Virginia Wine Board
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Fungicide sensitivity and resistance; continuation of monitoring and evaluation of powdery and downy mildew and Botrytis bunch rot

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Objectives

1. Continue research on impact of quinoxyfen (Quintec) resistance in grape powdery mildew
2. Continue research on phosphite sensitivity of grape downy mildew, with particular attention to a documented control failure in 2015
3. Continue Botrytis survey as needed to keep track of emerging resistances, and conduct field trial on efficacy of polyoxin-D (Ph-D, Oso), a recently labeled different mode of action against Botrytis.
4. Respond to emerging reports and concerns about fungicide resistance in grape pathogens

Activities and Results

Powdery Mildew – Quinoxyfen (objective 1)

A field test was conducted in 2016 at a commercial vineyard in western Virginia, where a powdery mildew population resistant to quinoxyfen (Quintec) had been documented since the fall of 2013 with continuing presence through 2015. The field trial was set up in two rows of Pinot Noir with plots consisting of 4-5 vines. Trials at this location in 2014 and 2015 had indicated that quinoxyfen was still fairly effective for powdery mildew control despite the presence of quinoxyfen-resistant isolates at a frequency of greater than 50%. Our main purpose in 2016 was (1) to confirm and quantify quinoxyfen's continued effectiveness for the control of powdery mildew; and 2) determine whether the number of applications and the application timing would affect the degree of control. The trial also included treatments aimed at evaluating downy mildew control (see objective 2, below) and consisted of ten treatments, as shown in Table 1, each replicated four times. Six fungicide applications were carried out with backpack sprayers during the season (Table 2). Treatments with Quintec were supplemented with Rally+sulfur rotated with Endura+sulfur applications to bring the total number of applications up to six. The pre-bloom grower spray program consisted of mancozeb and sulfur. Applications included Prophyt, Revus, mancozeb, or captan for control of downy mildew. Abound (10.4 oz/A) was included in the first three applications of all treatments for control of black rot (both powdery and downy mildew at this location were QoI resistant (i.e., resistant to Abound).

Table 1. Pinot noir powdery and downy mildew field trial treatments, 2016.

	Anti-powdery mildew fungicides Application date						Anti-downy mildew fungicides Application date					
	6.09	6.22	7.06	7.20	8.05	8.19	6.09	6.22	7.06	7.20	8.05	8.19
T1	-	-	-	-	-	-	Pro	Rev	Pro	Rev	Pro	Rev
T2	Q	Q	Q	Q	Ra-s	En-s	Ma	Ma	Ca	Ca	Rev	Rev
T5	Q	Q	Q	Q	Ra-s	En-s	Pro	Pro	Pro	Pro	Pro	Pro
T9	Q	Q	Q	Q	Ra-s	En-s	Pro	Pro	Pro	Rev	Rev	Rev
T3	Q	Q	Rs	Es	Ra-s	En-s	Pro	Pro	Pro	Rev	Rev	Rev
T4	Ra-s	En-s	Q	Q	Ra-s	En-s	Rev	Rev	Rev	Pro	Pro	Pro
T10	Ra-s	En-s	Q	Q	Ra-s	En-s	Pro	Pro	Pro	Rev	Rev	Rev
T6	Ra-s	En-s	Ra-s	En-s	Q	Q	Pro	Pro	Pro	Rev	Rev	Rev
T7	Viv	Viv	Viv	Viv	Viv	Viv	-	-	-	-	-	-
T8	Apr	Apr	Apr	Apr	Apr	Apr	Ma	Ma	Ca	Ca	Rev	Rev

Q = Quintec 4 fl oz
Ra-s = Rally 3 oz+sulfur (Microthiol Disperss) 1 lb
En-s = Endura 4.5 oz+sulfur 1lb.
Viv = Vivando 10.3 fl oz
Apr = Aprovia 8.6 fl oz

Pro = Prophyt 0.5%
Rev = Revus 8 fl oz
Ma = mancozeb, Dithane 75DF
Rainshield 1.5 lb
Ca = Captan 80WDG 1.25 lb

Table 2. Schedule of fungicide applications and disease evaluation

Fungicide application
June 9, approximately 40-50% bloom
June 22, approaching BB-sized berries
July 6, approaching cluster closing
July 20, berries starting to develop color
Aug 5, berries developing color
Aug 19, berry softening
Evaluation
Jul 14, powdery mildew cluster rating, and foliar downy mildew rating
Jul 20, powdery mildew cluster rating
Aug 13, foliar downy mildew rating
Sep 3, foliar powdery mildew and downy mildew rating

Both cluster and foliar powdery mildew infection developed in the Pinot noir trial, although disease pressure was low in the early part of the season. At the cluster ratings on July 14 and July 20 (Table 3 and 4), after 3 treatment applications, efficacy of treatments that included Quintec (T2 and T5 in Table 3, and T9 in Table 4) was slightly lower than efficacy of the best treatments (Aprovia, Vivando and Rally/Endura), but all treatments still provided decent control.

Table 3. Powdery mildew cluster infection rated on July 14, 2016 (8 days after the third anti-powdery mildew spray) (see Table 1 for treatment codes).

Treatment		Powdery treatment			Downy treatment			Cluster infection %	
T1	"Untreated" control	-	-	-	Pro	Rev	Pro	1.4	A
T8	Aprovia	Apr	Apr	Apr	Ma	Ma	Ca	0.1	B
T2	Quintec	Q	Q	Q	Ma	Ma	Ca	0.2	B
T5	Quintec	Q	Q	Q	Pro	Pro	Pro	0.6	AB
T7	Vivando	Viv	Viv	Viv	-	-	-	0.0	B

Cluster infection: 25 clusters per plot evaluated separately by one evaluator
Data not connected by the same letter are significantly different (Tukey's HSD).

Table 4. Powdery mildew cluster infection rated on July 20 (14 days after the third anti-powdery mildew spray) (see Table 1 for treatment codes).

Treatment		Powdery treatment			Downy treatment			Cluster infection %	
T1	"Untreated" control	-	-	-	Pro	Rev	Pro	9.6	A
T6	Rally/ Endura+Sulfur	Ra-s	En-s	Ra-s	Pro	Pro	Pro	0.7	B
T9	Quintec	Q	Q	Q	Pro	Pro	Pro	2.0	B
T7	Vivando	Viv	Viv	Viv	-	-	-	0.1	B

Cluster infection: 20 clusters per plot evaluated separately by each of two evaluators
Data not connected by the same letter are significantly different (Tukey's HSD).

The foliar rating on Sep 3 (Table 5), 15 days after the sixth application, showed that all treatments were reasonably successful. Vivando and Aprovia both provided complete powdery mildew control. Treatments with 4 Quintec applications (T2, T5, T9) tended to provide slightly less control of powdery mildew than treatments with 2 Quintec applications where the other treatments were replaced by a Rally/Endura alternation plus sulfur. Two early-season applications of Quintec provided slightly better control than two mid-season or late-season Quintec applications. It is worth noting that for T5 and T7, powdery mildew control may have been aided by better fungicide coverage of remaining leaves because of considerable defoliation due to downy mildew.

Overall, these results resemble the ones obtained in the previous two years: despite the presence of a Quintec-resistant population of powdery mildew, the effectiveness of Quintec for powdery mildew control was reduced only slightly.

Table 5. Powdery mildew leaf infection rated on September 3, 15 days after the sixth anti-powdery mildew spray (see Table 1 for treatment codes).

	Anti-powdery mildew fungicides						Anti-downy	PM infection %		Defoliation %	
T1	-	-	-	-	-	-	Pro/Rev	37.0	A		5.8
T2	Q	Q	Q	Q	Ra-s	En-s	Ma-Ca-Rev	2.2	B	B*	7.0
T5	Q	Q	Q	Q	Ra-s	En-s	Pro-Rev	1.0	B	BCD	60.0
T9	Q	Q	Q	Q	Ra-s	En-s	Pro-Rev	2.0	B	BC	4.3
T3	Q	Q	Rs	Es	Ra-s	En-s	Pro-Rev	0.1	B	D	2.3
T4	Ra-s	En-s	Q	Q	Ra-s	En-s	Rev-Pro	0.4	B	CD	2.5
T10	Ra-s	En-s	Q	Q	Ra-s	En-s	Pro-Rev	0.7	B	BCD	3.0
T6	Ra-s	En-s	Ra-s	En-s	Q	Q	Pro-Rev	1.4	B	BCD	2.5
T7	Viv	Viv	Viv	Viv	Viv	Viv	-	0	B	D	72.5
T8	Apr	Apr	Apr	Apr	Apr	Apr	Ma-Ca-Rev	0	B	D	5.3

PM infection as percent of leaf surface, two evaluators, rating 30 leaves per plot per evaluator; Defoliation, due to downy mildew, as percentage of the canopy of each plot was rated by one evaluator. Data not connected by the same letter are significantly different (Tukey's HSD). *Analysis of treatment differences with control excluded, to exclude the larger control variance from the within-treatment variance.

One hundred and nighty-nine (199) powdery mildew isolates were collected and bioassayed to determine their sensitivity to Quintec. Of these, 100 were resistant. The mean resistance frequency in non-Quintec plots was 46%, while the resistance frequency in Quintec-treated plots was 86%, indicating that a regular Quintec application increased the frequency of the Quintec resistance, as would be expected. For comparison, in 2015, the mean resistance frequency in non-Quintec plots was 49%, while the resistance frequency in Quintec-treated plots was 81%. As shown in Table 4, the resistance frequency declined only slowly over the past three seasons.

Table 6. Average resistance frequencies of non-treated plots from 2014-2016

Year	2014	2015	2016
Resistance frequency	65% (n=124)	50% (n=224)	46% (n=100)

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

Downy Mildew – Phosphite (objective 2)

In 2015, Prophyt, a phosphite fungicide was used in the powdery field trial as the downy mildew control agent. However, despite these treatments, the trial rows experienced a serious downy mildew outbreak after four applications. In 2016, a field trial was set up to examine the efficacy of Prophyt in the same location in comparison with other downy mildew fungicides. Prophyt was applied at 0.5%, which is the highest label rate. Isolates collected from trial vines were bioassayed along with our "standard" (reference) isolates that had never been exposed to Prophyt. Preliminary results indicated that some isolates from the trial plots were not as sensitive as the reference isolates, but the reduction in sensitivity was small (not statistically significant). We are maintaining and bioassaying these isolates on phosphite-treated (0.2% Prophyt) and untreated grapevine plants for further study.

In the field trial, at the first leaf rating on July 14, 14 days after the 3rd application, all treatments provided significant downy mildew control (Table 7). The Prophyt treatments T6 and T9 had higher disease levels than the non-Prophyt treatments (T2 and T4), but the differences were not significant. On August 13, 8 days after the 5th spray (Table 8), treatments consisting of Prophyt applications only (T5) were significantly less effective than the other treatments. Treatments where some of the Prophyt applications were replaced by Revus (T1, T4, T9) performed better. The mancozeb/captan/Revus rotation (T2) provided moderate disease control. On September 3, 15 days after the sixth spray, for mature leaves, the Prophyt-only treatment (T5) performed almost as poorly as the non-treated control (Table 9) whereas the mancozeb/captan/Revus rotation (T2) still provided moderate disease control, and treatments including additional Revus applications were significantly more effective. Infection of younger leaves (Table 10) was somewhat less severe as that of mature leaves but similar efficacy patterns were present.

Table 7. Downy mildew leaf infection rated on July 14, 2016 (8 days after the third anti-downy mildew spray) (see Table 1 for treatment codes).

	Anti-powdery	Anti-downy mildew treatments			Leaf infection %		*
T7	3 Viv	–	–	–	35.5	A	
T9	3 Q	Pro	Pro	Pro	10.3	B	B*
T6	Ra-s – En-s – Ra-s	Pro	Pro	Pro	7.1	B	BC
T2	3 Q	Ma	Ma	Ca	3.1	B	BC
T4	Ra-s – En-s – Q	Rev	Rev	Rev	0.8	B	C

Leaf infection: 30 leaves per plot evaluated separately by two evaluators

Data not connected by the same letter are significantly different (Tukey's HSD). *Analysis of treatment differences with control excluded, to exclude the larger control variance from the within-treatment variance.

Table 8. Downy mildew leaf infection rated on August 13, 2016 (8 days after the fifth anti-downy mildew spray) (see Table 1 for treatment codes).

	Anti-powdery	Anti-downy treatments					Leaf infection %		*
T7	5 Viv	–	–	–	–	–	57.9	A	
T5	4 Q – Ra-s	Pro	Pro	Pro	Pro	Pro	37.9	A	A*
T2	4 Q – Ra-s	Ma	Ma	Ca	Ca	Rev	19.7	B	B
T9	4 Q – Ra-s	Pro	Pro	Pro	Rev	Rev	15.0	B	B
T1	–	Pro	Rev	Pro	Rev	Pro	7.1	B	BC
T4	Ra/En-s – Q – Ra-s	Rev	Rev	Rev	Pro	Pro	7.0	B	C

Leaf infection: 30 leaves per plot evaluated separately by two evaluators
Data not connected by the same letter are significantly different (Tukey's HSD). *Analysis of treatment differences with control excluded, to exclude its larger variance from the within-treatment variance.

Table 9. Downy mildew infection of older, mature leaves rated on September 3, 2016 (15 days after the sixth anti-downy mildew spray) (see Table 1 for treatment codes).

	Anti-powdery	Anti-downy treatments						Leaf infection %	
T7	6 Viv	-	-	-	-	-	-	64.5	A
T5	4 Q – Ra/En-s	Pro	Pro	Pro	Pro	Pro	Pro	54.2	A
T2	4 Q – Ra/En-s	Ma	Ma	Ca	Ca	Rev	Rev	26.0	B
T1	-	Pro	Rev	Pro	Rev	Pro	Rev	17.5	BC
T4	Ra/En-s – 2 Q – Ra/En-s	Rev	Rev	Rev	Pro	Pro	Pro	12.8	C
T3	2 Q – 2 Ra/En-s	Pro	Pro	Pro	Rev	Rev	Rev	10.4	C

Leaf infection: 30 leaves per plot evaluated separately by two evaluators
Data not connected by the same letter are significantly different (Tukey's HSD).

Table 10. Downy mildew infection of younger leaves rated on September 3, 2016 (15 days after the sixth anti-downy mildew spray) (see Table 1 for treatment codes).

	Anti-powdery	Anti-downy treatments						Leaf infection %	
T7	6 Viv	-	-	-	-	-	-	39.0	A
T5	4 Q – Ra/En-s	Pro	Pro	Pro	Pro	Pro	Pro	26.3	B
T4	Ra/En-s – 2Q – Ra/En-s	Rev	Rev	Rev	Pro	Pro	Pro	13.2	C
T1	-	Pro	Rev	Pro	Rev	Pro	Rev	12.6	C
T2	4 Q – Ra/En-s	Ma	Ma	Ca	Ca	Rev	Rev	10.8	C
T3	2 Q – 2 Ra/En-s	Pro	Pro	Pro	Rev	Rev	Rev	8.5	C

Leaf infection: 30 leaves per plot evaluated separately by two evaluators
Data not connected by the same letter are significantly different (Tukey's HSD).

Botrytis and powdery mildew field trial (objective 3)

A second field trial was set up in a mature Riesling block in a commercial vineyard. The main purpose was to test several relatively new fungicides (PhD, Aprovia, Kenja) against Botrytis bunch rot, but since the same fungicides have activity against powdery mildew, the effect on that disease was evaluated as well. Table 11 summarizes the treatment and application schedule.

Table 11. Treatment and application schedule, 2016 Riesling field trial.

	Jun 10 50% bloom	Jun 19/22*	**Jun 29** Pre-cluster closing	Jul 14*	**Jul 29** early veraison	**Aug 13**
Untreated	--		--		--	--
PhD, 6.2 oz	PhD		PhD		PhD	PhD
Switch 11 oz / Elevate 16 oz +Sulfur 2.5 lbs	Switch		Elevate +sulfur		Switch +sulfur	Elevate +sulfur
Kenja 20 fl oz	Kenja		Kenja		Kenja	Kenja
Aprovia 8.6 oz	Aprovia		Aprovia		Aprovia	Aprovia
Volume/acre	60 gal		70 gal		80 gal	80 gal
Maintenance additions against downy and black rot	Prophyt 0.5%	Endura 2.13 oz Mancozeb 1 lb Pyraclostrobin Sulfur, 1 lb Prophyt	Ridomil Gold MZ, 2.5 lbs	Kocide DF 1 lb Presidio 4 fl oz Pyraclostrobin	Revus 8 fl oz	Presidio 4 fl oz

* Jun 19/22 and Jul 14 were maintenance sprays applied uniformly to all plots
** Sulfur: Microthiol Disperss (80% active ingredient)

Table 12. Disease evaluations, 2016 Riesling field trial.

Treatment	Cluster powdery mildew, Jul 29		Cluster powdery mildew Aug 5		Foliar powdery mildew Sep 10		Cluster rot Sep 10	
Control	12.7	A	11.4	A	17.7	A	17.7	A
PhD	3.7	AB	3.1	AB	15.5	A	20.5	A
Switch/Elevate +sulfur	2.1	BC	1.8	AB	11.0	A	15.6	A
Kenja	0.4	C	1.0	AB	5.5	AB	11.0	A
Aprovia	0.0	C	0.1	B	0.9	B	14.0	A

Numbers not followed by the same letter are significantly different (Tukey's HSD).

With respect to powdery mildew, PhD appeared somewhat effective in preventing cluster infection, although differences with the control were not statistically significant (Table 12). PhD had little effect on foliar powdery mildew rated late in the season. Aprovia was the most effective anti-powdery mildew treatment in this trial followed by Kenja and then the Switch or Elevate plus sulfur combination. None of the treatments were effective in reducing cluster rot. The nature of the rot was uncertain; a portion (could not be quantified) of it was due to Botrytis, but ripe rot and especially sour and unspecified other rots were major components as well.

Botrytis bioassays (objective 3)

Aprovia (benzovindiflupyr, from Syngenta) and Kenja (isofetamid, from SummitAgro) are fungicides that have become recently registered for use on grapes, and have FRAC group 7 mode of action (SDHI or succinate dehydrogenase inhibition). *Botrytis cinerea* resistance to the first Group 7 fungicide, boscalid (Endura, component of Pristine), is common in Virginia, but boscalid resistance has generally not entailed resistance to another group 7 fungicide fluopyram (Luna, from Bayer). We wanted to determine whether boscalid-resistant isolates might also have resistance to the two new fungicides in Aprovia and Kenja. Five boscalid-sensitive isolates tested were also sensitive to Aprovia and Kenja. Among 9 boscalid-resistant isolates, 7 were sensitive to the two other fungicides, and 2 had uncertain reactions, and will be repeated.

Downy Mildew – Revus (mandipropamid) (objective 4)

A sample of downy mildew-diseased leaves yielding one *P. viticola* isolate was received in July, and additional samples were collected in October 2016 from one vineyard in west-central Virginia, where Revus (23.4% SC mandipropamid) or Revus Top was reported to have failed to control downy mildew. Revus had been applied three times to this vineyard in 2016. Our bioassays included a sensitive isolate of *P. viticola*, collected before Revus was widely used in Virginia as a reference isolate. Sensitivity to mandipropamid was determined by a leaf disc bio-assay. Working solutions of 200, 20, 2, 0.2, 0.02, and 0 μg/ml (active ingredient) concentrations were prepared by appropriate dilution of a 1,000 μg/ml stock solution with sterile distilled water. The experiment was conducted in a completely randomized design with three replicates. Fungicides were applied to the lower surface of ~15 mm leaf discs 6 hours before inoculation, using a Preval Sprayer (Precision Valve Corporation). Ten μl of sporangial suspension (10^4 spores ml^{-1}) was inoculated to the center of each leaf disc. The inoculated leaf discs were incubated at 22°C with alternating periods of 12 h light and dark. After 6 to 7 days, the areas of the lesions were measured and the numbers of growing sporangiophores were evaluated with dissecting microscope. The diseased area was expressed as a percentage of that in the untreated control (considered as 100%), and plotted against the log10 of the fungicide concentration to calculate the EC50 value. The EC50 value of the reference *P. viticola* isolate was <0.2 μg/ml, showing sensitivity to mandipropamid. The EC50 values of all 8 isolates from the commercial vineyards were >240 μg/ml for mandipropamid, which was above the field rate (8 fl oz/acre, if applied in 70 gallons of water/acre would be 223 μg/ml active ingredient) showing their insensitivity to mandipropamid.

Summary

A field trial in a vineyard with a Quintec-resistant powdery mildew population confirmed the results from the previous two seasons: the type of resistance found has only a minimal effect on the efficacy of Quintec, as long as this fungicide is used preventatively and not too often. The resistant isolates did persist in the population for a fourth year at a frequency of close to 50%

A phosphite-based spray program with applications every 14 days was almost ineffective for downy mildew control, considerably less so than mancozeb followed by captan. However, no clear evidence was found that any kind of resistance is involved; the spray interval may just have been too long under the existing disease pressure

PhD, Aprovia, and Kenja were evaluated for powdery mildew and bunch rot control. Aprovia and Kenja worked well against powdery mildew while PhD had at best a marginal effect. None of the treatments were effective in preventing late-season rot

A single instance of mandipropamid (Revus)-resistant downy mildew was found in Virginia

Publications

Rallos, L.E.E and A. B. Baudoin. 2016. Co-occurrence of two allelic variants of CYP51 in *Erysiphe necator* and their correlation with over-expression for DMI resistance. PLoS ONE 11: e0148025. doi:10.1371/journal.pone.0148025.