

## Progress Report for July 1, 2009 – December 31, 2009

To the

**Virginia Wine Board**

### ***Characteristics and Monitoring of Fungicide-Resistant Grape Powdery Mildew***

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#### **Objectives**

1. Evaluate the effect(s) of moderate ergosterol biosynthesis inhibitor (EBI) resistance of powdery mildew (PM) on effectiveness of EBI spray program, with emphasis on spray rate or frequency needed for adequate control.
  - a. Determine field performance in vineyards with contrasting PM EBI sensitivities.
  - b. Relate field performance to EC50 values obtained in standard bioassays, and lab analysis of components of infection (PM germ tube elongation, latent period, sporulation rate).
2. Continue to monitor fungicide resistance of grape fungal pathogens, with emphasis on vineyards reporting unexpected problems, and vineyards with heavy use of boscalid or quinoxyfen.
3. Estimate fitness of QoI-resistant PM population by initiating field experiments in commercial vineyards to determine possible decline of QoI resistance in absence of any QoI application.
4. Examine potential mutations in the *CYP51* gene and promoter region of PM isolates with contrasting EBI sensitivities.

## Objective 1. Relating DMI resistance to control efficacy

Field tests were implemented at Surry Community College, NC, in Botetourt County, near Roanoke, VA, and at Winchester, VA. Powdery mildew disease pressure developed at Winchester (Table 1) and Surry (Table 2), but not in Botetourt County. At the latter site, despite a season-long spray program, only the activity of a few treatments against downy mildew could be evaluated.

Powdery mildew had a slow start in 2009 in much of Virginia, at least in part due to a cool spell (nighttime temperatures near or below freezing) on May 18-20. At Winchester, powdery mildew incidence on untreated vines was rated at less than 1% on June 11, but developed strongly in late June and early July, incidence having increased to 43% by June 27, and becoming especially severe on bunches. Rally and Mettle provided good control. (Table 1) Powdery mildew isolates were obtained and are being bioassayed for degree of sensitivity to these fungicides. At Surry, powdery mildew was first noted on July 2 and became severe by early August. Some fungicides did not perform as well in this trial as expected, for example, Rubigan, Mettle, and the 14-oz rate of Inspire Super. The relative performance of Elite (tebuconazole) compared to Rubigan (fenarimol) is puzzling since powdery mildew isolates obtained in the previous years were considerably less sensitive to tebuconazole than fenarimol (Table 3)

In 2009, we expanded trials with “sentinel vines” which had been initiated in a preliminary fashion in 2008 (prioritizing this over components analysis: relating EC<sub>50</sub>, inhibition based on surface area, with various components of infection, e.g. sporulation, length of latent period and germination rate). Chardonnay cuttings were rooted at Surry Community College, potted up and distributed to several locations and collaborators. These vines were deployed as follows:

1. Surry Community College, location as in 2008. This portion of sentinel vine study did not yield results in 2009 due to the following: downy mildew became a threat early in the 2009 season, and to prevent infection, the fungicide Prophyt was misapplied at an excessive rate that caused phytotoxicity, which defoliated those plants and greatly delayed their availability for the purposes of the study. By the time the vines grew sufficient new leaves, the main infection period for powdery mildew had lapsed.
2. Winchester AREC, as in 2008, plants deployed outdoors in early July, sprayed July 14-Sep 2, 16 treatments. Powdery mildew appeared in the vineyard in June, and on the sentinel plants in mid-August. Rating in early September (Table 4). Powdery mildew isolates were obtained for resistance assay.

Table 1. Powdery mildew Chardonnay field test at Winchester, VA, 2009.

Treatment name (rate/A) <sup>1</sup>	Days after first application	Disease incidence LS mean <sup>2</sup>	Disease severity LS mean <sup>2</sup>	Disease severity (bunch) LS mean <sup>2</sup>
Quintec (4 fl oz/A) .....	39, 54, 68	0.83 b	0.01 b	1.89 b
Rally 40WPS (3 oz/A) .....	39, 54			
Then Microthiol D (4 lb/A) ..	68	0.42 b	0.02 b	6.96 b
Rally 40WPS (3 oz/A) .....	39, 54, 68	2.08 b	0.04 b	6.08 b
Mettle 125ME (5 fl oz/A) .....	39, 54, 68	0.83 b	0.01 b	4.30 b
LEM-17 (16 fl oz/A) .....	39, 54, 68	1.67 b	0.05 b	0.68 b
Untreated .....		92.08 a	32.92 a	94.36 a

<sup>1</sup> Treatments were tank mixed with 3 lb/A Penncozeb 75DF in order to prevent downy mildew.

<sup>2</sup> Disease incidence = percentage of diseased leaves. Disease severity = percentage of area of leaves or bunches that is diseased. Assessment on July 10, 4 days after treatment termination and 72 days after first application. LS mean = least square mean of percentage. The same letter indicates there were no significant difference between treatment (Tukey-Kramer adjustment method,  $\alpha=0.05$ )

3. A commercial vineyard in Linden, Fauquier County, northern VA, Jul 11-Sep 2, 11 treatments. Powdery mildew was present by mid-August, and ratings were conducted in early September (Table 4). Powdery mildew isolates were obtained for bioassay.
4. Blacksburg, location as in 2008. Plants were sprayed weekly, or in some treatments every 14 days from June 22 through Sep 21, 34 treatments. Light powdery mildew developed by early August, and, after a rating, some of the plants were cut back to stimulate new growth and some fungicide rates were adjusted. However, no additional powdery mildew developed later, and colonies on plants that had them in mid-August mostly became inactive and “disappeared”.
5. Rockbridge County, same site as 2008 field trial and sentinel plants, 8 treatments, July 18-Oct 3. Sentinel vines deployed in 2008 has yielded results but these could not be compared directly with those from other sites because fungicides were applied every 10 days here rather than every week. Weekly applications were planned for 2009. Care of the plants was entrusted to an intern working at this vineyard, who appeared helpful and cooperative, but who, either through misunderstanding or neglect, did not apply treatments properly. Unlike 2008, powdery mildew disease pressure was very light at this location in 2009. Some powdery mildew did develop late in the season on control plants, but due to incorrect treatment application, no valid data were obtained.
6. Botetourt County commercial vineyard (site of 2009 field trial), 8 treatments. Sentinel plants were sprayed weekly from June 20 through Sep 27 and inspected for the last time on Oct 11. Very light powdery mildew was observed only on control plants in late July-early August, but failed to advance further. No powdery mildew developed on any treated plants. Powdery mildew also did not develop in the accompanying field trial, but downy mildew was extensive.

Table 2. Leaf disease severity in Chardonnay powdery mildew field trial at Surry Community College, NC, Aug 26, 2009

Treatment	Powdery mildew % leaf coverage	
Control	61	b
Inspire Super, (difenoconazole + cyprodinil), 14 oz, 14d schedule	22	cd
Inspire Super (difenoconazole + cyprodinil), 20 oz, 14d schedule	2	g
Revus Top, (difenoconazole + mandipropamid), 7 oz, 14d schedule	1	g
Elite+Rubigan combination, 14d schedule	16	cde
Elite, <b>7d</b> schedule, 4 oz	1	g
Elite, 14d schedule, 4 oz	12	def
Revus 250 (mandipropamid), 14d schedule, 8 oz	95	a
Rubigan, <b>7d</b> schedule, 3 oz	5	efg
Rubigan, 14d schedule, 4 oz	31	c
Rubigan, 14d schedule, 6 oz	15	cde
Standard Pristine, 14d schedule	3	fg
LEM-17 (penthiopyrad), 14d schedule	3	fg
Mettle (tetraconazole), 14d schedule	18	efg

Values followed by the same letter are not significantly different, Waller-Duncan test, P=0.05

Table 3. Powdery mildew sensitivity of population at 2008 sentinel vine locations (Bioassay data obtained in 2009)

	Blacksburg	Rockbridge	Surry CC	Winchester
Bioassay mean EC50 tebuconazole	0.03	11.0	7.5	--
Bioassay mean EC50 fenarimol	0.01	0.17	0.24	--

7. A commercial vineyard in Hillsboro, Loudon Co, northern VA, June 24-Sep 24, 8 treatments. Plants were sprayed weekly, but downy mildew caused considerable defoliation by early August. Powdery mildew developed on control plants, and a number of colonies were also observed on plants treated with Quintec 2.5 ppm active ingredient. Powdery mildew isolates have been obtained from these plants and will be bio-assayed. Isolates from control plants will be bio-assayed to confirm their sensitivity to test fungicides.

At several of these sites we included treatments with newly registered (Mettle, tetraconazole) and experimental fungicides (difenoconazole, flutriafol, fluopyram, and penthiopyrad), in order to collect “baseline” information, and to relate sentinel vine results to those of laboratory bioassays. Application frequencies of 7 and 14 days were compared at one location (Blacksburg, Table 5).

Problems experienced included heavy downy mildew disease pressure that resulted in partial or complete defoliation of some of the plants. Plants were sprayed with Prophyt, which is thought to be inactive against powdery mildew, but we became concerned that there might be slight activity because powdery mildew seemed to cease development in some instances after a Prophyt spray; alternatively this might have been due to weather conditions. We are planning winter lab experiments to determine which fungicide available for downy mildew control (e.g., Prophyt, Ridomil, Revus, Presidio) would have least effect on powdery mildew. Insect (Japanese beetle) pressure was light in 2009, but testing insecticides such as Sevin for possible anti-powdery mildew effects appears worthwhile as well. Occasional spray drift from the vineyard might be a possible explanation for lack of powdery mildew development at some sites as well.

At some sites, deer feeding was a problem and plants had to be enclosed in fencing. Some sites had trouble keeping the plants watered, esp. when pots fell over in windy conditions. Placing pots inside other pots that were bolted to the ground helped in this case, and retained the ability to move pots easily when plants needed to be sprayed.

## **Objective 2. Fungicide resistance monitoring**

Several growers expressed concern about the performance of Prophyt in 2009, which in many areas was a wet growing season very conducive to downy mildew. One grower submitted samples that had repeatedly been treated with both Ridomil and Prophyt, and where the downy mildew still looked surprisingly viable. However, attempts to transfer downy mildew from these leaves to other leaves failed. Additional samples were obtained from locations where Prophyt had been heavily used; these will be assayed in 2010.

## **Objective 3: Determine fitness costs associated with QoI-resistance**

It has proven to be difficult to find vineyards where (a) we know that QoI resistance (G143A mutation) was present in the past, (b) the grower has not applied QoI fungicides for some time or will

agree not to apply QoI fungicides, and (c) powdery mildew was present in 2009. One vineyard where the grower has not used QoI fungicides since 2007 (when 5 of 5 monoconidial powdery mildew isolates were found to have G143A) has provided samples in both 2008 and 2009. In 2009, the mean G143A of the samples was 40%, with individual samples ranging from 0.1 to 99% suggesting substantial spatial heterogeneity. In 2009, powdery mildew samples were also obtained from a vineyard that had discontinued QoI use since 2007; these samples are still being tested.

In light of this difficulty, to supplement these field data, a series of experiments on individual plants grown in plastic tubes (Fig. 1) have been started. Nine pairs of QoI-resistant isolates with G143A mutation and sensitive strains without G143A were co-inoculated on grape plants and cycled on the same plants 4 times. In addition, bulked inoculations were done where several resistant and sensitive isolates were mixed in equal proportion. In most comparisons, the percent G143A increased (Fig. 1), suggesting a lack of fitness penalty.

We also have a number of *E. necator* isolates with moderate QoI resistance that do not have the G143A mutation. Attempts are being made to detect possible mutations in these isolates.

#### Objective 4. Exploratory study to detect mutations in the *CYP51* gene.

The *CYP51* gene of two isolates was amplified with the primers described by Délye et al. (1997, Appl Environ Microbiol 63:2966-2970). Direct sequencing revealed the Y136F mutation described by Délye et al. only in an isolate (IVP-11) that was relatively resistant to the DMI fungicides tested and not in a DMI-sensitive strain (BLP4). In additional amplifications of several strains, two bands were generated, ~1.7kb and ~1.5kb. Using Délye's primers, the 1.7kb band is the expected size for *E. necator* CYP51. These bands will be sequenced to determine their identity.

**Outreach component:** An article (lesson) on fungicide resistance for The Plant Health Instructor (APSnet Education Center) is being drafted. Completion will be also be needed for a course taught by A. Baudoin this spring. Completion of internal review and submission is scheduled for May 2010.

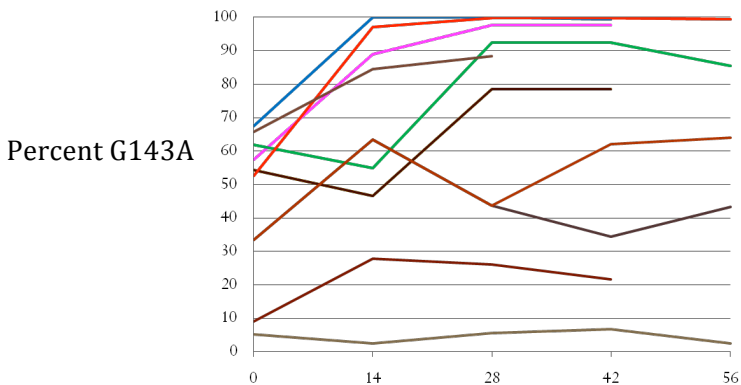


Figure 1. QoI-resistant and sensitive isolates of *E. necator* were paired on small, fungicide-free plants in plastic tubes (left). Percent G143A (QoI resistance) tended to increase or not change (right).

Table 4. Sentinel vine powdery mildew ratings in Linden and Winchester, VA, Sep 1, 2009.

Fungicide	Concentration (active ingredient, ppm)	Powdery mildew rating % of leaf surface Winchester	Powdery mildew rating % of leaf surface Linden
Control	na	8.7	7.6
Abound	300	2.7	0.1
Elite	25	0	0
Elite	5	3.3	0
Endura	5	0	0
Endura	1	0.1	--
Inspire	1	5.3	--
LEM-17	5	4.4	--
Mettle	5	0	--
Quintec	2.5	0.2	0.2
Quintec	0.5	3.9	0.1
Rally	25	0.2	0
Rally	5	4.7	0
Rubigan	10	0	0.1
Rubigan	2	0.4	0.1
Topguard	5	0.1	--

Two plants per treatment, 10 leaves per plant

Table 5. Sentinel vine powdery mildew ratings in Blacksburg, Aug 7, 2009

Fungicide	Concentration (active ingredient, ppm)	Spray frequency (days)	Powdery mildew rating 0-5 scale
Control	--	7	3.0
Elite	10 and 2	14	0
Elite	5 and 1	7	0
Endura	10	14	2.0
Endura	5	7	0.8
Endura	2	14	3.0
Endura	1	7	1.5
Flint	50	7	0
Fluopyram	5 and 1	7	0
Inspire	5 and 1	7	0
LEM-17	5	7	2.0
LEM-17	1	7	4.0
Mettle	5 and 1	7	0
Quintec	5	14	2.5
Quintec	2.5	7	0.3
Quintec	1	14	3.5
Quintec	0.5	7	0.5
Rally	5 and 1	7	0
Rubigan	4	14	0.5
Rubigan	0.8	14	1.0
Rubigan	2 and 0.4	7	0
Topguard	5 and 1	7	0

Two plants per treatment, rating on a scale of 0-5

Appendix  
***Characteristics and Monitoring of Fungicide-Resistant Grape Powdery Mildew***  
**July 1, 2008 – June 30, 2010**

**Publications**

- Baudoin, A., Olaya, G., Delmotte, F., Colcol, J.F., and Sierotzki, H. 2008. QoI resistance of *Plasmopara viticola* and *Erysiphe necator* in the mid-Atlantic United States. Plant Health Progress  
[doi:10.1094/PHP-2008-0211-02-RS](https://doi.org/10.1094/PHP-2008-0211-02-RS)
- Colcol, J.F. 2008. Fungicide Sensitivity of *Erysiphe necator* and *Plasmopara viticola* from Virginia and nearby states. MS Thesis, Virginia Tech, 67 pp. . <http://scholar.lib.vt.edu/theses/available/etd-08152008-144239/>
- Colcol, J.F. and Baudoin, A. 2008. Sensitivity of grape downy and powdery mildew to commonly used fungicides. Phytopathology 98:S214 (abstract).
- Colcol, J.F. and Baudoin, A. 2008. Fungicide resistance of *Erysiphe necator* in the U.S. Mid-Atlantic region. Phytopathology 98:S40 (abstract).
- Baudoin A. 2008. Fungicide resistance update 2008 (powdery and downy mildew), Viticulture Notes, Vol. 23, No. 1, March-April 2008

**Presentations**

- Baudoin A. 2007. Powdery and downy mildew fungicide resistance survey. Research update to Virginia Vineyards Association, Charlottesville, VA.
- Colcol J.F. and A. Baudoin. 2008. Survey of Downy and Powdery Mildew Sensitivity to Commonly Used Fungicides. Research update to Virginia Vineyards Association. Charlottesville, VA
- Baudoin A. 2009. Field assay of fungicide resistance in the vineyard? Annual Meeting of Virginia Vineyards Association. Charlottesville, VA. February 21, 2009.