Virginia Wine Board
Project #12-1982-03

Botrytis cinerea fungicide sensitivity evaluation in Virginia crops, and evaluation of powdery mildew advisory

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Results, by objective

Objectives 1 and 2. Original wording:

1. Survey *Botrytis cinerea* in Virginia crops (grapes, berries, greenhouse crops, ornamentals, vegetables, apples) for sensitivity to commonly used groups of fungicides.
2. Compare sensitivity patterns and genetic similarities of *B. cinerea* from different crops to estimate rate of exchange.

The Virginia Wine Board reduced the budget of the project with the apparent intent of not funding the comparison with other crops. Therefore, Botrytis isolates from other crops have received little attention, and only a few such isolates have been collected or received by mail, and tested.

The fall of 2012 was generally not favorable for the development of Botrytis grape bunch rot. Only about 20 additional isolates were collected from 4 vineyards, and several vineyards were visited where Botrytis collection was completely unsuccessful. No new samples were received from growers. Partial testing of new samples and additional testing of older samples generally confirmed results reported previously (July 2012).

Since boscalid resistance was found to be common, a few dozen isolates were tested against fluopyram, which is the major component of Luna Experience, a new fungicide introduced to the grape market in 2012. This material is in the same mode-of-action group (same FRAC code) as boscalid. However, all boscalid-resistant isolates tested sensitive to fluopyram, which is similar to what others have reported recently. The plan is to test all boscalid-resistant isolates against this new fungicide.

The degree and frequency of resistance to the active ingredients in Vangard and Scala has been somewhat difficult to determine due to inconsistency of the growth of different isolates on different culture media. In order to clarify the practical impact of the reduced sensitivity fund, inoculation experiments have been started with table grapes (Fig 1). These are much more labor- and time-consuming then germination tests on agar plates. It has become clear that at least some of the resistance detected is highly significant: a full field rate (calculated as 10 oz per 50 gallons) provided practically no control of the sensitive isolate, even under conditions that should optimize its effect (inoculation immediately after treatment) (Fig. 1).

With respect to Elevate (fenhexamid) resistance, several Elevate-resistant isolates were obtained from flower debris in a northern Virginia vineyard. Isolates obtained from the same location in the preceding fall had been uniformly sensitive. It is possible that the spring-collected isolates represent a recently described different species of Botrytis, *Botrytis pseudocinerea* (Walker et al. 2011. *Botrytis pseudocinerea*, a new cryptic species causing gray mold in French vineyards in sympatry with *Botrytis cinerea*. Phytopathology 101:1433-1445). This species is naturally resistant to Elevate and has been reported only from western Europe. It appears to be relatively unimportant as a grape pathogen, and cannot be distinguished from *Botrytis cinerea* by outward appearance but must be identified by molecular methods. In addition to these three spring-collected isolates, one fall-collected isolate has turned out to be resistant to Elevate. Species identification of these isolates will be attempted in the next few months.

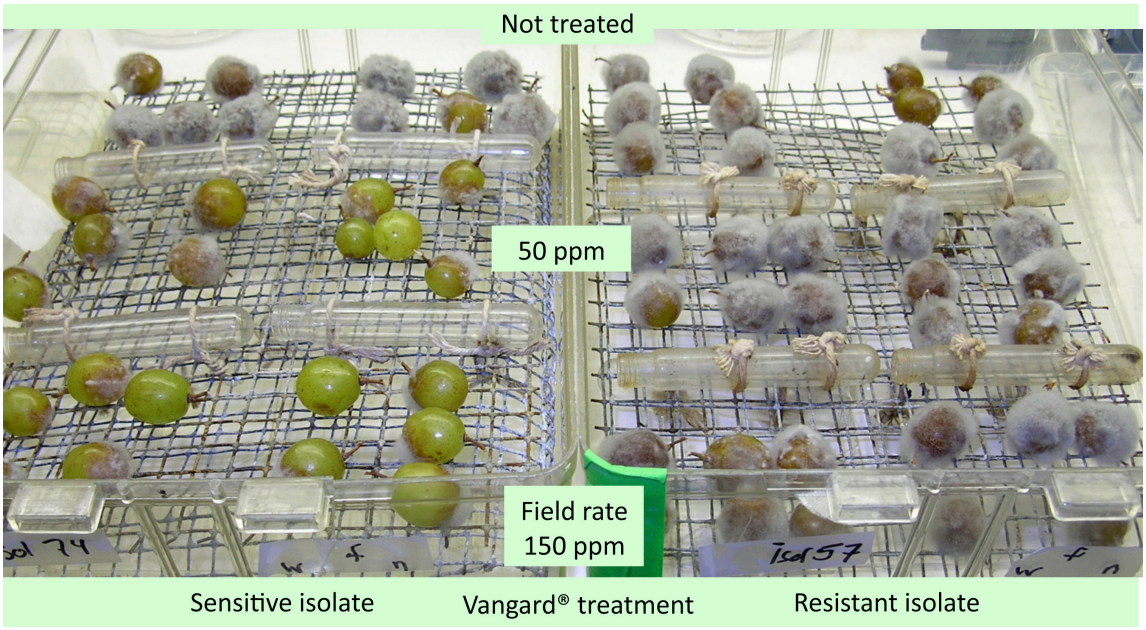


Fig. 1. Botrytis development on table grapes 4 days after treatment with Vangard (field rate and one-third of the field rate) followed by inoculation with a sensitive (left) or resistant (right) isolate. Within each treatment, the leftmost 5 or 6 grapes had been punctured before inoculation, whereas the 5 or 6 grapes on the right had not been wounded.

Isolates from other crops that were included in fungicide sensitivity tests include several isolates from a pelargonium plants which my spouse had purchased from the Christiansburg Lowe's store for planting around the house, and samples from three strawberry growers, one in Floyd County and two in Virginia Beach that were received by mail. Botrytis isolates from three of the four sources, the pelargonium, the Floyd Co. strawberries, and from one of the two Virginia Beach locations, had high levels of resistance to Elevate. This indicates that Elevate resistance in Botrytis of other crops is much more common than what has been found in Virginia grapes so far.

Objective 3. Explore EBI resistance mechanisms in our powdery mildew collection other than target site mutations.

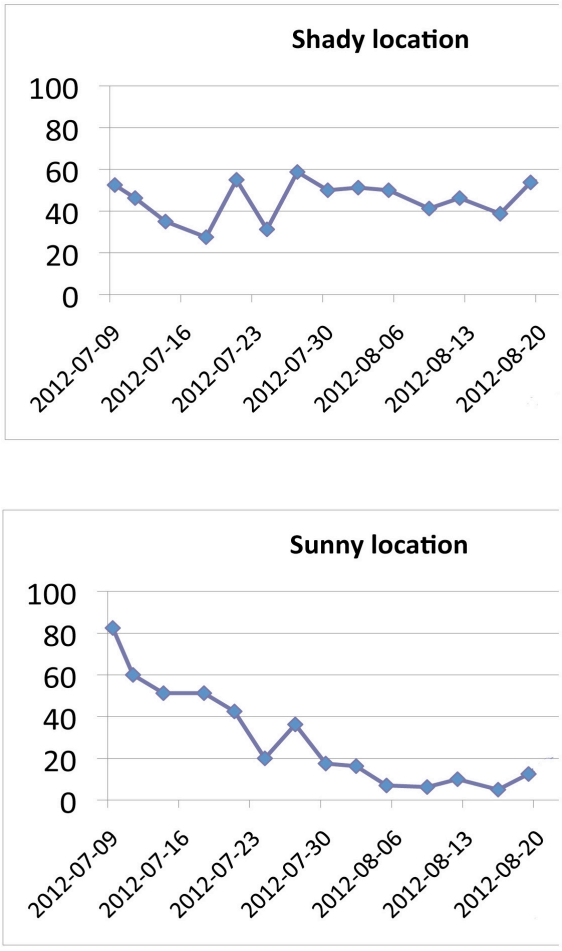
Only one *cyp51* target site mutation associated with DMI resistance has been found in Virginia isolates, and the level of resistance is variable in isolates with this mutation (Table 1). Gene expression was examined for three wildtype isolates and nine mutant isolates. The expression of *cyp51* was found to be higher in all isolates possessing Y136F than in isolates lacking the mutation, but the increase was only marginal in two of the isolates, while being significantly higher in seven out of nine isolates possessing Y136F ($P<0.0001$) (Table 1). As a group, the TWT-genotype had a slightly higher expression level than TTT, but this difference was not statistically significant (Table 1). A significant correlation was also found between *cyp51* expression level and resistance factor to the three fungicides. A strong correlation was obtained for myclobutanil ($r=0.80$, $P<0.0001$) and tebuconazole ($r=0.74$, $P<0.0001$), but slightly lower correlation for fenarimol ($r=0.64$, $P<0.0001$). This indicates a tendency toward increased up-regulation of cyp51 with increased resistance level to DMI fungicides.

We have attempted to detect efflux pump activity in *E. necator* by employing two common inhibitors, verapamil and CCCP, of the ABC and MFS transporters, two groups of efflux pumps known to be involved in DMI resistance. The inhibitor is expected to render resistant strains susceptible, when combined with the fungicide, because the fungus loses the ability to pump out the toxic material from the cell. Our preliminary trials showed a lack of inhibition of germ tube elongation by these chemicals (data not shown), indicating the need for optimizing experimental conditions and testing of other inhibitors.

Table 1. Relative gene expression of *cyp51* in *Erysiphe necator* isolates with different genotypes at the 136th codon. Relative expression was determined using the ΔΔCT method with the *En* β-tubulin gene as endogenous reference and BLP4 as the calibrator.

Isolate	Genotype (sequencing)	SNP assay	Resistance level, Rf-teb	Mean RQ*	n (RQ)
MVP9	TAT	Wildtype	0.6	1.0 ± 0.05 e	6
BLP4	TAT	Wildtype	1.0	1.0 ± 0.02 e	6
PBP1	TAT	Wildtype	1.0	1.4 ± 0.01 e	6
IVP4	TWT	Mix	21.3	10.7 ± 0.16 c	6
VAHP4	TWT	Mix	23.6	11.6 ± 0.60 bc	6
JRP4	TWT	Mix	35.5	18.6 ± 0.91 a	6
AMP1	TWT	Mix	60.9	12.0 ± 0.13 bc	5
IVP3	TTT	Pure mutant	1.5	1.4 ± 0.10 e	6
BXPIA	TTT	Pure mutant	6.2	6.0 ± 0.19 d	6
VAHP1	TTT	Pure mutant	9.2	1.8 ± 0.08 e	6
MDMRP5	TTT	Pure mutant	20.5	11.0 ± 0.39 c	6
MDMRP7	TTT	Pure mutant	23.8	12.9 ± 0.37 b	6

*Comparison of mean relative quantitation of gene expression (RQ) means by Tukey's test (JMP v. 9, SAS Institute, Inc. Means with same letters are not significantly different at ∝=0.05, $P<0.001$



Objective 4. Initiate evaluation of epidemiological powdery mildew infection models under Virginia conditions.

Potted grape plants were grown in a greenhouse maintained at too high a temperature to allow powdery mildew development. Fresh plants were taken outdoors starting in early July of 2013 on an every 3- to 4-days schedule, and placed near a group of large potted grape plants heavily infected with powdery mildew. The test plants were carefully checked for powdery mildew development after 7, 10, and 14 days. Temperature, humidity, wind speed, solar radiation, and rainfall data were obtained from a VT College of Agriculture automated weather station and from the Blacksburg National Weather Service Office, and were supplemented by temperature, leaf wetness duration, rainfall, and pan evaporation data the location where the grape plants were kept.

Initial analysis of the powdery mildew data revealed fluctuations but not any clear pattern that could easily be related to weather conditions. Under shady conditions, the

Fig. 2. Powdery mildew ratings (% of leaf surface, mean of four most heavily infected leaves of each test plant), 14 days after the dates shown on the x-axis which was the date of first outdoor exposure. Test plants were in a location with near-continuous shade, or with sun exposure for at least 6 hours of the day.

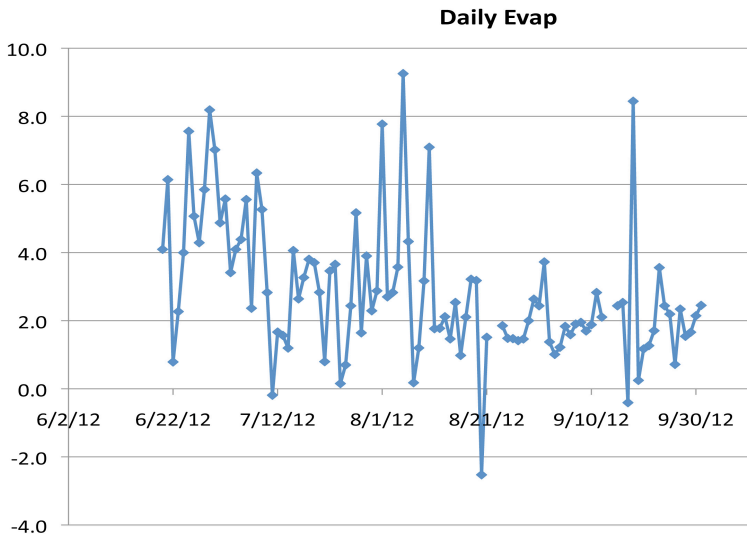


Fig. 3. Daily pan evaporation in Blacksburg, VA from Jun 20 to Sep 30, 2013. Water levels in the pan were corrected for rainfall, but some of the outlier data points occurred during heavy rain events, which may have caused losses due to splashing.

amount of infection sustained by test plant cohorts stayed fairly steady through July and August (Fig. 2), but under sunny conditions, disease levels declined, perhaps due to declining amounts of inoculum being available.

Pan evaporation (Fig. 3), the variable that in a recent report was correlated with cluster infection on a whole-season basis, occasionally produced anomalously high or low values during heavy rains, even if the water level in the pan was corrected for rainfall. Calculating expected pan evaporation from temperature, humidity, windspeed and other weather data using the Penman-Monteith equation may get around this. Analysis of the infection and weather data is still incomplete.

Report on experiments initiated in previous fiscal year
(Fiscal years ends in middle of growing season)

Fitness of QoI resistant isolates, summer 2012. This was a repeat of the 2011 experiment with mixed inoculations of small groups of potted plants. In 2011, the resistance percentage dropped, which was though to be due to influx of inoculum from outside the experiment. Modifications included using a 50 resistant:50 sensitive inoculum and closely monitoring the first appearance and severity of disease on non-inoculated and inoculated plants. Four plants per treatment (mixed, resistant only, sensitive only, non-inoculated control) were used, and new plants were added two weeks after inoculation to provide new host tissue for colonization. Leaves were sampled from all plants on June 15 and June 28. DNA samples were subjected to real time PCR for quantitation of the G143A mutation that is associated with resistance.

Severity ratings almost two weeks after inoculation were generally higher for plants inoculated with resistant population (R) than with sensitive population (S), suggesting a higher inoculum deposition for the former. No disease was detected on non-inoculated plants at this time. The non-inoculated control plants were checked regularly for the onset of infection. The final evaluation was on July 15, and only a few leaves showed visible powdery mildew growth (<1% surface area, data not shown).

Late June to mid-July samples taken from the non-inoculated plants did not have the G143A (0%) except for one leaf sample (5% G143A, Table 2), which indicates that some spore migration did occur in the field over 100 m despite the physical barriers. The R and S populations remained the same throughout the infection season, except for one R and one S leaf population, with 70% and 13% G143A, respectively (Table 2.6). This further supports the occurrence of spore migration in the field within four weeks of infection.

The plants receiving the mixed population consisting initially of 63% G143A developed a heterogeneous infection (27-100% G143A, Table 2). On the average, the powdery mildew populations on the small plants harbored 84% and 93% G143A. On field plants, these were 95% and 100% G143A two weeks after inoculation,

Table 2. Changes in QoI resistance (%G143A) in mixed resistant and sensitive *Erysiphe necator* populations cycled on fungicide-free grape plants under 2012 field conditions.

Source	%G143A[a]				
	Resistant	Sensitive	Mixed Population		Control[b]
May 31, inoculum	100	0	62.7		
			Site 2-R/S	Site 3-R/S	
June 1, first generation	100	0	84 (27-100)	93 (65-100)	
June 15					
Field plant A	100	0-0.01	73-100		-
Field plant B	100	0	81-100		-
Field plant C	100	0		96-100	-
Field plant D	72-100	0-0.7		100	-
Mean	98.5	0.001	95.4	99.9	
June 28-29					
Field plant E	100	0-13	67-100		0-5
Field plant F	60-100	0	62-100		(July 15)
Field plant G	100			45-91	
Field plant H	100	0		51-99	
Mean	97	3	86	74	0.5

[a] Five leaf populations per test cane and field plant

[b] Ten leaf populations from the non-inoculated control plants; sampling on June 21, 29 and July 15. Rows with dash (-) means no analysis because there was no visible powdery mildew growth.

and 85% and 75% another two weeks later, for the first and second sites, respectively. Throughout the monitoring period, resistance (>95% G143A) did not disappear from the population.

2012 Sentinel vines were placed at a commercial vineyard and sprayed weekly with a discriminatory dose of Quintec and Endura, the powdery mildew fungicides of concern at this location. The experiment was continued from June into September. Powdery mildew on control plants was limited (downy mildew was a greater problem this year), and no powdery mildew of concern was detected on treated plants.

Publications

Rallos, L E. E. 2012. Characterizing resistance of the grapevine powdery mildew *Erysiphe necator* to fungicides belonging to quinone outside inhibitors and demethylation inhibitors. Ph.D. Dissertation, Virginia Tech. 135 pp.

Presentations

Baudoin, A, 2013. Grape Disease Management: Back-to-Basics I. Powdery Mildew and Botrytis: Biology, management and research updates. Presentation at annual technical meeting of the Virginia Vineyards Association, Feb 2, 2013, Charlottesville, VA