Virginia Wine Board
Project #14-1675-02
Annual Progress Report - July 2015

Botrytis cinerea fungicide sensitivity evaluation in Virginia crops

Investigators
Anton Baudoin, Associate Professor, email: abaudoin@vt.edu
and Noah Adamo, Graduate Research Assistant
Dept. of Plant Pathology, Physiology and Weed Science
Virginia Tech, Blacksburg, VA 24061-0331
Tel. 540-231-5757. Fax (departmental) 540-231-7477
Collaborator: M. Nita, Winchester AHS AREC

Results and activities, by objective

In the late summer and fall of 2014, 46 samples were collected from vineyards located primarily in the Shenandoah Valley and central VA. During this same period, fungicide sensitivity profiles were generated for 59 samples collected from grapes (47 isolates) and strawberries and ornamentals (12 isolates), all but one of which were collected in the 2014 growing season (Table 1). Additional samples were collected from flower debris in June 2015, from which 77 isolates were recovered and are currently being processed.

Additional isolates were subjected to a second round of bioassays to clarify ambiguous fungicide sensitivity data from past years and these data used to amend the cumulative results of this survey (Table 2). 558 isolates have been collected, the majority of which have been tested. Some isolates still have to be assessed for sensitivity to one or more modes of action. Cumulatively, it seems that fenhexamid resistance is significantly more common in ornamentals and small fruits (34%) than grapes (8%). In contrast, while QoI resistance was common in both groups, grapes had more isolates with reduced sensitivity (81%) than other crops (62%). Fludioxonil remains an effective fungicide from a resistance development standpoint, with the vast majority of isolates from grapes and other crops displaying no reduction of sensitivity to this mode of action (96% and 81%, respectively). The isolates that did display reduced sensitivity to fludioxonil did so only at very low levels with one or two exceptions and likely do not constitute a practical control issue at this point. As reports of multiple drug resistance phenotypes associated with fludioxonil resistance are appearing in the literature, monitoring for fludioxonil resistance should continue in VA. Until a more thorough understanding of the gene flow between *Botrytis* populations on other crops is attained, we must not discount the possibility of fludioxonil resistance moving from ornamental production facilities or other crops to nearby vineyards.

Table 1. New tests: numbers of isolates of *Botrytis sp.* with various fungicide resistance levels, collected from Virginia grapes, or from ornamentals and strawberries. Results are since July 2014, and do not include data from prior years.

	Grapes				Ornamentals and strawberries			
	Sens*	Less sens	Mod res	Res	Sens	Less sens	Mod res	Res
Thiophanate m	12			35	1			11
QoI	9			38	1			11
Fenhexamid	40			7	7			5
Boscalid	11		1	35	1		3	8
Fluopyram	44	2	1		12			
Cyprodinil	20		26	1	3		8	1
Iprodione	29	7	11		2	4	5	1
Fludioxonil	46	1			12			

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

Table 2. Cumulative numbers of isolates of *Botrytis sp.* with various fungicide resistance levels, collected from Virginia grapes, or from ornamentals and strawberries. Results are from 2011-2014 survey and bioassays.

	Grapes				Ornamentals and strawberries			
	Sens*	Less sens	Mod res	Res	Sens	Less sens	Mod res	Res
Thiophanate m	107			231	35			62
QoI	63			268	36			59
Fenhexamid	310			29	65			34
Boscalid	86		24	222	54		6	38
Fluopyram**	154	52			60	22		
Cyprodinil	144	21	104	16	45	2	38	9
Iprodione	204	88	28	1	40	33	19	5
Fludioxonil	311	17			79	18		

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

**Fluopyram was not included in initial bioassays for fungicide resistance, hence the lower number of data points

As part of an effort to understand sources of resistance to fenhexamid (Elevate) identified in in vitro bioassays, 57 isolates underwent molecular analysis to determine whether the cryptic species *Botrytis pseudocinerea* is present in Virginia. *B. pseudocinerea* is a weak

pathogen not usually associated with berry rot, with innate, non-target-site-based resistance to fenhexamid. Determining whether fenhexamid resistance is due to the presence of this cryptic species may inform fungicide use strategies in VA, as this cryptic species does not represent a disease or control problem in vineyard production.

Of the 57 isolates being investigated, 53 isolates have been sequenced and identified as *Botrytis cinerea* using NCBI's Basic Local Alignment Search Tool (BLAST). Poor quality sequencing reads prevented identification of 4 isolates as of this date. The Erg27 gene of these isolates will be sequenced to identify the point mutations conferring fenhexamid resistance and these point mutations will be compared to get a sense of whether the resistance is emerging independently at different locations.

Grape berry inoculation tests with isolates with various degrees of resistance to cyprodinil (Vangard, similar to Scala) are ongoing, in order to more reliably distinguish resistant (R) from moderately resistant (mR) isolates. Results have indicated that in vitro distinctions between different sensitivity categories may be of practical value in grapes. The ability of isolates classified as resistant to colonize grapes treated with cyprodinil was significantly greater than that of isolates termed moderately resistant and sensitive (Table 3).

Table 3. Relative fitness of isolates sensitive (s), moderately resistant (mR), and resistant (R) to cyprodinil.

		Average % of control	Average % coverage of berry surface			
Isolate	Type	Growth In Vitro	In Vivo 4 DAI-**CON**	In Vivo 4 DAI-**CYP**	In Vivo 8 DAI-**CON**	In Vivo 8 DAI-**CYP**
167	s	22	2.5	1.5	8	16.5
175	s	20	5	1.5	11	4
141	mR	45	5	2.5	22	18
241	mR	65	1.6	0	8.3	15
269	mR	56	12.5	6	33	15
317	mR	55	13.5	4	41	21
60	R	100	19	11	64	70
151	R	81	8	13.5	64	71

Additionally, several dozen isolates have been assessed for sensitivity to the eight fungicides we typically evaluate (boscalid, fluopyram, thiophanate-methyl, fludioxonil, fenhexamid, cyprodinil, iprodione, QoI) using the quick-turn-around Profile 24 system developed by Drs. Anchour Amiri and Guido Schnabel and employed by Schnabel's group at Clemson University, with a single discriminatory concentration (suggested by G. Schnabel's

group) for each fungicide. The correct classification rate overall (based on previously determined resistance status) was 80% (Fig. 1). Boscalid, cyprodinil and iprodione resistance was most often misclassified. Nevertheless, this system appears to be a potentially powerful tool for performing quick, preliminary fungicide sensitivity screening.

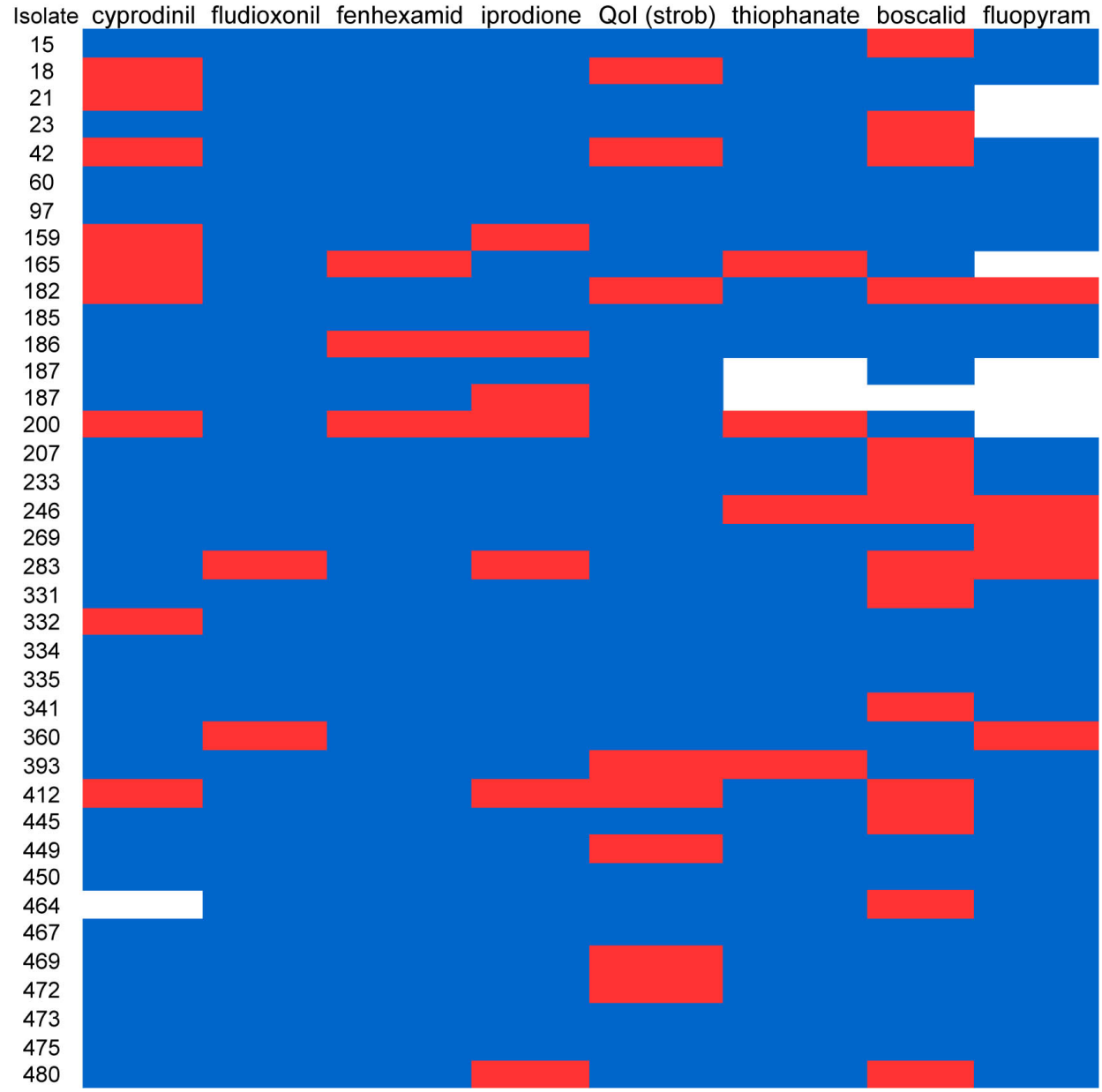

Fig 1. Heat map displaying correct (blue) and incorrect (red) fungicide resistance classifications as determined by Profile plate system. Blank cells had bacterial contamination issues or had no fluopyram data with which to compare Profile results.

Appendices

Publications about this and related research (entire year)

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 98: 1494-1502

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.

Rouxel, M., P. Mestre, A. Baudoin, O. Carisse, L. Delière, M.A. Ellis, D. Gadoury, J. Lu, M. Nita, S. Richard-Cervera, A. Schilder, A. Wise, and F. Delmotte. 2014. Geographic distribution of species of Plasmopara viticola causing downy mildew on wild and cultivated grapes in eastern North America. Phytopathology 104:692-701.

Colcol, J.F. and A. B. Baudoin. 2015. Sensitivity of *Erysiphe necator* and *Plasmopara viticola* in Virginia and nearby states to QoI fungicides, boscalid, quinoxyfen, thiophanate methyl, and mefenoxam. Plant Disease 100: (in press)

Impact statement

We have monitoring fungicide resistance in Virginia grape diseases for almost a decade, collecting samples from all areas of the state, as well as processing samples submitted by extension personnel and growers. Resistance of powdery and downy mildew as well as Botrytis bunch rot to a variety of commonly fungicides has been detected and its distribution documented. In 2013, we discovered quinoxyfen resistance in grape powdery mildew, the first detection in North America. Providing fungicide resistance information to growers will allow them to choose fungicides that are still effective at their location.