Virginia Wine Board

Progress Report for July 1, 2006 – June 30, 2007

Survey of grape powdery and downy mildew sensitivity to commonly used fungicides, 2006-07

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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

About 40 isolates of powdery and downy mildew had been collected previously in 2005 and an additional 189 isolates (Table 1) were obtained in 2006, mostly from Virginia but including samples from Pennsylvania, Maryland and North Carolina. A limited number of additional samples were frozen for later testing by PCR.

A Masters student, Ms. Jeneylyne Colcol, has started work on this project in mid-August of 2006. All 220 isolates are being transferred to fresh leaf disks every 2-3 weeks to maintain them. Attempts at live, frozen storage have been successful for downy mildew, but have not yet been reliable for powdery mildew; further variables are being investigated. Reliable frozen storage without loss of viability would greatly reduce the amount of time needed for culture maintenance.

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

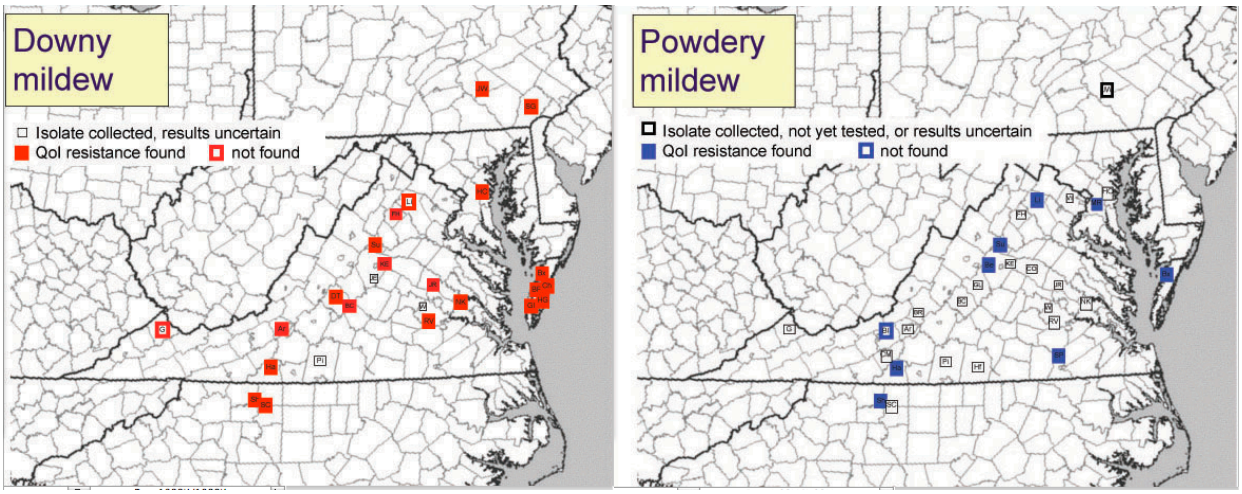

Figure 1. Geographic origins of downy and powdery mildew isolates, 2005 and 2006. Solid squares = isolates predominantly resistant to QoI fungicides, open squares with heavy lines indicate QoI sensitivity. Open squares with thin, black lines indicate isolates for which tests have not been completed (2006 isolates).

Table 2. QoI sensitivity of grape powdery and downy mildew isolates collected in 2005 from several locations in Virginia and North Carolina.

		Powdery mildew		Downy mildew	
Code	Source of isolates	QoI resistant in bioassay	PCR for G143A	QoI resistant in bioassay	PCR for G143A [1]
A	Eastern Shore, Va.			4 / 4	4 / 4
B	Montgomery Co..	0 / 2	0 / 1	0 / 1	0 / 1
C	Central Va.	8 / 8	0 / 1	3 / 3	2 / 2
D	Northern Va.	5 / 5	1 / 1	0 / 3	0 / 3
E	Central Va.	4 / 4	1 / 1		
F	South-Central Va.	3 / 3	1 / 1		
G	Western NC			2 / 2	2 / 2
H	Western NC			7 / 8	5 / 6

[1] PCR results positive for high-frequency presence of G143A mutation for strobilurin resistance. Data provided by Syngenta Corp. and INRA Bordeaux.

Objective 1, powdery mildew. In 2005, isolates were obtained from three commercial vineyards and one experimental vineyard as well as from minimally sprayed grapes in Blacksburg (Figure 1). Testing of these isolates was completed in 2006.

All isolates from the four commercial vineyards had QoI (strobilurin) resistance (Table 2). Resistance levels were high enough (over 50%, and for many isolates near 100% growth on leaf tissue treated with 1 mg/L azoxystrobin compared to nontreated controls) that they are expected to seriously compromise the efficacy of QoI fungicides against these isolates (Wilcox, WF, Burr, JA, Riegel DG, and Wong FP, 2003. Practical resistance to QoI fungicides in New York populations of *Uncinula necator* associated with quantitative shifts in pathogen sensitivities. Phytopathology 93:S90, Abstr.). A few isolates were tested by PCR at Syngenta Corporation. Three of the four resistant isolates had a high frequency of the G143A mutation, but it is interesting to note that the fourth isolate that was resistant in bioassay (from vineyard C in Table 2) was negative for this mutation.

Reduced sensitivity to DMI fungicides also was common in these isolates (Table 3). More isolates were tolerant of myclobutanil and tebuconazole than of triadimefon and fenarimol. All isolates from vineyards C and D (Fig 1) produced good growth on 1 mg/L of myclobutanil and tebuconazole, whereas sensitive isolates produced little growth at this concentration. Populations at sites B, E and F were more variable, but numbers of isolates are too small for firm conclusions. Contrary to expectations, tolerance to Bayleton was no higher than tolerance to the other fungicides in this group.

Testing of 2006 isolates is underway. Results so far trend in the same direction as those obtained with 2005 isolates: QoI resistance is common in isolates from commercial vineyards in Virginia and surrounding states. Reduced sensitivity to myclobutanil (Nova) and tebuconazole (Elite) is common, whereas sensitivity to triadimefon (Bayleton, to be discontinued) and fenarimol (Rubigan) tends to be clearly greater.

No sensitivity problems for quinoxyfen or boscalid have been found in the isolates tested.

Objective 2, downy mildew. The majority of 2005 isolates from 4 locations in Virginia and western North Carolina were resistant to the QoI fungicides ozoxystrobin and pyraclostrobin (Table 2), with ED50 values for disease incidence above 100 mg/L for azoxystrobin and around 25 mg/L for pyraclostrobin. Determination of ED50 values is made more difficult by often severe leaf tissue damage after treatment with the higher QoI concentrations. PCR results obtained at Syngenta Corporation (N. Kraus, G. Olaya and H. Sierotski) and INRA Bordeaux (F. Delmotte) indicated that the G143A mutation was present at high frequency in all resistant isolates for which results were obtained (Table 2). This type of resistance had not been previously detected in North America.

Tests with 2006 isolates (Table 4) indicate presence of resistance at many additional locations, including locations in Maryland and southeast Pennsylvania. Efforts to obtain spray history information from vineyards have been only partly successful. It would be useful to determine whether resistance is common in vineyards where strobilurins have been used three or fewer times per year (the currently recommended maximum). My impression is that this is the case.

None of the downy mildew isolates were found to be resistant to mefenoxam (Ridomil). Occasionally, some downy mildew isolates will produce a small amount of growth on leaf discs treated with 1 ppm mefenoxam, but we believe that this is due to fungicide leaching from the leaf disc into the agar. Such growth has not been reproducible in repeats of the experiment. The absence of Ridomil resistance in Virginia is probably due to limited use of this material. If one or more vineyards could be found where Ridomil has been used on a somewhat regular basis, it would be worthwhile to focus collection efforts on those.

Table 3. Tentative sensitivity of grape powdery mildew isolates (2005) from five Virginia locations (indicated by letters B-F in isolate designations) to four ergosterol biosynthesis inhibiting fungicides.

	Approximate concentration (mg/L) required for 50% inhibition			
Isolate	**Triadimefon**	**Myclobutanil**	**Tebuconazole**	**Fenarimol**
B-P1	0.3	0.3	0.3	0.3
B-P2	1	1	-	0.3
C-P1	1	3	10	0.3
C-P10	1	6	10	2
C-P11	0.3	0.6	0.6	0.1
C-P13	2	3	6	0.3
C-P2	2	6	10	3
C-P5	0.6	1	6	0.3
C-P6	-	3	10	0.6
C-P9	1	2	10	3
D-P1	2	6	6	6
D-P3	6	10	10	6
D-P5	2	10	10	1
D-P6	6	6	6	0.6
D-P7	3	3	10	6
E-P2	0.3	3	5	0.3
E-P4	2	6	10	1
E-P5	0.3	2	10	1
E-P6	1	0.5	3	0.3
F-P2	2	5	6	1
F-P3	0.3	0.3	0.5	0.1
F-P4	0.3	0.3	0.3	0.3
Mean	1.6	3.2	6.7	1.5

Table 4. QoI sensitivity of grape downy mildew isolates collected in 2006 from locations in Virginia, Maryland, and Pennsylvania

Code	Location	Results
Bx1	Accomack Co.	3R
Bx2	Accomack Co.	9R, 5?
G	Buchanan Co., home garden, Concord	4S
JE	Albemarle Co.	1S?
KE	Albemarle Co.	3R, 3?
HC	Maryland, Annapolis	3R
SG	Pennsylvania, Chester Co,	7R, 2?
FH	Rappahannock Co.	1R?
Ha	Patrick Co.	7R, 1?
Ar	Roanoke Co.	6R, 2?
GW	Northampton Co.	4R
Ch	Northampton Co.	6R
HG	Northampton Co	13R
DT	Amherst	4R, 1?
BC	Amherst Co.	1R
JR	Hanover Co.	1R
Pi	Pittsylvania Co. Prob. backyard, cv. unknown	1?
NK	New Kent Co.	1R
RV	Riverdale Vineyard, Chesterfield Co.?	3R
W	Chesterfield Co.	2S

* S=sensitive, R=resistant,? = results equivocal, contradictory, or not repeated

Objective 3. 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. Powdery mildew assays using a settling tower for inoculation appear to be less labor intensive and results less variable than assay by inoculation with single spores.

We have started testing PCR protocols ourselves for more rapid detection of known resistance mutations, especially for QoI fungicides. Dr. David Schmale, a plant pathologist at Virginia Tech, is collaborating with us in this area.

Technology transfer

Results were reported to Virginia growers through two articles in Viticulture Notes (http://www.ext.vt.edu/cgi-bin/WebObjects/Docs.woa/wa/getnews?cat=tt-news-viti) and a presentation at the February 2007 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, several grape extension pathologists in eastern states were kept informed of new discoveries, and the results were incorporated in the Virginia Extension Pest Management Guide (http://www.ext.vt.edu/pubs/pmg/hf3.pdf).