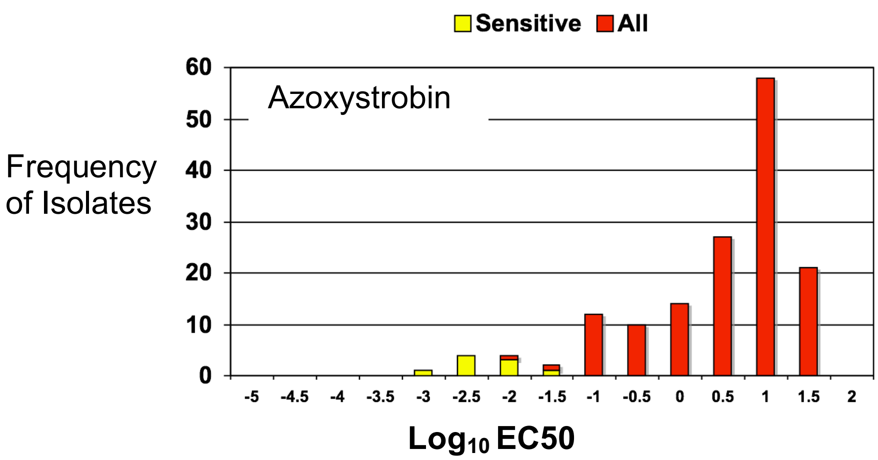

Figure 2. Frequency distribution of the log EC50 of azoxystrobin-sensitive (yellow portions of bars) and all (yellow+red bars) isolates of *E. necator* (n=155).

concentrations (based on 100 gallons/acre spray volume) of formulated azoxystrobin (Abound, 334 μg a.i./ml) or Flint (150 μg a.i./ml), little control was apparent.

SYBR-green real-time PCR was used to quantify the well-known QoI resistance mutation G143A in powdery mildew isolates. The majority of the QoI-resistant powdery mildew isolates contained a high level of the 143A allele. Most QoI-sensitive isolates contained less than 1% of 143A, but 14 out of 154 QoI-resistant isolates (defined as able to grow on azoxystrobin concentration ≥ 1 ppm), also contained less than 1% 143A; some of these had EC50 values in the 5-15 mg/L range. Different mutations conferring QoI resistance have been reported elsewhere but their presence has not yet been explored in our studies.

Ergosterol Biosynthesis Inhibitors

Figure 3 summarizes the response of the powdery mildew isolates tested in bioassays.

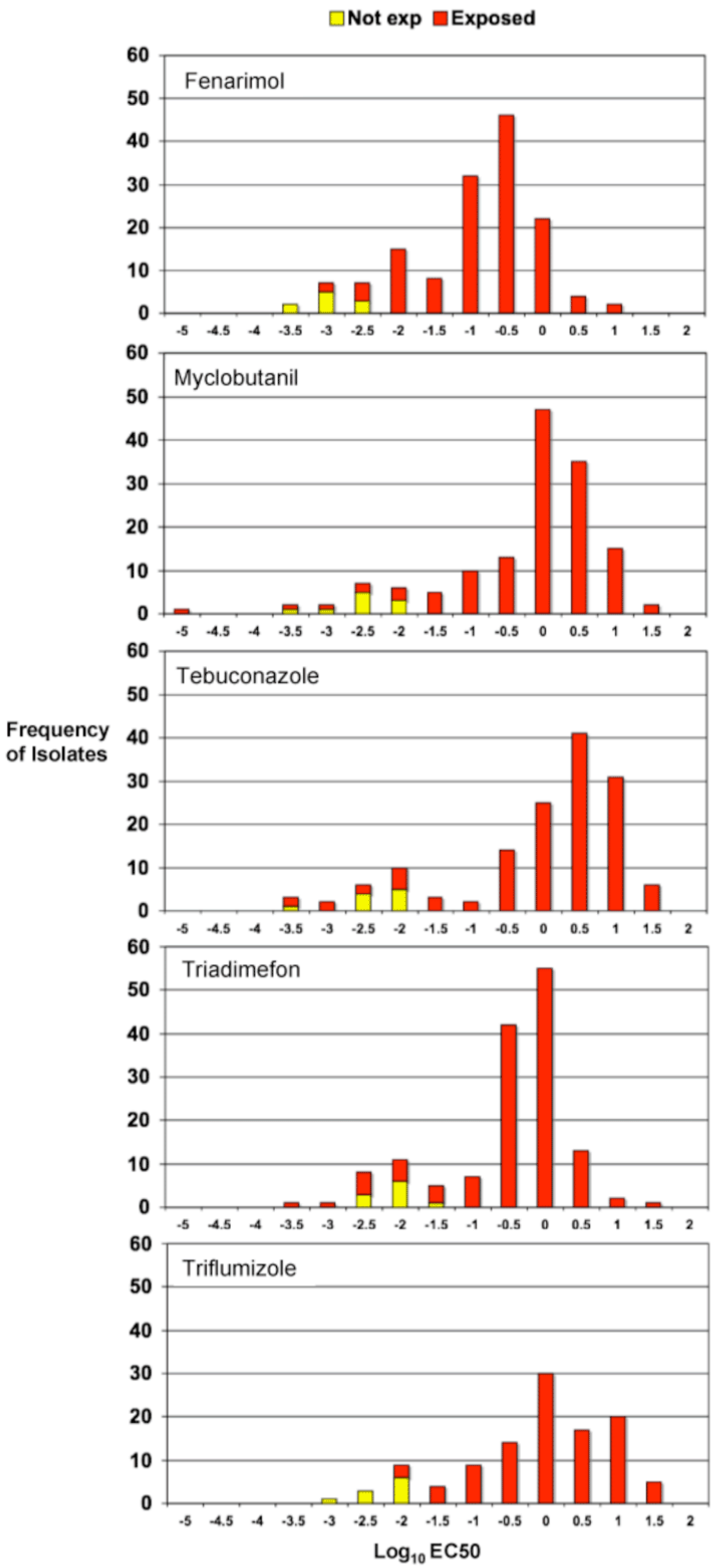

Figure 3. EC50 values of powdery mildew in bioassays with five ergosterol biosynthesis inhibiting fungicides. Yellow bars represent a sensitive subpopulation, namely isolates from two sites that appear to have had very little or no exposure to single-site fungicides; red and yellow-plus-red bars represent counts for all isolates.

Note: units on x-axis are on a log scale, so a change of, for example, -0.5 to +0.5 represents a 10-fold increase.

Table 1. The mean EC50 (mg/L), maximum label rates (based on 100 gal/A spray volume), and EC50 as % of maximum label rates of grape powdery mildew isolates tested for five ergosterol biosynthesis inhibiting fungicides.

	Fenarimol	Myclobutanil	Tebuconazole	Triadimefon	Triflumizole
Mean EC50 (see also Fig 3)	0.85	4.2	7.03	1.72	4.73
Mean resistance factor	**53**	**350**	**360**	**61**	**79**
Maximum label rate	56	150	135	225	300
EC50 as % of max label rate	**1.52**	**2.80**	**5.21**	**0.76**	**1.58**

The results continue to confirm the trend that was seen in our previous reports, namely that resistance levels against tebuconazole and myclobutanil tended to be higher than against triadimefon and fenarimol (Table 1). Probably, the latter two fungicides have not been used as much in recent years in Virginia. We believe that triflumizole has also not been commonly used on Virginia grapes. However, triflumizole is intrinsically less active against powdery mildew. Although the average of our isolates was not highly sensitive to triflumizole, when expressed in relation to the maximum label rate or as a resistance factor, the resistance shift for triflumizole was similar to that for fenarimol and triadimefon, and less than for tebuconazole and myclobutanil (Table 1)

Boscalid (Endura, component of Pristine)

All powdery mildew isolates tested have had normal sensitivity to this compound. This material is relatively new (introduced in 2003), so there has been limited time for resistance development. However, Alternaria blight of pistachio in California did develop boscalid resistance in 2007, supporting the notion that this may happen with other pathogens as well.

Quinoxyfen (Quintec)

All powdery mildew isolates tested have had normal sensitivity to this compound. This material is also relatively new in the US grape market (introduced in 2003), so there has been limited time for resistance development.

Objective 3. Assay development

We have started tissue-culturing grape plants, which can also be kept completely free of powdery mildew contamination, in order to determine whether susceptibility of tissue-cultured plant material to the diseases in question is more uniform than that of greenhouse-grown material. However, even though shoot-producing plantlets are now available, as a means of quickly producing plant material it has been slower than we had hoped.

Powdery mildew assays using a settling tower for inoculation were found to be less labor intensive and the results perhaps less variable than assay by inoculation with single spores.

We have compared the "incidence" evaluation method of Miller and Gubler (2004, Plant Disease 88:1205-1212), with the more traditional evaluation of severity. The authors claim major time savings from this approach, but for us it appears that the amount of time saved is small at best. Careful inspection to determine whether a leaf disk has any sporulating PM takes almost as much time as estimating percent of surface covered.

Although we use single-spored isolates for all our bioassays, we have reduced the time needed to test isolates for resistance to boscalid, quinoxyfen, and mefenoxam (fungicides for which we have not yet found resistance) by combining a number of isolates in a single inoculation. If growth is found, each isolate will subsequently be tested individually, but as long as resistance is rare, this approach consumes less time.

Our PCR protocols for detection of strobilurin resistance in both grape powdery and downy mildew now work effectively, but there is still a question about the significance of the group of powdery mildew isolates that was negative for the G143A mutation, but tested partially resistant in our bioassays.

Experiments started in the 2008 growing season include deploying into vineyards small grape plants that are regularly sprayed with fractional rates of ergosterol biosynthesis inhibiting fungicides as a means to "trap" powdery mildew and assess the fungicide resistance status of the vineyard's powdery mildew population.

In addition, we have started field tests to assess the level of field control that is obtained by ergosterol biosynthesis inhibiting fungicides (various rates and frequencies) in vineyards with partially resistant powdery mildew populations.

Technology transfer

Results were reported to Virginia growers through a presentation at the winter meeting of the Virginia Vineyards Association. Owners or managers of vineyards where the isolates originated were notified of the results by email. In addition, the results were incorporated in the Virginia Extension Pest Management Guide (http://www.ext.vt.edu/pubs/pmg/hf3.pdf).

A publication on the downy mildew results of 2005 and 2006 and powdery mildew results from 2005 were published in the peer-reviewed, online journal Plant Health Progress. Support from the Virginia Wine Board, Viticulture Consortium East, and the Virginia Agriculture Council was acknowledged.

Baudoin, Anton, Gilberto Olaya, François Delmotte, Jeneylyne F. Colcol, and Helge Sierotzki. 2008. QoI resistance of *Plasmopara viticola* and *Erysiphe necator* in the mid-Atlantic United States. Online. Plant Health Progress doi:10.1094/PHP-2008-0211-02-RS. http://www.plantmanagementnetwork.org/php/elements/sum2.asp?id=6820 (summary) http://www.plantmanagementnetwork.org/sub/php/research/2008/qoi/ (subscription required)