Virginia Wine Board

Progress Report for July 1, 2008 – June 30, 2009

Characteristics and Monitoring of Fungicide-Resistant Grape Powdery Mildew

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Gill Giese (2008 and 2009) and **Mizuho Nita** (2009)

Objectives

1. Evaluate effect 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 disease development (PM germ tube elongation, latent period, sporulation rate).
2. Continue to monitor fungicide resistance of grape pathogens (powdery and downy mildew), with emphasis on vineyards reporting unexpected problems, uncertainty about QoI (strobilurin) sensitivity, and vineyards with heavy use of boscalid or quinoxyfen, or metalaxyl (for downy mildew)
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. Exploratory study to detect specific point mutations in the *CYP51* gene and promoter region of PM isolates with contrasting EBI sensitivities.

Personnel

Ms. Jeneylyne Colcol defended her thesis in August 2008. A new Ph.D. student, Ms. Lynn Rallos has started work on this project in August of 2008. Two new collaborators were added to this project: Mr. Gill Giese, Surry Community College, who already participated in 2008 by managing a set of sentinel vines, and Dr. Mizuho Nita, who started in January of 2009 as Virginia's Research and Extension Grape Pathologist.

Objective 1.

One field test was conducted, a location with contrasting powdery mildew sensitivities to two different EBI fungicides. This field test was conducted in Rockbridge Co, VA, a commercial, long-existing vineyard with a powdery mildew population expected (based on previous year's collections) to have considerable tolerance to tebuconazole (Elite) but a much lower level of tolerance to fenarimol (Rubigan).

In the early part of the season, the test area was treated by the grower with mancozeb plus sulfur. On June 7, 2008 (early bloom) 10- and 15-day spray schedules were initiated with tebuconazole and fenarimol at several spray rates, see Table 1. Sprays were applied with Solo 410 backpack mistblower, with spray volume increasing from 50 (bloom) to 95 gallons (after late June) per acre. These spray schedules were continued until July 21. At that point a severe powdery mildew epidemic was well underway, and because of the state of fruit development, little additional mildew development was expected on the clusters. Plots were rated on July 25, and were visited again on August 14, but no additional rating was deemed useful.

Label rates of Elite on a 10-day schedule gave poor (59%) powdery mildew control (Table 1). Even increasing the rate to 6 oz (early sprays) and 8 oz (later sprays) did not result in commercially acceptable control (78%). Rubigan at the maximum labeled rate (6 oz) or 4 oz Elite plus 2 oz Rubigan provided over 90% control of cluster infection. The rationale for the latter treatment was that Elite should play an additional role in improved black rot control.

Field trials that have been initiated for the 2009 season are one trial in Botetourt County, one trial managed by cooperator Gill Giese at Surry Community College in North Carolina, and one trial by new cooperator Mizuho Nita at Winchester Virginia. These include several different treatments of several EBI fungicides, including newly registered and experimental compounds, and all trials will be accompanied by sentinel vines as outlined below.

In addition to the field test, we initiated what we call "**sentinel vine**" tests at four locations in 2008 and additional locations in 2009 (Figure 1). We intend to develop this approach into a practical method for growers and/or consultants to monitor powdery mildew populations themselves. Greenhouse-grown, potted vines are placed near commercial vineyards, and sprayed on a regular basis with a range of low rates of a single fungicide. The plants are monitored for powdery mildew development, and the lowest spray rate that still prevents mildew development provides information on the fungicide sensitivity at the site. The "discriminating rates" have to be determined for each fungicide individually

In 2008, two plants for each fungicide and spray rate were set out in the first half of July (Figure 1), and sprayed every 1 to 2 weeks (depending on site and time of season, Table 2) for the course of the growing season. Powdery mildew severity was rated repeatedly; selected ratings are shown in Table 2, demonstrating distinct differences between sites that corresponded with what was expected based on previous lab-based bioassays. Powdery mildew isolates were collected from the untreated sentinel plants, or (Rockbridge County) from untreated field plots; these isolates were bioassays for tebuconazole and fenarimol sensitivity (Table 2)

Table 1. Powdery mildew field test in Rockbridge Co, VA, 2008.

Treatment	Percent of clusters covered with powdery mildew and, in parentheses, percent control, Jul 25 [1]	Statistical significance[2]
Untreated Check	78 (0)	A
Elite, 10d schedule, 4 oz	32 (59)	BC
Elite, 10d schedule, 6-8 oz	17 (78)	D
Elite, 15d schedule, 4 oz	39 (50)	B
Elite, 15d schedule, 6-8 oz	31 (60)	C
Rubigan, 10d schedule, 4 oz	27 (66)	C
Rubigan, 10d schedule, 6 oz	7 (91)	E
Rubigan, 15d schedule, 4 oz	26 (66)	C
Rubigan, 15d schedule, 6 oz	16 (79)	D
Rubigan 2 oz + Elite 4 oz, 10d	8 (89)	E

[1] Based on rating of 40 clusters per plot. Powdery mildew was about 2% (translates to 97% control) in an adjacent downy mildew test where powdery mildew was controlled with combinations of sulfur and Rubigan rotated with Elite.

[2] Differences between treatments followed by the same letter were not statistically significant (Analysis of variance followed by Waller's test, P=0.05).

Figure 1. Potted sentinel vines treated weekly with low rates of fungicides and located near commercial grapevine block to monitor fungicide sensitivity of local powdery mildew population.

Sentinel vine locations, 2008

1. Blacksburg, VA, not near any commercial vineyards, potted plants in location with a history of powdery mildew on backyard vines, but very little fungicide use. Isolates from this location had been sensitive to all QoI and DMI fungicides in previous bioassays.
2. Surry Community College, Dobson, NC (one prior-year powdery mildew isolate had a low level of DMI tolerance and was QoI sensitive)
3. Winchester Agricultural Research and Extension Center, VA (no sensitivity data available)
4. The commercial vineyard in Rockbridge County that was the location of the summer field test, with powdery mildew resistant to QoI fungicides and considerable tolerance to DMI fungicides in bioassays, poor field control by Elite and mediocre field control by Rubigan (Table 1).

Sentinel vine locations, 2009

1. Blacksburg, as above
2. Surry Community College, as above
3. Winchester AREC, as above
4. Botetourt County commercial vineyard (site of field trial)
5. Two commercial vineyards in North Carolina
6. Two additional commercial vineyards in Virginia, in Loudon and Fauquier County
7. Still to be deployed in July: Rockbridge County, same site as in 2008, see above

At several of these sites we have included treatments with newly registered (Mettle, tetraconazole) and experimental fungicides (Inspire, difenoconazole; Topguard, flutriafol; fluopyram, and DPX LEM17), in order to collect “baseline” information, and to relate sentinel vine results to those of laboratory bioassays. Application frequencies of 7 and 14 days are being compared at one location. One of the sentinel vine sites was specifically chosen because QoI resistance is known to have been prevalent in 2005-2006, but no QoI fungicides have been used in that vineyard in more recent years (see objective 3, below)

Table 2. Powdery mildew severity, as estimated percentage of leaf surface covered. Each number represents a potted vine.

Treatment: Fungicide, μg active ingredient per ml[1]	Blacksburg Rated Sep 20-21. Sprayed weekly until Aug 23, then 10-day interval, then 14 days	Rockbridge Rated Aug 14. Sprayed every 10 days	Surry CC Rated Sep 6. Sprayed weekly	Winchester Rated Sep 30. Sprayed weekly until early September
Untreated	95, 95	70, 60	35, 26	0, 0
Elite 135	-	1, 1	-	0, 0
Elite 50	-	10, 3, 1	0	0, 0
Elite 10	0, 0	35, 15	18, 1	0, 0
Elite 2	0, 0	-	51, 42	0, MTC[3]
Elite 0.5 [2]	0, 3	-	-	-
Bioassay mean EC50 tebuconazole	**0.026**	**0.132**	**0.113**	-
Flint 50	trace, 0	60, 50	0, 3	0, 0
Rubigan 25	-	5, 1	0, 0	0, 0
Rubigan 10	0, 0	25, 15, 3	1.4, 0	0, 0
Rubigan 2	0, 0	20, 5	20, 6	0, 0
Rubigan 0.5 [2]	0, 2	-	-	-
Bioassay mean EC50 fenarimol	**0.009**	**7.841**	**5.315**	-
Endura 20 [2]	0, 0	-	-	-
Endura 4 [2]	3, 1	-	-	-
Endura 0.8 [2]	30, 25	-	-	-
Quintec 10 [2]	0, 0	-	-	-
Quintec 2 [2]	2, 1	-	-	-
Quintec 0.5 [2]	3, 1	-	-	-

[1] Label rate (based on spray volume of 100 gallons per acre) for Elite (tebuconazole) is 4 oz/A or 135 μg a.i./ml; Rubigan (fenarimol) 6 oz/A or 56 μg a.i./ml; Flint 2 oz/A or 75 μg a.i./ml; Quintec 6.6 oz/A or 116 μg a.i./ml; and Endura 8 oz/A or 419 μg a.i./ml.

[2] Elite 0.5 and Rubigan 0.5 treatments were added on Aug 9, Endura and Quintec on Aug 16.

[3] MTC = many tiny colonies, on only one plant.

Objective 2: In 2008, we investigated one situation with suspected boscalid resistance of powdery mildew, and one set of isolates for possible reduced sensitivity of downy mildew to phosphonate (Prophyt). In both cases, the results indicated that the alarm was false, and that no fungicide resistance was present. There may be a slight difference in Prophyt sensitivity of different downy mildew isolates; this will be the subject of further tests. No new situations have arisen yet in 2009.

A number of bioassays were done with Topsin M (thiophanate methyl) to see if this material, which is registered for use against grape powdery mildew, might provide an alternative for occasional use. Due to the use of Benlate (benomyl) in the 1970-1990s, grape powdery mildew populations in the eastern United States developed resistance to this group (the benzimidazoles), but there appears to be no published information on how widespread and prevalent this resistance was in Virginia. Observations from California indicated that benomyl resistance was very uncommon in that state (Ypema et al. 1997, Plant Disease 81: 293). Also, use of benomyl on grapes has probably been uncommon for at least 10 years (all US benomyl sales ended in 2001), and although Topsin M was registered in its place, it has probably not been widely used on grapes.

Data were obtained with 55 isolates from 23 locations, although only 19 of these representing 15 locations were repeated. Fifty-one of 55 isolates grew well on leaf tissue treated with 50 ppm a.i. of formulated Topsin M, two of them did not grow, and two had an intermediate reaction. Since resistance appeared to be unexpectedly widespread, a number of isolates were also tested against a different formulation of thiophanate methyl (Cleary 3336 Plus) and against an old sample of Benlate 50DF. Results with Cleary were similar, but several isolates were more strongly inhibited by Benlate than by Topsin M. There appeared to be at least two different levels of resistance: isolates inhibited by 250 ppm a.i. benomyl, but not by 50 ppm, and isolates inhibited by neither. Results with benomyl are of no practical relevance since the compound is not available anymore, and grape powdery mildew resistance to Topsin M appears to be so widespread that it will be useless as an alternative.

Objective 3: We received powdery mildew samples from one vineyard in nelson County, VA with previously confirmed QoI (strobilurin) resistance where no QoI fungicide had been used in the last season. Several isolates were recovered and appear to be still QoI resistant with the G143A mutation. We have initiated lab competition experiments of QoI-resistant versus sensitive isolates of grape powdery mildew.

Objective 4: Work on amplifying and sequencing the CYP51 gene of *E. necator* is scheduled to be initiated in summer and fall of 2009.

Outreach

Results were incorporated in: Pfeiffer D., A. Baudoin, J. Bergh. Grapes: Diseases and Insects in Vineyards. In: 2009 Pest Management Guide for Horticultural and Forest Crops. Va. Coop. Ext. Pub. 456-017. Pages 3-1 – 3-14

Baudoin, A., 2009. Field assay of fungicide resistance in the vineyard?
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