Incidence of the G143A Mutation for QoI Resistance in Grapevine Powdery Mildew Populations

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What is Powdery Mildew

Erisyphe necator

- Destructive grapevine pathogen

Obligate biotroph

- Infects all green tissue (interferes with photosynthesis)
- Effects quality and quantity of grape yield
  - Low sugar
  - Poor colour
  - Increased acidity
  - Cracked berries have a mouldy flavour

Significant economic loss

- Exceeds 30% of production costs

Stummer et al. (2000), Rantsiou et al. (2020), Pool et al. (1984), Gadoury et al. (2001), Calonnec et al. (2004), Fuller *et al.* (2014) & Sambucci et al. (2019)

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Life Cycle

Sexual

- Leads to **overwintering** structures **(chasmothecia) –** ensure the survival of inoculum for the growing season (sexual stage can remain dormant)
- **Heterothallic** mating system
  - *MAT1-1* & *MAT1-2*
- Allow organisms to **adapt** to changing **environments**
  - Associated with shifts in **fungicide sensitivity**
  - Qol's not as effective anymore

Concerns

- From 1996 – 2025 we have seen a significant increase in the presence of chasmothecia
- This is concerning because
  - Chasmothecia- **survival**
  - Forcefully discharge - **long distance dispersal**
  - New possible **favourable genetic make-up**
    - Less responsive to management – **fungicide resistant**
    - **More adapted** to wider climatic conditions

Billiard et al., 2012, Drenth et al., 2019, Otto, 2009, Bell, 1982, Nieuwenhuis and James, 2016, Ni et al., 2011, Gadoury et al., 2012, Cochrane, 1974, Hamilton et al., 2013, Sephton-Clark and Voelz, 2018, Huang and Hull, 2017, Redl et al., 2021a

QoI Sensitivity globally

Fungicide applications (preventative)

- Viticulture has one of the highest use of fungicides – increased risk of fungicide sensitivity shifts
- Registered QoI actives
  - Azoxystrobin
  - Pyraclostrobin
  - Trifloxystrobin

First report of QoI resistance in New York – 5 years after introduction

- Eastern United States (Baudoin 2008)
- France (Dufour 2011)

Extensive global studies

- USA (Miles et al., 2012; Baudoin et al., 2008, Wilcox et al., 2003, Miller et al., 2004, Colcol et al., 2015, Rallos et al., 2014)
- Europe (Dufour et al,. 2011)
- New Zealand (Beresford et al., 2016)
- **But no QoI studies done in SA**

Baudoin et al. (2008); Wilcox et al. (2003); Miles et al. (2012); Sierotzki et al. (2007), Miles et al. (2021)

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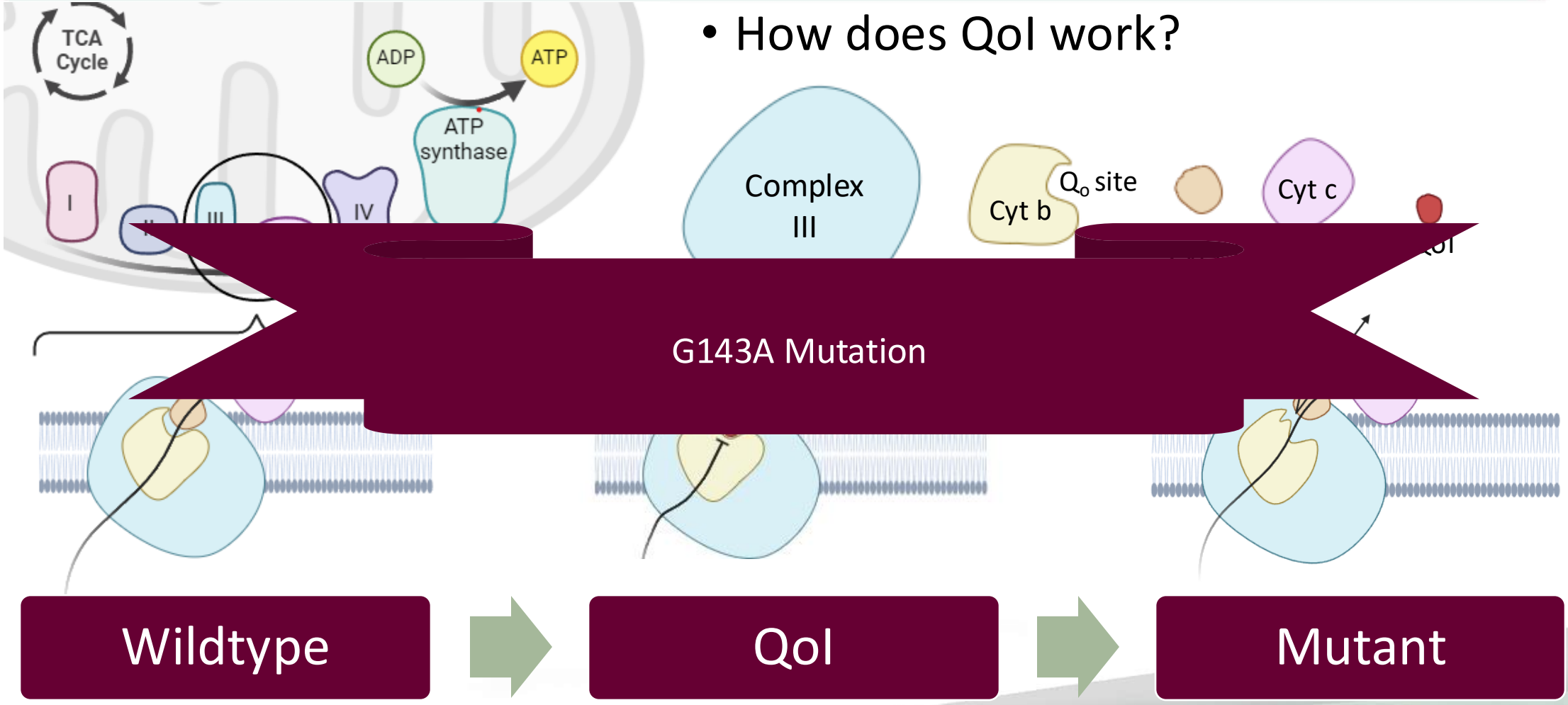
QoI sensitivity mechanism

- How does QoI work?



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Problem statement

- The level of sensitivity and the distribution of WT and MT individuals to QoI actives in SA vineyards is unknown.

Aim

- To investigate the geographical and temporal distribution of the G143A mutation in *Erysiphe necator* and explore the role of sexual reproduction in resistance.

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Objectives

1. Determine the geographical distribution of the WT and MT individuals from 27 vineyards

2. Temporal distribution of the WT and MT individuals in four vineyards

3. Determine the EC_{50} values associated with WT and MT individuals in South African populations

Objective 1

1. Determine the geographical distribution of the WT and MT individuals from 27 vineyards

2. Temporal distribution of the WT and MT individuals in four vineyards

3. Determine the EC_{50} values associated with WT and MT individuals in South African populations

Geographical distribution of G143A mutation

27 vineyards

- Table grape cultivars
- Wine grape cultivars

6 Köppen-Geiger climate classification areas

- BSk, BWk, Cfa, Cfb, Csa, Csb

20 Single colonies collected per site with “scotch tape” **in the field**

- Chelex 100 DNA extractions (Miles et al. 2021)
- 493 isolates
  - Species-specific PCR (Brewer and Milgroon, 2011)
  - *MAT 1-1* (EnαR3 and EnαF2) & *MAT 1-2* (ENHGMF and ENHGMR) (Brewer and Milgroon, 2011)

20 symptomatic leaves per vineyard

Inspected under a stereomicroscope for chasmothecia

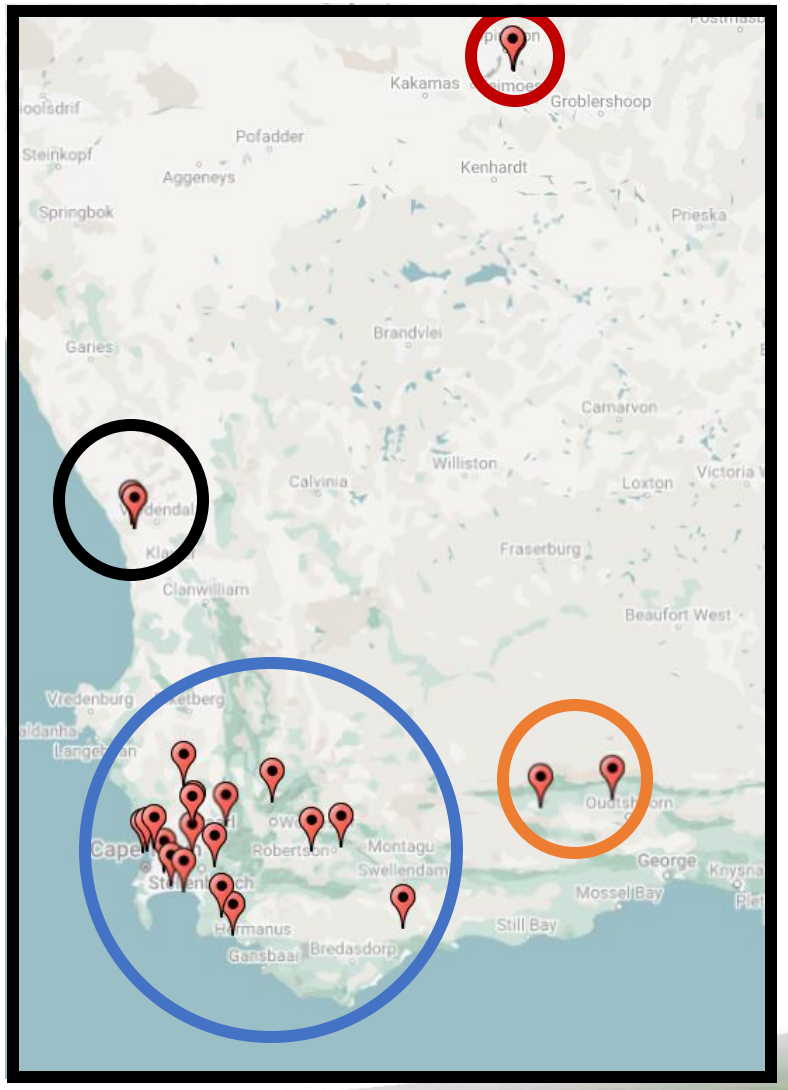
qPCR assay

Determine presence of mutant (MT) or wildtype (WT) allele (G143A):

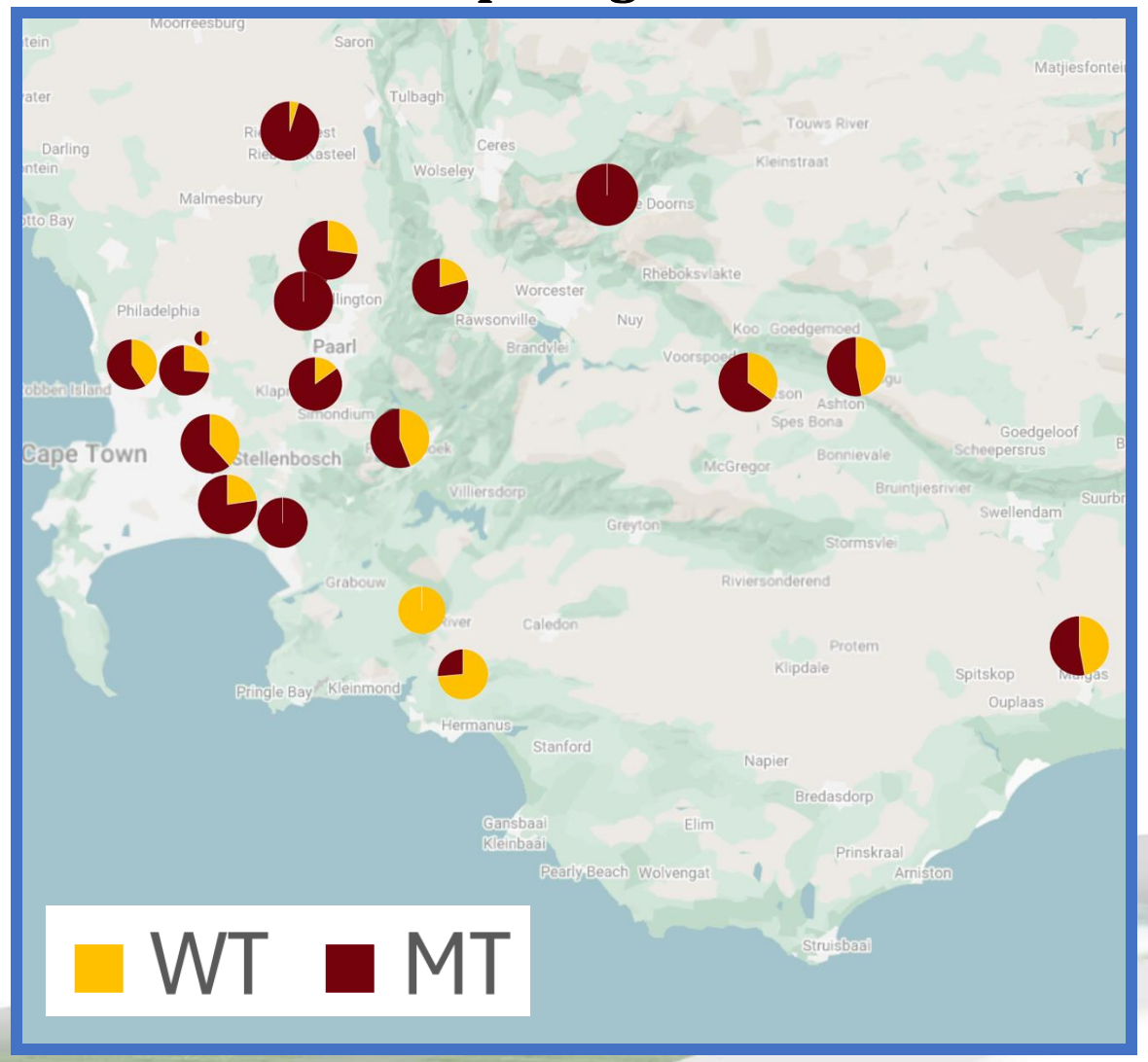
- Quant studio
- Taqman probe **(Miles et al., 2021)**
  - 83F and 517R primers
  - WT FAM and MT HEX probes
- Distribution of WT:MT per vineyard determined with Chi-square (R-studio)

27 vineyards

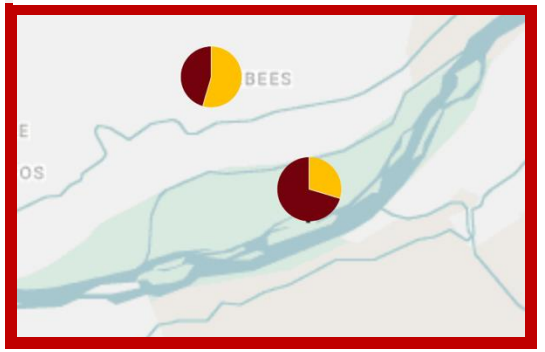
27 Farms



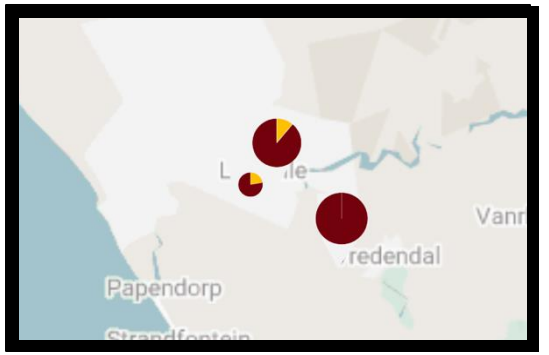
Cape region



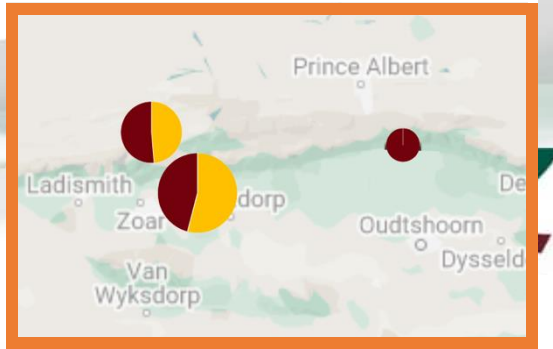
Orange river



Olifants river



Klein Karoo

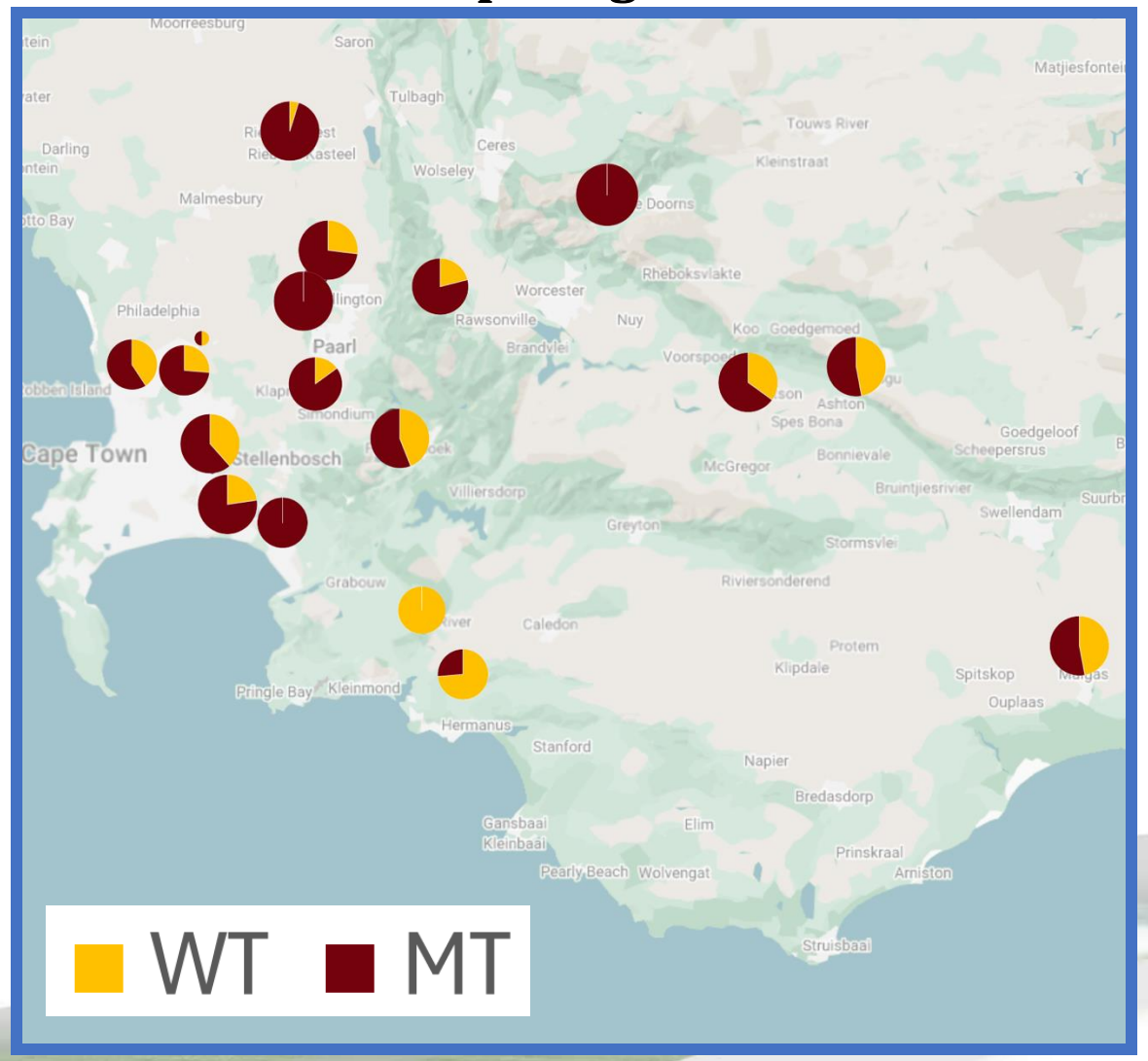


MT:WT ratio over all populations

Mutant : Wildtype

- **Dominant MT**
  - 66.34% MT
  - 33.66% WT
- **Populations**
  - MT dominant – 14
  - Even – 11
  - WT dominant – 2

Cape region



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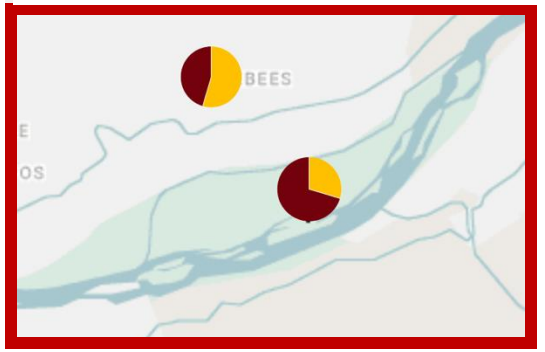

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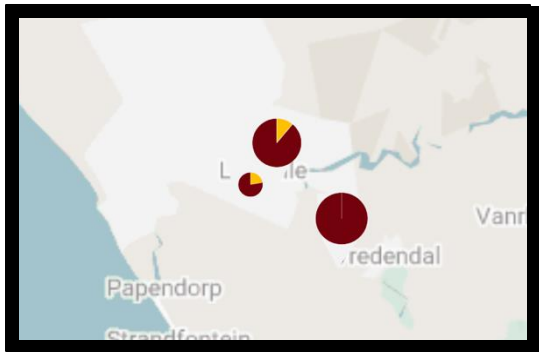

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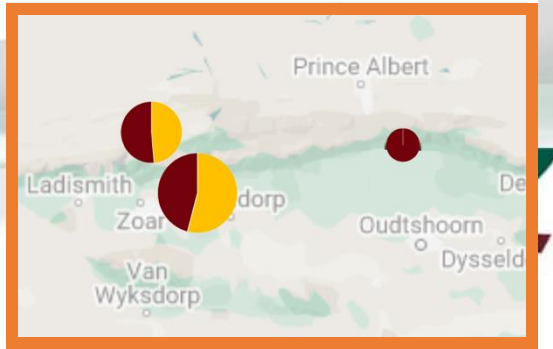
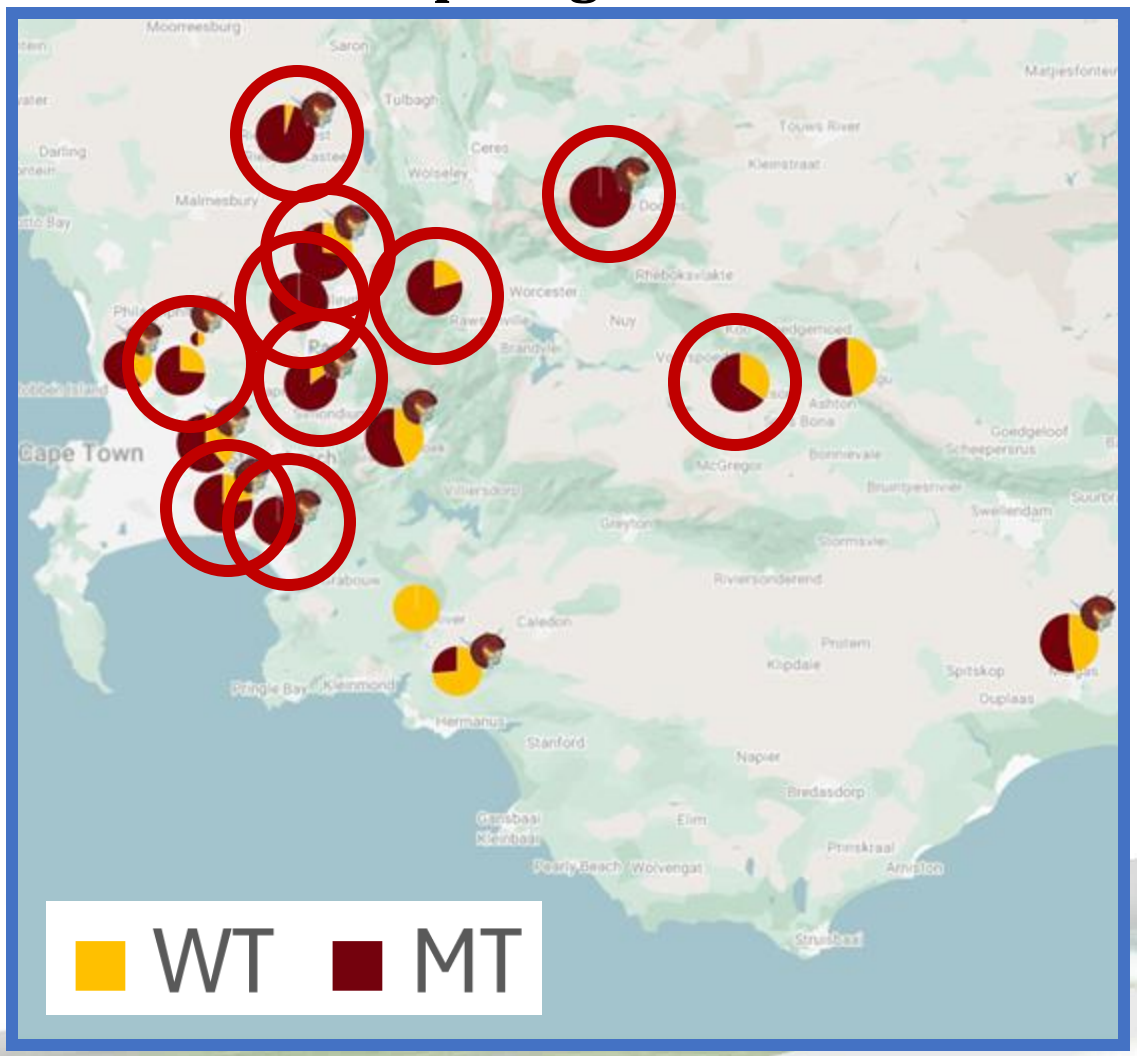


MT dominant populations

MT dominant

- 14/27 MT dominant
  - 5 vineyards only MT
- All populations had either:
  - Both *MAT1-1* & *MAT1-2* (14)
  - Chasmothecia (10)
    - Could have sampled too early

Cape region



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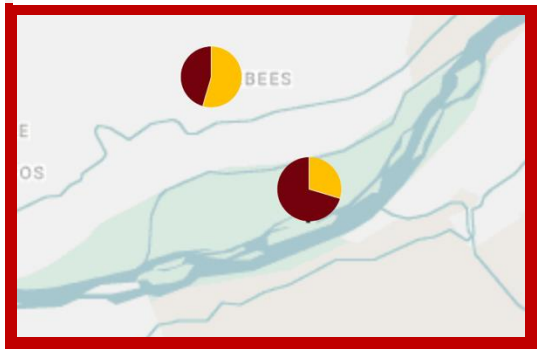
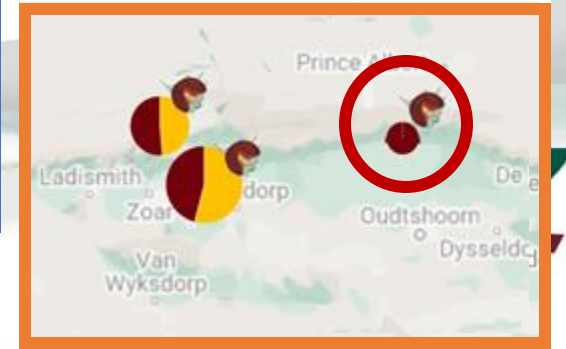


Olifants river

Klein Karoo

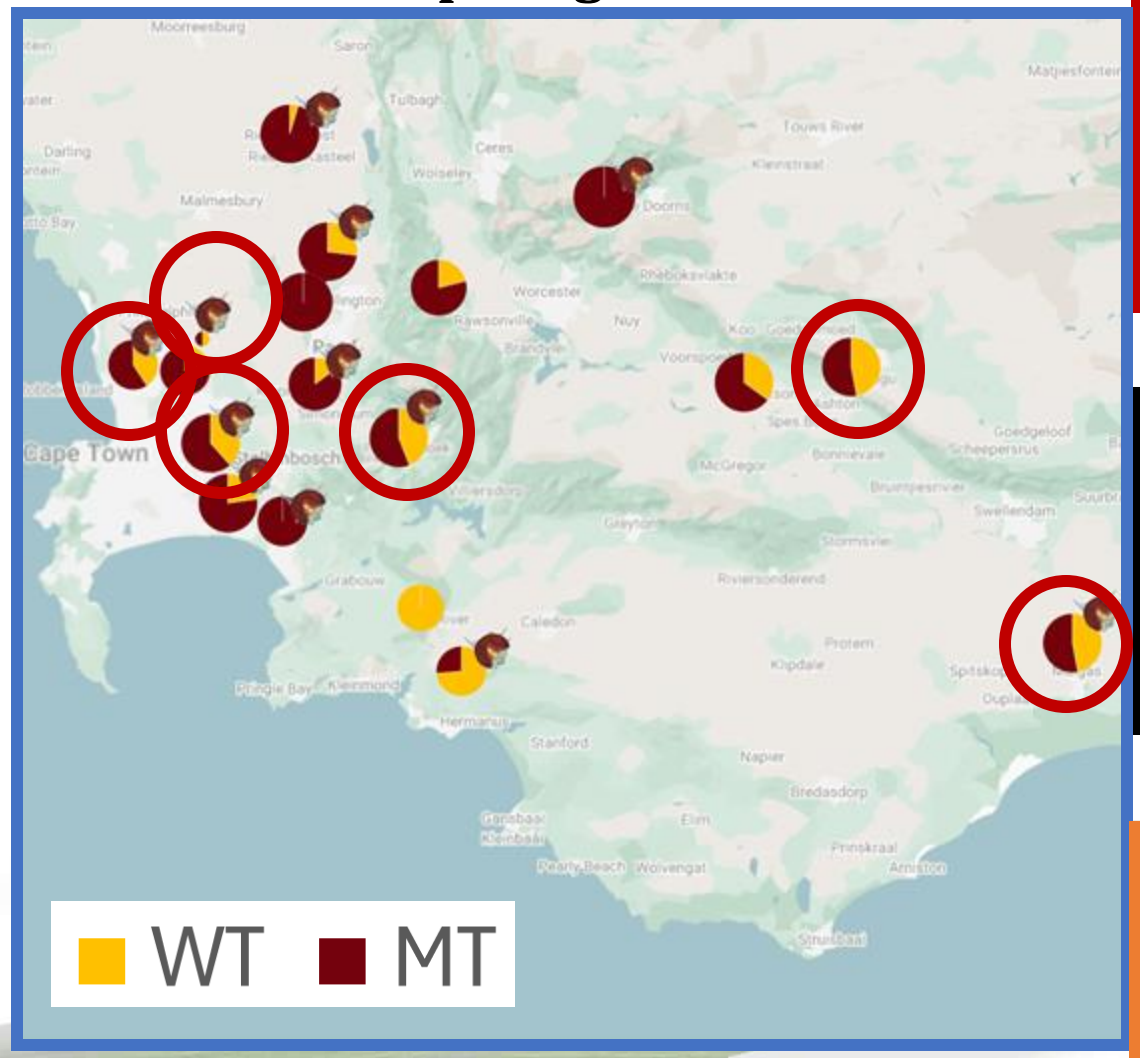


Populations with even MT and WT ratio

Even MT and WT ratio

- 11/27 even WT and MT
  - Chasmothecia (6) or both *MAT1* idiomorphs observed (8)
- Except for the two Upington vineyards
  - No chasmothecia and only *MAT1-2*
  - Janse van Rensburg (2025) surveyed chasmothecia in surrounding blocks

Cape region



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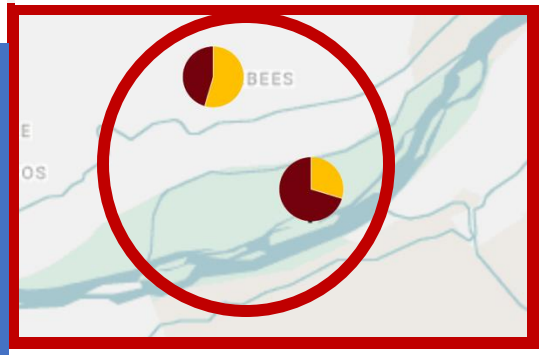

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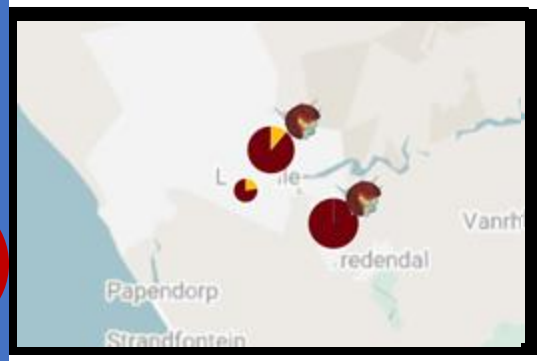

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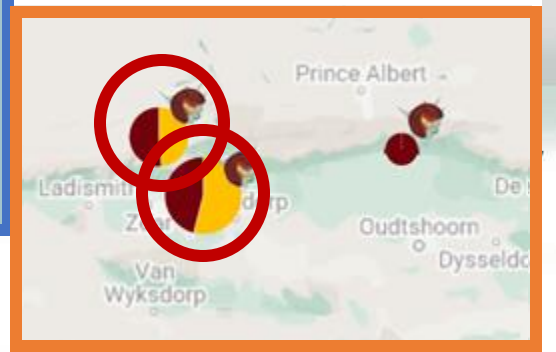
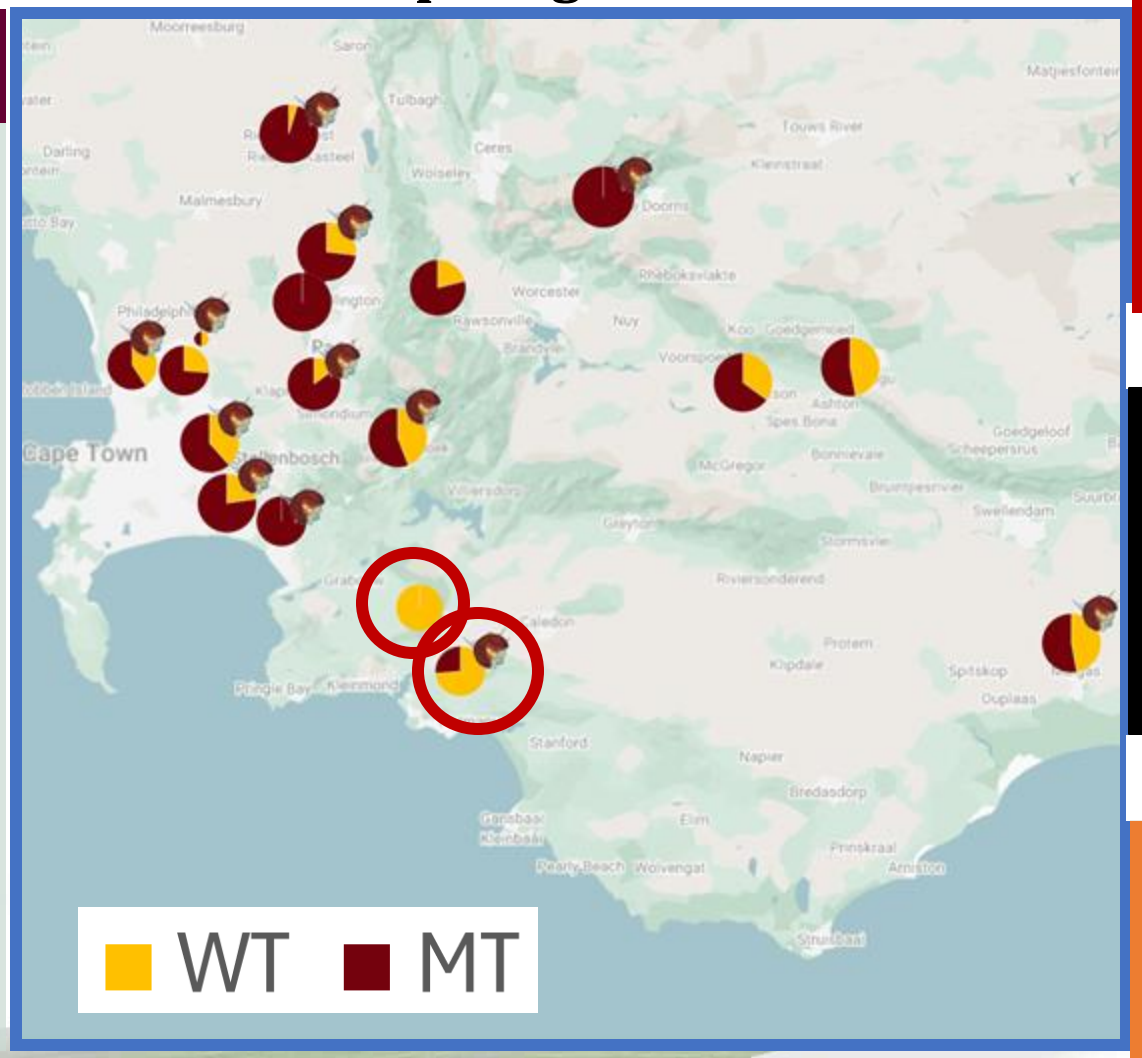


WT dominant populations

WT dominant

- **Two vineyards WT dominant**
  - In Hermanus and Botrivier
- **Only one WT only population (Botrivier)**
  - No chasmothecia
  - Only *MAT1-2*
- **Other WT dominant**
  - Chasmothecia present
  - Both *MAT1* idiomorphs

Cape region



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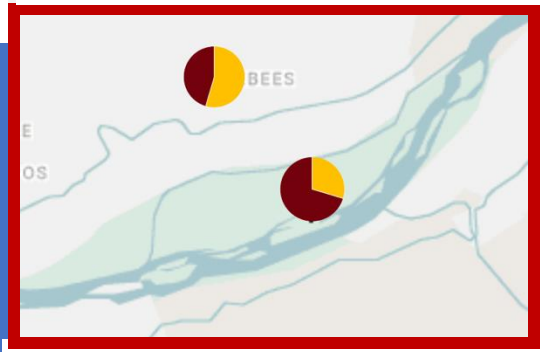

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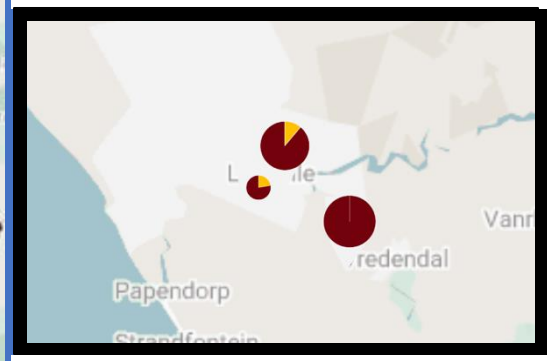

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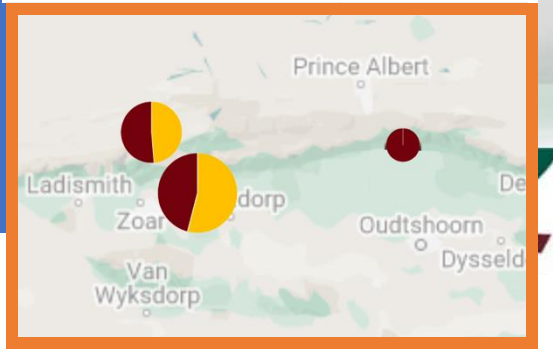


Objective 2

1. Determine the geographical distribution of the WT and MT individuals from 27 vineyards

2. Temporal distribution of the WT and MT individuals in four vineyards

3. Determine the EC_{50} values associated with WT and MT individuals in South African populations

Sample four vineyards over time (Oct 2023-May 2024)

Vineyards selected (history of PM)

- Two wine grape cultivars
  - Simondium
  - Stellenbosch (organic-sulphur)
- Two table grape cultivars
  - de Doorns
  - Wellington

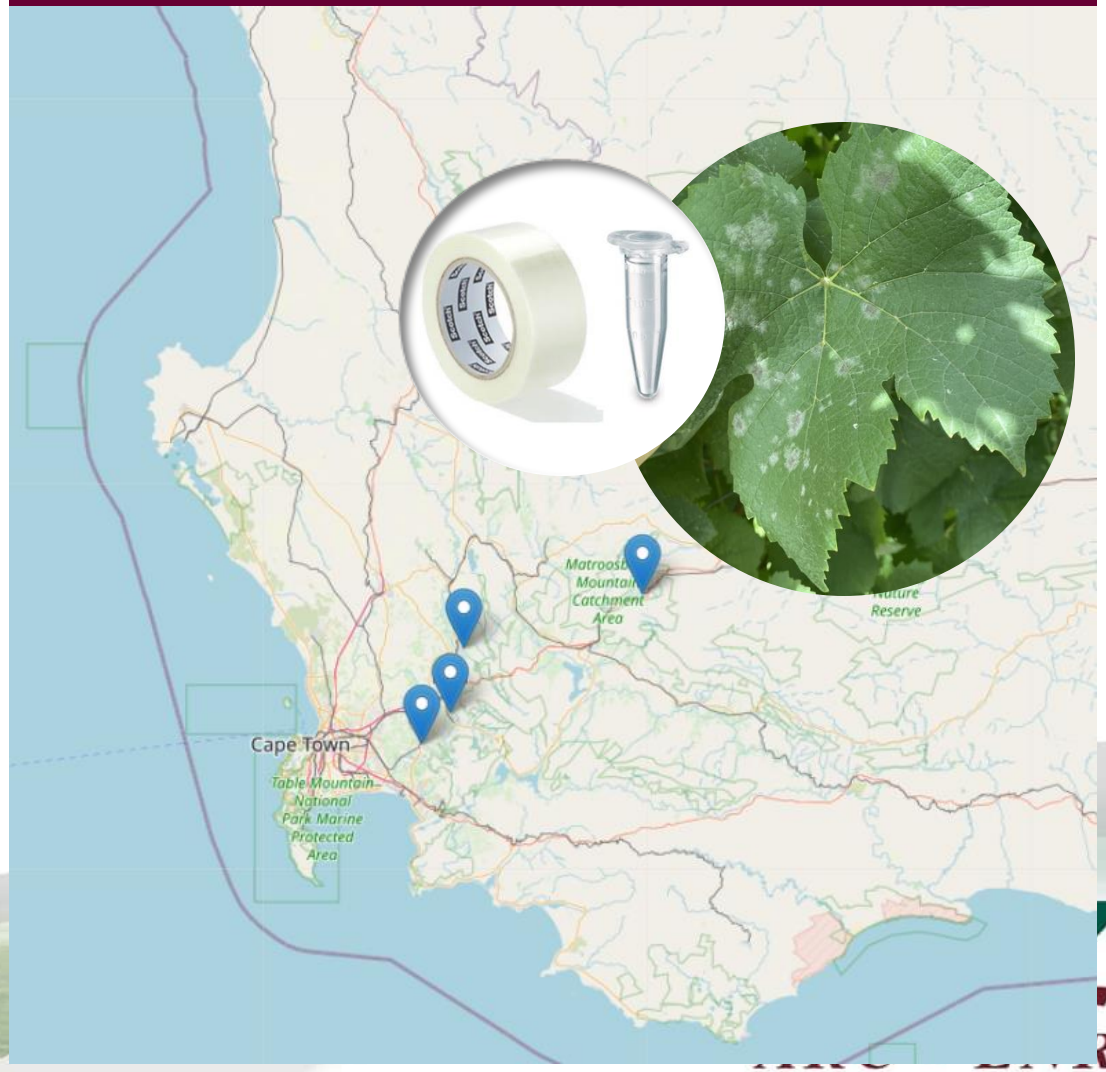
Sampled biweekly (leaves observed for chasmothecia)

- Colonies collected using Scotch tape
- Chelex 100 DNA extractions (Miles et al. 2021)
- Species-specific PCRs (Brewer and Milgroom., 2011)

qPCR assay (Chi-square analysis)

- Taqman probe assay (Miles et al., 2021)

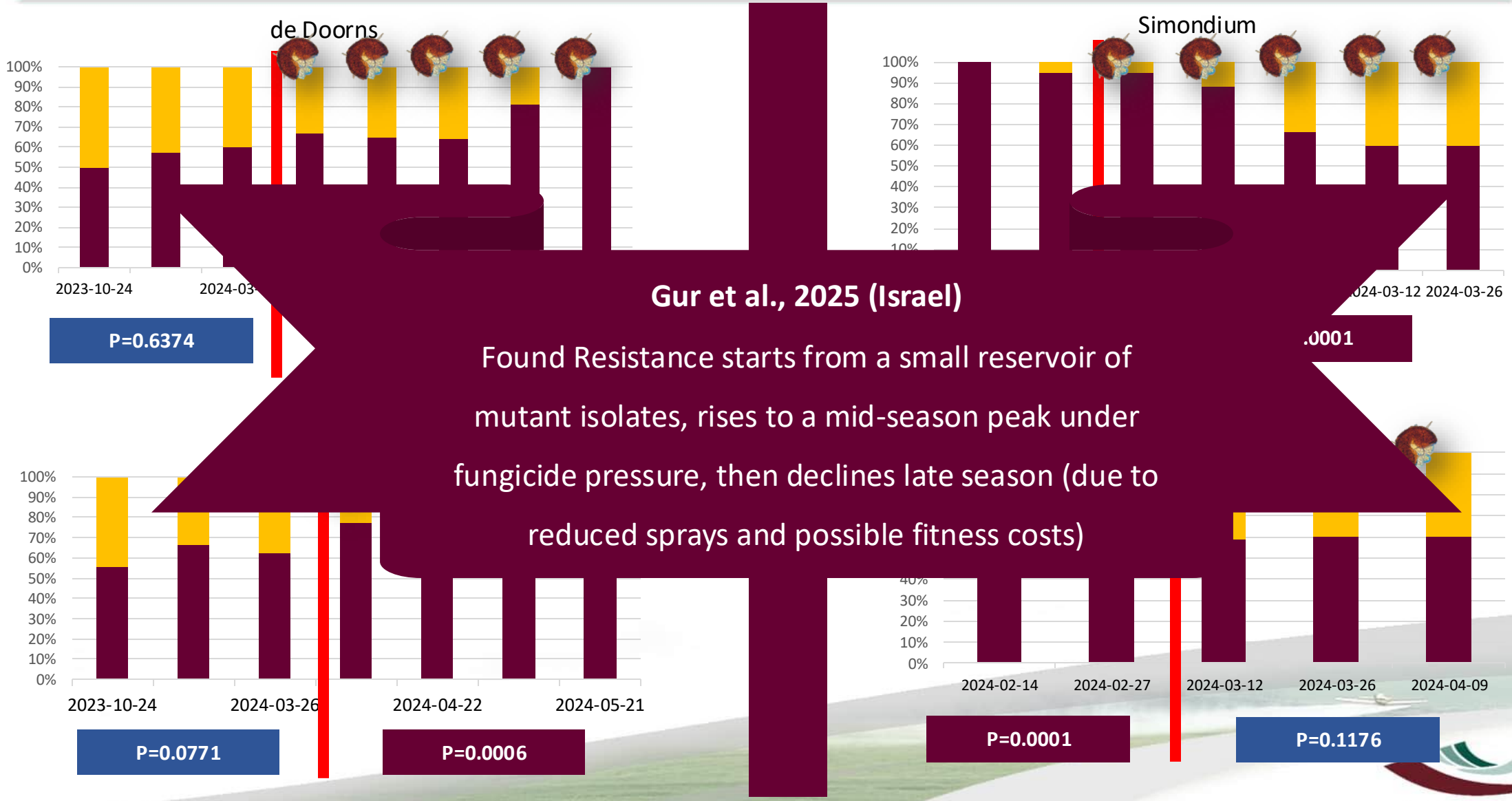
Sampled single colonies



Temporal distribution

WT MT

Table Grape



Gur et al., 2025 (Israel)

Found Resistance starts from a small reservoir of mutant isolates, rises to a mid-season peak under fungicide pressure, then declines late season (due to reduced sprays and possible fitness costs)

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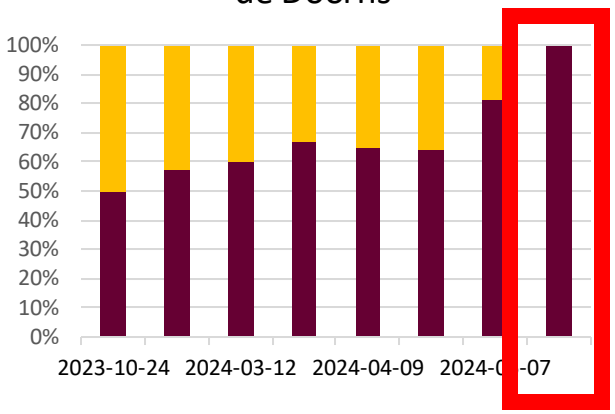
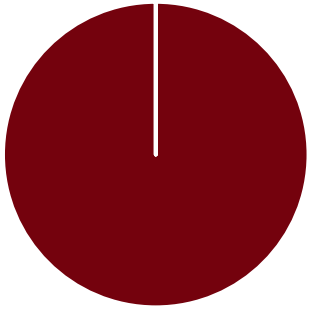
Snapshot

WT MT

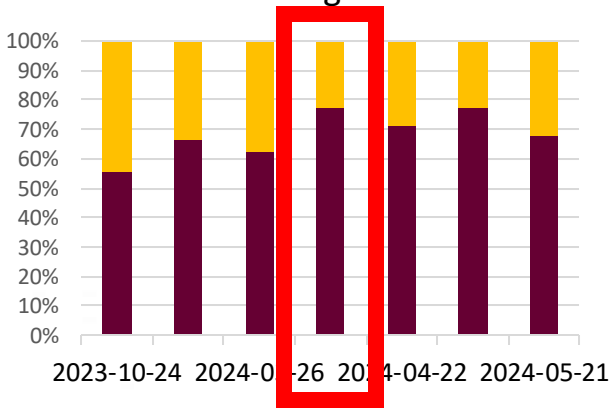
Table Grape

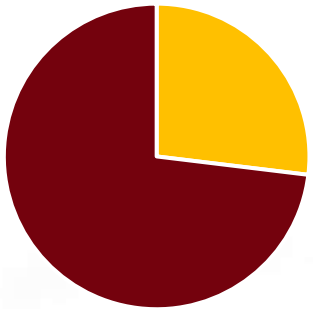
de Doorns





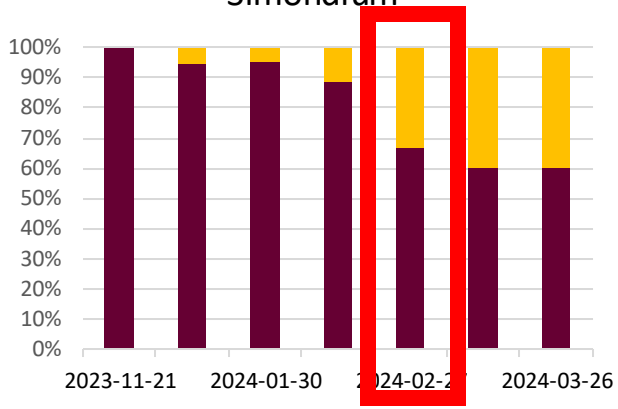
Wellington

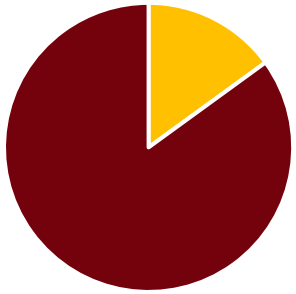




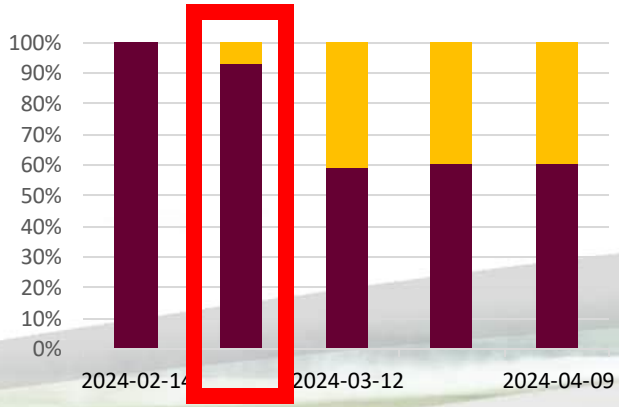
Wine Grape

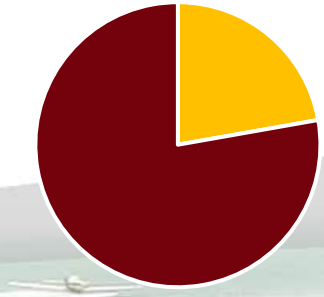
Simondium





Stellenbosch





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Objective 3

EC_{50} values for MT reported in other countries:

- **New Zealand**
  - > 100µg/mL (Beresford et al., 2016)
- **USA**
  - > 60 µg/mL (Rallos et al., 2008)
  - >100 µg/mL (Miles et al., 2012)
- **Israel**
  - > 100µg/mL (Gur et al., 2025)

3. Determine the EC_{50} values associated with WT and MT individuals in South African populations

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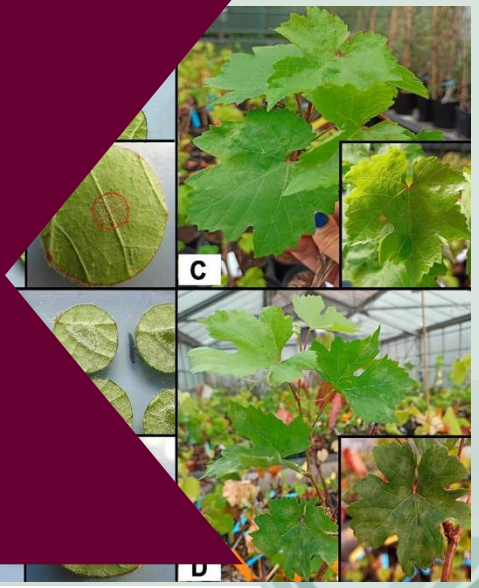
Challenges of working with an obligate biotroph

- EC_{50} values are generally determined with mycelial growth on amended media
  - *Erysiphe necator* is an obligate biotroph

Single conidia chain transfer	Detached leaf on water agar	EC_{50} on leaf disks



Leaf discs too laborious

- Would require too many leaf disks (> 4 000)
- Contamination
- Don't have 100% germination efficacy

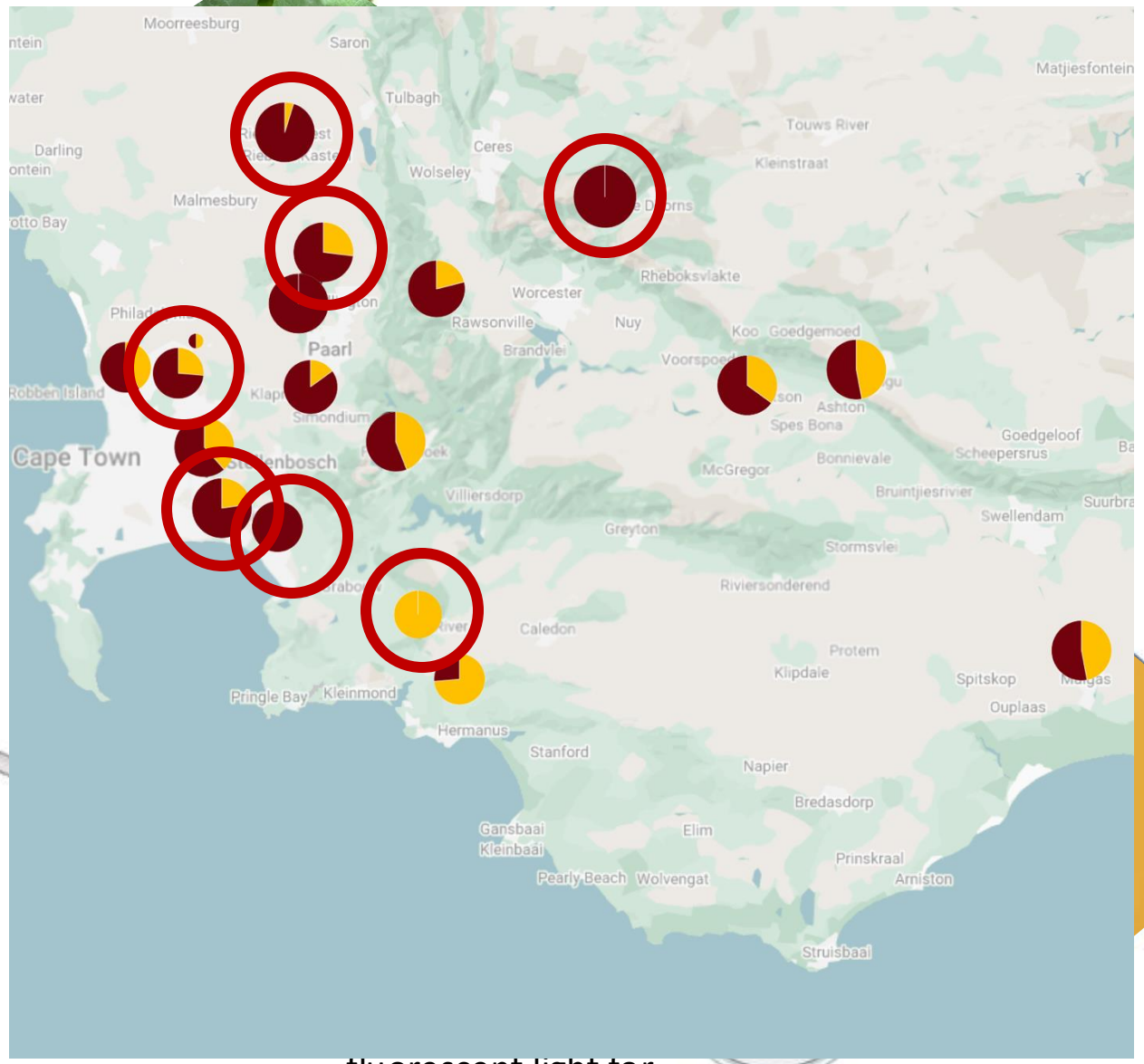
Opted for WA assays (Miles et al., 2012)

- PM can germinate for 48hrs until host is found, germination ceases if no host is present

e: Ruiz-Garcia et al. (2021)

Reference: Evans et al. (1996) *Mycological Research* 100: 675-680

3. Materials and Methods: EC_{50} assays



fluorescent light for 48hrs

Collect 20 diseased leaves per site

- 3 Table grape cultivars
- 4 Wine grape cultivars

Single-spore onto leaf disks

Brush onto amended WA

- Azoxystrobin and Pyraclostrobin
  - 0.1, 1, 10, 100 µg/mL
- Count the number of germinated spores
- Four reps per isolate per concentration

qPCR

- Taqman probe assay (Miles et al., 2021)

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EC_{50} Data

Isolate	Area	Cultivar	QoI EC50		WT/MT	Sanger Sequencing (G143A)
			Pyraclostrobin	Azoxystrobin		
			Wine grape vineyards			
G.11	Durbanville	Chenin Blanc	<0.1	-	WT	G
G.2	Durbanville	Chenin Blanc	<0.1	-	WT	G
G.3	Durbanville	Chenin Blanc	<0.1	<0.1	WT	G
R.14	Stellenbosch	Shiraz	0.1	<0.1	WT	G
R.15	Stellenbosch	Shiraz	0.1	<0.1	WT	G
V.12	Somerset West	Merlot	<0.1	<0.1	-	-
V.13	Somerset West	Merlot	0.1	-	WT	G
V.14	Somerset West	Merlot	<0.1	<0.1	WT	G
V.16	Somerset West	Merlot	<0.1	<0.1	WT	G
V.5	Somerset West	Merlot	<0.1	<0.1	WT	G
W.12	Botrivier	Chardonnay	1	>0.1	WT	G
W.16	Botrivier	Chardonnay	0.1	-	WT	G
W.7	Botrivier	Chardonnay	0.1	<0.1	WT	G
W.8	Botrivier	Chardonnay	<0.1	0.1-1	WT	G
			Table grape vineyards			
Ro.4	Wellington	Arra15	0.1	>0.1	WT	G
Ro.5	Wellington	Arra15	<0.1	-	WT	G
Ro.7	Wellington	Arra15	<0.1	10	WT	G
H.1	Halfmanshof	Autumn Royal	<0.1	<0.1	WT	G
H.10	Halfmanshof	Autumn Royal	<0.1	<0.1	both	G & A
H.14	Halfmanshof	Autumn Royal	<0.1	<0.1	WT	G
H.3	Halfmanshof	Autumn Royal	<0.1	<0.1	WT	G
M.4	De Doorns	Arra15	1	>100	WT	G
M.6	De Doorns	Arra15	1	1	WT	G

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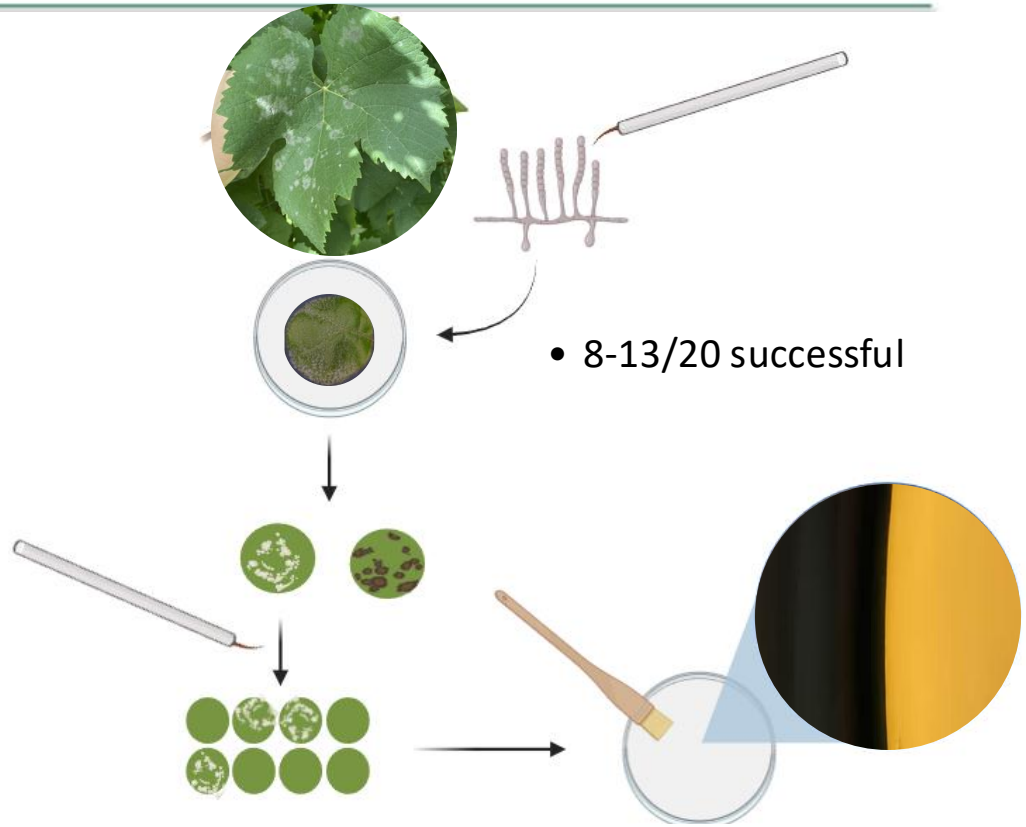
EC_{50} Data

All isolates WT

- **Isolation pipeline selected for the isolates with the highest fitness**
- Rallos et al. (2014) – no fitness penalty in their populations
  - "Fitness is a result of interacting factors in the whole genome, which implies that not all observed differences in fitness can be attributed to the resistance phenotype."
  - Population genetics



Fitness of *Erysiphe necator* with G143A-Based Resistance to Quinone Outside Inhibitors

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Conclusions to date (Obj 1&2)

QoI MT are widespread through WC and NC (66.34%)

- **New Zealand**
  - Beresford et al, (2016) –100%
- **USA**
  - Baudoin et al. (2008) – 90.91%
  - Rallos et al. (2008) – 25%
  - Miles et al., 2012) – 31.4%
- **France**
  - Dufour et al. (2011) – 9.05% - 20.25% in different areas
- **Germany**
  - Thesis (2025)– 90%
- **First report of QoI mutation in South Africa – only had EC_{50} for sensitive isolates**

Evidence of sexual reproduction in majority of vineyards, except for 3 populations

- **Botrivier:** Only vineyard with no QoI resistance and no sexual reproduction: sexual reproduction can lead to less sensitive individuals
- **Upington:** Mixed WT and MT present as clonal lineages (*MAT1-2*) without sexual reproduction; population genetics needed to confirm diversity.

Resistance dynamics are shaped by reproduction and spray programs, not just QoI fungicide pressure.

- In wine grapes sexual reproduction and absence of QoIs allow WT to re-emerge late season
- More frequent spray applications can select for MT, like in table grapes

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Conclusions to date (Obj 3)

Snapshot

- Monitoring once does not give the accurate representation of what populations are like
  - Need to monitor over time to understand distribution dynamics

Fitness penalty

- **There** was an observed fitness penalty for the G143A mutation in the South African populations (contrary to Rallos et al., 2014)
  - Population genetics
  - Spray other actives for better control of GPM

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Next steps?

SDHIs

- Already have EC_{50} values
  - Fluopyram and Boscalid
- Run SDH PCRs
  - Determine mutations associated (not well characterised)
  - *Sdhb, Sdhc* and S*dhd* subunits

Sequence genomes

- Optimise extraction protocol
- Develop microsatellite markers
- Population genetics

Baudoin et al. (2008); Wilcox et al. (2003); Miles et al. (2012); Sierotzki et al. (2007), Miles et al. (2021)

Acknowledgements

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South African Table Grape Industry

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