GRDC Grains Research & Development Corporation

A review of the implementation of rotation of resistance genes

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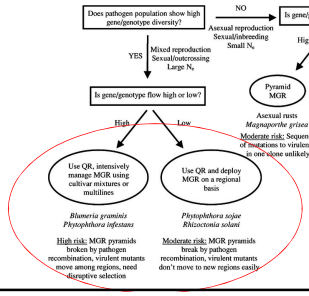
Resistance groups concept introduced 5 years ago

Sylvestris resistance

Non-sylvestris resistance

Suggested rotation of resistance genes can be used to minimise disease

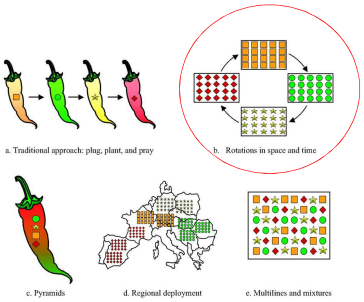
Literature suggested rotation, mixtures or multilines were a good option for high risk pathogens



- McDonald and Linde (2002) Pathogen population genetics, evolutionary potential, and durable resistance. *Phytopathology*

Proposed as best model by French researchers

- (B) Rotations of R-genes in time or space. Each R-gene is deployed over a limited number of years or area, and is withdrawn before the corresponding virulence allele achieves a high frequency in the pathogen population.



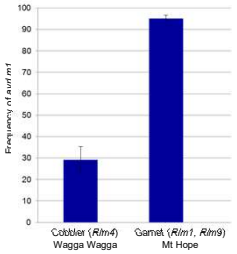
Glasshouse experiments confirm field data

Rlm1 on Rlm1 stubble

Rlm1 on Rlm4 stubble

Cultivars exposed to stubble of a different cultivar have less blackleg disease compared to being exposed to their own stubble

Molecular experiments confirm field and glasshouse data



Inoculated with ATR-Cobbler stubble

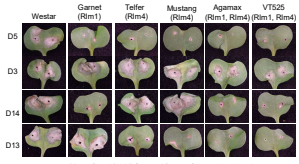
Inoculated with AV-Garnet stubble

Frequency of virulent isolates varies at different sites and is influenced by the resistance genes present in individual cultivars

Need to know what resistance genes are in commercial cultivars

Resistance groups determined for all commercial cultivars

- Currently differential set = 16 isolates
- Identify major gene resistance (*Rlm1-Rlm9*, *LepR1-4*) using differential isolates
  - Issues with *LepR2* (two different seed sources, both giving different results)
- Information provided to growers



Marcroft et al (2012) Crop and Pasture Science 63: 338-350

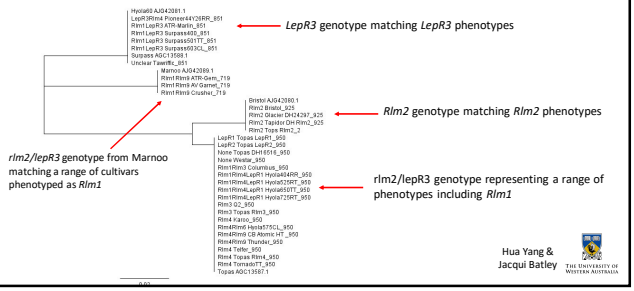
8 resistance groups including:

Group A – *Rlm1*
Group B – *Rlm4*
Group C – *Rlm3*
Group D – *LepR1*
Group F – *Rlm6*
Group G – Juncea (*Rlm5* + *Rlm6*)
Group H – *Rlm7*
Group S – *LepR3*
Many cultivars have multiple groups e.g. ABD

Identification of avirulence genes and development of DAIs

- Cloning of avirulence genes allows molecular markers to be made for monitoring populations
  - *AvrLm1, AvrLm2, AvrLm4-7, AvrLm5 (AvrLmJ1), AvrLm6*
- Generate Differential Addition Isolates for characterising "unknown" sources of resistance
  - *Rlm6* in Group F
  - *Rlm1* and *Rlm4* in Group D
  - *Rlm1*, *Rlm4*, *Rlm6* in Group F
- Must have PC2 facilities

Genotypic data using the *LepR3*/*Rlm2* alleles correlates with phenotypic data

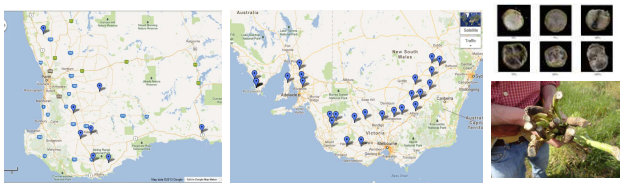


Hua Yang & Jacqui Batley

THE UNIVERSITY OF WESTERN AUSTRALIA

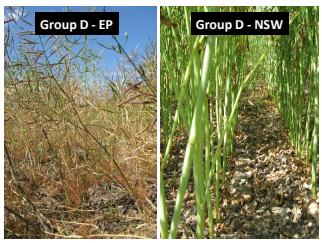
Blackleg resistance group monitoring sites

- Representative cultivars from each resistance group are sown at each monitoring site
- Disease assessed at the end of the year
- Isolates collected from sites each year (6000+ representing 2000-2017)

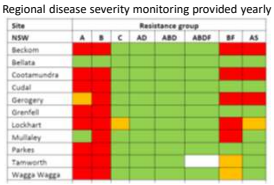


Warnings and regional advice provided to growers

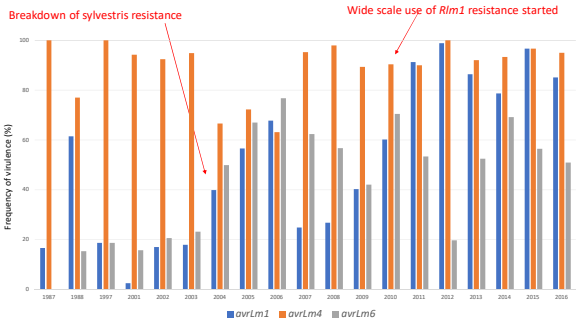
Resistance breakdown warning in 2012



Regional disease severity monitoring provided yearly

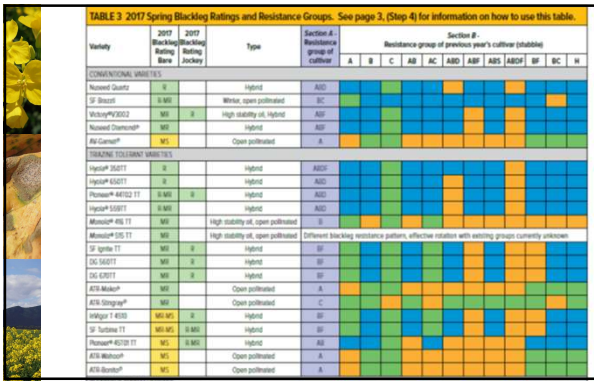


Molecular analysis of populations supports field data



Blackleg management guide

- Released biannually with all management options for minimising disease
- Step by step guide



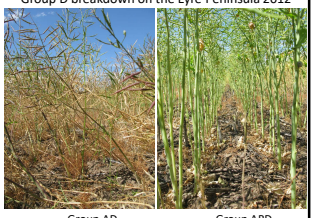
Does rotation of resistance genes work?

Evidence from the boom and bust on the Eyre peninsula

Group S breakdown on the Eyre Peninsula 2003

Group B Group AS

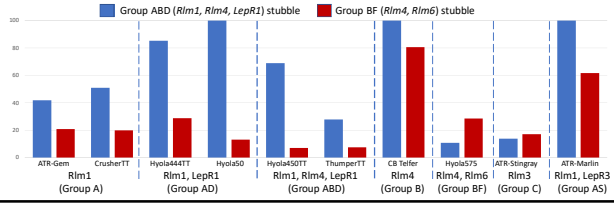
Group D breakdown on the Eyre Peninsula 2012



Group AD Group ABD

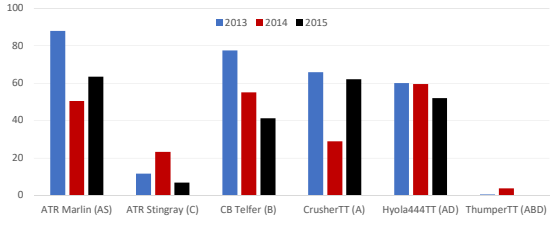
Preliminary field evidence that rotation of resistance genes works

- Verification sites on the Eyre Peninsula where Group D resistance was overcome in 2012
- Cultivars with different resistance sown into two different stubble types
- Different levels of disease are detected between cultivars depending on the stubble source.

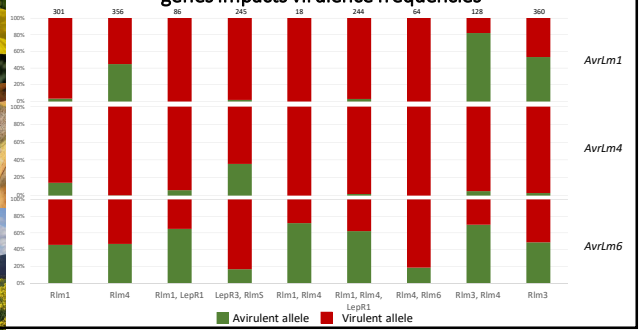


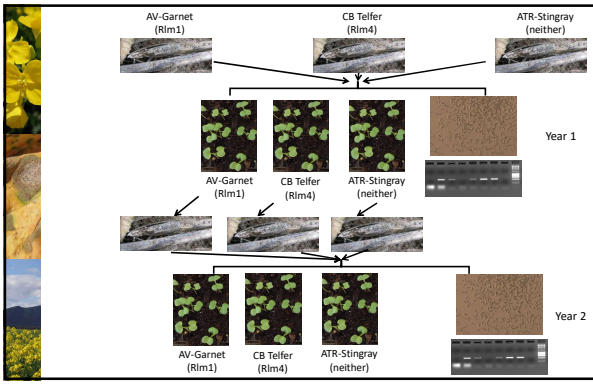
Rotation is not a perfect system

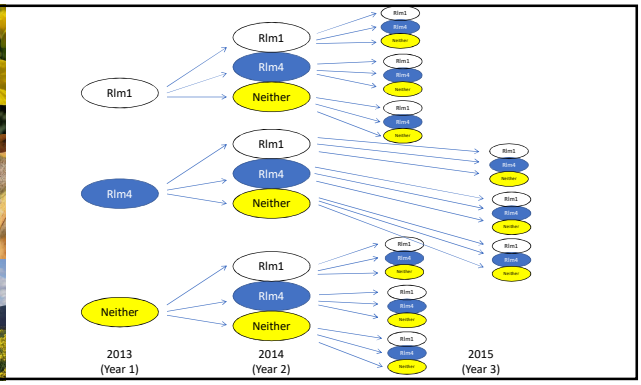
- Three years of trials where cultivars with different resistance genes are sown into Group C stubble
- Most cultivars consistently get high levels of disease on Group C stubble



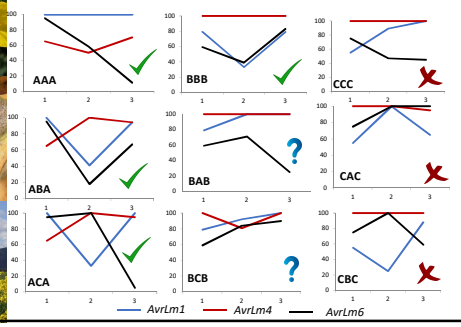
Analysis of allele frequencies suggests that rotation of resistance genes impacts virulence frequencies







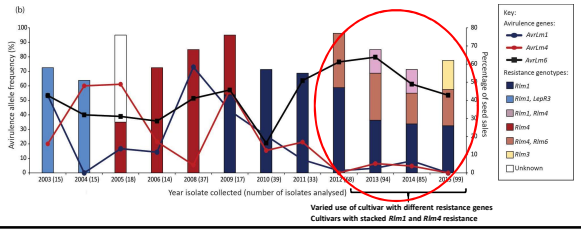
Not all rotations are equal or behave as expected



- Some rotations always gave the result we expected. Others never did
- Small scale (20 isolates/population), no replication and didn't know starting allele frequency
- Hoping to repeat in new project

Other factors influencing the impact of resistance gene rotation

- Use molecular markers for *AvrLm1*, *AvrLm4* and *AvrLm6* to track changes in virulence
- Large fluctuations in allele frequencies observed when cultivars with single resistance are sown and on large scale
- Isolates virulent towards both *AvrLm1* and *AvrLm4* are predominant in later years
  - Stacking of resistance genes prevent rotation of resistance?
  - Block sowing of cultivars needed for rotation to be effective?



R-Avr interactions: What we thought 5 years ago

Rlm1
Rlm2
Rlm3
Rlm4
Rlm5
Rlm6
Rlm7
Rlm9
LepR1
LepR2
LepR3
LepR4

- Surpass 400, Surpass501TT, Surpass603CL = *Rlm1* and *LepR3* (based on phenotypic data)
- *Rlm5* and *Rlm6* identified in *B. juncea*
- There are other genes reported by French researchers but no one has access to the material

R-Avr interactions: What we thought 5 years ago

Rlm1	*AvrLm1*
Rlm2	*AvrLm2*
Rlm3	*AvrLm3*
Rlm4	*AvrLm4*
Rlm5	*AvrLm5*
Rlm6	*AvrLm6*
Rlm7	*AvrLm7*
Rlm9	*AvrLm9*
LepR1	*AvrLepR1*
LepR2	*AvrLepR2*
LepR3	*AvrLepR3*
LepR4	*AvrLepR4*

R-Avr interactions: What we now know

Rlm1
Rlm2
Rlm3
Rlm4
Rlm5
Rlm6
Rlm7
Rlm9
LepR1
LepR2/RlmS?
LepR3
LepR4

- *LepR3* and *Rlm2* are cloned. They are alleles of the same gene
- Surpass400 et al have *LepR3* and a second gene (*RlmS*) but NOT *Rlm1*
- Mapping suggests *RlmS* and *LepR2* may be same gene
- *Rlm6* identified in *B. napus* lines (based on phenotypic data)

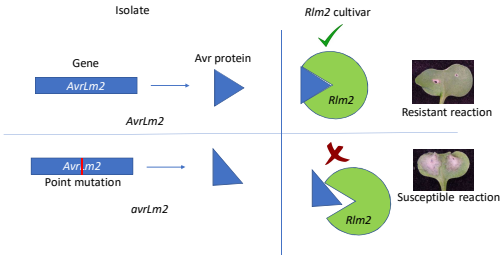
R-Avr interactions: What we now know

Rlm1	*AvrLm1*
Rlm2	*AvrLm2*
Rlm3	*AvrLm3*
Rlm4	*AvrLm4-7*
Rlm5	*AvrLm5-9*
Rlm6	*AvrLm6*
Rlm7	
Rlm9	
LepR1	*AvrLepR1*
LepR2/RlmS?	*AvrLepR2/AvrLmS?*
LepR3	
LepR4	*AvrLepR4*

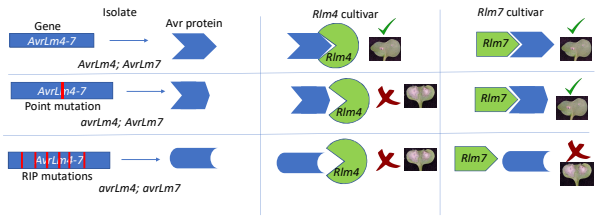
Not a simple gene-for-gene interaction

Cloned Avr genes Still to be identified

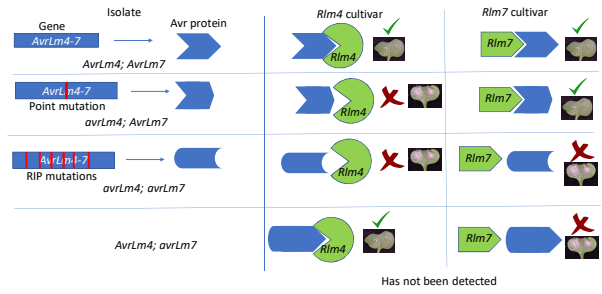
Simple gene for gene interaction



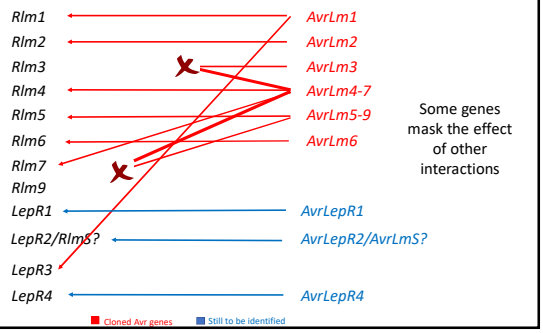
Single avirulence genes are recognising multiple R genes



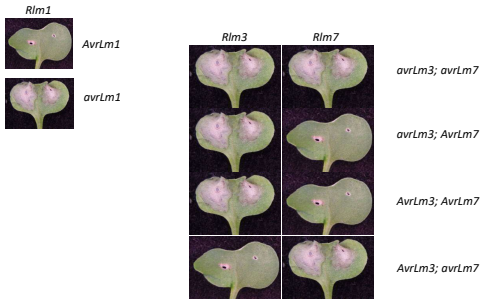
Single avirulence genes are recognising multiple R genes



R-Avr interactions: What we now know

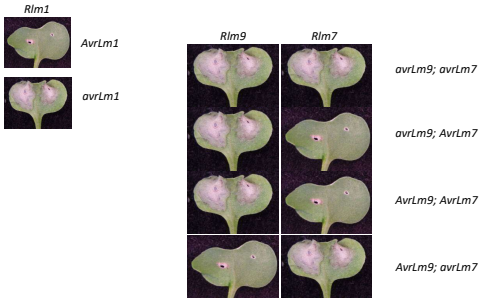


AvrLm7 masks the *AvrLm3-Rlm3* interaction



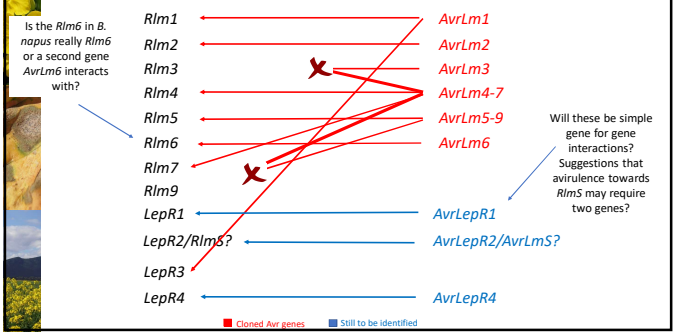
AvrLm7 is epistatic over *AvrLm3*

AvrLm7 masks the *AvrLm9-Rlm9* interaction



AvrLm7 is epistatic over *AvrLm9*

R-Avr interactions: Still lots of unknowns



Industry implications

- Rotation of resistance genes influences fungal populations and can minimise disease
  - Unclear what is the best method for rotation (rotate every year or every three years)
  - Are all rotation patterns equal? Probably not
  - Is block sowing needed? Does stacking of resistance genes influence selection?
  - Can defeated resistance genes be reintroduced to the system?
- Monitoring resistance genes in the field/lab and subsequent warnings for industry have been hugely successful
  - Knowing which cultivars are affected i.e. resistance groups
  - Is the breakdown region specific?
- Need to understand both host and pathogen – not a simple system