

BE ALERT: Is fungicide resistance coming to your vineyard?



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SARDI

Why do you think you may have resistance?

- Fungicide not working

Possible causes?

- Coverage
- Fungicide choice
- Correct rate
- Application timing
- Resistance

What is fungicide resistance?

Resistance = a stable, heritable trait that results in a reduction in sensitivity to a fungicide by a fungus

Practical resistance = label rates of a fungicide no longer provide commercially acceptable control of a disease

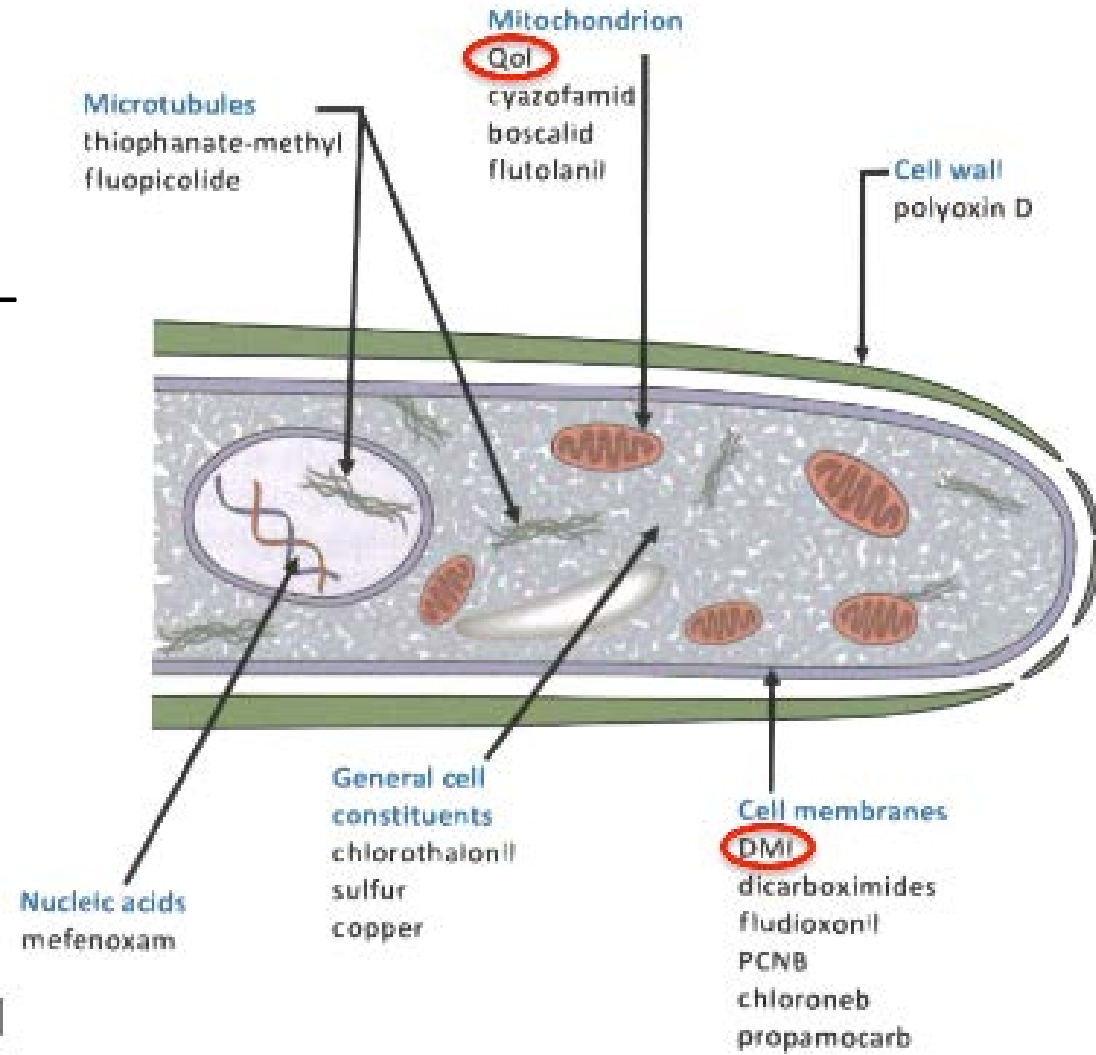
Time scale for development of resistance

- Mode of Action of fungicide
- Genetics of fungus- *target site*
- Fungus life cycle
 - generation time/reproduction*
- Fungicide applications
 - number*
 - timing*

➤ **Difficult to predict development of resistance**

Mechanisms of resistance

Altered target site – prevents fungicide from accessing the target site



A: Nucleic Acid Synthesis

A1: RNA polymerase I
3 RNA polymerase (Dm) fungicides

A2: adenosin deaminase
8. Sydney (A2) fungicides

A3: DNA / RNA synthesis (prop.)
22. Antimetabolites

A4: DNA topoisomerase type I (grisea)
31. Carboxylic acids

Mode of Action of Fungicides

FRAC classification on mode of action 2017 (www.frac.info)

B: Cytoskeleton and Motor Proteins

B1: > 8-tubulin assembly in mitosis
1. 3-DC fungicides (-Benzil, Epoxystyrenes, Carbazoles)

B2: > 8-tubulin assembly in mitosis
10. 8-tubulin carbamates

B3: > 8-tubulin assembly in mitosis
22. Benzimidazole and thiazole carbamates

B4: cell division (prop.)
20. Phenylamides

B5: delocalisation of spectrin-like proteins
6. Benzimidazole, pyridinylmethyl benzimidazole

B6: actin/myosin/ fibrin function e.g. in vesicle trafficking
47. Cyanoacrylates, heterocyclic amides

C: Respiration

C2: Inhibition of complex II: succinate-dehydrogenase
7. SCD fungicides (Quinazolinone, Quinazolinone)

C3: Inhibition of complex III cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene)
13. Qo fungicides (Quinone, Quinone)

C4: Inhibition of complex III cytochrome bc1 (ubiquinol oxidase) at Qi site
24. Qi fungicides (Quinone, Quinone)

C5: Inhibitors of oxidative phosphorylation, ATP synthase
40. Fungicides

C7: ATP production (prop.)
33. Fungicides (Carbamate)

C8: uncouplers of oxidative phosphorylation
27. Fungicides

C1: Inhibition of complex I NADH oxidoreductase
20. Fungicides (Quinone, Quinone)

C4: Inhibition of complex III cytochrome bc1 (ubiquinol oxidase) at Qi site
24. Qi fungicides (Quinone, Quinone)

C5: Inhibitors of oxidative phosphorylation, ATP synthase
40. Fungicides

C7: ATP production (prop.)
33. Fungicides (Carbamate)

C8: uncouplers of oxidative phosphorylation
27. Fungicides

D: Amino Acid and Protein Synthesis

D1: methionine biosynthesis (cys gene) (prop.)
9. Anilino fungicides

D2: protein synthesis type I (grisea)
21. Anilino fungicides

D3: protein synthesis type II (grisea)
24. Anilino fungicides

D4: protein synthesis type I (grisea)
21. Anilino fungicides

D5: protein synthesis type II (grisea)
24. Anilino fungicides

E: Signal Transduction

E1: signal transduction (mechanism unknown)
11. Fungicides

E2: osmotic signal transduction > MAP / histidine kinase (os-1, Dst)
2. Dicarboximides

E3: osmotic signal transduction > MAP / histidine kinase (os-2, HOG1)
47. Quinazolinone, pyridinylmethyl benzimidazole

F: Lipid Synthesis or Transport / Membrane Integrity or Function

F2: phospholipid biosynthesis > methyltransferase
3. Phospholipidase & thiazolopyridines

F3: lipid peroxidation (prop.)
14. 14-oxo fungicides (Quinone, Quinone)

F4: cell membrane permeability, fatty acids (prop.)
23. Carbamates

F6: microbial disruptors of pathogen cell membranes
43. Microbial disruptors

F7: cell membrane disruption (prop.)
46. Plant site fungicides

F8: ergosterol binding
3. Ergosterol binding

F9: lipid homeostasis and transport
49. Ergosterol binding

I: Melanin Synthesis in Cell Wall

I1: tyrosinase in melanin biosynthesis
14. Tyrosinase inhibitors

I2: polyketide synthase in melanin biosynthesis
15. Polyketide synthase inhibitors

I3: ornithine decarboxylase in melanin biosynthesis
16. Ornithine decarboxylase inhibitors

I4: polyketide synthase in melanin biosynthesis
15. Polyketide synthase inhibitors

G: Sterol Biosynthesis in Membranes

G1: C14-demethylase in sterol biosynthesis (erg11/cyp51)
3. DMF fungicides (Quinazolinone, Quinazolinone)

G2: Δ¹⁴ reductase and Δ¹⁴ → Δ⁷ isomerase in sterol biosynthesis (erg2, erg 24)
4. Steroid biosynthesis (DMF, Class II)

G3: 3-keto reductase in C4-26-methylation (erg27)
17. DMF (Class III)

G4: squalene epoxidase in sterol biosynthesis (erg7)
18. DMF (Class IV)

H: Cell Wall Biosynthesis

H4: chitin synthase
10. Fungicides

H5: cellulose synthase
6. Cellulose synthase

P: Host Plant Defence Induction

P01: salicylic pathway
1. Salicylic acid

P02: salicylic pathway
1. Salicylic acid

P03: salicylic pathway
1. Salicylic acid

P04: salicylic pathway
1. Salicylic acid

P05: salicylic pathway
1. Salicylic acid

P06: salicylic pathway
1. Salicylic acid

M: Chemicals with Multi-Site Activity

M01: Multi-site activity

M02: Multi-site activity

M03: Multi-site activity

M04: Multi-site activity

M05: Multi-site activity

M06: Multi-site activity

M07: Multi-site activity

M08: Multi-site activity

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M96: Multi-site activity

M97: Multi-site activity

M98: Multi-site activity

M99: Multi-site activity

M100: Multi-site activity

BM: Biologicals with Multiple Modes of Action

BM01: Biologicals with Multiple Modes of Action

BM02: Biologicals with Multiple Modes of Action

Unknown Mode of Action

U1: Unknown Mode of Action

U2: Unknown Mode of Action

U3: Unknown Mode of Action

U4: Unknown Mode of Action

U5: Unknown Mode of Action

U6: Unknown Mode of Action

U7: Unknown Mode of Action

U8: Unknown Mode of Action

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U93: Unknown Mode of Action

U94: Unknown Mode of Action

U95: Unknown Mode of Action

U96: Unknown Mode of Action

U97: Unknown Mode of Action

U98: Unknown Mode of Action

U99: Unknown Mode of Action

U100: Unknown Mode of Action

NC: Not Classified

NC: Not Classified

Legend:

- = mode of action group
- = sub-group
- = target site of action
- = carbon source or pathway
- = target site of action
- = FRAC code
- = mode of action group
- = chemical class group

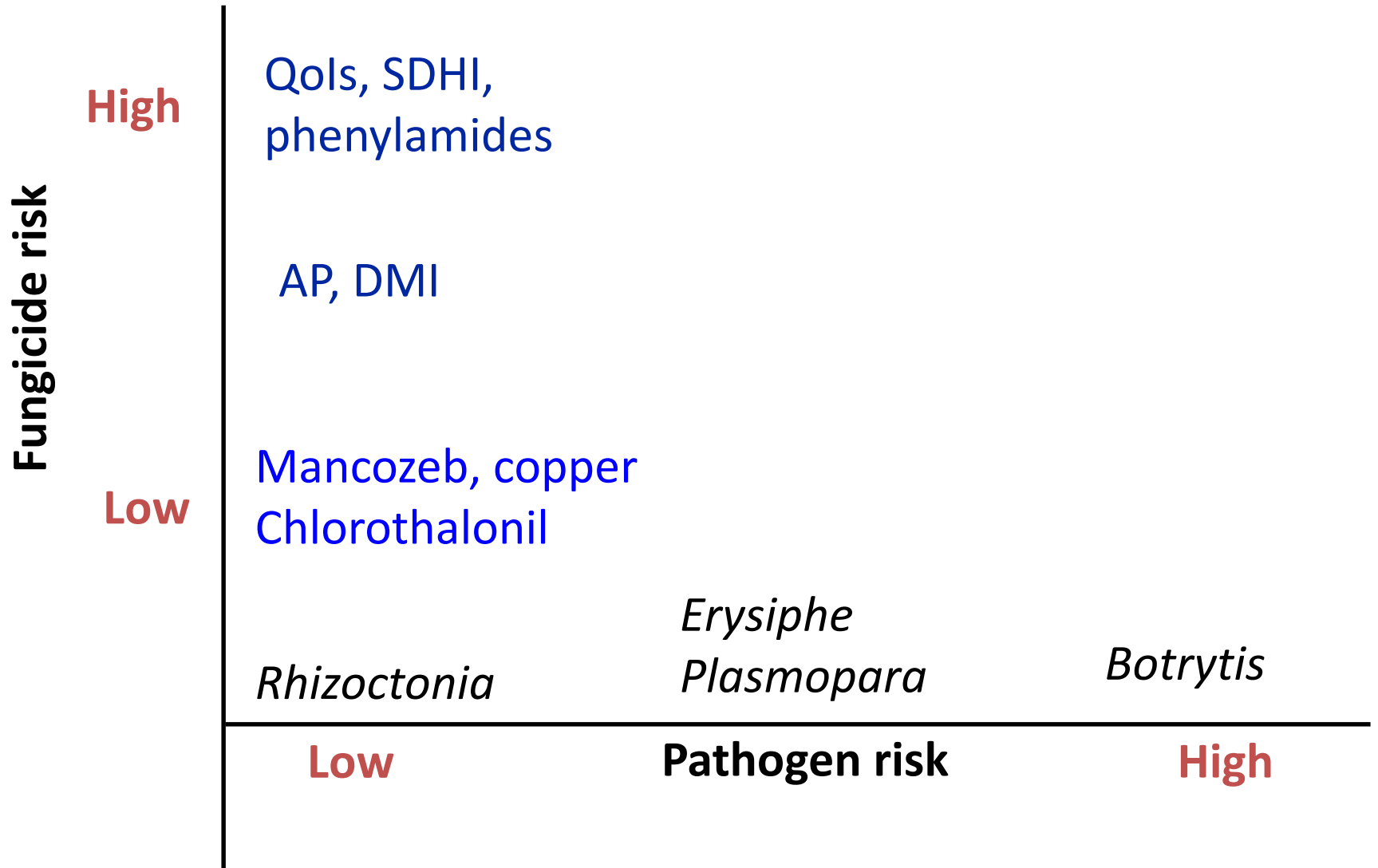
C: Respiration

- = Inhibition of complex I
- = Inhibition of complex II
- = Inhibition of complex III
- = Inhibition of complex IV
- = ATP production
- = Uncouplers of oxidative phosphorylation

FRAC
FUNGICIDE RESISTANCE ACTION COMMITTEE

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Fungicide / target site risk

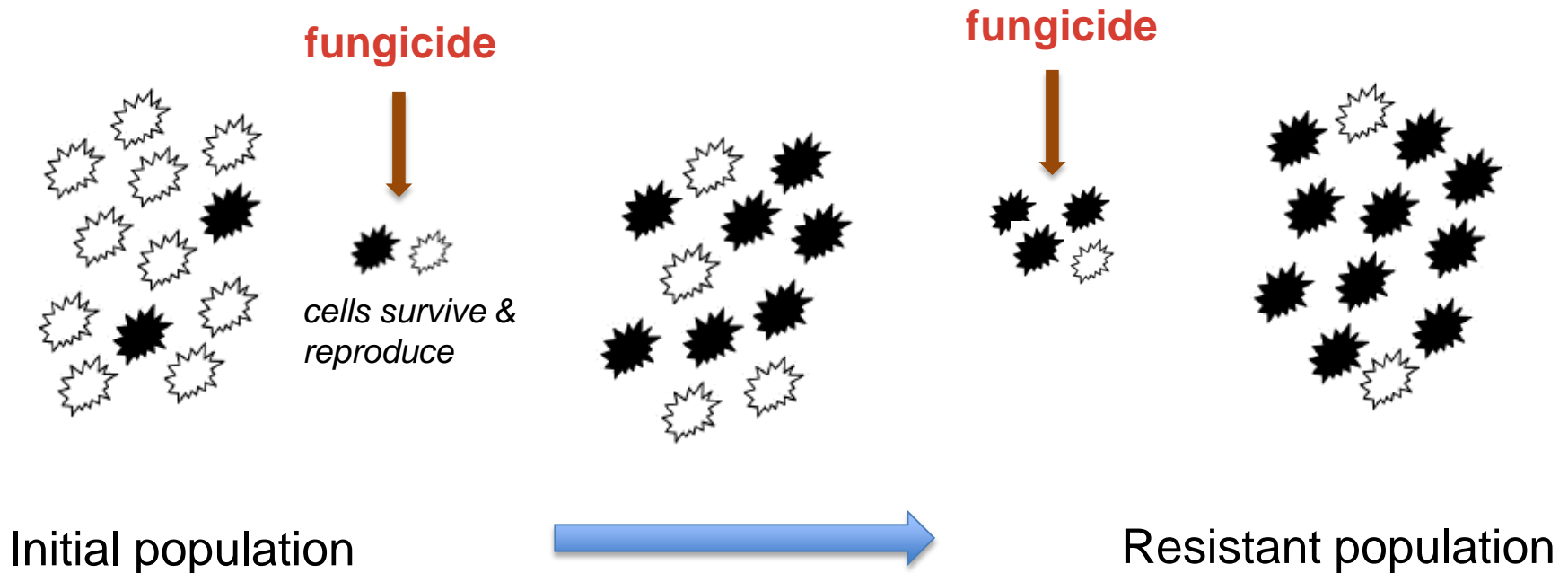


How does resistance develop?

DNA mutations affect fungicide activity

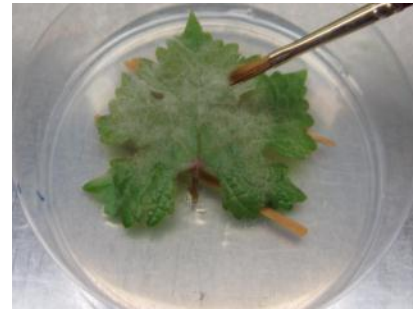
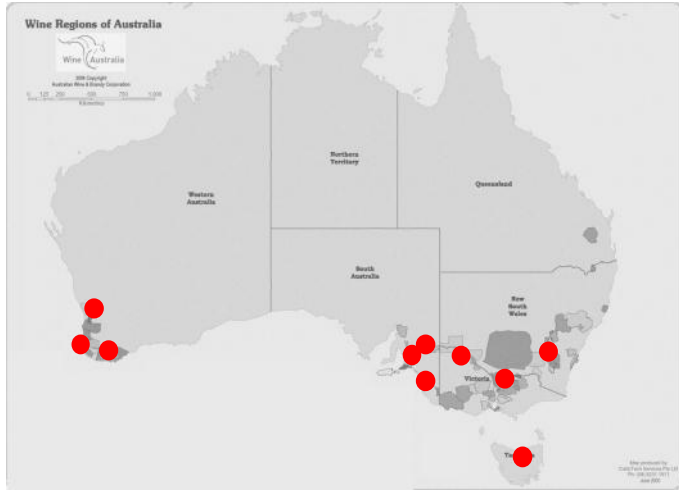
 Sensitive: no mutation

 Resistant: naturally occurring mutation

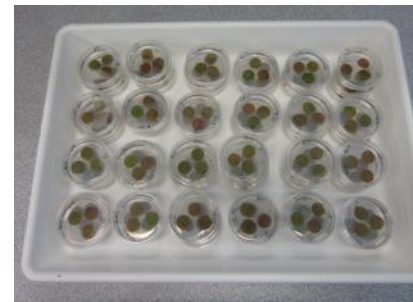


Testing for resistance

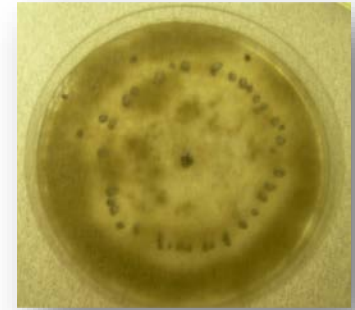
Methods



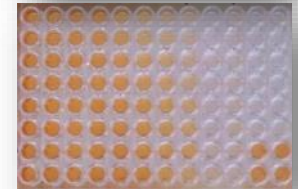
Powdery & downy



Lab tests for sensitivity



Botrytis



**Sensitive
OR
Resistant?**



DNA

— = Adenine

— = Thymine

— = Cytosine

— = Guanine

— = Phosphate backbone


+

Detect mutation(s)

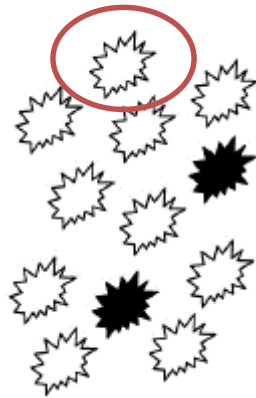
Caveat.....

Lab results on small numbers of samples cannot be directly equated to field performance

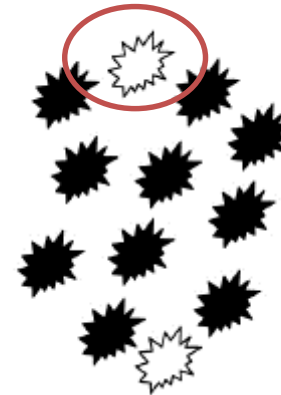
Resistance detection

 Sensitive: no mutation

 Resistant: naturally occurring mutation



Sensitive population



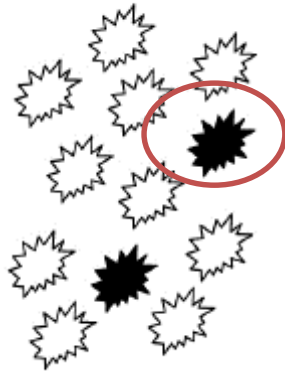
Resistant population

One test may only detect sensitive: not representative of population

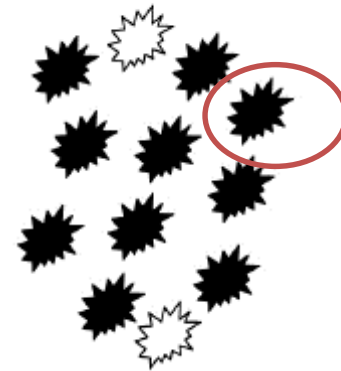
Resistance detection

 Sensitive: no mutation

 Resistant: naturally occurring mutation




Sensitive population



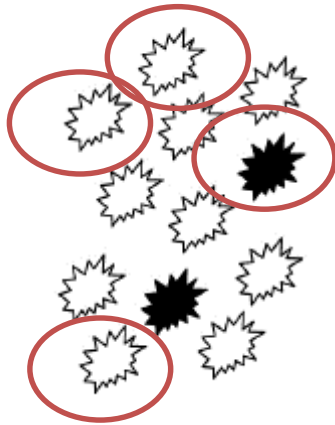
Resistant population

One test may only detect resistant: not representative of population

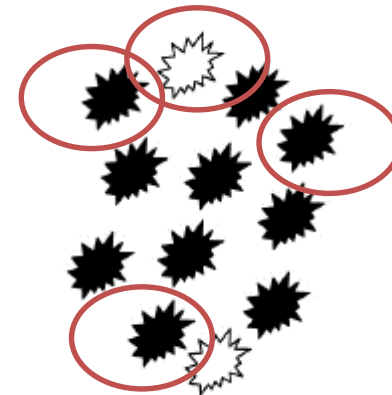
Resistance detection

 Sensitive: no mutation

 Resistant: naturally occurring mutation



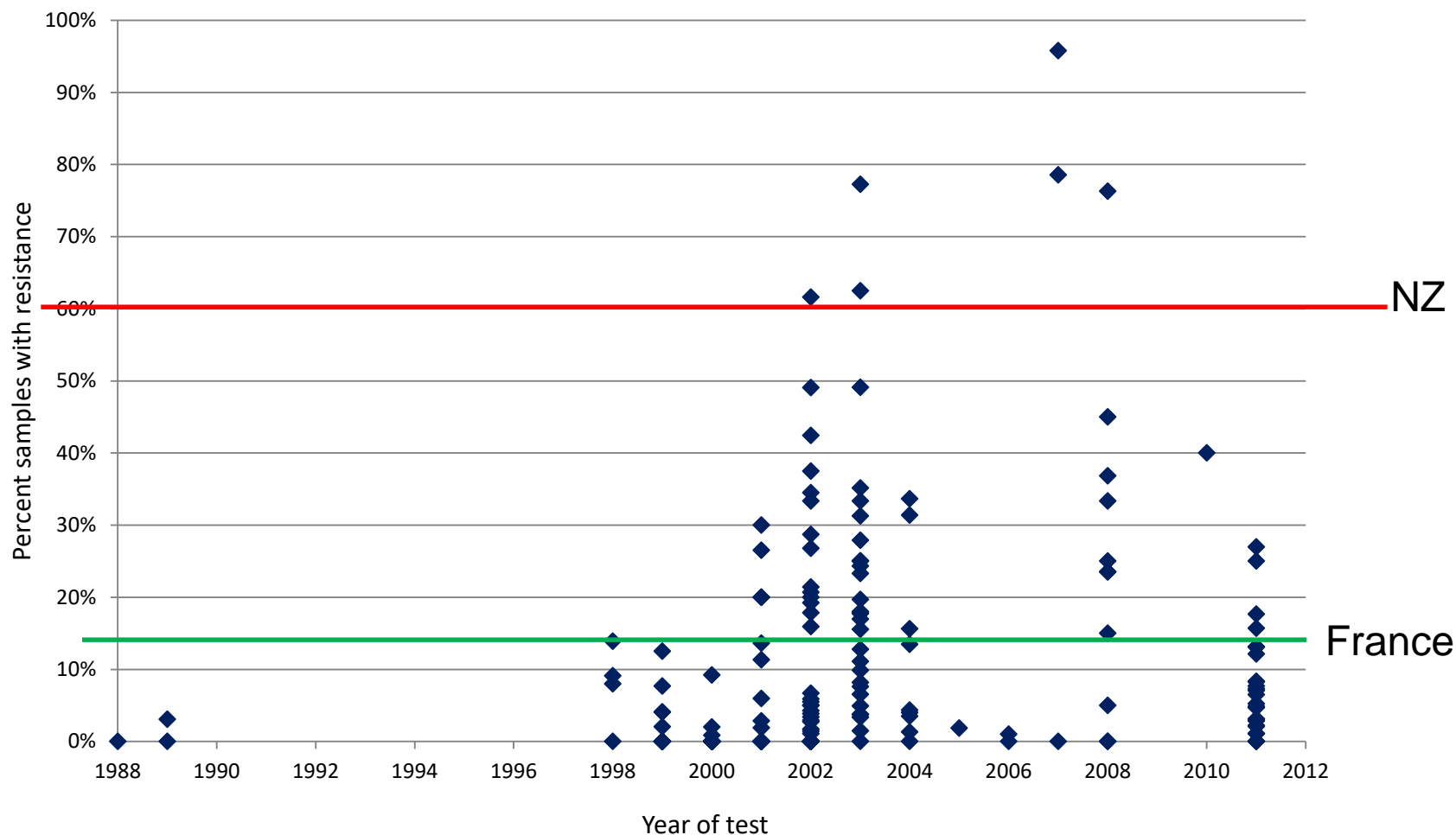
Sensitive population



Resistant population

Multiple tests are needed to be representative of population

Botrytis - dicarboximide



Results of where field performance is affected vary:

France: 15% of 100 bunches resistant, have reduced field performance

NZ suggest needs to be 60%

What's new with powdery mildew?

- Testing of Talendo
 - Field trial with apparent failure : possibility of reduced sensitivity but unlikely to be resistant
 - Also tested against Legend – all sensitive
- SDHI base line testing (commercial) – all sensitive

Powdery mildew fungicides

FRAC group	Activity group	Product examples	Active ingredient
QoIs (Quinone outside Inhibitors)	11	Amistar® Cabrio® Flint®	azoxystrobin pyraclostrobin trifloxystrobin
Demethylation Inhibitors -DMIs	3	Topas® Mycloss® Domark® Bayfidan® Triadimefon® Digger® Viva® Orius®, Folicur®	penconazole myclobutanil tetraconazole triadimenol triadimefon difenoconazole* hexaconazole tebuconazole
Morpholines, SBI Class II	5	Prosper®	spiroxamine
SDHI*	7	Filan® coded product	boscalid -
Aza-naphthalenes*	13	Legend® Talendo®	quinoxifen* proquinazid
Phenyl-acetamide	U6	Flute®	cyflufenamid
Aryl-phenyl-ketone	U8	Vivando® Kusabi®	metrafenone pyriofenone

Red = resistance detected or assumed

Green = not detected

Black = not tested

Purple = possible - more testing needed

* Limited samples tested

What's new with downy mildew?

Yarra Valley: metalaxyl and pyraclostrobin resistance confirmed (including in abandoned vineyard)

King Valley: metalaxyl resistance confirmed, pyraclostrobin not detected but two samples slightly higher EC_{50}

Hunter Valley: metalaxyl resistance confirmed, pyraclostrobin suspected

More work will start 2019/20 with additional funding from WA

Metalaxyl resistant isolates wanted for AWRI work

Downy mildew- fungicides

FRAC group	Activity group	Product examples	Active ingredient
PhenylAmides	4	Ridomil®	metalaxyl
QoIs (Quinone outside Inhibitors)	11	Amistar® Cabrio®	azoxystrobin pyraclostrobin
Carboxylic acid amides	40	Acrobat® Revus®*	dimethomorph mandipropamid*
Phosphonates	33	Aliette® Foli-R-Fos®	fosetyl-aluminium phosphorous acid

Red = resistance detected or assumed

Green = not detected

Black = not tested

Purple = possible - more testing needed

* Limited samples tested

What's new with Botrytis?

- Retesting fludioxonil with lower discriminatory dose – have found some potentially less sensitive isolates
- SDHI base line testing (commercial) – all sensitive

Botrytis fungicides

FRAC group	Activity group	Product examples	Active ingredient
Dicarboximides	2	Rovral®	iprodione
SDHI	7	Filan® coded new product* coded new product	boscalid - -
Anilino-Pyrimidines	9	Scala® Solaris®, Switch®	pyrimethanil cyprodonil
Qols	11	Amistar®	azoxystrobin
PhenylPyrroles	12	Switch®	fludioxonil
SBI Class III	17	Teldor® Prolectus®	fenhexamid fenpyrazamine

Red = resistance detected

Green = not detected

Black = not tested

Purple = possible - more testing needed

* Initial showed suspect resistance, repeat testing showed not resistant, but less susceptible

Where are we now with testing?

Botrytis:

- Relationship between lab test and field performance known for older fungicides
- Good relationship between phenotypic and genotypic tests
- Potential for in field testing eg LAMP assays, dPCR
- Commercial testing available (plate assays)

Powdery mildew:

- Phenotypic tests work but laborious and sample size needed unknown
- Relationship between phenotypic and genotypic tests not clear
- Commercial testing not available

Downy mildew:

- Phenotypic tests work but consistency of results an issue
- Metalaxyl has no known mutation
- Commercial testing not available

Manage poor performance

- Good coverage
- Fungicide choice
 - Correct rate
 - Timing
- Manage resistance risk

Managing resistance risk

Always follow resistance management guidelines

Rotate or mix different modes of action

Use label rates

Limit total number of applications per fungicide group

Educate yourself about fungicide activity, modes of action, resistance groups & management practices

Start a fungicide program with a multi-site MOA to reduce populations – **DO NOT** use single-site MOA to control well-established infections



Thanks to Wine Australia, growers & viticulturists



Project team & industry collaborators



Industry reference group



Vitisolutions



SARDI