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Botrytis cinerea fungicide sensitivity evaluation in Virginia crops

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Results and activities, by objective

Most of the second half of the 2015-2016 grant period was spent on data analysis and writing of graduate student Noah Adamo's thesis, which he successfully defended in May 2016.

The overall fungicide resistance frequencies for 488 isolates collected from 2011 through 2015 are shown in Table 1, separately for samples collected from wine grapes or from strawberries and ornamentals. These frequencies have not changed a great deal over these years (Table 2). In grapes, isolates resistant to thiophanate methyl, the QoI fungicides (Abound, Flint, etc.), and boscalid (Endura, Pristine) are common and widespread. Fenhexamid (Elevate) resistance remains less common in grapes than in ornamentals and strawberries.

Table 1. Cumulative percentages of isolates of *Botrytis cinerea* with various fungicide resistance levels, collected from Virginia grapes, or from ornamentals and strawberries from 2011 to 2015.

	Grapes				Ornamentals and strawberries			
	Sens [a]	Less sens	Mod res	Res	Sens	Less sens	Mod res	Res
Thiophanate m	33			67	35			65
QoI	19			81	39			61
Fenhexamid	95			5	64			36
Boscalid	26		19	55	57		5	38
Fluopyram [b]	86	14			69	31		
Cyprodinil	52		45	3	46		50	4
Iprodione	67	24	8	1	46	30	20	4
Fludioxonil	95	5			81	19		

[a] Sens=sensitive, Less sens=less sensitive, Mod res=moderately resistant, Res=resistant
[b] Fluopyram was not included in initial bioassays for fungicide resistance, hence a lower number of data points

Table 2. Changes in resistance frequencies: percentage of *B. cinerea* isolates collected in Virginia and North Carolina from grapes, strawberries, and ornamentals from 2011 to 2015 that were resistant (R), resistant or moderately resistant (R/MR), or less sensitive (Ls) to eight fungicides.

Fungicide (resistance level)		Isolates (%) 2011 (n=153)[a]	2012 (n=46)	2013 (n=161)	2014 (n=66)	2015 (n=61)
Thiophanate-methyl	(R)	67	57	64	73	72
Trifloxystrobin (QoI)	(R)	82	62	70	79	87
Fenhexamid	(R)	1	11	19	20	3
Boscalid	(R/mR)	72	52	60	76	84
Fluopyram	(Ls)	17	4	26	3	3
Cyprodinil	(R/mR)	43	68	53	65	26
Iprodione	(R/mR)	6	14	15	25	4
Fludioxonil	(Ls)	6	5	14	3	5

[a] Within parentheses, numbers of isolates of *B. cinerea* collected and characterized each year**.**

The isolates collected and evaluated in 2015-16 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. Resistance to QoI and thiophanate 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 (Table 3). 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 late in the previous season.

Table 3. Fungicide sensitivities of isolates of *B. cinerea* collected from wine grape clusters or wine grape flowers.

	2014 Cluster isolates (%) (n=46)[a]				2015 Flower isolates (%) (n=58)			
	s[b]	ls	mR	R	s	ls	mR	R
Thiophanate- methyl	28			72	28			72
Trifloxystrobin	20			80	12			88
Fenhexamid	89			11	97			3
Boscalid	24		7	69	16		81	3
Fluopyram	96	4			97	3		
Cyprodinil	43		57		74		26	
Iprodione	64	14	22		72	26	2	
Fludioxonil	96	4			95	5		

[a] Within parentheses: number of isolates of *B. cinerea* collected and characterized
[b] s=sensitive, ls=less sensitive, mR=moderately resistant, R=resistant

The resistance to fenhexamid reported in Tables 1-3 is resistance during germ tube elongation, and was found in only 5% of wine grape isolates, but in 33% of isolates from strawberries and ornamentals. All of the fenhexamid-resistant isolates were identified as *B. cinerea* carrying various mutations in the *erg27* gene. An additional subset of isolates was identified with moderate resistance to fenhexamid during mycelial growth, but not germination and germ tube growth. These were identified as *B. cinerea* HydR2 isolates, which possess an unknown mechanism of resistance towards fenhexamid in mycelial growth, and, based on experience in Europe, may be of little practical concern. *Botrytis pseudocinerea* was not identified in any of our tests; it is a species reported only in Europe that can be distinguished only by molecular tests, is most common early in the season, and has a natural resistance of mycelial growth to fenhexamid, which we thought might cause confusion in our interpretation of fenhexamid results, but doesn't appear to be a factor.

In our bioassays, both the germ tube elongation assay of Weber and Hahn 2011 (J. Plant. Dis. Protect. 118: 17-25) and the multiwell assay by Schnabel et al. 2012 (Schnabel, G., Amiri, A., and Brannen, P.M. 2012. Field kit- and internet-supported fungicide resistance monitoring. In: Fungicide Resistance in Crop Protection: Risk and Management. Ed. T. S. Thind. CAB International, Wallingford Oxfordshire, UK, p 116-132) were compared (Table 4). The same answer was usually obtained with both techniques; only borderline resistance types (e.g., less sensitive versus moderate resistance) were sometimes classified differently.

Table 4. Attributes of germ tube elongation-type and 24-well plate-type bioassays for fungicide sensitivity against *B. cinerea*.

	Germ tube elongation	**24-well plate**
Media Preparation[a]	equivalent	equivalent
Assay Setup[b]	60 min.	30 min.
Incubation	12-18 h.	72 h.
Evaluation	90-180 min.	15-30 min.
Data	quantitative; incidence can be inferred by approximating proportion of non-germinated conidia	qualitative

[a] When preparing 100 ml of medium per treatment
[b] When evaluating six isolates per assay

Moderate resistance to cyprodinil (Vangard) was common (45% of grape isolates, Table 1) but in grape inoculation tests, moderately resistant isolates were controlled almost as well as sensitive isolates by field rates of cyprodinil (Table 5). Resistance levels that could not be controlled by field rates were found in only 3% of isolates. That doesn't mean that moderate resistance is of no concern: fungicide concentration on the grapevine immediately after application (which is when the isolates were tested) will be much higher than after 1 or 2 weeks exposure to weather, so some erosion of efficicacy can be expected in the presence of moderaly resistant isolates.

Table 5. Control of isolates s, mR, and R to cyprodinil on table grapes treated with different rates of cyprodinil, 4 days after inoculation.

		Lesion coverage (% of surface)			Incidence (% diseased berries)		
Isolate	Type	Control	Cyprodinil – Low[a]	Cyprodinil – High[a]	Control	Cyprodinil – Low[a]	Cyprodinil – High[a]
43	s	81	5	0	100	60	0
156	s	80.5	2.5	0	100	40	0
444	s	68	2	1	100	30	20
188	mR	81	8.5	2.5	100	80	40
256	mR	86.5	10	2	100	90	30
446	mR	78	5	2	100	50	40
71	R	82.5	37	19	100	100	70
151	R	96	85	75	100	100	100
344	R	79	33.5	17	100	100	70
Mean	s	76.5 b[b]	3.3 e	0.3 e	100 a	43.3 b	6.7 c
Mean	mR	81.8 ab	7.8 e	2.2 e	100 a	73.3 a	36.7 b
Mean	R	85.8 a	51.2 c	37.0 d	100 a	100.0 a	80.0 a

[a] Low = 150 µg/ml cyprodinil. High = 560 µg/ml cyprodinil, equivalent to 10 oz/100 gal
[b] Numbers followed by the same letter are not significantly different at α=0.05

Diminished sensitivity (perhaps a very low degree of resistance) to fludioxonil and fluopyram was found in only a few isolates. Fluopyram (Luna, from Bayer), as well as benzovindiflupyr (Aprovia, from Syngenta) and isofetamid (Kenja, from SummitAgro), two newly registered fungicides with activity against Botrytis as well as powdery mildew, are thought to have the same mode of action (succinate dehydrogenase inhibition) as boscalid (Endura); all of these are designated as FRAC Group 7. Although boscalid resistance is widespread, fluopyram resistance is not, and initial bioassays of Virginia Botrytis isolates against the new fungicides indicate that several of our boscalid-resistant isolates are also still sensitive to Aprovia and Kenja. Additional isolates will be tested.

Preliminary evaluation of baseline sensitivity of *Botrytis* from VA crops to the FRAC 19 biofungicide polyoxin-D suggest that this chemistry may be a promising botryticide for VA growers. EC50s for 29 of our isolates were in line with values from populations elsewhere without historical use of polyoxin-D as reported in other studies, with the exception of one isolate, which was a little less sensitive at 8.34 µg/ml (Figure 1). A field trial including polyoxin-D, benzovindiflupyr, and isofetamid has been initiated.

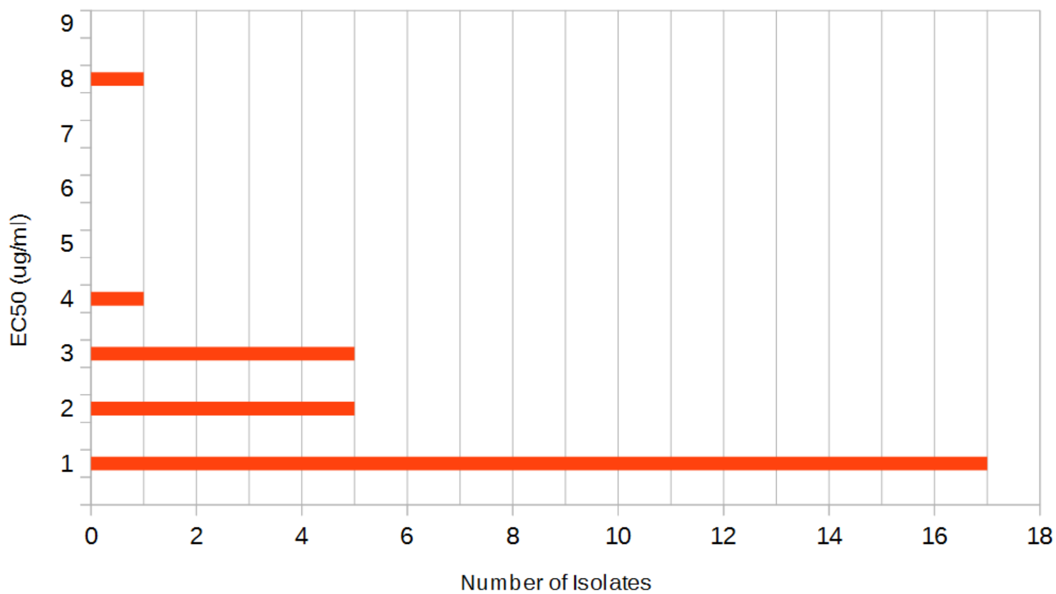

Figure 1. Frequency distribution of polyoxin sensitivity (EC50) of 29 isolates of *B. cinerea* collected from VA grapes, strawberries, ornamentals, and herbaceous plants from 2011 to 2015.

Publications

Adamo, N. and Baudoin, A. 2016. Fungicide resistance of *Botrytis cinerea* from Virginia grapes, strawberries, and ornamentals. Phytopathology 106: S3.1 (Abstr.) http://dx.doi.org/10.1094/PHYTO-106-5-S3.1

Adamo, Noah R. 2016. Fungicide resistance of *Botrytis cinerea* from Virginia wine grapes, strawberry, and ornamental crops, MS Thesis, Virginia Tech, 52 pp. (will in due time become available via http://vtechworks.lib.vt.edu/handle/10919/5534, and may be requested from nadamo1@vt.edu or abaudoin@vt.edu.