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GRAINS PATHOLOGY

THE UNIVERSITY OF  
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## Influence of rotation of blackleg resistance genes on frequencies of avirulence genes

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Australian Government  
Grains Research and Development Corporation

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### Major gene resistance is a gene-for gene interaction

- Plant contains resistance genes (R genes)
- Pathogen contains avirulence genes (Avr genes) corresponding to R genes

Plant Genotype: ***Rlm1***

Fungus Genotype: ***AvrRml1* (Avirulent)**

Plant recognises fungus  
- Defence mechanisms  
- **NO INFECTION**

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Plant Genotype: ***Rlm1***

Fungus Genotype: ***avrRml1* (Virulent)**

Fungus undetected by plant  
**INFECTION/DISEASE**

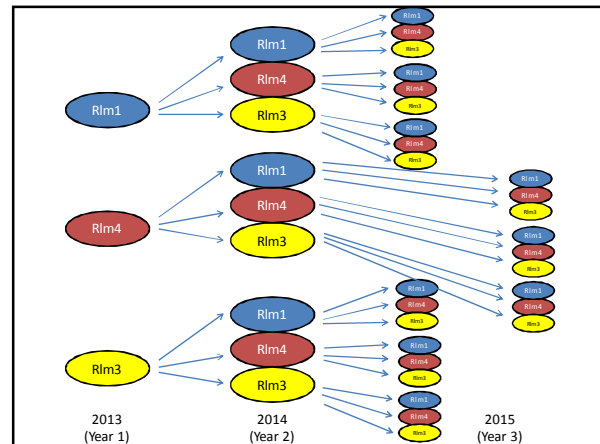
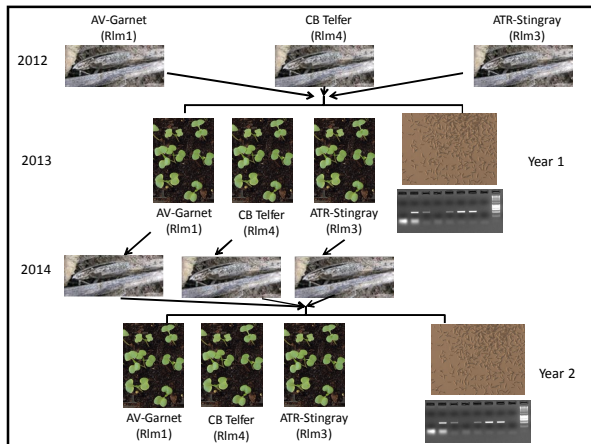
	cv. AV-Garnet ( <i>Rlm1</i> )	cv. CB Telfer ( <i>Rlm4</i> )
D13 ( <i>avrLm1</i> ; <i>AvrLm4</i> )		
D14 ( <i>AvrLm1</i> ; <i>avrLm4</i> )		

### Rotation of resistance genes

- Previous field and molecular data suggested that rotation of resistance genes reduces level of blackleg disease
- Resistance genes characterised in all released cultivars
  - Use differential set of isolates
- Classified into resistance groups and released to industry via blackleg management guide
- Has proven successful
  - Hyola50 (Group D) warning for EP
- System becoming complicated
  - stacked resistance genes
  - Wide scale deployment of particular R genes (Group A and B)
- Are we doing rotation right?

### 3 year experiment to monitor effect of cultivar rotation on allele frequencies

- Recurring selection experiment established in 2012
- Cultivars with known single R genes (*Rlm1*, *Rlm4* or *Rlm3*)
- Stubble releases ascospore inoculum onto seedlings in shadehouse experiments
- Frequency of avirulence alleles determined by high through put molecular assay



**Five avirulence genes have been cloned from *L. maculans***

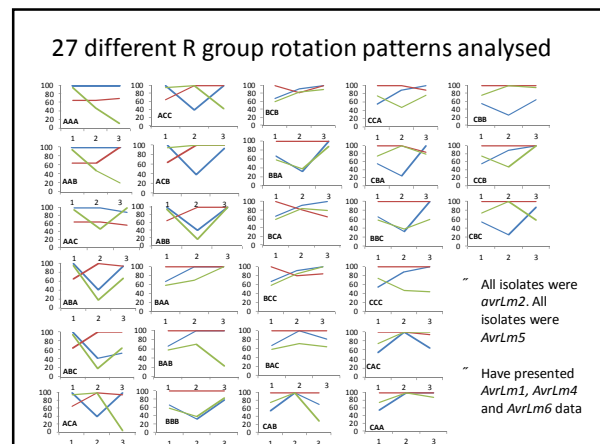
- “ *AvrLm1 - Rlm1* (AV-Garnet, Group A)
- “ *AvrLm4-7 - Rlm4* (CB Telfer, Group B)  
- *Rlm7* (Hyola970, Group H)
- “ *AvrLm2 - Rlm2* (presence unknown in current cultivars)
- “ *AvrLm5 - Rlm5* (Juncea R gene)
- “ *AvrLm6 - Rlm6* (Hyola575CL, Group F, Juncea)

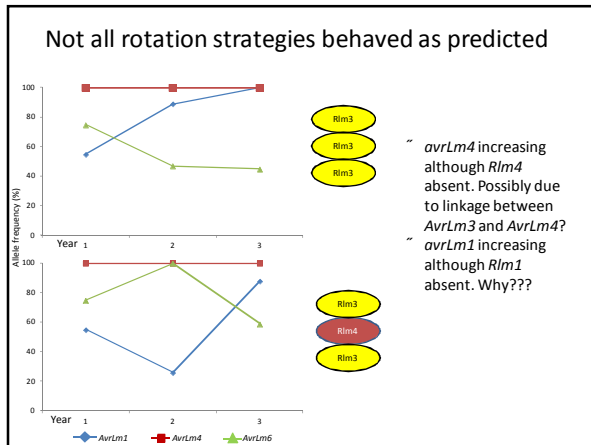
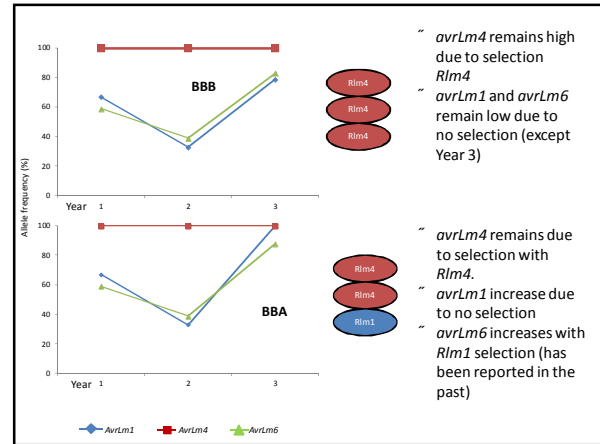
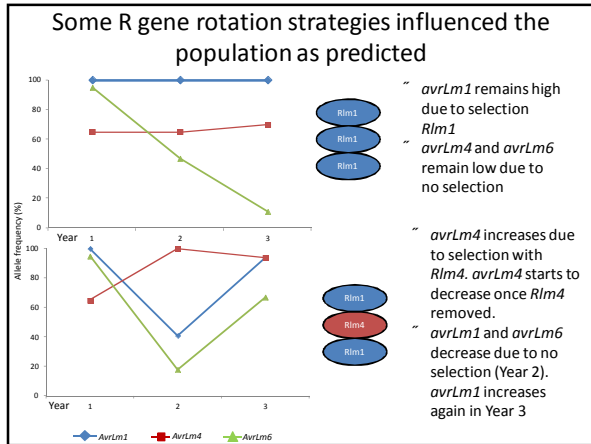
**Deletion, point mutations and RIP mutation confer virulence towards R genes**

- “ Different mutation events occur in different avirulence genes.
  - Can develop molecular markers to screen isolates to determine virulence genotype.
- “ Deletion of entire gene
  - Presence/absence PCR screen
  - *AvrLm1*
  - *AvrLm6*
- “ Single point mutation (confers amino acid substitution or stop codon)
  - PCR and restriction digest
  - *AvrLm4*
  - *AvrLm2*
  - *AvrLm5*
- “ RIP mutations (generate many stop codons and amino acid mutations)
  - Sequence gene
  - *AvrLm7*

**Influence of rotation R genes on frequencies of Avr genes determined**

- “ All isolates screened with molecular markers for *AvrLm1*, *AvrLm4*, *AvrLm6*, *AvrLm2* and *AvrLm5*
- “ 780 isolates screened in total
  - 20 isolates collected from each rotation regime
  - 60 Isolates in 2013
  - 180 isolates in 2014
  - 540 isolates in 2015





### Not all rotation strategies are equal

- ~ Rotation of Group A (*Rlm1*) and Group B (*Rlm4*) mostly behave as expected
- ~ Rotation of Group C (*Rlm3*) results in increased virulence towards *Rlm1* and *Rlm4*
  - . Should Group C be used in the rotation?
- ~ *AvrLm6* influenced by *Rlm1*
  - . Already known from sylvestris
  - . *AvrLm1* and *AvrLm6* linked in the pathogen
- ~ *AvrLm2* remains 100% virulent in all populations despite *Rlm2* absent in all selection regimes.

### Field data support allele frequency data for Group C (*Rlm3*)

- ~ R group verification sites sown on the EP for past three years.
  - . Cultivars representing different R groups sown into Group A, Group C and Group ABD stubble
- ~ Group A, B and AS more disease on Group C stubble

Cultivar	Stubble		
	Stingray (Gp C)	Garnet (Gp A)	Thumper (Gp ABD)
ATR Marlin (AS)	64	31	31
ATR Stingray (C)	7	4	7
CB Telfer (B)	41	11	26
CrusherTT (A)	62	25	34
Hyla444TT (AD)	52	20	94
ThumperTT (ABD)	0	1	8

% Internal infection from 60 plants

### Survival data from blackleg ratings support field and allele frequency data

- ~ Compared survival data from 8 BL rating sites for 28 cultivars representing 12 R groups
- ~ Group A, AB, B, AS low survival on Group C stubble

Resistance group	Stubble of Blackleg nursery		
	A	BF	C
A	23	40	21
AB	26	25	15
B	29	22	27
AS	13	25	2
BF	75	57	65
ABF	58	68	37
ABS	70		
AD	70	23*	73
ABD	79	56	74
BC	67		
C	27	30	23
H	83		
Site averages	45	41	37

\* Both sites located on EP where Group AD resistance overcome

## Conclusions

- “ Rotation of cultivars with different resistance genes influence frequencies of avirulence genes
- “ Not all avirulence genes respond similarly to this rotation
- “ Not all rotation strategies equal – Group C
- “ Need to understand the interaction between Avr genes and R genes better
  - . Linkage of Avr or R genes influences allele frequencies

## Industry recommendations

- “ Currently suggested rotation benefits in Blackleg management guide based on theory
- “ Do we need to update blackleg management guide to include these new findings?
  - . Impact of Group C in rotation?
  - . Group F (*Rlm6*) following Group A (*Rlm1*)
  - . Group S increased survival after Group D (previous data)
  - . Group S increased survival after Group B (previous data)
  - . Group ABD versus AD?

## Acknowledgments

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