Virginia Wine Board
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Evaluation of Powdery Mildew Quinoxyfen Resistance and Assessment of Variability of Grape Downy Mildew Sensitivity to Fungicides

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Objectives

1. Determine the frequency and geographic extent of quinoxyfen (Quintec) resistance (QR) of grape powdery mildew
2. Characterize QR isolates with respect to survival and competitiveness
3. Continue to collect grape downy mildew isolates from vineyards with histories of heavy phosphite fungicide use, and immediately assay their sensitivity to phosphite fungicide.
4. If resistance or reduced sensitivity is found, determine its stability by periodically assaying and comparing isolates maintained on treated as well as untreated plants.
5. Maintain the capability and conduct analyses of potential cases of resistance to at-risk fungicides, such as mandipropamid (Revus), fluopicolide (Presidio), and mefenoxam (Ridomil) for downy mildew, and boscalid (Endura, Pristine) for powdery mildew.

Activities and Results

Quintec – Powdery Mildew: In 2014, a field trial was completed at the western Virginia vineyard where quinoxyfen-resistant grape powdery mildew isolates had been collected in the fall of 2013 (QR vineyard). The trial was set up in two rows of Chambourcin with plots consisting of 4 vines. The main objective was to determine to what extent quinoxyfen might still be able to control or contribute to the control of powdery mildew; the trial consisted of 5 treatments as shown in the tables, each replicated 4 times. The early-season grower spray program consisted of mancozeb and sulfur. The first trial spray was applied on June 12 at approximately 60% bloom, 19 days after the last sulfur application.

However, on June 21, a large portion of the trial area was mistakenly treated with Endura plus sulfur. In order to even things out, the remaining parts of the trial were sprayed 5 days later with the same mix. Trial sprays were resumed on July 9, and were reapplied on Jul 23 and Aug 16 (when Rally at 3 oz/A was used instead of sulfur for the sake of rotation). Sulfur alone at 1.5 lb/A was applied as a rotation spray on Aug 7. Sprays were supplemented with 0.2 or 0.3% Prophyt and 1 lb/A mancozeb or (later applications) 10.4 oz/A Abound (powdery and downy mildew at this location were QoI resistant) for control of downy mildew and black rot.

Cluster infection was evaluated on July 29 (when berries were beginning to change color which would make it increasingly difficult to recognize powdery mildew cluster infection); two evaluators each rated 15 clusters on each side of each plot (60 cluster ratings per plot) (Table 1).

Leaf infection was slow to develop. The last application targeting powdery mildew was made on August 16, and foliar powdery mildew was rated on September 14 (Table 1).

Table 1. Powdery mildew infection of clusters and leaves, 2014.

	Cluster severity* July 29		Leaf infection* colonies/2 min** Sep 14	
T1 Non-treated control ***	14.3	A	74.6	A
T2 Quintec, 4 fl oz	0.3	C	3.5	B
T3 Endura, 4.5 oz or 3 oz Rally + 1 lb Microthiol Disperss	0.8	BC	1.4	B
T4 Vivando, 10.4 fl oz/A	1.6	B	0.4	B
T5 Quintec, 4 fl oz + Endura, 4.5 oz / 3 oz Rally + 1 lb Microthiol Disperss	0.9	BC	0.2	B

* Both analyses based on log-transformed data; levels not connected by same letter are significantly different.

** Powdery mildew colonies on leaves per 2-minutes search for each side of each plot.*** Anti-downy and -black rot materials in all treatments.

Disease levels were fairly low, part of which may have been due to the accidental Endura application in late June that was applied to all treatments including the check. Even so, surprisingly, quinoxyfen still provided striking powdery mildew control, just as effective or almost as effective as the other treatments.

Powdery mildew isolates were collected between June 26 and September 27 in this vineyard. They were single-spored, and then individually bioassayed by inoculating them onto leaves treated with 10 or 30 ppm quinoxyfen. The majority (89 out of 141 isolates tested, or 63%) were quinoxyfen-resistant in these bioassays. The frequency of resistance as a proportion of the total number of isolates varied from 53% to 100% at individual

collection times, but displayed no increasing or decreasing trend in the course of the season.

In 2015, field trials were set up again at the same vineyard in two separate blocks. One set of treatments was initiated in the same Chambourcin rows that were used in 2014, and identical treatments are being applied to a row of Pinot noir with divided canopy. The treatment plan is essentially the same as in 2014, with the first trial spray applied on June 2 at 10-20% bloom, the second spray on June 15, and the third spray on June 29. Low levels of powdery mildew were found at that vineyard on Jun 29, but very little in the plots so far.

In 2014, powdery mildew isolates were collected at two additional vineyards, one about 5 miles to the west, the other one about 10 miles to the northeast of the QR vineyard. Four potted "sentinel" Chardonnay vines were stationed at each of these locations; two of them were sprayed on a regular basis with 10 ppm quinoxyfen and the remainder were non-treated controls. These plants were visited approximately every 14 days, and powdery mildew was collected as disease developed. A very limited number of powdery mildew colonies developed on the Quintec-treated plants, whereas moderate (northeast) to severe (west location) powdery mildew developed on non-treated plants. At the northeastern location 5 out of 31 isolates tested, or 16%, were able to grow on Quintec-treated leaves; at the western location 2 of 42 isolates tested, or 5%, were resistant. Surprisingly, QR isolates appeared to be just as likely to be collected from non-treated as from Quintec-treated plants.

In order to find molecular markers that can be used to differentiate powdery mildew isolates with resistance to QoI (strobilurin) fungicides and quinoxyfen, previously collected powdery mildew isolates were tested against 30 ppm trifloxystrobin (Flint) and 30 ppm quinoxyfen (Quintec) solutions. Ten resistant and ten sensitive isolates for each fungicide were selected, spores were collected and stored for DNA extraction and sequencing.

Downy mildew

In 2014, we obtained or collected downy mildew samples from 8 different vineyards. One set of samples came from a northern Virginia vineyard where phosphite fungicides may have been applied 10 or more times during the 2014 season, and there was nevertheless severe downy mildew development by the end of the season. However, when bioassayed 3 or 4 weeks after collection, this downy mildew collection did not exhibit detectable phosphite resistance. Phosphite application schedules (not replicated plots) were completed in two separate commercial vineyards in 2014. Thirty downy mildew isolates from these plots as well as past collections have been tested against mandipropamid (Revus, 10 ppm), mefenoxam (Ridomil, 1 ppm), phosphite (Prophyt (0.00625-0.1%), and fluopicolide (Presidio, 0.001-10 ppm) to establish discriminatory concentrations and quantify any variability in sensitivity. So far, no obvious reduction of sensitivity was found in tehse collections.

In an attempt to reduce variability in the bioassay method for grape downy mildew fungicide sensitivity, the role of inoculum volume was examined, comparing 10µl, 20µl, and

40µl sporangial suspension per inoculation. The result suggested that with a high sporangial concentration ($1x10^5$/ml), the higher volumes produced larger lesions even if the total number of sporangia in each inoculum drop was the same, but with a lower sporangial concentration ($1x10^4$/ml), the volume appeared to make little difference.

A mixture of four downy mildew isolates suspected of possible reduced phosphite sensitivity was inoculated onto plants treated with 0.2% and 0.1% phosphite (Prophyt), but we were not able to maintain the pathogen on he plants through sequential transfers.

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: S3.160. (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; it's geographic distribution and impact on practical quinoxyfen efficacy is being investigated. Providing fungicide resistance information to growers will allow them to choose fungicides that are still effective at their location.