Virginia Wine Board Project #13-1732-02 Progress Report - July 2014

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

With addendum: Characterization of Quintec-resistant powdery mildew isolates

Investigators

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Results and activities, by objective

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

A limited number of samples, primarily from grapes and strawberries, have been collected in the first half of 2014. One site with both strawberries and grapes in close proximity to each other was revisited, in order to estimate changes in Botrytis population since the previous year. Email messages were sent to the Viticulture Notes email list, and Virginia County Extension agriculture agents, but, as before, few responses have been received as of yet. Cumulative results of all 2013-14 testing are shown in Table 1

Grape berry inoculation tests are being continued with isolates with various degrees of resistance to cyprodinil (Vangard, similar to Scala), in order to mnore reliably distinguish resistant (R) from moderately resistant (mR) isolates.

Objective 3. Explore ergosterol biosynthesis inhibitor (EBI) resistance mechanisms in our powdery mildew collection other than target site mutations.

This was part of the 2-year project, but activities ended with graduate student graduation in December 2012, results reported previously.

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

All experiments have been reported in the January 2014 progress report

since june 2015, and do not mendee data from prior years.									
	Grapes			Ornamentals and strawberries					
	Sens*	Less sens	Mod res	Res	Sens	Less sens	Mod res	Res	
Thiophanate m	31			55	19		4	27	
QoI	15			68	25		3	21	
Fenhexamid	77			9	33			19	
Boscalid	20			66	32			18	
Fluopyram	74	10			45	6			
Cyprodinil	25		25	8	12		13	8	
Iprodione	48	26	10		22	14	12	3	
Fludioxonil	73	3			39	10			

Table 1. Numbers of isolates with various fungicide resistance levels of *Botrytis sp.* from Virginia grapes, as compared to isolates from ornamentals and strawberries. Results are since June 2013, and do not include data from prior years.

*Sens=sensitive, Less sens=less sensitive, Mod res=moderately resistant, Res=resistant

Objective 5, Characterization of Quintec-resistant powdery mildew isolates, and determining extent of their distribution (Added Dec 2013)

In the fall of 2013, we collected powdery mildew samples from a commercial vineyard in western Virginia where the grower complained of difficulties in maintaining powdery mildew control with Quintec. All isolates collected turned out to be resistant to Quintec, and are referred to here as "QR". QR isolates developed extensive powdery mildew infections on plants treated with commercial labeled rates of Quintec. Germination of QR spores was still inhibited to a variable extent on Quintec-treated leaves compared to non-treated leaves (Figure 1), but normally sensitive powdery mildew isolates do not grow at all on leaves treated with any of the concentrations shown (except 0).

Single-spored isolates were prepared from the field-collected powdery mildew. Some were maintained continuously on untreated leaves (QRC) and some were maintained on leaves treated with 30 ppm quinoxyfen. Both approaches allowed for maintenance of the isolates for 8 months as of this writing, so they appeared to be quite stable.

Another question to be addressed is how competitive the QR isolates are. If their capability for growth, sporulation, or infection is reduced by the quinoxyfen resistance, they may gradually disappear in a field population if quinoxyfen is not applied. In order to gauge competitiveness, QR isolates were paired with sensitive isolates and applied to leaves as 50-50 spore suspensions. The mixtures were allowed to grow and spore suspensions harvested approximately every 2 weeks to start a new generation. In order to determine whether quinoxyfen resistance survived in the mixture after a number of such generations, spore suspensions of the mixture as well as the constituting pure isolates were applied to

quinoxyfen-treated as well as untreated leaves. In all tests completed so far, quinoxyfen resistance persisted in the mixtures for up to 7 generations, often in substantial amounts Table 2). Since the genetic basis of quinoxyfen resistance is not known, it is not possible to use the molecular techniques that we previously used to measure QoI resistance. However, we will attempt to develop techniques to generate more precise estimates of percent quinoxyfen resistance.

A small-plot field trial was initiated in the commercial vineyard where quinoxyfen resistance had been detected, with the purpose of determining whether Quintec still retained any residual efficacy against powdery mildew. Other than in this trial, no Quintec will be applied to this vineyard this season. Applications were made on Jun 12, Jun 26, Jul 9 and Jul 23. Unfortunately a mistaken vineyard application in late June affected a portion of the plots. Powdery mildew samples have been collected at the time of each of these applications, and in very preliminary tests, 3 of 7 isolates were sensitive to quinoxyfen, indicating that there is still a mix at this location.

In addition, potted plants have been deployed at two vineyards located 5-10 miles away from the affected location. Some of the plants are sprayed weekly with a low rate of quinoxyfen and some are left untreated, in the hope of "catching" any QR or other mildew isolates without interference from the vineyard spray record. The very first isolates from these plants were collected in July (no detectable powdery mildew yet in the vineyards).

Publications

- Rallos, Lynn Esther E., Johnson, Nels G., Schmale David G. III, Prussin Aaron J. II, and Baudoin Anton B. 2013. Fitness of G143A-based Resistance to QoIs in *Erysiphe necator* Populations. Plant Disease (accepted)
- Baudoin, A. 2013. Survey of fungicide resistance of *Botrytis cinerea* in Virginia vineyards. (Abstr.) Phytopathology 103(Suppl. 2):S2.1. http://dx.doi.org/10.1094/PHYTO-103-6-S2.1 Annual Meeting of the Potomac Division of the American Phytopathological Society.
- Baudoin, A. 2014. First confirmation of resistance to quinoxyfen in grape powdery mildew in North America. (Abstr.) Phytopathology 104 in Press. Annual Meeting of the Potomac Division of the American Phytopathological Society.



Figure 1. Percent germination and relative germ tube length of quinoxyfen-resistant *E. necator* on treated grape leaf pieces.

Expt	Generations	Comparison	QR estimate
1	2	Mix3 - QRC3 - Li9-3	High percent QR
2	3	Mix1 - QRT1 - BLP11	High % QR
3	2 or 3	Mix2 - QRC1 - BLP14	Very low % QR
4	4	Mix3 - QRC3 - Li9-3	Moderate % QR
5	5	Mix3 - QRC3 - Li9-3	Moderate % QR
6	6	Mix1 - QRT1 - BLP11	25-50% QR
7	3	Mix4 - QRT4 - SuP13	Substantial % QR
8	7	Mix2 - QRC1 - BLP14	Very high % QR

Table 2. Estimated percent quinoxyfen resistance in mixtures of QR and sensitive isolates after stated number of generations on untreated grape leaves.