Downy mildew resistance to QoI fungicides is rampant in Georgia vineyards

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Georgia's wine grape industry is relatively young and fragile. Though vinifera and hybrid vineyards have been in production in Georgia for approximately 40 years, the industry has only shown significant growth in the last 10-15 years. Wine grapes are contributing significantly to the Georgia economy even though acreage pales in comparison to that of North Carolina and Virginia. Georgia vineyards are concentrated in the Valley and Ridge and Blue Ridge geographic regions of northern Georgia and the Piedmont of west Georgia. However, hybrid grapes are now being grown in the southern Coastal Plains, so wine grapes are actually becoming a statewide enterprise. Many grape species and hybrids share one significant issue in common – susceptibility to a disease called downy mildew. Arguably, downy mildew may be the most difficult to control disease in Georgia grapes, and this is true for most if not all of the Southeast.

Downy mildew is caused by the oomycete *Plasmopara viticola*. Though downy mildew is an important disease for grape growers worldwide, the southeastern environment is perfect for disease development – generally humid and wet for much of the growing season. *P. viticola* infects and reproduces on berries (Fig. 1), pedicels, and the undersides of grape leaves (Fig. 2), reducing photosynthesis and rendering the fruit unusable. In severe cases, leaf drop (Fig. 2) will decrease the vine's overwintering potential because of nutrient deprivation, which leads to winter injury/kill. Downy mildew also lowers yield and has a substantively negative impact on the resulting wine quality due to both direct (infected fruits) or indirect (reduced photosynthates and poor grape quality) effects.

Figure 1. Downy mildew sporulation on young grape clusters (photo courtesy of C.F. Hong; University of Georgia Plant Pathology Department).

Figure 2. Downy mildew sporulation on the underside of leaves (left) and subsequent defoliation (right).

Downy mildew thrives on *V. vinifera*, hybrids and even some natives, which account for most of the cultivars grown in Georgia's wine industry. To minimize downy mildew and other diseases, grape growers spray fungicides throughout the season and even after harvest (to protect leaves). Due to the season-long infection potential of *P. viticola*, vineyard managers may employ as many as thirteen to seventeen downy mildew sprays in any given growing year. Because of the numerous applications of oomycete-active materials applied, resistance can readily develop to different chemical classes utilized to control downy mildew.

Spraying fungicides with the same single-site mode-of-action active ingredient repeatedly selects for individuals who are less sensitive. These less sensitive individuals and their progeny remain in the vineyard and cause more damage – often as if no fungicide had been applied. Fungicide resistance in *P. viticola* is well studied for many classes of fungicides and a variety of geographic locations. The main classes of fungicides studied for resistance are the quinone outside inhibitors (QoI), carboxylic acid amides (CAA), phenylamides (PA), and cyano-acetamide oximes, all of which utilize a single mode of action to impede downy mildew (Gisi and Sierotzki 2008). These fungicide classes have been evaluated for grape growing regions in Europe, China, Japan, and the US. However, a study of fungicide resistance had not been conducted in Georgia.

In 2017, a survey of fungicide sensitivity was performed on downy mildew isolates from throughout the state. Leaves with downy mildew were collected from multiple commercial Georgia vineyards and tested for genetic mutations known to confer resistance and/or tested in bioassays to further confirm resistance. The main mutation known to cause QoI resistance is the G143A mutation, which is a point mutation in the cytochrome b gene that changes the resulting amino acid from glycine to alanine (Baudoin et al. 2007). This mutation is known to confer total resistance to the entire QoI fungicide class. The known mutations for CAA resistance, point mutations in the PvCesA3 gene, also show cross resistance among the CAA fungicides (Nanni et al. 2016; Zhang et al. 2017). The presence of these mutations was evaluated using PCR for the samples collected in 2017 and on DNA isolated from Georgia downy mildew samples collected in previous years by Cheng-Fang Hong, a Ph.D. student at the University of Georgia.

To further confirm the molecular results for fungicide sensitivity, isolates were also tested against a discriminatory dose of a formulated commercial QoI and CAA fungicide in a leaf disc bioassay. The QoI active ingredient azoxystrobin (Abound) and the CAA active ingredient mandipropamid (Revus) were utilized. This bioassay was largely derived from the Fungicide Resistance Action Committee's (FRAC) microtiter plate test for fungicide sensitivity (Sierotzki and Kraus 2003). A bioassay test was also conducted against a range of fungicide concentrations of the PA fungicide mefenoxam (Ridomil), to determine sensitivity of this class. Testing the PA fungicides against a range of concentrations is necessary, as no molecular markers for resistance have been identified. Results indicated that QoI resistance is widespread among *P. viticola* populations throughout Georgia (Table 1 and Fig. 1). However, neither CAA nor PA resistance was observed at any vineyard in Georgia (Table 1). This is fortunate, as CAA resistance has been found in Virginia and North Carolina within the last few years.

Table 1. Downy mildew resistance to QoI (Abound and Pristine; azoxystrobin and pyraclostrobin; FRAC 11), CAA (Revus and one component of Zampro; mandipropamid and dimethomorph; FRAC 40), and PA (Ridomil; mefenoxam; FRAC 4) fungicide classes in 2017 Georgia surveys.

Percent Resistance by County

		Bioassay			PCR	
County	N*	QoI	CAA	PA	QoI	CAA
Cobb	8 (1)	100	0	0	100	0
Colquitt	28 (1)	0	0	0	28.6	0
Fannin	8 (1)	100	0	0	100	0
Gilmer	9 (2)	100	0	0	100	0
Haralson	4 (1)	100	0	0	-	-
Rabun	8 (1)	100	0	0	100	0
White	13 (4)	100	0	0	100	0

*Number of isolates (vineyards sampled)

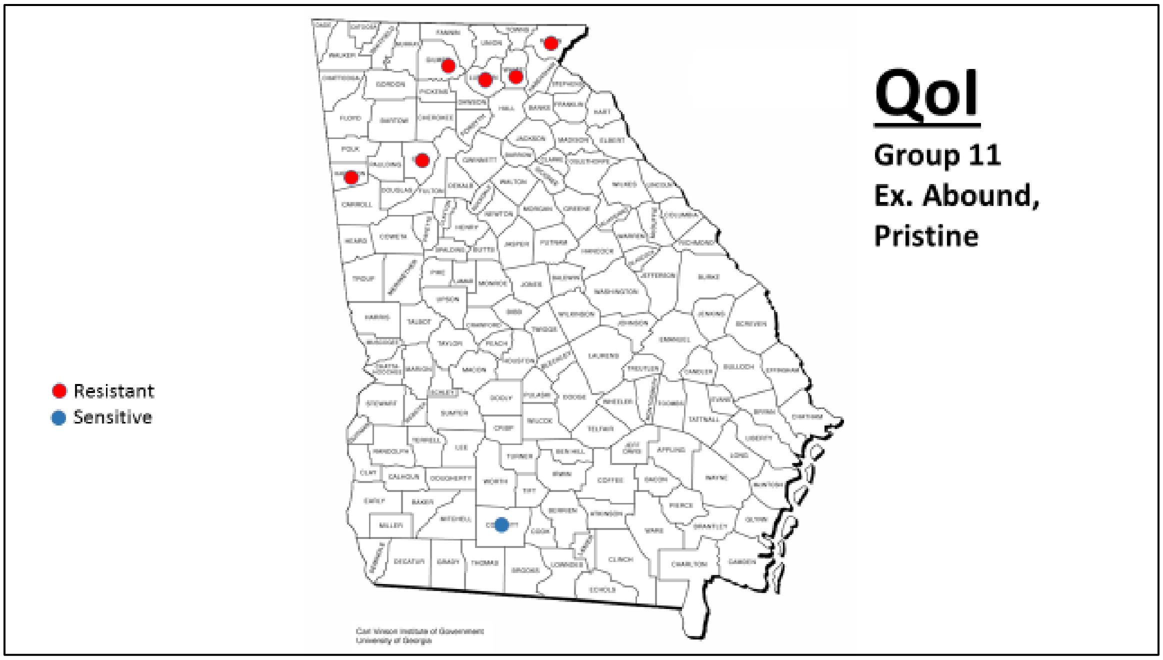

Figure 3. Survey results by county of *Plasmopara viticola* (downy mildew) resistance to QoI (FRAC 11) fungicides in Georgia (2017).

In addition to field surveys for downy mildew resistance, efficacy of eight different fungicides and two combinations (10 total treatments) were tested for downy mildew control at three locations (the University of Georgia Research and Education Center in Blairsville, GA and two commercial vineyards). Rates were calculated to correspond with a 50 gallon per acre spray volume, and applications were made at bloom, post-bloom, bunch closure, and second cover. Treatments included: (1) Abound, (2) Captan, (3) Pristine, (4) Prophyt, (5) Revus, (6) Revus Top, (7) Ranman, (8) Zampro, (9) Prophyt + Captan, and (10) Prophyt + Ranman.

In these trials, fungicides separated into three efficacy categories: (1) high efficacy – Revus, Zampro, Revus Top, Prophyt + Captan and Prophyt + Ranman; (2) good efficacy – Ranman, Captan, and Prophyt; and (3) no efficacy – Abound and Pristine (essentially the same as an untreated control) (Fig. 4). Downy mildew from these sites showed significant resistance to the QoI fungicides, as evidenced by the G143A mutation in the mitochondrial genome and bioassays. These trials further clearly document field resistance of downy mildew to the QoI fungicides azoxystrobin (Abound) and pyraclostrobin (Pristine) at these sites and confirm the total lack of activity by these fungicides in most sites in Georgia.

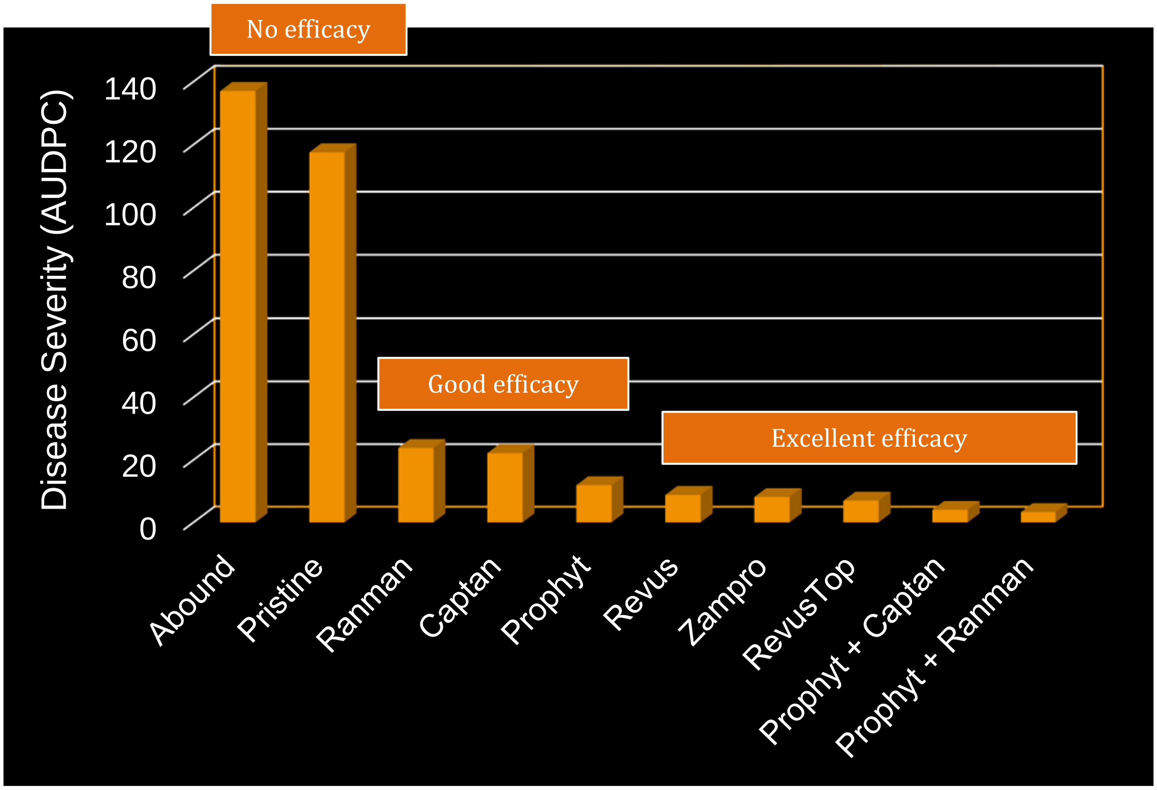

Figure 4. Efficacy (disease severity response as measured by the area under the disease progress curve [AUDPC]) of downy mildew active materials averaged across three trial sites in 2017. The QoI-containing products Abound and Pristine did not provide downy mildew control, and all three sites experienced a "field failure" where these materials were applied. Ranman, Captan, and Prophyt provided good control, but it is advisable that these materials be utilized in tank mixes such as Prophyt + Captan or Prophyt + Ranman for optimal efficacy and resistance management.

In conclusion, resistance to the strobilurin (quinone outside inhibitor [QoI]; Fungicide Resistance Action Committee [FRAC] class 11) is widespread, and these fungicides (Abound, Pristine, Sovran, Flint), when utilized for control of other diseases, should always be mixed with mancozeb (early season) or Captan products at a minimum to increase or provide downy mildew control. Ridomil (mefenoxam) is still active, as is Revus and Zampro. Neither mancozeb nor Captan products are known to develop resistance, so maximum use of these products for downy mildew management should be encouraged. For the other classes with activity against downy mildew (Table 2), it is recommended that vineyard managers limit themselves to one application per season when possible – targeting periods with increased and sustained precipitation. Rotation among all active chemical classes will require producers to purchase multiple chemicals that will be utilized only once per season, but alternation of chemical classes is critical to maintaining these fungicides for years to come. We have already essentially lost the QoIs, and we simply can't afford to lose more classes if we are to manage this aggressive disease in the future.

Table 2. List of downy mildew active materials, Fungicide Resistance Action Committee (FRAC) codes, and efficacy ratings with notes.

Fungicides	FRAC Code	Efficacy
Ametocradin + dimethomorph (Zampro)	40 + 45	+++++ Systemic
Azoxystrobin (Abound)	11	??? Systemic (Resistance prevalent; always mix with mancozeb or Captan)
Boscalid + Pyraclostrobin (Pristine)	7 + 11	??? Systemic (Resistance prevalent; always mix with mancozeb or Captan)
Captan	M4	++++ Contact protectant; combine with Phosphonates
Cyazofamid (Ranman)	21	++++ Locally systemic; combine with Phosphonates
Famoxadone + Cymoxanil (Tanos)	11 + 27	++++ Use with Captan or mancozeb (required)
Mancozeb	M3	++++ Contact protectant
Mandipropamid (Revus)	40	+++++ Translaminar protectant
Mandipropamid + Difenoconazole (Revus Top)	3 + 40	+++++ Translaminar protectant
Mefanoxam + Mancozeb (Ridomil Gold MZ)	4 + M3	+++++ Systemic + contact protectant
Phosphonates (Prophyt, etc.)	33	++++ Systemic (combine with Captan)
Ziram	M3	++++ Contact protectant
Zoxamide + Mancozeb (Gavel)	22 + M3	++++ Contact protectant fungicides

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