Virginia Wine Board
Project #15-1643-01
Semi-annual Progress Report - December 2015

Botrytis cinerea fungicide sensitivity evaluation in Virginia crops

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Results and activities, by objective

During late summer and fall of 2015, fungicide resistance profiling was completed for 61 isolates collected during spring and early summer of 2015 (see Table 1). The isolates collected and evaluated during this period largely comprise *Botrytis cinerea* recovered from grape flower debris collected from sites at which isolates had previously been isolated from clusters in fall of 2014 (54 isolates). Resistance to QoI and thiohanate methyl was comparably common in isolates isolated both early and late in the growing season from flower or clusters, while resistance to cyprodinil and lessened sensitivity (but not true resistance) to fludioxonil and fluopyram was similarly uncommon at both points in the growing season. Resistance to fenhexamid, iprodione, boscalid, and moderate resistance to cyprodinil were considerably less common in isolates isolated early in the growing season from flower debris than in isolates collected from clusters later in the growing season.

Table 1. Number and percentage of isolates of *Botrytis cinerea*, with various fungicide resistance levels, collected from Virginia grapes (n=59), or from ornamentals (n=2). Results are since July 2015.

	All *Botrytis*			
	Sensitive	Less sensitive	Moderately resistant	Resistant
Thiophanate methyl	17 (28%)			44 (72%)
QoI	8 (13%)			53 (87%)
Fenhexamid	59 (97%)			2 (3%)
Boscalid	10 (16%)		48 (79%)	3 (5%)
Fluopyram*	59 (97%)	2 (3%)		
Cyprodinil	45 (74%)		16 (26%)	
Iprodione	42 (71%)	15 (25%)	1 (2%)	1 (2%)
Fludioxonil	58 (95%)	3 (5%)		

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

Cumulative results from the fungicide resistance survey conducted over the 2011-2015 growing seasons are displayed below (Tables 2 and 3). Overall, the fungicide resistance trends in one crop are generally mirrored in the fungicide resistance phenotypes present in other crops. However, fenhexamid resistance was considerably more common in *Botrytis* recovered from the strawberries, ornamentals, and herbaceous crops sampled than in grapes. Similarly, phenotypes somewhat less sensitive to fludioxonil and the SDHI fungicide fluopyram were more prevalent in *Botrytis* from strawberries, ornamentals, and herbaceous crops than from grapes, but no moderate or high levels of resistance to these two chemistries were found in any of our isolates (data not shown).

Table 2. Cumulative numbers of isolates of *Botrytis cinerea* with various fungicide resistance levels, collected from Virginia grapes, ornamentals, and strawberries. Results are from 2011-2015 survey and bioassays.

	All *Botrytis*			
	Sensitive	Less sensitive	Moderately resistant	Resistant
Thiophanate methyl	164 (34%)			324 (66%)
QoI	112 (23%)			370 (77%)
Fenhexamid	437 (90%)			51 (10%)
Boscalid	154 (32%)		82 (17%)	249 (51%)
Fluopyram*	293 (85%)	52 (15%)		
Cyprodinil	241 (51%)		219 (46%)	15 (3%)
Iprodione	301 (63%)	122 (25%)	48 (10%)	8 (2%)
Fludioxonil	440 (92%)	37 (8%)		

Table 3. Table. Percentages of isolates of *Botrytis cinerea* with various fungicide resistance levels, collected from Virginia grapes, or from ornamentals and strawberries. Results are from 2011-2015 survey and bioassays.

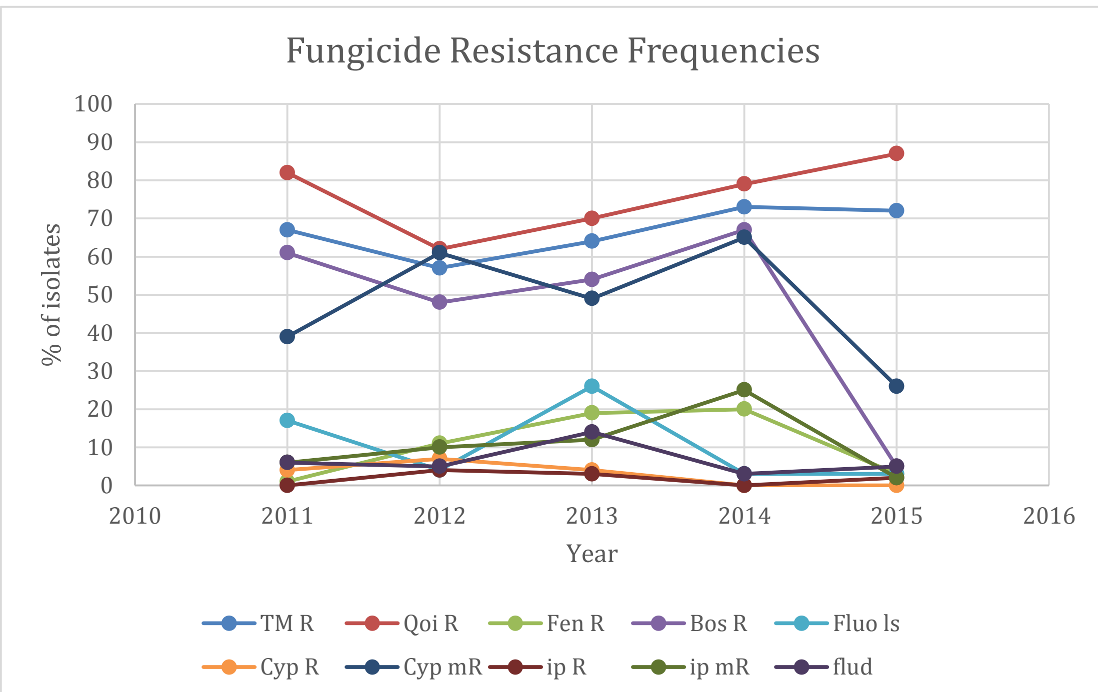
	Grapes				Ornamentals and strawberries			
	Sens*	Less sens	Mod res	Res	Sens	Less sens	Mod res	Res
Thiophanate m	32			67.7	35.4			64.6
QoI	18.7			81	39.2			60.8
Fenhexamid	93			7.2	63.6			36.4
Boscalid	25.9		18.7	55.6	56.6		5.1	38.3
Fluopyram**	81.5	18.5			69.2	30.8		
Cyprodinil	52.4		44.6	3.0	45.7		50	4.3
Iprodione	66.3	24.6	8.1	1	45.8	30.2	19.8	4.2
Fludioxonil	94.9	5.1			81.4	18.6		

*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

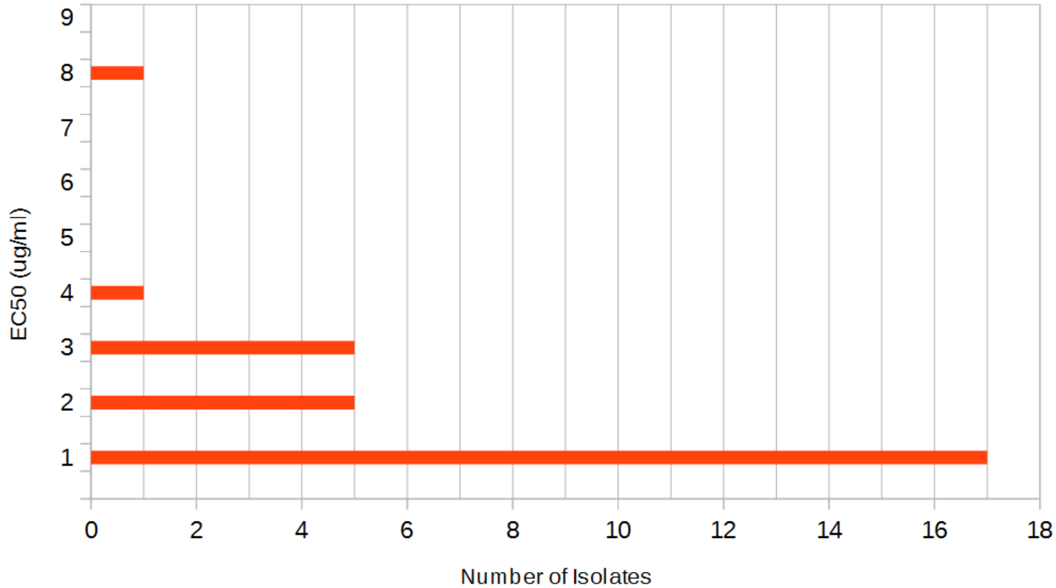
While there was considerable variability between the fungicide resistance phenotypes present in *Botrytis* collected from grape flower debris as opposed to clusters (2014-2015), and while some differences were present between populations isolated from host crops, for the majority of this study's duration, cumulative resistance frequencies remained relatively static from 2011 to 2014 (Figure 1). Resistance to trifloxystrobin (QoI) and thiophanate-methyl was common and widespread, as was boscalid resistance and moderate resistance to cyprodinil. While other forms of resistance were less common, the presence of reduced sensitivity (if not strong resistance) to every chemistry we evaluated in this study suggestes the need for ongoing fungicide resistance monitoring in VA vineyards. The relative abundance of fenhexamid resistance in strawberries and other host crops argues for targeted sampling in areas with heavy strawberry production side by side with wine grape cultivation, such as in the Eastern Shore/Hampton Roads region, as the effect of host crop on genetic exchange and fungicide resistance development in *Botrytis cinerea* is not yet clearly understood.

Figure 1. Histogram of various fungicide resistance phenotypes in *Botrytis cinerea* from VA crops from 2011-2015.



Preliminary evaluation of baseline sensitivity of *Botrytis* from VA crops to the FRAC 19 biofungicide polyoxin-D indicates that this chemistry may be a promising Botriticide for VA growers. EC50s for 29 isolates were in line with values from populations without historical use of polyoxin-D as reported in other studies, with the exception of one isolate, at 8.34 ug/ml (Figure 2). Further characterization of *in vitro* and *in vivo* activity of this fungicide against VA *Botrytis* should be conducted to determine the durability of this mode of action and to provide better recommendations for future use at the field scale.

Figure 2. Frequency distribution of polyoxin sensitivity (EC50) of 29 isolates of *B. cinerea* collected from VA grapes, strawberries, ornamentals, and herbaceous plants from 2011 to 2015.



Additionally, 27 of 55 isolates resistant to fenhexamid have undergone molecular analysis of the Erg27 gene to determine the mechanisms of fenhexamid resistance present in VA and to determine whether evidence exists that this resistance is being transported from one site to another around the state (Figure 3). While the F412S point mutation was by far the most prevalent fenhexamid-resistance-conferring point mutation identified, there were isolates with mutations including F412I and T63I as well. This indicates that resistance to fenhexamid may be emerging independently at different sites in response to fenhexamid use, as opposed to having a single origin point, and calls for ongoing monitoring of the fenhexamid resistance situation around the state.

Figure 3. Alignment of isolates resistant and sensitive to fenhexamid showing resistance conferring point mutations at position 412, 108, and 63 of the Erg27 gene.