Fungicide resistance status of powdery and downy mildew in Australia

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In a project funded by Wine Australia, the current resistance status of key fungicide groups used to control foliar diseases of winegrapes in Australia is under investigation. For powdery mildew, research has revealed a high incidence of resistance to pyraclostrobin, some loss of efficacy to penconazole and myclobutanil and widespread detection of the genetic mutation associated with DMI resistance. For downy mildew, resistance to metalaxyl and, to a lesser extent, pyraclostrobin has been detected in vineyards with high disease pressure. Practical methods for monitoring fungicide resistance are being developed.

IN BRIEF

- In Australia, the control of powdery mildew and downy mildew relies on fungicides from nine and 13 different mode of action groups, respectively; resistance to several of these groups has been reported.
- Since 2013, the incidence and severity of fungicide resistance in Australian viticulture has been investigated.
- Between 2014 and 2019, samples of powdery mildew and downy mildew were obtained from most wine regions in South Australia, New South Wales, Victoria, Tasmania and Western Australian from a variety of vineyards with poor field performance of fungicides due to unknown causes or suspected resistance, those with hot spots of disease, organically managed vineyards and home gardens.
- This article presents the results for fungicide resistance in powdery and downy mildew to a range of selected fungicides.

INTRODUCTION

Fungicides are a key part of an integrated pest management program; essential to maintain healthy and productive vines. However, development of resistance to fungicides in grapevine pathogen populations poses a major challenge to the industry's sustainability. Fungicide-resistant populations reduce fungicide efficacy and lead to disease management failure, reducing yields, fruit quality and, hence, economic returns.
The delay of resistance development in grapevine pathogen populations is critical to maintain fungicide efficacy for as long as possible. Fungicide resistance problems are widespread around the world, so ongoing monitoring of resistance is vital to determine the cause of poor disease control, check if disease management strategies are working, monitor the development or change in resistance status of individual fungicides and, ideally, to gain baseline data before the commercial introduction of a new fungicide (Brent and Hollomon 2007).

Resistance develops through the survival and spread of initially rare, naturally-occurring, mutants in the pathogen population, after which the mutant is selected by consistently spraying the same fungicide/s or fungicide group/s, eventually leading to field failure (Brent 2007, Figure 1). Although the resistance mechanisms vary, mutations in pathogens can cause modifications in the primary target site of action of a fungicide. This decreases the ability of the fungicide to bind to the target site in the pathogen cell, resulting in a loss of sensitivity or even complete resistance to that fungicide. Resistance can result from a single gene mutation, as is the case for resistance to Quinone outside Inhibitor (QoI, group 11) fungicides in powdery and downy mildew pathogens, where the mutation G143A is associated with sudden and complete loss of effectiveness. Multiple mutations or mechanisms are associated with the gradual loss of effectiveness in some fungicide groups, e.g. the Demethylation Inhibitor (DMI, group 3) fungicides. In grapevine powdery mildew, the mutation Y136F is associated with a gradual decrease in sensitivity to the DMIs but other, yet unidentified, mutations and mechanisms are also involved. However, for many fungicide groups, mutations or mechanisms associated with resistance are not yet known, e.g. metalaxyl resistance in the downy mildew pathogen.

In Australia, control of grapevine powdery mildew is reliant on fungicides from nine different mode of action groups (MOAs) and downy mildew on 13 MOAs. Resistance to several of these groups has been reported worldwide as well as in Australia (Table 1, see page 56).

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2013, the incidence and severity of fungicide resistance in Australian viticulture has been investigated. Here, results for fungicide resistance in powdery and downy mildew to a range of selected fungicides are presented. Fungicides were prioritised in conjunction with an Industry Reference Group.

METHODS

Pathogen samples

Samples of powdery mildew and downy mildew were obtained from most wine regions in South Australia (SA), New South Wales (NSW), Victoria (Vic), Tasmania (Tas) and Western Australian (WA) between 2014 and 2019. Sampling was not targeted and came from a variety of vineyards with poor field performance of fungicides due to unknown causes or suspected resistance, those with hot spots of disease, organically managed vineyards and home gardens. Although results for each viticulture region are presented, they are not intended to be representative of a region.

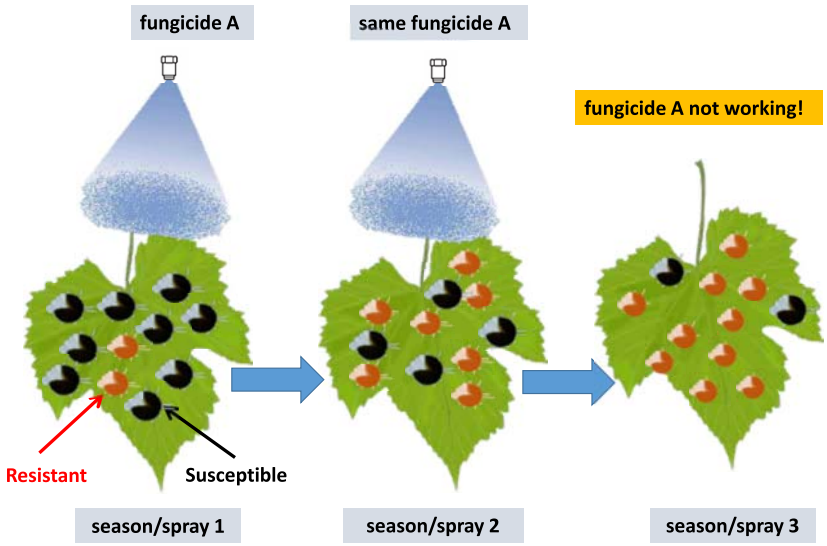

Figure 1. Simplified diagram showing the resistant population of a plant pathogen before fungicide use (left), when most of the population is sensitive (black), but a few individuals are resistant (orange). After consecutive sprays of the same fungicide, the resistant population builds up (middle) and eventually the fungicide becomes ineffective (right).

Resistance testing

Fungicide resistance can be characterised in two ways: phenotypically and genotypically. **Phenotyping** involves exposing a culture to a particular fungicide, and quantifying the culture's response. **Genotyping** involves sequencing genes associated with resistance to identify known or novel mutations. Both methods are complementary and provide different types of information on resistance status. ▸

Table 1. Reduced sensitivity or resistance reported worldwide and in Australia to the fungicide groups used for control of powdery mildew (PM) and downy mildew (DM).

Mode of action group (FRAC group)	Active constituent(s)	Resistance potential (FRAC)*	Resistance or reduced sensitivity	
			Worldwide	Australia
Multi-site activity (M1, M2, M3, M4, M5 and M9)	copper, sulphur, mancozeb, thiram, ziram, captan, chlorothanonil and dithianon	low	not detected	not detected
Demethylation inhibitors (3)	various	medium	PM	PM
Phenylamides (4)	mefenoxam, metalaxyl	high	DM	DM
Amines (5)	spiroxamine	low - medium	PM	not detected
Succinate dehydrogenase inhibitors (7)	boscalid	medium - high	PM	not detected
Quinone outside Inhibitors (11)	pyraclostrobin, azoxystrobin trifloxystrobin, kresoxim-methyl	high	PM, DM	PM, DM
Aza-napthalenes (13)	Quinoxyfen, proquinazid	medium	PM	PM
Quinone inside Inhibitors (21)	cyazofamid	Resistance risk unknown but assumed to be medium to high	not detected	not detected
Unspecified (29)	fluazinam	low	not detected	not detected
Carboxylic acid amines (40)	mandipropamid, dimethomorph	low - medium	DM	DM#
Quinone x inhibitors (45)	ametoctradin	medium to high	not detected	not detected
Aryl-phenyl-ketones (50; reclassified from U8)	metrafenone, pyriofenone	medium	DM	not detected
Unknown (U6)	cyflufenamid	unknown	not detected	not detected
Unknown (U1)	potassium salts of fatty acids	low	not detected	not detected

*** Fungicide Resistance Action Committee (www.frac.info) # To be confirmed**

Powdery mildew, caused by the fungus *Erysiphe necator*, and downy mildew, caused by the organism *Plasmopara viticola*, are both host-specific obligately biotrophic organisms. This means that they only grow on live, healthy, green grapevine material, therefore phenotypic resistance tests typically involve leaf disc bioassays (Figure 2). In bioassays for *E. necator* and *P. viticola*, healthy leaves are detached from susceptible Cabernet Sauvignon and Sultana vines, respectively, and soaked in or sprayed with various fungicide dilutions, typically 0.001, 0.01, 0.1, 1.0, and 10.0µg/mL. Leaf discs (10mm diam.) are cut from treated leaves and placed on a water agar medium. The leaf discs are then inoculated with spores of the appropriate pathogen. After incubation for 14 days for *E. necator* and seven days for *P. viticola*, the percentage of each leaf disc covered with pathogen growth for each fungicide concentration is recorded. From these data, the phenotypic sensitivity of that pathogen to a particular fungicide is determined. Cultures of *E. necator* were tested in bioassays using the following fungicides: pyraclostrobin 250g/L (Cabrio®, BASF), azoxystrobin 250g/L (Amistar 250 SC®, Syngenta), trifloxystrobin 500g/kg (Flint® 500 WG, Bayer), penconazole 100g/L (Topas® 100EC, Syngenta), myclobutanil 200g/L (Mycloss® Xtra, Corteva), tetraconazole 40g/L (Domark® 40ME, Sipcam), difenoconazole 250g/L (Digger®, Nufarm), proquinazid 200g/L (Talendo®, Corteva) and quinoxyfen 250g/L (Legend®, Corteva). Cultures of *P. viticola* were tested with; pyraclostrobin 250g/L, metalaxyl 480g/L (Ridomil® Gold 480 EC, Syngenta) and mandipropamid 250g/L (Revus® SC, Syngenta).

DNA sequencing was used to detect and quantify the mutations associated with resistance:

- G143A mutation associated with resistance of both *E. necator* (Delyé *et al*. 1997) and *P. viticola* to the QoIs

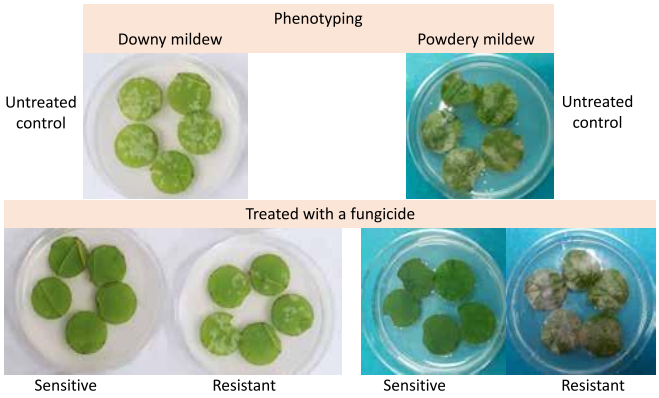

Figure 2. Leaf disc bioassay used to phenotype resistant cultures of *Plasmopara viticola* (left) and *Erysiphe necator* (right). Resistant pathogens grow on discs treated with a concentration of fungicide that inhibits sensitive pathogens.

- Y136F mutation associated with decreased sensitivity of *E. necator* to the DMIs (Delyé *et al*. 1997, Colcol *et al*. 2012)
- nG1105s mutation linked with resistance of *P. viticola* to mandipropamid (Blum *et al*. 2010)

RESULTS AND DISCUSSION

Powdery mildew

Powdery mildew samples were collected from over 200 vineyards, from which 150 viable cultures of *E. necator* were established and used in testing.

Quinone outside Inhibitors (QoIs; group 11)

Pyraclostrobin was the QoI tested on the greatest number of cultures. Of the 123 cultures tested, 66% were phenotypically resistant to pyraclostrobin (Table 2, see page 58), the majority of which were detected with the G143A mutation (data not shown).

Thirteen cultures were tested for response to azoxystrobin, eight of which were phenotypically resistant; these originated from the following regions: Barossa (1), Langhorne Creek (1), Adelaide Hills (2), Margaret River (1), Tamar Valley (1), Orange (1) and Mornington Peninsula (1; data not shown). Only two of these phenotypically resistant cultures were genotyped and both contained the G143A mutation. The five remaining cultures were sensitive and these were from the King Valley (1), Riverina (2), Riverland (1) and the Adelaide Hills (1).

Five cultures were tested for response to trifloxystrobin; one culture from a vineyard in the Adelaide Hills with reported field failure to this fungicide, and four other cultures from the Adelaide Plains (2), Adelaide Hills (1) and McLaren Vale (1) (data not shown). The suspected 'field failure' culture was confirmed to be phenotypically resistant, as was the other culture from the Adelaide Hills, and all other cultures were sensitive.

The majority of phenotypically resistant cultures were detected with the G143A mutation. In view of this direct relationship, future research will aim to develop an in-field genotype method to provide a quick and reliable test of field resistance.

Demethylation Inhibitors (DMIs; group 3)

In contrast to the QoIs, there was little relationship between phenotype and genotype for the DMIs. Fifty-six cultures were

phenotypically tested with penconazole and only 12 showed a low-level loss of sensitivity, with the remainder sensitive (data not shown). For myclobutanil, 80 cultures were phenotyped, and seven of these, from commercial vineyards in Margaret River, Langhorne Creek, Mornington Peninsula and Yarra Valley, and a research vineyard in the Adelaide Plains, showed reduced sensitivity (data not shown). Forty-two cultures showed a low-level loss of sensitivity and the remaining 31 cultures were sensitive. Fifteen cultures were tested with difenoconazole and 24 with tetraconazole, and all were sensitive (data not shown).

Overall, 124 cultures were genotyped and 70% were detected with the Y136F mutation (Table 2). The contrast between phenotype and genotype with regard to DMIs reflects the lack of relationship reported elsewhere (Délye *et al.* 1997, Miazzi *et al.* 2011, Frenkel *et al.* 2015). This indicates that there are other mechanisms associated with the loss of sensitivity in the DMIs, such as the copy number of the gene that contains the Y136 mutation. In this project, research is now underway on gene copy number and the association with loss of sensitivity to the DMIs. In addition, there are likely to be other, not yet characterised, molecular mechanisms associated with DMI resistance in powdery mildew.

What does this mean for a grower? The presence of the Y136F mutation acts as a warning signal to follow resistance management guidelines and manage the DMIs carefully. Phenotype tests give a good indication of decreased effectiveness for a particular DMI fungicide or that field failure is related to resistance. For the DMIs, a combination of both phenotype and genotype tests is required to understand decreased sensitivity.

Aza-napthalenes (AZN; group 13)

Testing of AZNs has been less extensive than the QoIs and DMIs. Twenty-four cultures were phenotyped with proquinazid, with only four showing decreased sensitivity (data not shown). Field failure was reported in the four vineyards from which the cultures originated; Adelaide Hills (1), Hilltops (2) and Orange (1), and spray records showed applications of the fungicide to have exceeded the recommended number. The remaining 20 cultures were sensitive.

Ten cultures, including the culture from the Adelaide Hills with proquinazid 'field failure', were tested with quinoxyfen, but no loss of phenotypic sensitivity was observed (data not shown). It is generally understood that cross-resistance exists between these two group 13 fungicides (Genet and Jaworska 2009). The current study does not support the cross-resistance theory, perhaps because

Table 2. Cultures of *Erysiphe necator* (powdery mildew) that were obtained from various wine regions in Australia and phenotypically tested by exposure to Quinone outside Inhibitor (QoI) fungicide pyraclostrobin (250g/L a.i.; Cabrio®, BASF), and genotyped for presence of the Y136F mutation associated with a decrease in sensitivity to Demethylation Inhibitor (DMI) fungicides.

State	Wine region	QoI - pyraclostrobin		DMI - Y136F mutation	
		Total tested	Resistant	Total tested	Y136 present
South Australia	Adelaide Hills	14	8	17	14
	Adelaide Plains	16	5	22	14
	Barossa	13	9	10	9
	Coonawarra	1	0	1	1
	Langhorne Creek	7	7	10	6
	Mount Benson	1	0	1	1
	Padthaway	2	2	2	1
	Riverland	14	8	7	5
Victoria	Bendigo	1	1	3	0
	Geelong	1	1	1	0
	Goulburn Valley	1	1	2	0
	King Valley	5	4	5	2
	Mornington Peninsula	4	2	4	3
	Strathbogie Ranges	3	3	3	3
	Yarra Valley	5	5	5	4
Tasmania	East Coast	1	1	1	1
	Tamar Valley	3	3	3	1
New South Wales	Hilltops	3	2	3	3
	Hunter Valley	3	2	3	2
	Orange	7	6	7	6
	Riverina	4	1	3	3
Western Australia	Great Southern	4	4	3	2
	Margaret River	9	6	7	6
	Swan District	1	0	1	0
TOTAL		123	81 (66%)	124	87 (70%)

resistance to proquinazid carries a fitness penalty, meaning sensitivity developed over time in the absence of exposure. Further work is underway to better understand the fitness of resistance in proquinazid.

The molecular mechanism of resistance is not known for the group 13 fungicides, therefore phenotype testing remains the only method to determine reduced sensitivity to this group.

Downy Mildew

Of the 111 samples of downy mildew received, 47 viable cultures of *P. viticola* were established for phenotype testing using metalaxyl, pyraclostrobin and/or mandipropamid. For metalaxyl, 74% of cultures had reduced phenotypic sensitivity; these originated from various regions in NSW, Vic, Tas and WA (Table 3). The highest incidence of metalaxyl resistance was found in regions with high downy mildew pressure. The mechanisms of resistance in *P. viticola* to metalaxyl are not known, therefore genotyping is not yet possible.

Resistance to pyraclostrobin was found for the majority of samples from the Yarra Valley but was not recorded for cultures from any other region, although there were no results for the Hunter Valley and Wagga Wagga due to technical issues (Table 3). The G143A mutation was detected in all but one of the cultures from the Yarra Valley, but this mutation was not detected in any of the phenotypically sensitive cultures.

Of the 32 cultures tested for resistance to mandipropamid, only one culture, from Wagga Wagga, showed a possible low-level of reduced sensitivity to mandipropamid (data not shown). However the associated mutation, G1105S, was not detected.

CONCLUSION

This research has revealed the current resistance status of key fungicide groups used to control powdery and downy mildew on winegrapes in Australia. A high incidence of QoI fungicide resistance and the associated mutation G143A was found for powdery mildew. For the DMIs, the Y136F mutation associated with decreased sensitivity was detected frequently in cultures of *E. necator*, yet phenotypical resistance was uncommon, except for some cultures in response to myclobutanil and penconazole. There was some loss of effectiveness for proquinazid where use of this fungicide exceeded the recommended number of applications per season. In regions where downy mildew pressure was high, there was a high incidence of resistance to metalaxyl and pyraclostrobin.

Ongoing monitoring of resistance is critical to detect changes in resistance status and to ensure that current resistance management strategies are working. Croplife Australia (www.croplife.org.au) produce resistance management guidelines for each registered fungicide in viticulture. These guidelines are also available in the AWRI Dog Book (www.awri.com.au). All growers are strongly encouraged to follow the resistance management guidelines.

A key outcome of this research will be the development of quick, reliable, in-field tests for those fungicide groups where the mechanism of resistance is known (e.g. QoIs). However, for those fungicides where the molecular mechanism is unknown, phenotype assays remain the only option at this point in time. This is particularly slow and time consuming for the obligately biotrophic pathogens *E. necator* and *P. viticola*. This research also aims to better understand the resistance status of other fungicide groups with regard to powdery and downy mildew.

The project team requires more samples of powdery and downy mildew for testing this season, particularly from vineyards with reduced fungicide efficacy, and where resistance issues are suspected. If you can help, please contact Dr Ismail Ismail at SARDI (ismail.ismail@sa.gov.au).

REFERENCES

Brent, K.J. (2007) Fungicide resistance in crop pathogens: how can it be managed? FRAC Monograph No. 1 Second (revised) edition. Croplife International, Brussels, Belgium.

Brent, K.J. and Hollomon, D.K. (2007) Fungicide resistance: the assessment of risk. FRAC Monograph No. 2, Second (revised) edition. Croplife International, Brussels, Belgium.

Table 3. Cultures of *Plasmopara viticola* (downy mildew) that were obtained from various wine regions in Australia and phenotypically tested for resistance to metalaxyl (480g/L a.i.; Ridomil® Gold 480 EC, Syngenta) and pyraclostrobin (250g/L a.i.; Cabrio®, BASF).

State	Wine region	Metalaxyl		Pyraclostrobin	
		Total tested	Resistant or reduced sensitivity	Total tested	Resistant
South Australia	Adelaide Hills	1	0	0	-
	Adelaide Plains	1	0	1	0
	Clare Valley	1	0	1	0
	McLaren Vale	1	0	1	0
Victoria	Yarra Valley	17	16	17	14
	King Valley	5	5	3	0
	Alpine Valley	1	1	1	0
New South Wales	Hunter Valley	11	7	7	*
	Wagga Wagga	1	1	1	*
Tasmania	Tamar Valley	2	2	2	0
Western Australia	Margaret River	2	2	2	0
	Swan District	4	1	2	0
Total		47	35 (74%)	38	14 (37%)

***Resistance status was unable to be determined due to technical issues**

Colcol, J.F.; Rallos, L.E. and Baudoin, A.B. (2012) Sensitivity of *Erysiphe necator* to demethylation inhibitor fungicides in Virginia. Plant Disease 96:111-116.

Délye, C.; Laigret, F. and Corio-Costet, M-F (1997) A mutation in the 14α-demethylase gene of *Uncinula necator* that correlates with resistance to a sterol biosynthesis inhibitor. Applied and Environmental Microbiology 63:2966-2970.

Genet, J-L and Jaworska, G. (2013) Characterization of European *Plasmopara viticola* isolates with reduced sensitivity to cymoxanil. European Journal of Plant Pathology 135:383-393.

Frenkel, O.; Cadel-Davidson, L.; Wilcox, W.F. and Milgroom, M.G. (2015) Mechanisms of resistance to an azole fungicide in the grapevine powdery mildew fungus, *Erysiphe necator*. Phytopathology 105:370-377.

Miazzi, M. and Hajjeh, H.R. (2011) Differential sensitivity to triadimemol of *Erysiphe necator* isolates belonging to different genetic groups. Journal of Plant Pathology 93:729-735.

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