The effect of rotation of R gene on frequencies of avirulence alleles in blackleg populations

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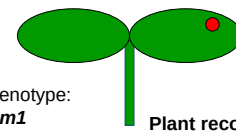
Major gene resistance is a gene-for gene interaction

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



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

Plant Genotype: ***Rlm1***

Plant recognises fungus
- Defence mechanisms
- NO INFECTION

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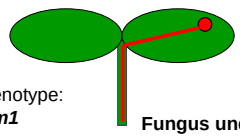
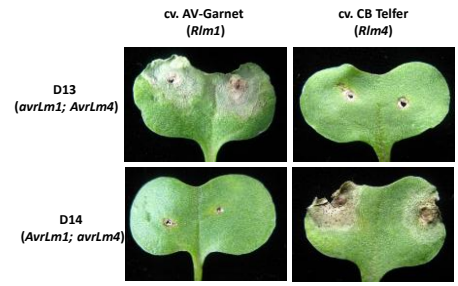


Fungus Genotype: ***avrRml1*** **(Virulent)**

Plant Genotype: ***Rlm1***

Fungus undetected by plant
INFECTION/DISEASE



Take home messages

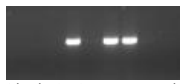
- Monitoring changes in frequencies of avirulence alleles using molecular markers
  - Enables risk of resistance breakdown to be determined
- Rotation of cultivars with different R genes can influence allele frequencies
  - Affects both the corresponding avirulence gene and linked avirulence genes
- Rotations of some R genes will have more benefit than others
- Genomic location (linkage) of R genes in plant and Avr genes in pathogens may influence selection pressure on pathogen by sowing particular R genes

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

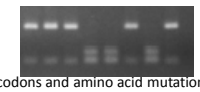
- *AvrLm1* - *Rlm1* (AV-Garnet, Group A)
- *AvrLm4-7* - *Rlm4* (CB Telfer, Group B)
  - *Rlm7* (not in current cultivars)
- *AvrLm2* - *Rlm2* (presence unknown in current cultivars)
- *AvrLm5* - *Rlm5* (Juncea R gene)
- *AvrLm6* - *Rlm6* (Juncea R gene)

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

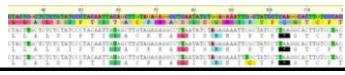
- Different mutation events occur in different avirulence genes.
  - Can develop molecular markers to screen isolates to determine virulence genotype.
- Deletion of entire gene
  - Presence/absence PCR screen
    - *AvrLm1*
    - *AvrLm6*



- 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*

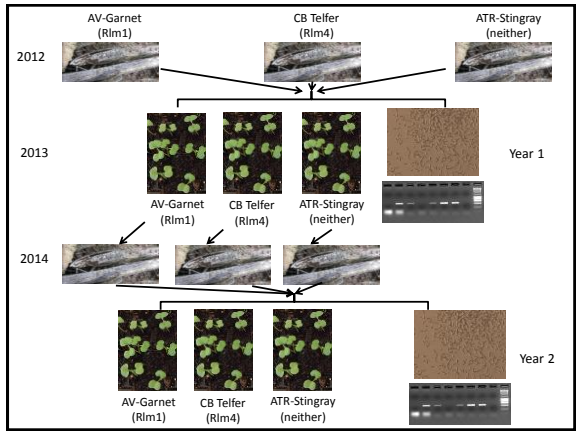


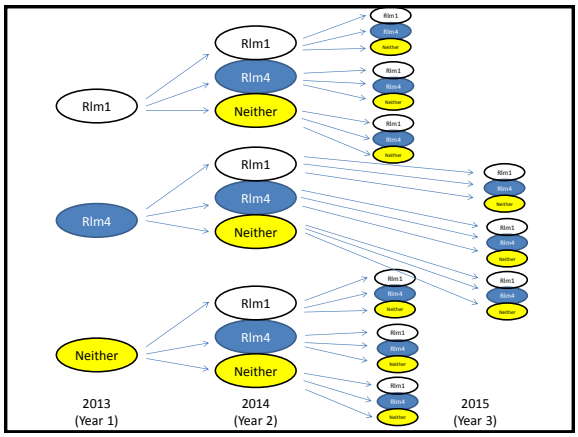
Molecular markers used to monitor changes in avirulence allele frequencies

- Blackleg isolates (ascospores) collected from stubble since 2000
- DNA prepared and tested with molecular markers for each avirulence gene
  - 1901 isolates in total
    - 657 from Vic
    - 427 from NSW
    - 675 from SA
    - 142 from WA
- These data used to confirm disease severity in field and glasshouse experiments
  - Supported the findings that R gene rotation influences Avr allele frequencies.
  - **However, isolates always selected randomly from different locations under different environmental conditions**

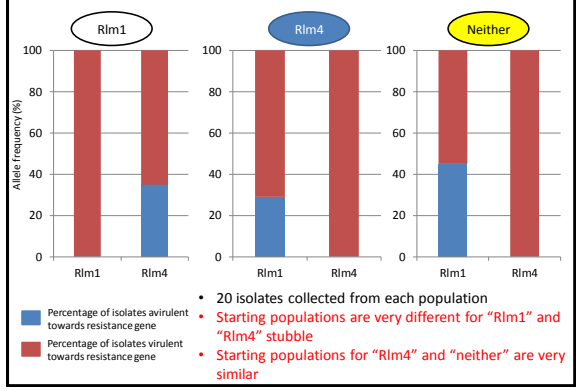
3 year experiment to monitor effect of cultivar rotation on allele frequencies

- Recurring selection experiment established in 2012
- Cultivars with known R genes (*Rlm1*, *Rlm4* or neither)
- Stubble releases ascospore inoculum onto seedlings in shadehouse experiments
- Frequency of alleles of *AvrLm1* and *AvrLm4* determined by high through put molecular assay





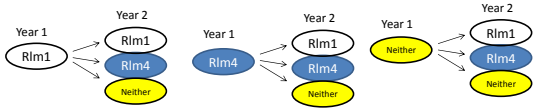
Allele frequencies for each population at Year 1



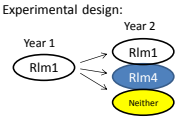
- 20 isolates collected from each population
- Starting populations are very different for "Rlm1" and "Rlm4" stubble
- Starting populations for "Rlm4" and "neither" are very similar

Allele frequencies for each population at year 2

- 20 isolates collected from each selection regime (year 2 population)
- Analysed for virulence towards *Rlm1* and *Rlm4*

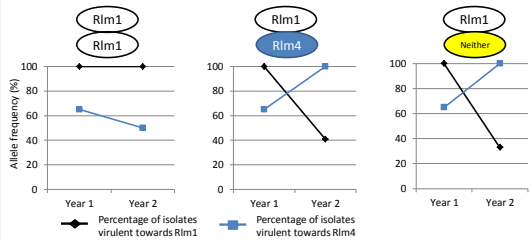


Experimental design:



Findings:

- When Rlm1 selection is continued (graph 1), all isolates remain *avrLm1* whilst the frequency of *avrLm4* isolates decreases.
- When *Rlm1* selection is removed the frequency of virulence towards *Rlm1* decreases (graphs 2 and 3)
- When *Rlm4* selection is added, the frequency of *avrLm4* isolates increases as expected.
- However, *avrLm4* frequency increases in the ATR-Stingray (neither) population.



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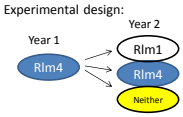
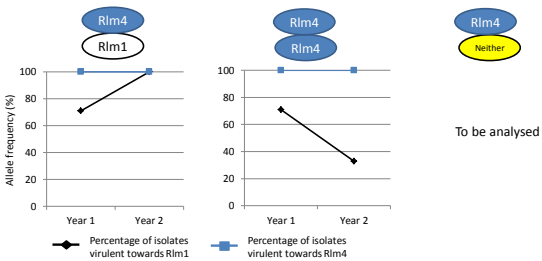
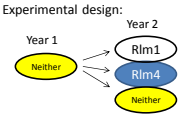


Findings:

- When *Rlm4* selection has been removed, the frequency of *avrLm4* did not decrease (graph 1)
- In the absence of *Rlm1* selection the frequency of *avrLm1* isolates continues to decrease (graph 2)



Experimental design:



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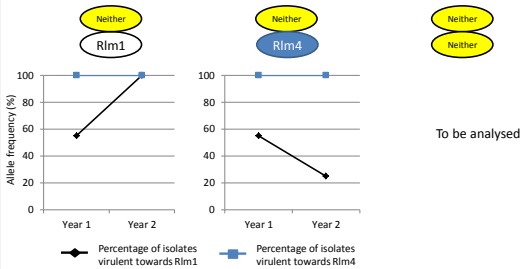
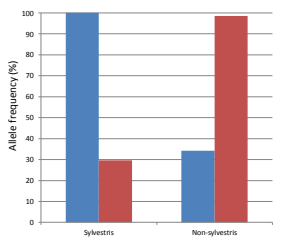


Changes in allele frequencies differ for different avirulence genes

- Changes to *avrLm1* allele frequencies as expected (decreased when *Rlm1* selection removed)
- Frequency of *avrLm4* isolates did not decrease when *Rlm4* selection was removed
  - No fitness cost to the pathogen?
  - Changes in frequency of this avr gene are slow to occur?
  - Know that *avrLm4* frequency can change with rotation (sylvestris situation)

Frequency of *avrLm4* decreases after selection with sylvestris resistance

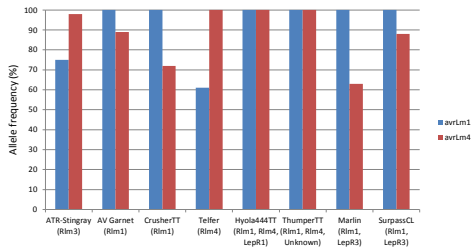


- Sylvestris resistance broke down on EP in 2003
- Two R genes – LepR3 and Rlm1
- Isolates collected from either sylvestris stubble (54) or non-sylvestris stubble (70) from EP during 2004-2008
- Only 30% of isolates derived from sylvestris stubble can attack *Rlm4*
- Reverse is seen in isolates derived from non-sylvestris (*Rlm4* containing) stubble

Percentage of isolates virulent towards *Rlm1*

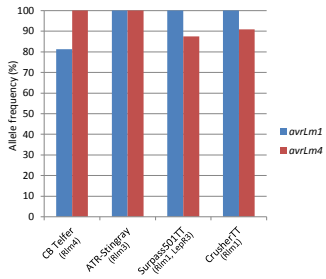
Percentage of isolates virulent towards *Rlm4*

Similar differences in allele frequency are seen in a single year



577 isolates collected from stubble of different cultivars across Australia in 2014

Allele frequencies collected at a single site can vary



- 10 isolates cultured from stubble of various cultivars collected from Parkes, NSW
- Allele frequencies for both *AvrLm1* and *AvrLm4* vary, depending on the stubble from which isolates are cultured
- Findings similar to experiments using ascospore shower technique

Changes in allele frequencies may depend on gene location in the host or pathogen

- Blackleg populations derived from stubble of ATR-Stingray (neither *Rlm1* or *Rlm4*) behaved very similarly to those derived from *Rlm4* stubble
  - ATR-Stingray has *Rlm3*
  - *Rlm3-4-7-9* are linked in the plant
  - *AvrLm3-4-7-9* are linked in the fungus
- Does gene location influence Avr allele frequencies?
  - *AvrLm1* and *AvrLm6* are genetically linked in the pathogen
  - *LepR3* (*Rlm1*?), *Rlm2* and *Rlm6* are reported as alleles of the same gene (Larkan et al.)
  - Selection from sylvestris cultivars (*Rlm1*) influences frequency of *AvrLm6* in blackleg populations (Van de Wouw et al. 2010).

Conclusions

- Rotation of cultivars with different resistance genes influence frequencies of avirulence genes
- Not all avirulence genes respond similarly to this rotation
- Need to understand the interaction between Avr genes and R genes better
  - Linkage of Avr or R genes influences allele frequencies
  - Some rotations will have more benefit than others e.g. *LepR3* followed by *Rlm4*
- We still don't know
  - What frequency of virulent isolates leads to resistance being overcome?
  - Whether we should rotate R genes every year or every 3 years?