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Fungicide Resistance Survey



Fungicide Use in Australia

- ▶ Actives
 - ▶ DMI
 - ▶ SDHI
 - ▶ Q_oI
- ▶ Application
 - ▶ Seed dressing
 - ▶ Fertiliser
 - ▶ Foliar
- ▶ Reports of up to 5 fungicide applications in a growing season
 - ▶ Seed dressing + fertiliser + 4-8 leaf + 10% bloom + 50% bloom



In Planta Method

- ▶ 8 commercial fungicides
- ▶ Fungicides applied as seed treatment or as foliar 7 days after sowing
- ▶ Inoculate for 2 days, 8 days after sowing
- ▶ Score disease 14 days post inoculation

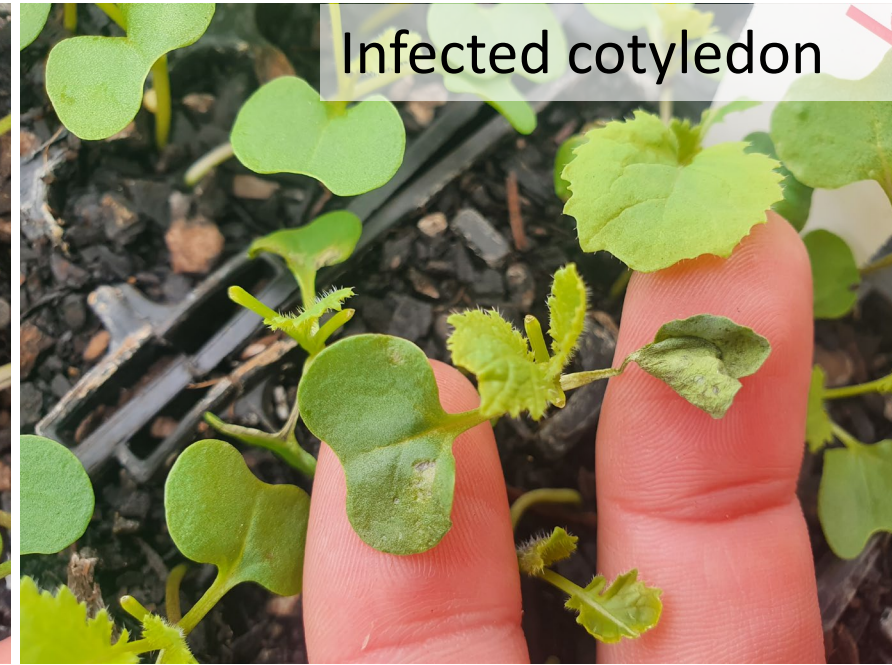
Fungicide	Class	Group	Actives
Jockey Stayer	DMI	3	Fluquinconazole
Proviso EC	DMI	3	Prothioconazole
Prosaro 420SC	DMI	3	Tebuconazole + Prothioconazole
Veritas Opti	Qol + DMI	11 + 3	Azoxystrobin + Tebuconazole
Maxentis	Qol + DMI	11 + 3	Azoxystrobin + Prothioconazole
ILevo	SDHI	7	Flupyram
Saltro 200FS	SDHI	7	Pydiflumetofen
Aviator XPro	SDHI + DMI	7 + 3	Bixafen + Prothioconazole



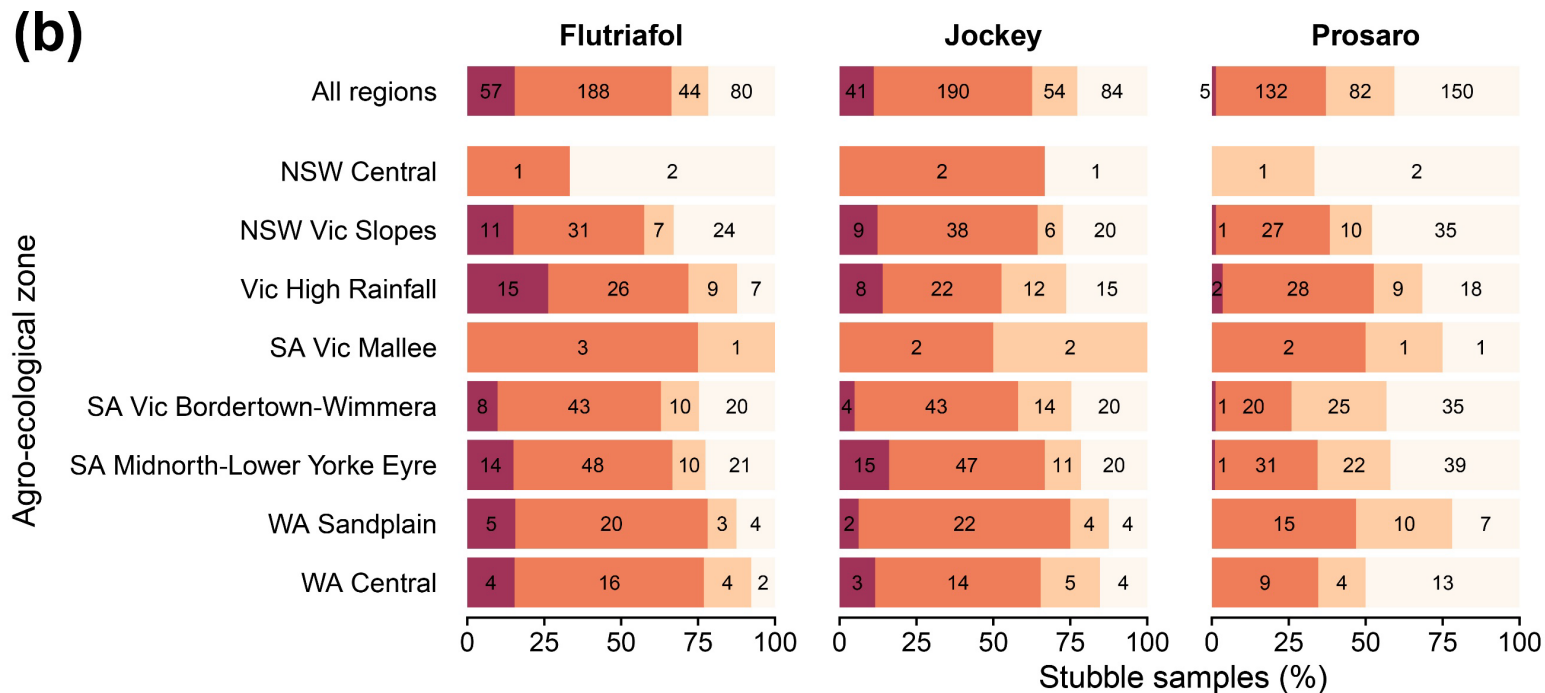
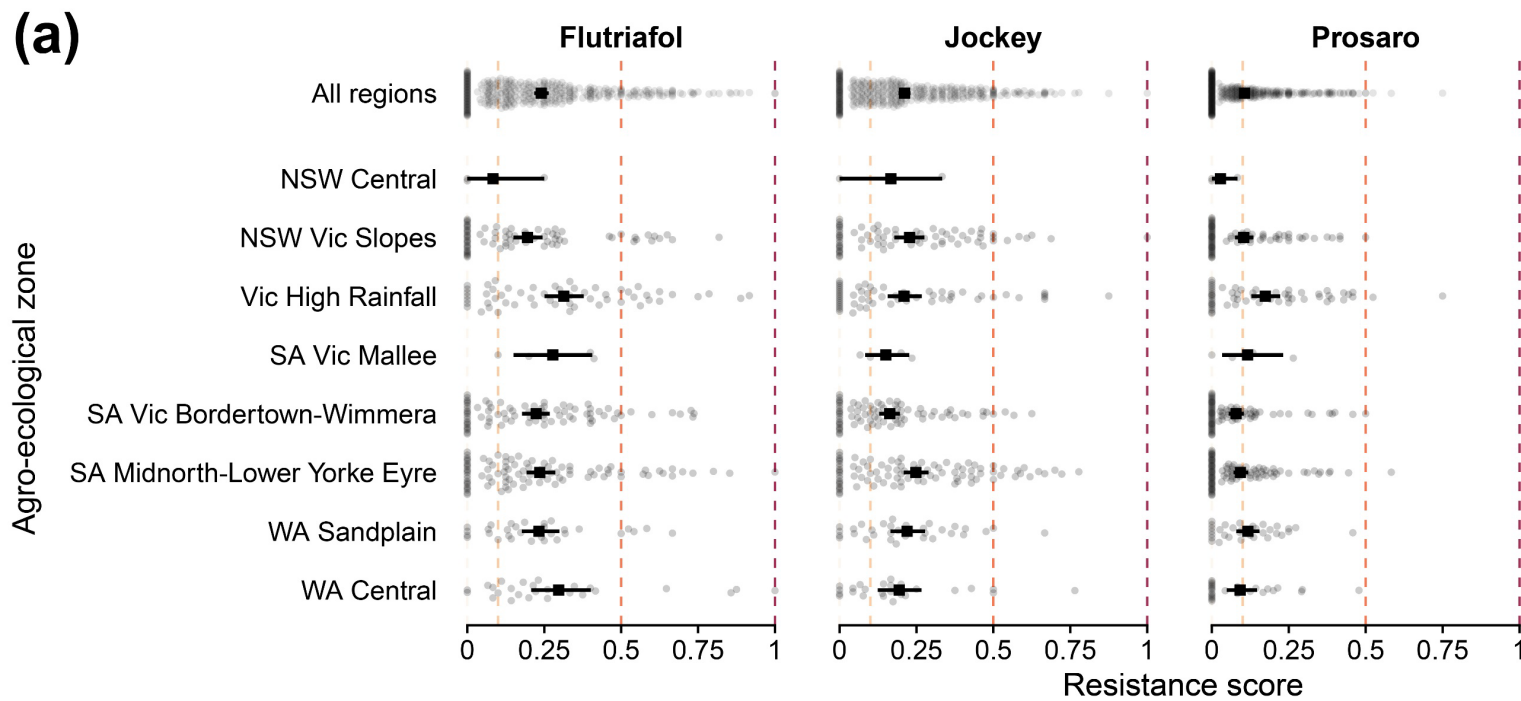
Method

Data

- ▶ No. of lesions
- ▶ Infected cotyledons
- ▶ Dead cotyledons
- ▶ Infected petioles

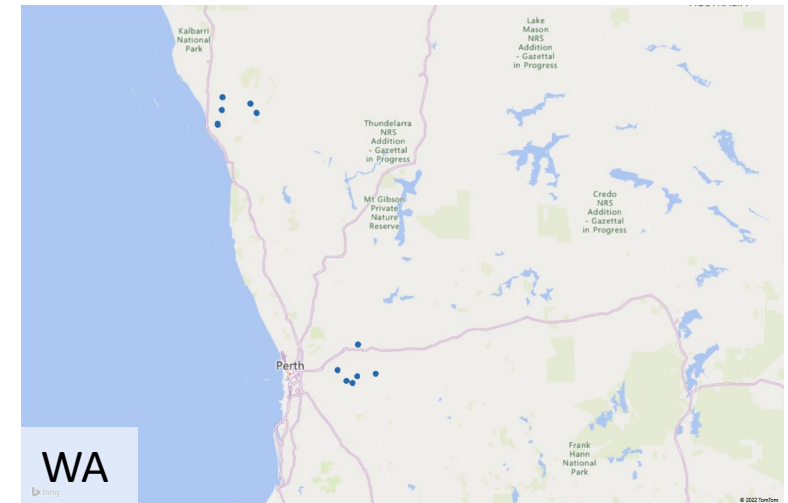
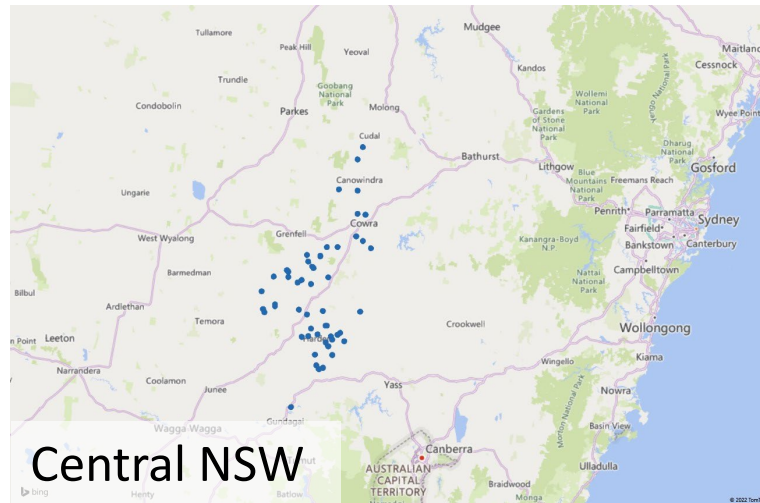
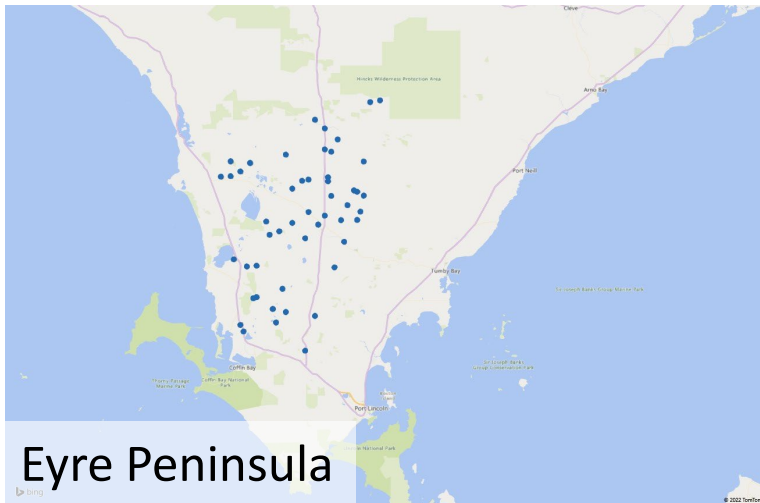


2018 - 2020
Widespread,
high levels of
DMI resistance
detected



2022 Stubble Populations

- ▶ 123 blackleg populations from canola growing regions with high fungicide use
 - ▶ Eyre Peninsula
 - ▶ Central NSW
 - ▶ WA
- ▶ Target different fungicide strategies within these regions
 - ▶ Does fungicide strategy affect resistance?



Results

- ▶ Resistance rating based on disease severity compared to untreated
- ▶ Slow increase in resistance over time in the DMIs
- ▶ No resistance to SDHIs, low in Q₀Is

	Percentage of populations with high, moderate and low levels of resistance											
	2022 results			2020 results			2019 results			2018 results		
Fungicide	High	Moderate	Low/None	High	Moderate	Low/None	High	Moderate	Low/None	High	Moderate	Low/None
Flutriafol	not screened			29.3	24.4	46.3	25.1	22	52.9	28.6	31.6	39.8
Jockey	32.0	20.4	47.6	29.3	11	59.8	20.4	24.6	55	22.4	31.6	45.9
Prosaro	7.8	15.5	76.7	15.9	15.9	68.3	7.3	13.1	79.6	7.1	17.3	75.5
Proviso	7.8	17.5	74.8	not screened			not screened			not screened		
Veritas	7.8	9.7	82.5	2.4	39	58.5	0	3.1	96.9	0	1	99
Maxentis	1.9	1.0	97.1	not screened			not screened			not screened		
Saltro	0	0	100	0	0	100	0	0	100	0	0	100
Aviator	0	0	100	0	0	100	0	0	100	0	0	100
ILeVo	0	0	100	0	0	100	0	0	100	0	0	100
Miravis	not screened			0	0	100	0	0	100	0	0	100

Results

- ▶ Similar levels of resistance between regions
- ▶ No correlation between fungicide use and resistance rating

Percentage of populations with high, moderate and low levels of resistance in 2022						
	Eyre Peninsula, SA			Central NSW		
	high	moderate	low/none	high	moderate	low/none
Jockey	35.4	20.8	43.8	28.8	21.1	50.0
Proviso	4.2	16.7	79.2	9.6	15.3	75.0
Prosaro	4.2	12.5	83.3	11.5	19.2	69.2
Veritas	10.4	14.6	75.0	5.7	5.7	88.4
Maxentis	4.2	2.1	93.8	0	0	100
Ilevo	0	0	100	0	0	100
Saltro	0	0	100	0	0	100
Aviator	0	0	100	0	0	100

Ascospore liberation,
capture and germination



DNA extraction



PCR amplification of
erg11 promoter or
coding region



Illumina 150 bp
paired-end sequencing



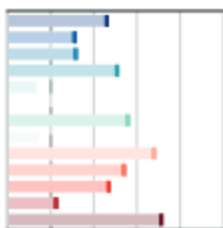
Allelic contig
assembly



Flow variation graph
haplotype assembly



Estimation of indel
allele frequencies



Deep amplicon sequencing as a method for detecting fungicide resistance

- Allows detection of all known alleles as well as novel alleles

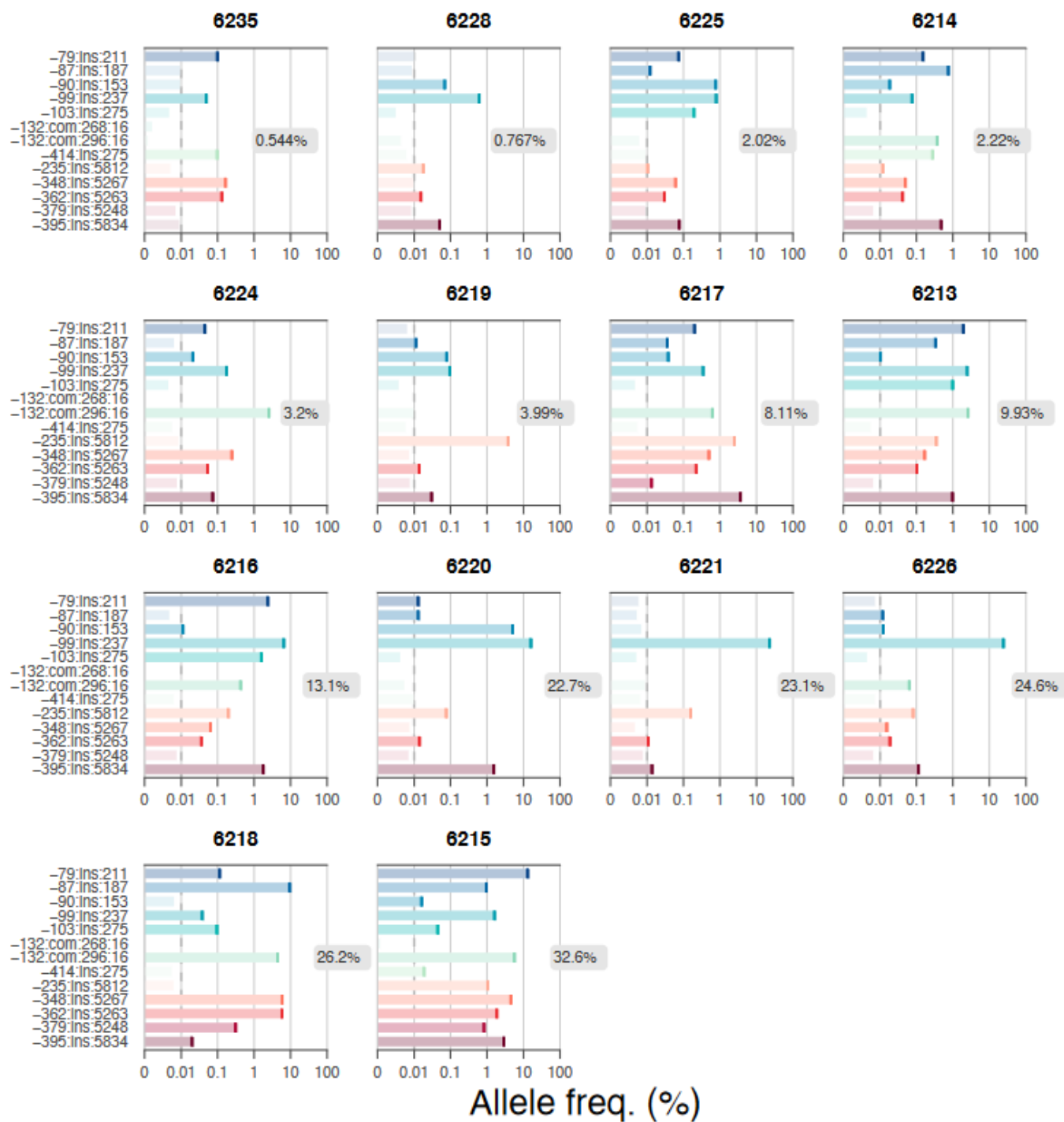
Deep amplicon sequencing
workflow

syngenta

Australian Government
Australian Research Council



Indel alleles

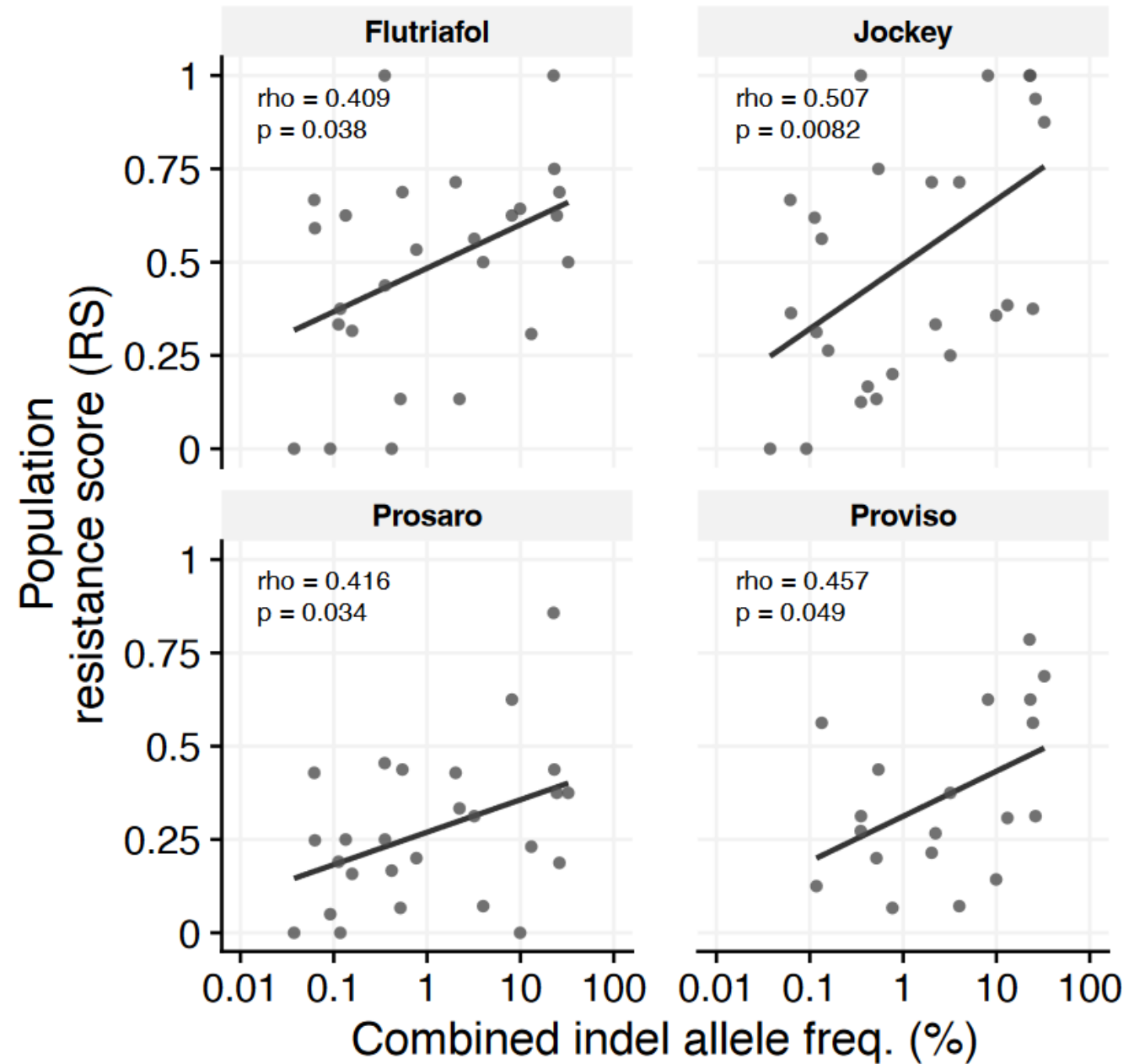


Fungicide resistance ranges from <math><0.05</math> – 32%

- Highest individual allele was detected at 13%



Level of resistance alleles correlated with *in planta* disease responses



Future Work

- ▶ Continue monitoring populations for resistance
 - ▶ Early detection of SDHI and Q_oI resistance
- ▶ What does this mean for growers?
 - ▶ Management of resistance?
- ▶ What level of resistance will result in field failure of the fungicide?
- ▶ Does timing of infection or fungicide application impact the evolution of resistance?