Virginia Wine Board

Progress Report for July 1-Dec 31, 2007

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

Anton Baudoin

Jeneylyne F. Colcol

Dept. of Plant Pathology, Physiology & Weed Science, Virginia Tech, Blacksburg, VA 24061.
Phone 540-231-5757, Fax (departmental) 540-231-7477, Email: abaudoin@vt.edu

And

Tony K. Wolf
AHS AREC, Virginia Tech, 595 Laurel Grove Rd., Winchester VA 22602-2852.
Email: vitis@vt.edu, Phone: 540-869-2560, extn 18, Fax: 540-869-0862

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

During 2007, 53 new powdery mildew isolates were obtained from 12 Virginia locations. These were in addition to isolates from the two previous seasons (Table 1). Due to the dry weather, downy mildew was much less prevalent in 2007; only 12 additional isolates from 2 locations were obtained.

Most downy mildew isolates that are not needed for immediate testing are now in ultra-cold storage at -80C. Over 200 powdery mildew isolates (a few have been lost) are being transferred to fresh leaf disks every 2-3 weeks to maintain them until testing can be completed, because attempts at live, frozen storage have not been successful.

Table 1. Numbers and origin of pathogen isolates collected.

	Powdery mildew		Downy mildew	
Year	Isolates	Locations	Isolates	Locations
2005	20	5 - VA	21	5 - VA NC
2006	71	17 – VA NC MD	118	19 - VA NC PA MD
2007	53	12 -VA	12	2 - VA

Sensitivity testing, downy mildew

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

A total of 109 isolates have been tested, and 100 of those were resistant to strobilurin fungicides. In most vineyards, all samples tested were resistant. 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. But it is clear that in vineyards were strobilurins have been used previously, they are unlikely to be effective against downy mildew anymore.

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 does 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 (it appears likely that commercial vineyards in Virginia received at least 6-11 fungicide applications per season).

Mefenoxam or metalaxyl (Ridomil)

None of the isolates tested has shown evidence of resistance to mefenoxam.

Sensitivity testing, powdery mildew.

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

A majority of the 129 isolates tested was resistant to strobilurin fungicides, although the percentage is not quite as high as with downy mildew. Results suggest that there are several different levels of resistance. In some cases, it appears that the same vineyard can have resistant downy and sensitive powdery mildew populations (although it is possible that these occurred in different blocks).

Table 2. Reaction of 129 grape powder mildew isolates to strobilurin fungicides.

Reaction to strobilurins	Number of isolates
Highly resistant	55
Resistant, level uncertain	7
Moderately resistant	26
Sensitive	21
Uncertain	20

Ergosterol Biosynthesis Inhibitors

Over 120 powdery mildew isolates have been bioassayed for sensitivity against the five EBI compounds. Figures 1 and 2 show the response of the most and the least sensitive quarter of isolates tested.

Sensitive isolates have very little or no ability to grow on leaf tissue treated with 0.3 ppm of any of the fungicides (Figure 1). The most resistant isolates (Figure 2) grow well on leaf tissue treated with more than 30 times as much.

Fen = fenarimol (Rubigan)
My = myclobutanil (Nova=Rally)
Teb = tebuconazole (Elite, Orius),
Tri = triadimefon (Bayleton)
Trif = triflumizole (Procure)

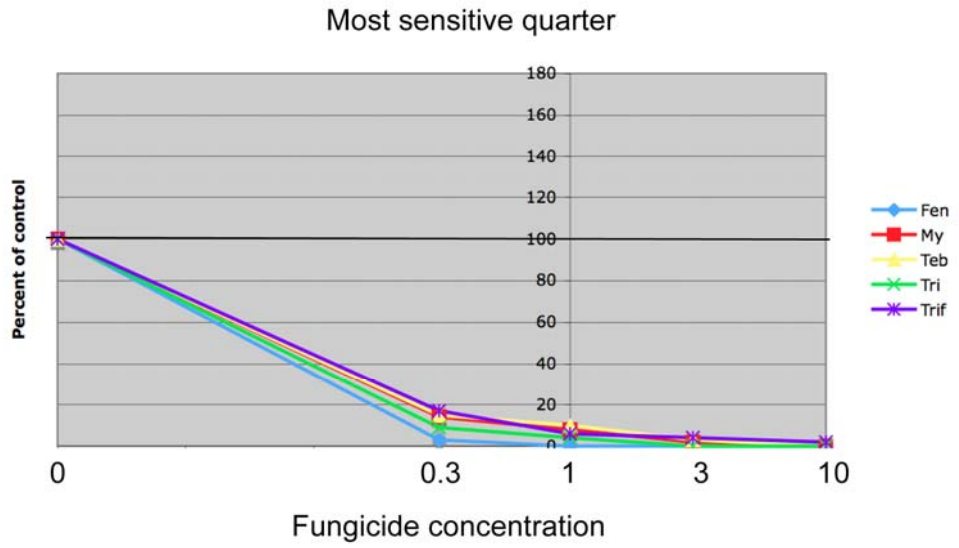

Figure 1. Percent control of powdery mildew (most sensitive quarter of mid-Atlantic isolates) on leaf disks treated with different concentrations of ergosterol biosynthesis inhibiting fungicides.

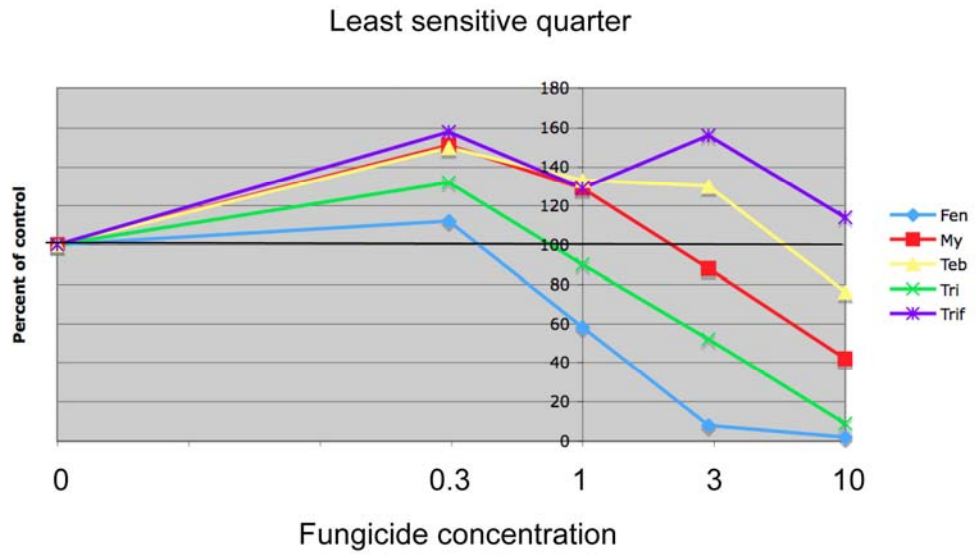

Figure 2. Percent control of powdery mildew (least sensitive quarter of mid-Atlantic isolates) on leaf disks treated with different concentrations of ergosterol biosynthesis inhibiting fungicides.

The results confirm the trend that was seen in our 2006 data, namely that resistance levels against tebuconazole and myclobutanil tended to be higher than against triadimefon and fenarimol. 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, and started to include it in our bioassays to test the hypothesis that Virginia powdery mildew populations would be more sensitive to it than to tebuconazole and myclobutanil. However, this turned out **not** to be the case: most of our isolates were less sensitive to triflumizole than to the others.

Boscalid (Endura, component of Pristine)

All PM isolates tested have had normal sensitivity to this compound. This material is relatively new (introduced in 2003), so there has not been much time for resistance development yet. 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 PM isolates tested have had normal sensitivity to this compound.

Objective 2. Assay development

We have started tissue-culturing grape plants in order to determine whether susceptibility of tissue-cultures plant material to the diseases in question is more uniform than that of greenhouse-grown material, and is guaranteed free of powdery mildew contamination. However, even

though shoot-producing plantlets are now available (Figure 3), as a means of quickly producing plant material, it is slower than we had hoped.

Figure 3. Tissue-cultured, pathogen-free grape plant.

Powdery mildew assays using a settling tower for inoculation were found to be less labor intensive and results 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), but 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.

With respect to PCR protocols, we still have problems with the protocols for strobilurin resistance. However, due to the great prevalence of such resistance in Virginia, these tests, although good to have, may not be of great practical use. For ergosterol biosynthesis inhibiting fungicides, no practical molecular resistance tests have been developed yet, but depending on the molecular nature of the resistance, such test may be possible. This is under consideration for future research.

Technology transfer

Results from these tests were incorporated in the Virginia Extension Pest Management Guide (http://www.ext.vt.edu/pubs/pmg/hf3.pdf). Owners and managers of vineyards from which samples were obtained have been and will be notified about results as they become available.

A publication on the downy mildew results of 2005 and 2006 and powdery mildew results from 2005 has been accepted for publication in the peer-reviewed, online journal Plant Health Progress. The accepted manuscript is attached. Support form the Virginia Wine Board is acknowledged on page 8.

> Anton Baudoin, 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. *Plant Health Progress* (In Press)