Virginia Wine Board
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Project Report
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Sentinel vines to evaluate powdery mildew sensitivity to fungicides on winegrapes

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Note: this report should be read in conjunction with the semiannual report from Feb 2011

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.

Objectives 1 and 2. Relating DMI resistance to control, and continued resistance monitoring

Two field test tests have been initiated for the 2011 growing season. One is located in a commercial vineyard in southwest Virginia with high powdery mildew disease pressure and considerable DMI resistance to determine how recently registered DMI fungicides (Inspire Super and Mettle) perform relative to the poor performance of the "older" ones, Elite and Vintage (which performed poorly in 2010 at this site). Only fungicides actually registered for use on grapes are included at this site. Results of a first disease rating are shown in Table 1 (statistical

analysis remains to be completed). The second experiment, at the Winchester AREC, includes additional, not yet commercial but soon-to-be-registered fungicides. Both locations also "host" a set of sentinel vines in order to further link results from sentinel vines to field performance.

In addition, sets of sentinel vines have been placed at two commercial vineyards, and are being managed by those growers, and an additional large set is being maintained at Blacksburg.

Experiments have been initiated to determine discriminatory dosages for downy mildew fungicides as well (Ridomil, Revus, Forum, Presidio, etc.)

Table 1. Powdery mildew severity on clusters and main-shoot leaves of grape, 12 July 2011. Treatments had been applied June 7 (full bloom) and 21 and July 5.

	Percent of surface covered	
Treatment	Clusters	Leaves[1]
Untreated (water)	85	60.7
Elite, 4 oz/A	53	10.1
Vintage, 6 fl oz/A	66	8.1
Inspire Super 16 fl oz/A	16	0.4
Mettle 5 fl oz/A	52	2.4
Vivando, 15.4 fl oz	4	0.2
Endura, 4.5 oz/A	54	0.5
Quintec, 6 fl oz/A	18	0.7
Sulfur, 5 lbs/A	68	2.7

[1] Only basal leaves on main shoots were rated. Additional powdery mildew is developing on laterals, and will be evaluated in the future.

Objective 3: Determine fitness costs associated with QoI-resistance

We have conducted competition assays under controlled conditions (laboratory scale) to determine whether or not QoI resistance carries a fitness cost. Bioassays using mixed inocula (resistant and sensitive isolates) showed that resistant isolates are not compromised in their growth and reproduction, suggesting the lack of a fitness cost. In addition, QoI resistance was found to persist for three years in a field site where QoIs have not been used since 2007. To further test the hypothesis, we ventured to find out if resistant isolates inoculated on grape plants would behave similarly under field conditions. A competition assay employing a mixed inoculum was started recently. Potted grape plants (3 per site, two sites) were situated at the Glade Road Research Center, Virginia Tech and inoculated with a suspension of spores from resistant and sensitive isolates in a ratio of 1:9. Control plants (not inoculated, inoculated with sensitive spores only, or with resistant spores only) were also placed at different locations in the area. Plants are watered every 2-3 days, and the development of powdery mildew is monitored. Sampling of powdery mildew is done by rubbing disinfested grape leaves on lesions/growth (5 per plant, 15 per treatment). Sampling will be done at least two times, the first at the start of the infection period and throughout the growing season when new infection (on new shoots) appear. The rubbed leaves are placed on water agar and incubated until sporulation is profuse. Spores will be harvested by scraping with a sterile spatula and collecting spores in a tube. DNA will be

extracted from these spores using the BioSprint Plant DNA Kit (Qiagen®). The changes in population composition will be determined based on the proportion of the mutant allele (A143 in *cytochrome b*) that is responsible for a high-level strobilurin resistance. Individual resistant strains possess very high %G143A (>90%), whereas sensitive strains have very low values (<1%). The fitness of resistant strains can be determined by monitoring the changes in %G143A. If the %G143A does not decline, but stays constant or increases, this is an indication that a fitness penalty is not associated with QoI resistance in grape powdery mildew, and supports the findings in our laboratory-scale (controlled conditions) experiments.

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

We have reported earlier that the mutation (Y136F) in the target molecule (CYP51) associated with DMI resistance has been confirmed in some of our resistant isolates. Our next goal was to obtain a complete section of the transcribed region for isolates with different sensitivities. Additional DNA samples obtained from isolates (n=28) with various levels of DMI resistance were subjected to PCR. For this sample set, three new primer pairs were designed based on our earlier sequences and the reference sequences to generate overlapping amplicons so that the whole *CYP51* sequence could be assembled. PCR products were cleaned up using enzymatic removal of non-target products, and sent to the University of Chicago Sequencing Facility. A total of 198 sequences were generated. Assembly and alignment using the Seqman Pro (ver.8.1.3) of the DNAStar Lasergene program revealed that 18 out of 21 isolates with decreased sensitivity to DMIs (resistance factor as follows: myclobutanil >83, tebuconazole >77, triadimefon >6, triflumizole >34, and fenarimol ≥10) had the Y136F change. Another two isolates had moderate to high resistance levels to the first four DMIs, but very low RF to fenarimol (RF_{fen} = 0.1) and yet possessed the mutation. The last isolate lacked the mutation but had low to high tolerance to DMIs (RF_{myc}=300; RF_{teb}=256; RF_{tria}=34; RF_{trif}=5.7; RF_{fen}=5.7). These results suggest that other mechanisms as reported for other pathogens might also be involved in DMI resistance in *E. necator*. Another single nucleotide polymorphism (SNP) within the *CYP51* sequence was associated with some of the resistant isolates, and none of the sensitive isolates carried this SNP. We have yet to determine if this SNP leads to a significant change in the deduced amino acid sequence of the protein. The prevalence of this mutation and how the introduction of the selection pressure drives its evolution in field populations are also being investigated, as this will provide a basis for developing the Y136F as a molecular tracking tool for DMI resistance.

Presentations 2011

Rallos, L.E. and A. Baudoin. 2011. Sentinel Vines for Assessing Fungicide Resistance, and Resistance Update 2011. Annual meeting of Virginia Vineyards Association. Charlottesville, VA. February 18 and 19, 2011.

Rallos, L.E. and Anton Baudoin. 2011. Stability of QoI resistance of grapevine powdery mildew in competition experiments and in the field. Annual meeting of the Potomac Division of the American Phytopathological Society, March 2011, Rehoboth Beach, DE.