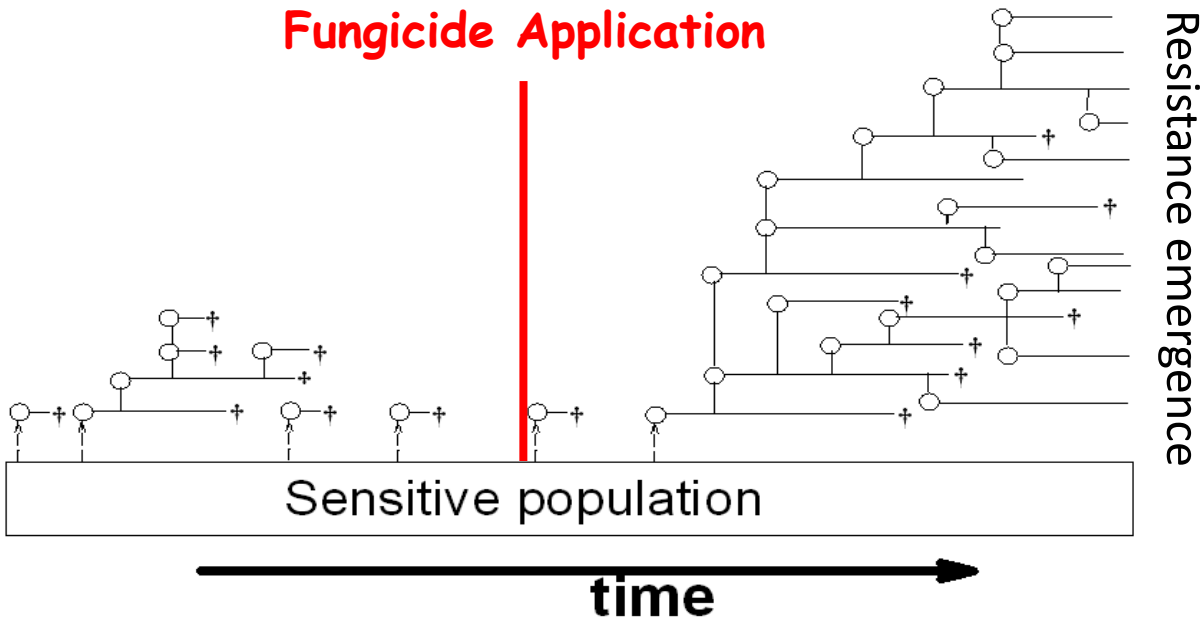
Fungicide resistance – discoveries in barley net blotches pave the way to better understanding of the resistance

Fran Lopez, CCDM


Resistant?
How much?
Optimum
Minimum
Maximum
When?
Calendar
Friend or foe?
Which product?

How does resistance occur?

- Naturally occurring
- May be present before fungicide application



Courtesy of Frank van den Bosch, Rothamsted Research, UK

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Resistance detection
Or how to win the lotto

Imagine that you need to find a coin that somebody has in this room

Imagine that you have to find it by random search

The probabilities of finding the coin would be quite low

$$\left(\frac{1}{n}\right) x100$$

Now imagine that instead we have 1 million spores of 10 different diseases

What would be the odds of finding the resistant spore?

0.00001%

So how is it possible that we find resistance almost everywhere we look?

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Detecting resistance
A genetic needle in a haystack

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Resistance cases confirmed 2012-2017

Disease and fungicide
Barley Powdery Mildew – Group 3 (DMI)
Wheat Powdery Mildew – Group 3
Wheat Powdery Mildew – Group 11 (strobilurins)
Canola Blackleg – Group 2 (Map-kinase)
Canola Blackleg – Group 3
Barley Net Form – Group 3
Barley Spot Form – Group 3
Legume chocolate spot – Group 1 (MBC)
Ascochyta blight – Group 1
Wheat Septoria Blotch – Group 3

Resistance to DMI (Group 3) in SFNB

	Fungicide in µg/mL					
	Tebuconazole	Epoxiconazole	Prothioconazole	Propiconazole	Difenoconazole	Prochloraz
Mean EC_{50} (Sensitive)	0.28	0.15	0.07	0.09	0.05	0.05
16FRG073 EC_{50}	2.57	1.29	0.34	0.35	0.05	0.05
Esperance (RF)	9.33	8.50	4.97	3.88	1	1
17FRG089 EC_{50}	10.49	1.19	1.08	3.14	2.70	2.86
S. Stirling (RF)	38.11	7.84	15.67	34.88	54	57.2

South Stirling resistant SFNB population has higher resistance factors (RF) than the population isolated from Esperance

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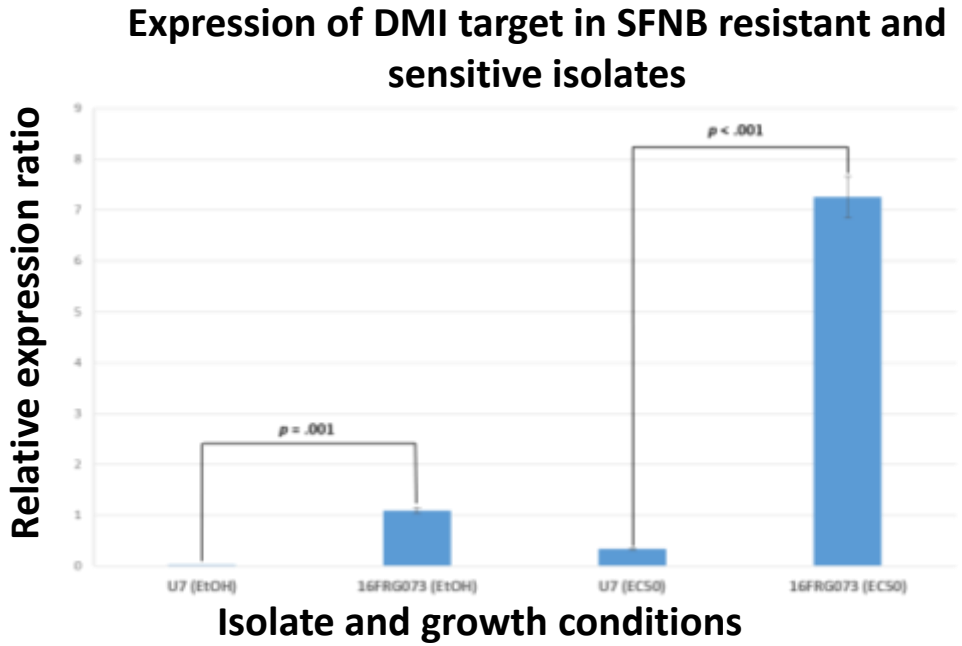
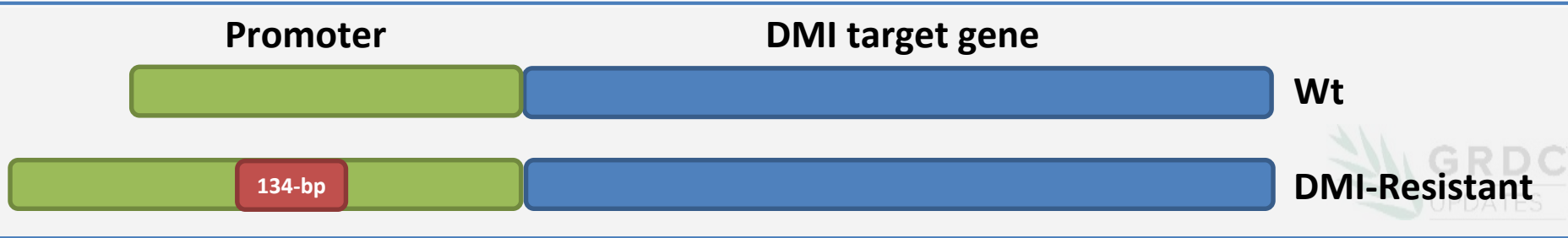
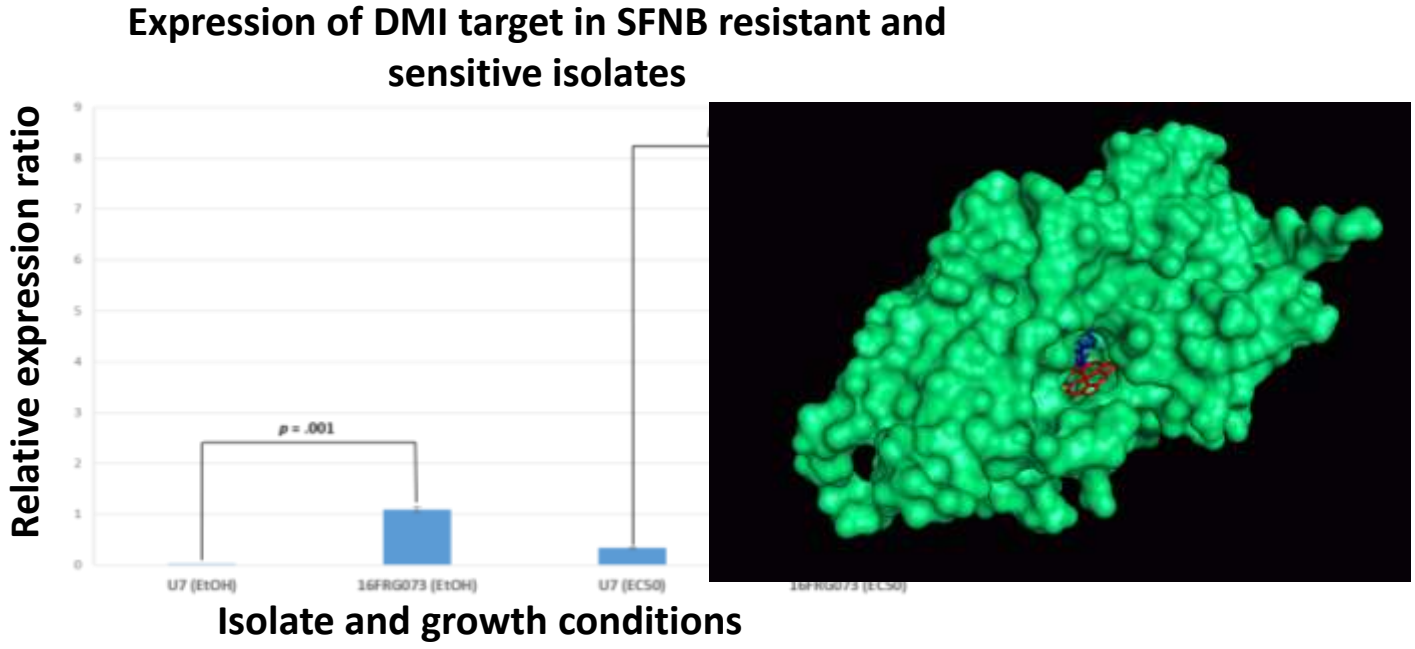
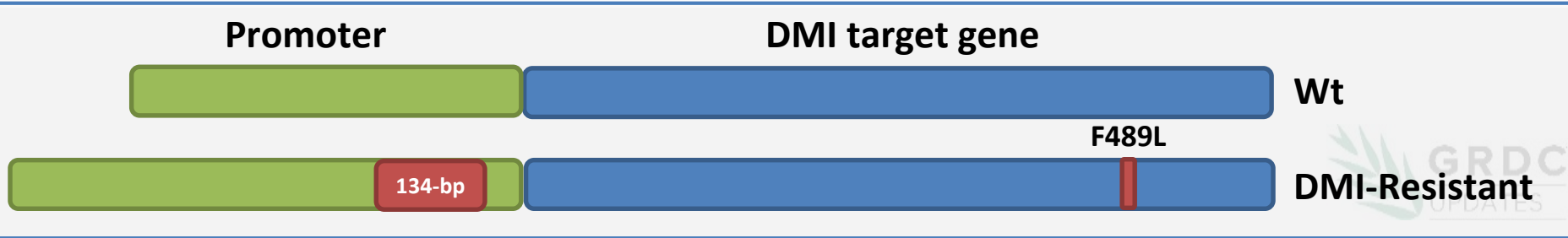
Mechanism of Esperance DMI-resistant SFNB





Mechanism of S. Stirling DMI-resistant SFNB





A few facts about DMI resistance in SFNB

- **The overexpression of the DMI target has the potential to affect all DMI**
- **The South Stirling population was growing quite happily after a heavy DMI spray program:**
    - Hombre (SD) 400mL/100kg seed
    - Propiconazole @ 325 mL/ha at Z25
    - Amistar Xtra @ 400 mL/ha at Z31
    - Epoxiconazole @ 250 mL/ha at Z39
    - Propiconazole @ 500 mL/ha at Z52
- **No control failure in the case of the population from Esperance but two populations now (Gibson and Munglinup)**

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High throughput genetics detection

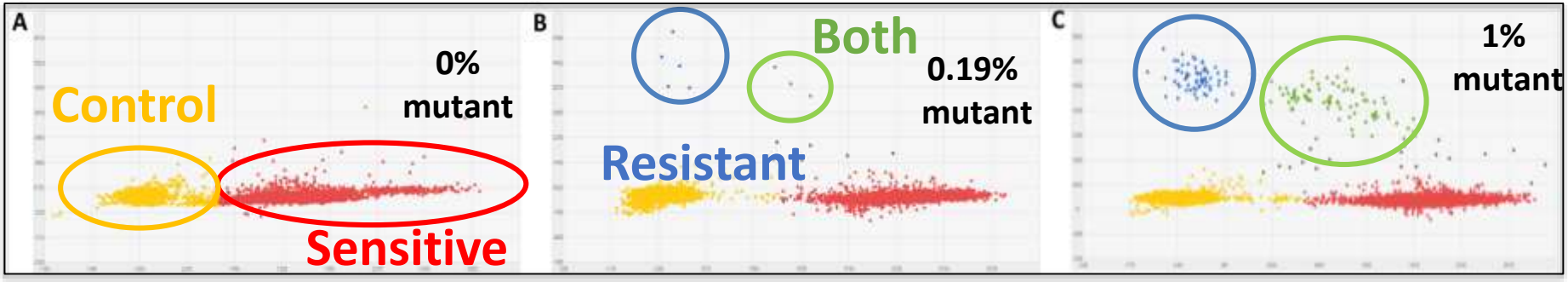
Bgh infected leaf samples pooled and DNA extracted

DNA partitioned into thousands of reaction wells on dPCR chip

Chips undergo PCR thermal cycling with probes targeting SNP

Readout of end point fluorescence on chip

Absolute quantification of mutant alleles present in sample



Methodology is fast, accurate, sensitive, high throughput and affordable

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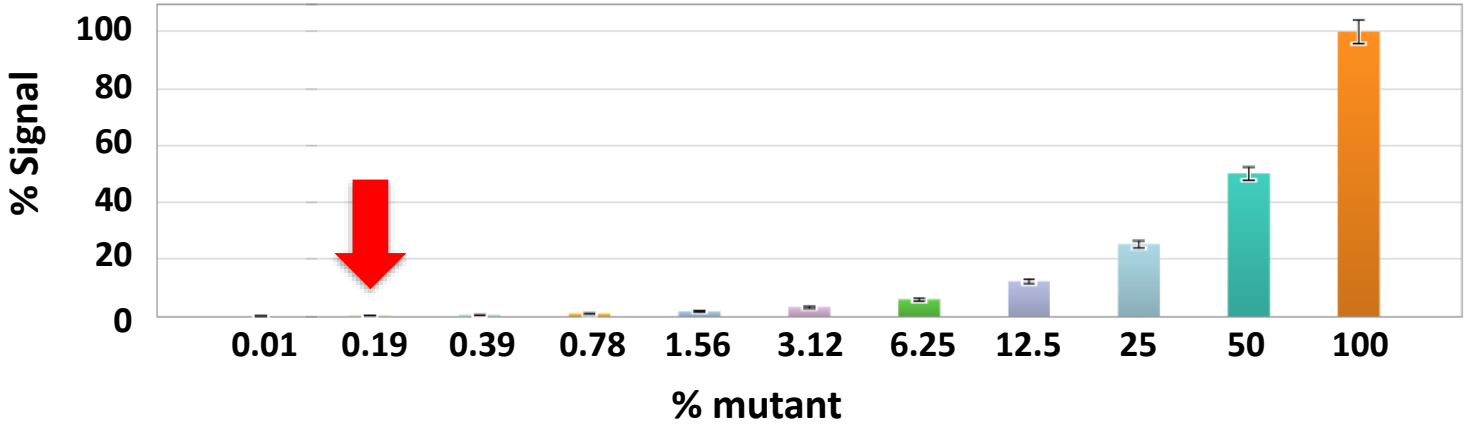
In planta high throughput SFNB detection



100% wild-type	0.0122% mutant	0.195% mutant	0.39% mutant	0.78% mutant	1.56% mutant	3.125% mutant	6.25% mutant	12.5% mutant	25% mutant	50% mutant	100% mutant
0.0002%	0.0003%	0.19%	0.34%	0.85%	1.69%	3.06%	5.77%	12.21%	25.21%	50.08%	99.97%

Able to accurately quantify mutant levels when present at as low as 0.19% in infected leaf material

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What's happening this season with SFNB

- **Distribution –** SFNB sample collection by DPIRD, CCDM and the industry
- **Field assessment** – assessment of fungicide efficacy to compare *in-planta* the lab outcomes
- **Local extension** – informing WA growers and specifically southern WA zone growers about the issue (messages about avoiding standalone DMI, etc.)
- **Virulence analysis –** are resistant isolates as virulent as wild types?
- **Restrict stubble movement –** avoid collecting and moving stubble from the identified sites

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Resistance management strategies

Grow resistant cultivars

Removal/burning of stubble

Crop rotation

Good farm hygiene

- Chemical methods
  - Only spray if necessary – limit applications
  - Avoid repetitive use of single formulation
  - Tank mix 2 modes of action or alternate sprays
  - Apply protectant sprays before wide infection
  - Use lowest registered dose

Should never compromise effective control

What can we all do?

Integrated Disease Management Strategies

Select resistant crop varieties when possible

Rotate modes of action whenever possible

Select a fungicide mixture with different modes of action whenever possible

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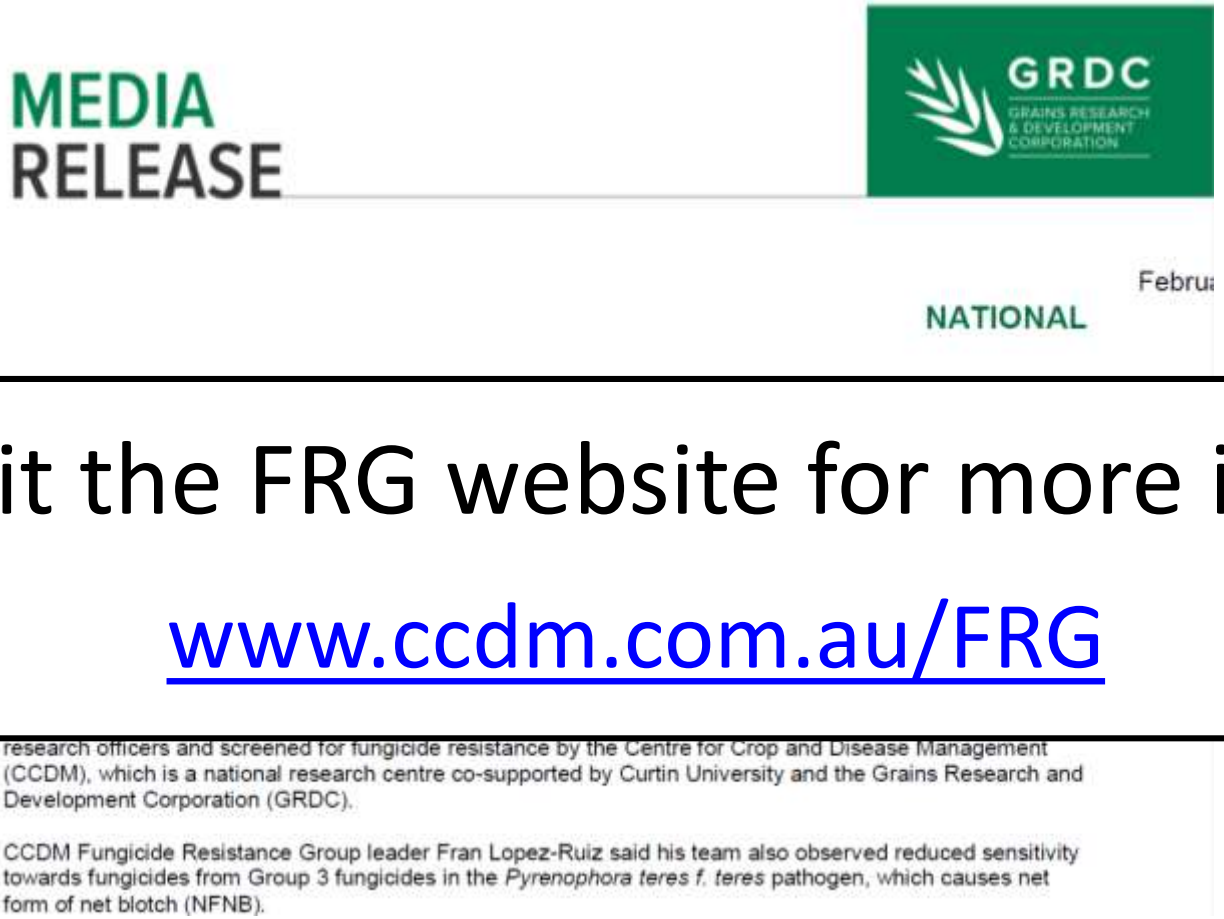
Let's communicate the message



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research officers and screened for fungicide resistance by the Centre for Crop and Disease Management (CCDM), which is a national research centre co-supported by Curtin University and the Grains Research and Development Corporation (GRDC).

CCDM Fungicide Resistance Group leader Fran Lopez-Ruiz said his team also observed reduced sensitivity towards fungicides from Group 3 fungicides in the *Pyrenophora teres f. teres* pathogen, which causes net form of net blotch (NFNB).

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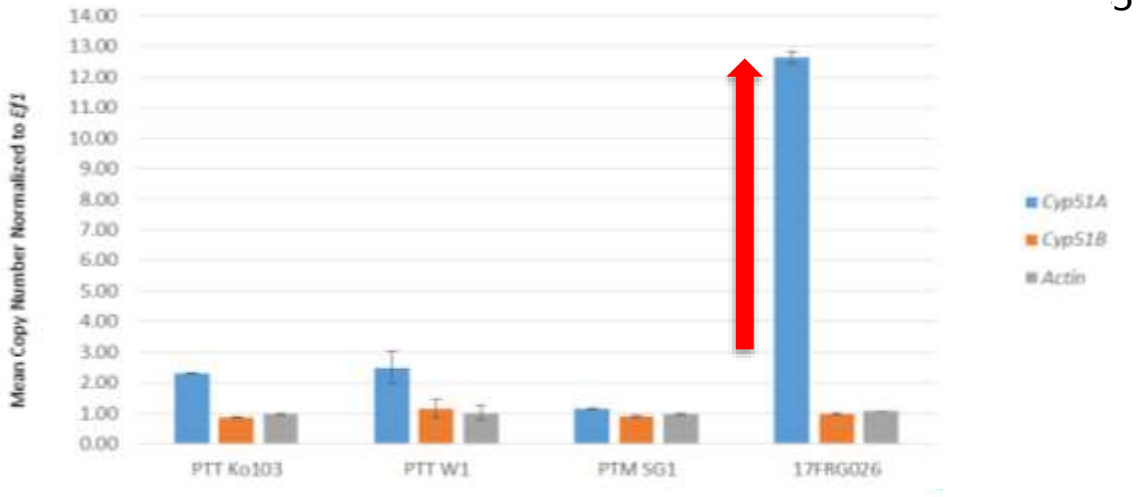
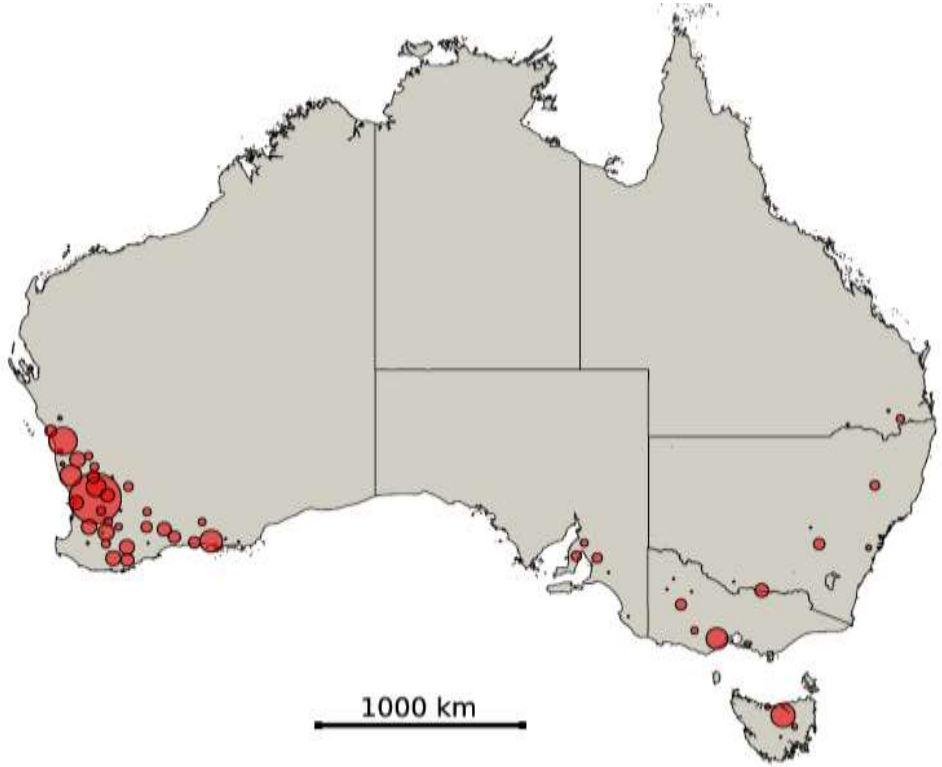
Resistance to DMI in NFNB 2.0 (Esperance)

	Fungicide in µg/mL					
	Tebuconazole	Epoxiconazole	Prothioconazole	Propiconazole	Difenoconazole	Prochloraz
Mean EC_{50} (Sensitive)	0.23	0.11	0.07	0.09	0.01	0.01
Mean EC_{50} (Resistant)	3.69	0.16	0.18	0.69	0.11	0.25
Resistant factor (RF)	16	1.45				26.45

Amount of DMI target produced



Monitoring: We need you to find them



Courtesy of Darcy Jones and James Hane, Centre for Crop and Disease Management (CCDM), Perth

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Who does the job & who pays the bills

The Fungicide Resistance Group
Centre for Crop and Disease Management

School of BioSciences
University of Melbourne

Angela Van de Wouw
Alexander Idnurm
Steve Marcroft

Institute of Life Science
Swansea University, UK

Jonathan Mullins

FAR Australia

Nick Poole
Tracey Wylie

Horticulture Pathology
SARDI

Barbara Hall
Suzanne McKay

Computational & Systems Biology
Rothamsted Research, UK

Frank Van Den Bosch

Collaborating Companies

BASF · syngenta · Nufarm · BAYER · DOW Dow AgroSciences

CCDM

Richard Oliver
Kar-Chun Tan
Simon Ellwood

DPIRD

Geoff Thomas
Kith Jayasena
Andrea Hills

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Thank you

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