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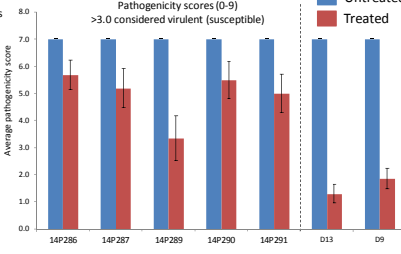
THE UNIVERSITY OF MELBOURNE

Fungicide tolerance in Australian populations of *Leptosphaeria maculans* – An update

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University of Melbourne



Fluquinconazole tolerance identified in Australia in 2014

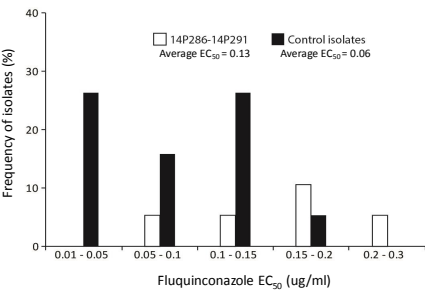



Isolate	Untreated (Average score)	Treated (Average score)
14P286	~7.0	~5.8
14P287	~7.0	~5.2
14P289	~7.0	~3.5
14P290	~7.0	~5.5
14P291	~7.0	~5.0
D11	~7.0	~1.5
D9	~7.0	~1.8

Pathogenicity scores (0-9)
>3.0 considered virulent (susceptible)

Individual isolates inoculated onto cotyledons of treated and control seedlings
Lesions scored for disease development

in vitro assays with fluquinconazole show average EC₅₀ for fungicide-tolerant isolates are significantly higher than the control isolates



Fluquinconazole EC ₅₀ (ug/ml)	14P286-14P291 (%)	Control isolates (%)
0.01 - 0.05	0	~26
0.05 - 0.1	~5	~16
0.1 - 0.15	~5	~26
0.15 - 0.2	~10	~5
0.2 - 0.3	~5	0

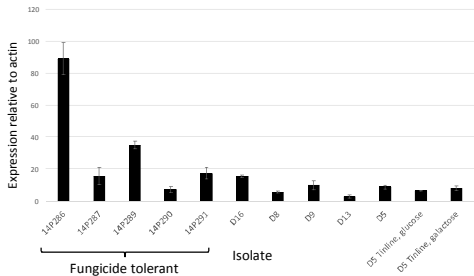
Average EC₅₀ = 0.13 (14P286-14P291)
Average EC₅₀ = 0.06 (Control isolates)

Steven Chang, Fran Lopez (Curtin University)

No mutations detected within the *Cyp51* gene in the fungicide-tolerant isolates

- “ *Cyp51* gene sequenced from fluquinconazole tolerant isolates and control isolates
 - . No mutations detected
- “ Expression of *Cyp51* examined *in planta* fluquinconazole tolerant isolates and control isolates

Expression differences detected but not consistent with increased tolerance phenotype

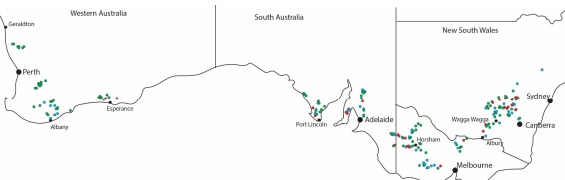


Isolate	Expression relative to actin
14P286	~90
14P287	~15
14P289	~35
14P290	~10
14P291	~20
D16	~15
D8	~5
D9	~10
D13	~5
D5	~10
D5 (Triticum glaberrime)	~10
D5 (Triticum glaberrime)	~10

14P286-14P291: Fungicide tolerant
D16, D8, D9, D13, D5, D5 (Triticum glaberrime): Isolate

Survey of Australian populations

- “ GRDC funded a survey of 200 paddocks from across Australia
- “ Samples submitted by agronomists and farmers
- “ Samples from a range of cultivars and fungicide regimes



Stubble used to inoculate seedlings treated with jockey.
Two punnets with no fungicide as control (circled in red)

Counted number of cotyledons with lesions and determined percentage of infected seedlings
Designated populations:
no tolerance
low tolerance
high tolerance

No tolerance High Tolerance

No correlation between tolerance and fungicide use, cultivar or location

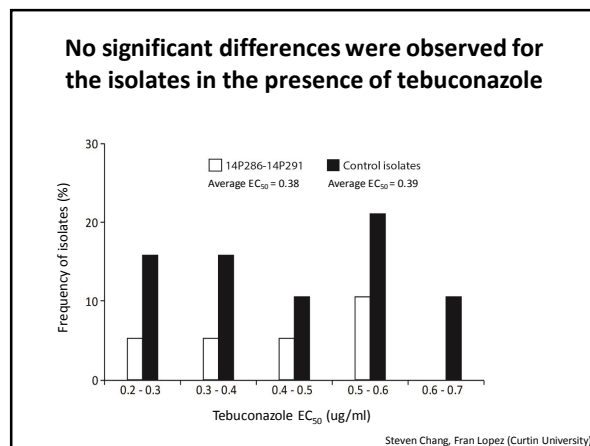
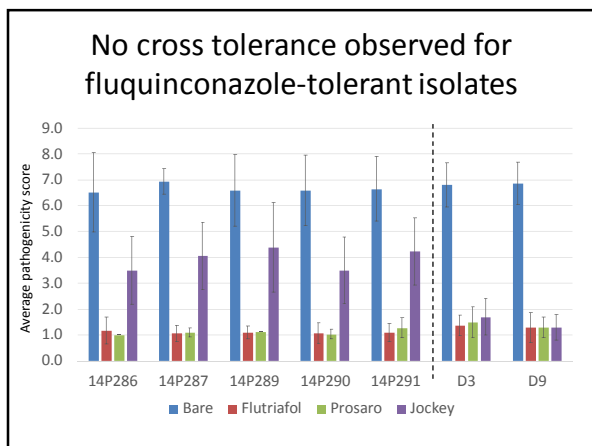
State	# samples submitted	% of population (# isolates)		
		No tolerance	Low tolerance	Tolerance
VIC	50	60 (30)	26 (13)	14 (7)
SA	42	60 (25)	23 (10)	17 (7)
NSW	66	54 (36)	26 (17)	20 (13)
WA	42	76 (32)	17 (7)	7 (3)
Total	200	62 (123)	23 (47)	15 (30)

Screening for tolerance to DMIs

- Industry reliant on fungicides to maintain current intensity and production
- Fungicides available:
 - Seed dressing (Fluquinconazole - Jockey)
 - Fungicide amended fertilizer (Flutriafol – Impact in Furrow)
 - Foliar application (Tebuconazole and Prothioconazole - Prosaro)
 - All DMI class of fungicides
- Do we have cross tolerance/resistance to these other fungicides?
 - Developed *in planta* screen to look at cross tolerance

in planta screen for Flutriafol and Tebuconazole/Prothioconazole tolerance

- Seedlings of cultivar Westar grown in punnets were sprayed with flutriafol or Tebuconazole/Prothioconazole as per registered rates
- Seedlings allowed to dry overnight
- Cotyledons wounded and inoculated as per standard inoculations.
- Disease progression assessed at 21 dpi



Industry implications

- “ No cross tolerance between Jockey (fluquinconazole) and Impact-in-furrow (flutriafol) or Prosaro (Tebuconazole/Prothioconazole)
- “ Tolerance (insensitivity) not resistance?
 - . Have mapping population to look at inheritance of the tolerance
- “ First time such a survey has been done
 - . Do not know if this tolerance has always been present, is increasing or decreasing

Industry recommendations

- “ Try to reduce reliance on fungicides:
 - . Cultivar resistance rating
 - . Avoid previous year's stubble
 - . Blackleg resistance groups
- “ Is still effective in 85% of paddocks.



Acknowledgements

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- “ Steven Chang, Fran Lopez (Curtin University)
- “ Agronomists/farmers who submitted stubble samples

