Virginia Wine Board Project #15-1644-01 **Semi-annual Report - December 2015**

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

Investigators

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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

Powdery Mildew - Quinoxyfen (objectives 1 and 2)

In 2015, two field tests were conducted at a commercial vineyard in western Virginia, where quinoxyfen-resistant powdery mildew isolates had been collected in the fall of 2013 and again in 2014.

One trial was set up in two rows of Chambourcin with plots consisting of 4 vines; the other in a row of Pinot noir at the same vineyard, about 200 m distant from the Chambourcin. 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 five

treatments, as shown in table 1 and 2, each replicated four times. The early-season grower spray program consisted of mancozeb and sulfur.

Trial sprays were applied:

June 2 at approximately 5-10% bloom (Rally for the rotation in Treatments 3 and 5) June 15, approaching BB-sized berries (Rally for the rotation in Treatments 3 and 5) Jun 29, approaching cluster closing (Endura for the rotation in Treatments 3 and 5) July 14 (Endura for the rotation in Treatments 3 and 5) July 28 (Pinot noir only) (Rally for the rotation in Treatments 3 and 5)

Applications were supplemented with 0.4% Prophyt and 1 lb/A mancozeb or (later applications) 10.4 oz/A Abound (both powdery and downy mildew at this location were QoI resistant) for control of downy mildew and black rot.

Little powdery mildew developed in the Chambourcin plots until late in the season. No powdery mildew could be detected on July 28, 14 days after the last application, and only one cluster with a very small amount of infection on Aug 12. Foliar infection developed later, and a September 5 rating is summarized in Table 1. As expected so long after the last treatment (53 days), none of the differences were statistically significant.

Both cluster and foliar powdery mildew infection developed in the Pinot noir trial (Table 2). At the July 14 cluster rating, after 3 treatment applications, all treatments still provided good control. However, a foliar rating on Aug 12, 15 days after the last application, showed distinctly more powdery mildew in the Quintec plots than in the other treatments with the exception of the nontreated control.

Table 1. Foliar powdery mildew infection, Chambourcin, September 5, 2015, 53 days after the last anti-powdery mildew spray.

Treatment and per acre rate	Leaf infection %	
1 Control	0.31	
2 Vivando, 10.3 fl oz	0.38	
3 Rally, 3 oz OR Endura 4.5 oz + Sulfur 1 lb	0.12	
4 Quintec, 4 fl oz	0.22	
5 Quintec, 4 fl oz + Rally, 3 oz OR Endura 4.5 oz + Sulfur 1 lb	0.13	

None of the differences were statistically significant (Tukey's HSD)

Table 2. Powdery mildew infection of clusters and leaves, Pinot noir 2015.

		Cluster infection	Foliar colonies per	Leaf infection
		%, 7/14	4-min search, 8/12	%, 8/23
1	Control	10.8 A	127.8 A	5.3 A
2	Vivando	0.1 B	0.1 C	0.0 C
3	Rally/Endura+Sulfur	1.1 B	1.3 C	0.1 C
4	Quintec	1.7 B	35.3 B	2.9 B
5	Quintec+Rally/Endura+Sulfur	0.5 B	1.1 C	0.1 C

Data not connected by the same letter are significantly different (Tukey's HSD).

Four hundred and two (402) powdery mildew isolates were sampled and bioassayed to determine the sensitivity to Quintec. Of these, 253 were resistant. The mean resistance frequency in non-Quintec plots was 49%, while the resistance frequency in Quintec treated plots was 81%, indicating that a regular Quintec application significantly increased the frequency of the Quintec resistance, as would be expected. For comparison, the mean reistance frequency was 63% in 2014. The resistance frequency was maintained at a fairly stable level for the whole season (Fig. 1).

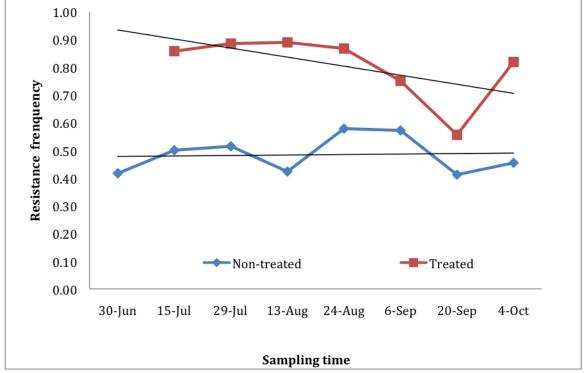


Fig 2. Frequency of quinoxyfen-resistant powdert mildew isolates in quinoxyfen-treated plots and vineyard-wide, 2015

^{7/14} cluster infection: 30 clusters per plot evaluated separately by two evaluators

^{8/12}: foliar colonies were counted in a 2-minute search by two evaluators, one on each side of the row

^{8/23:} leaf infection as percent of berry cluster surface, two evaluators, one checking 50 leaves per plot, the other 15 leaves per plot.

In 2015, 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. 13 out of 19 isolates tested, were able to grow on Quintec-treated leaves; 1 QR isolate was collected from non-treated plants while 12 resistant isolates were collected from treated plants.

In order to find characteristic molecular markers to differentiate powdery mildew isolates resistant and sensitive to strobilurin fungicides and to quinoxyfen, lab-stored powdery mildew isolates were tested against 30 ppm trifloxystrobin (Flint) and 30 ppm quinoxyfen (Quintec). Ten resistant and 10 sensitive isolates were chosen each fungicide. Conidia of each isolate were collected by washing them off leaf tissue with sterile water and storing the suspensions at minus 20C for future DNA extraction and sequencing. DNA extracted from 10 Quintec-resistant and 10 sensitive isolates has been sent off for sequencing.

Downy Mildew - Phosphite (objectives 4 and 5)

In 2015, we obtained or collected downy mildew samples from 5 different vineyards. These downy mildew samples did not exhibit detectable phosphite resistance. Bioassays of remaining isolates from these collections and some from previous years are still in progress. We have started to collect spores for extracting DNA from downy mildew samples for whole genome sequencing for clade identification.

In the above-mentioned powdery mildew trial, we used Prophyt, a phosphite fungicide, as the material included for downy mildew control. It was used at a label rate of 0.4%, and increased to 0.5% in late July when downy mildew developed. However, despite these treatments, the trial rows experienced a serious downy mildew outbreak after four applications. Isolates collected from these vines were able to grow on leaf tissue treated with higher rates that our "standard" isolates, but results are still incomplete on how much higher. We are maintaining these isolates on phosphite-treated (0.2%) and untreated grapevine plants for further studies.

Publications

Colcol, J.F. and A. B. Baudoin. 2016. 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)