



Monitoring of virulence in Australian populations of the blackleg fungus

Angela Van de Wouw, Anton Cozijnsen, Jo Rayner and Barbara Howlett

School of Botany
The University of Melbourne
26 Feb 2009



Thierry Rouxel
Mylene Balesdent



Steve Marcroft



Phil Salisbury



Australian Government
Grains Research and Development Corporation



Grains Research &
Development Corporation

***Brassica - Leptosphaeria maculans* interaction**

<i>L. maculans</i> avirulence gene	<i>Brassica</i> resistance gene	Varieties
<i>AvrLm1</i>	<i>Rlm1</i>	Columbus
<i>AvrLm6</i>	<i>Rlm6</i>	Aurea
<i>AvrLm4-7</i>	<i>Rlm4</i>	Pollen
<i>AvrLm4-7</i>	<i>Rlm7</i>	Line 23-1-1

- Fungal populations evolve very quickly due to outcrossing and large population size
- Major gene resistance results in strong selection pressure on populations to increase frequency of virulent isolates

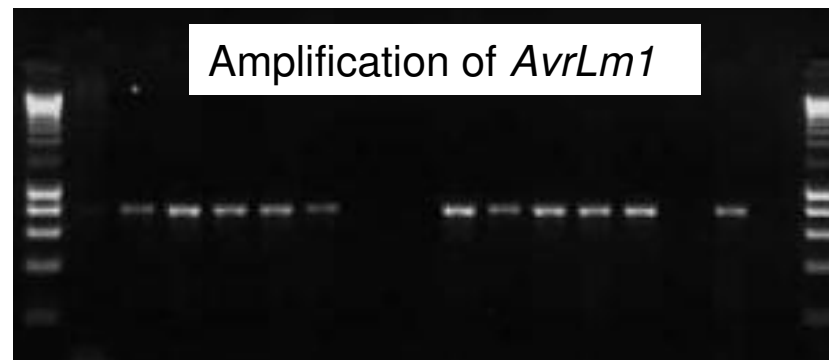
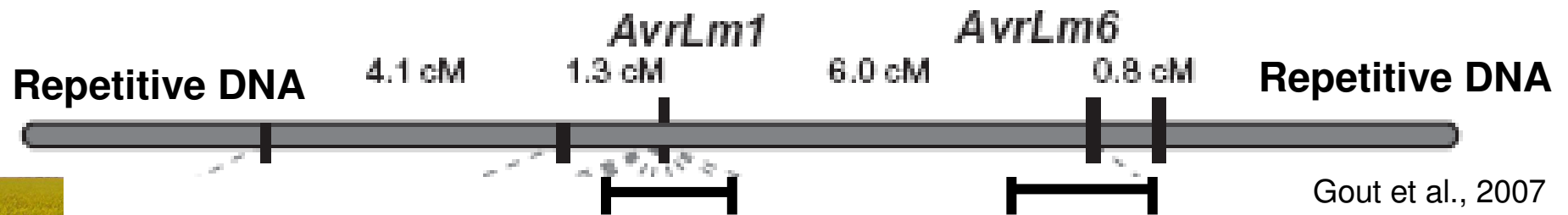


Markers for fungal avirulence genes can inform

- Presence and number of resistance genes in a variety
 - Mapping studies with markers for *AvrLm1* showed that varieties with *sylvestris*-derived resistance contain both *Rlm1* and *LepR3*
- Change in frequencies towards particular resistance gene
 - This information can be used to maximise durability of resistance
 - *AvrLm1*, *AvrLm6*, *AvrLm4-7* sequences published (Rouxel et al)

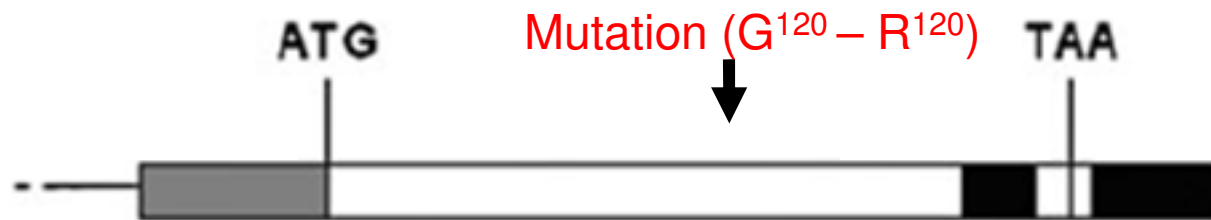
L. maculans avirulence genes - I

- *AvrLm1* and *AvrLm6*
 - Genetically linked in the fungus
 - Virulence often via deletion (>85%)

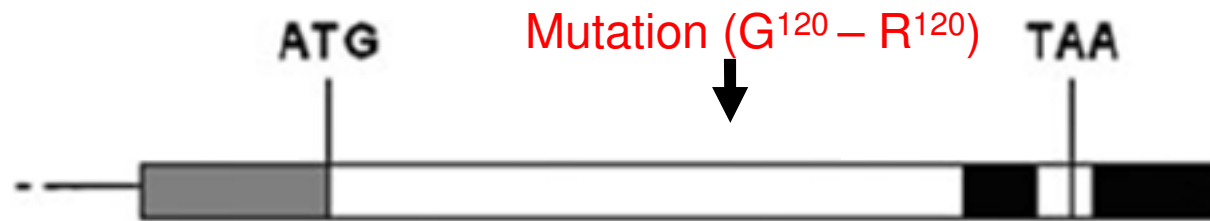


L. maculans avirulence genes - II

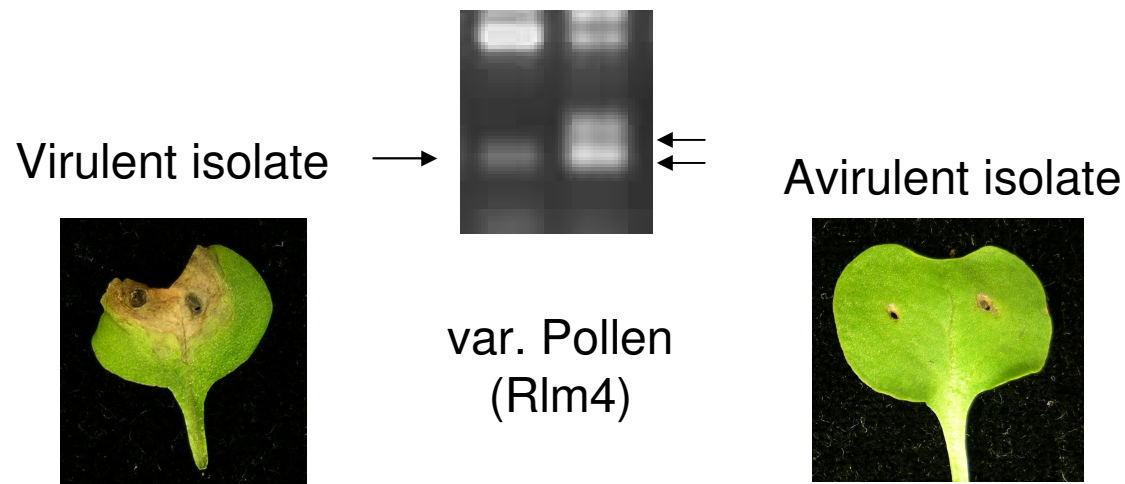
- *AvrLm4-7*
- A single avirulence gene confers virulence towards both *Rlm4* and *Rlm7*
 - Basis of virulence towards *Rlm7* is complicated
 - Virulence towards *Rlm4* is associated with an amino acid change (Glycine (G)¹²⁰ – Arginine(R)¹²⁰)
 - Molecular marker discriminates isolates that are avirulent or virulent towards *Rlm4*



L. maculans avirulence genes - III



Amplify gene by PCR
Cut DNA with enzyme (*HaellI*)
Different banding pattern in avirulent and virulent isolates



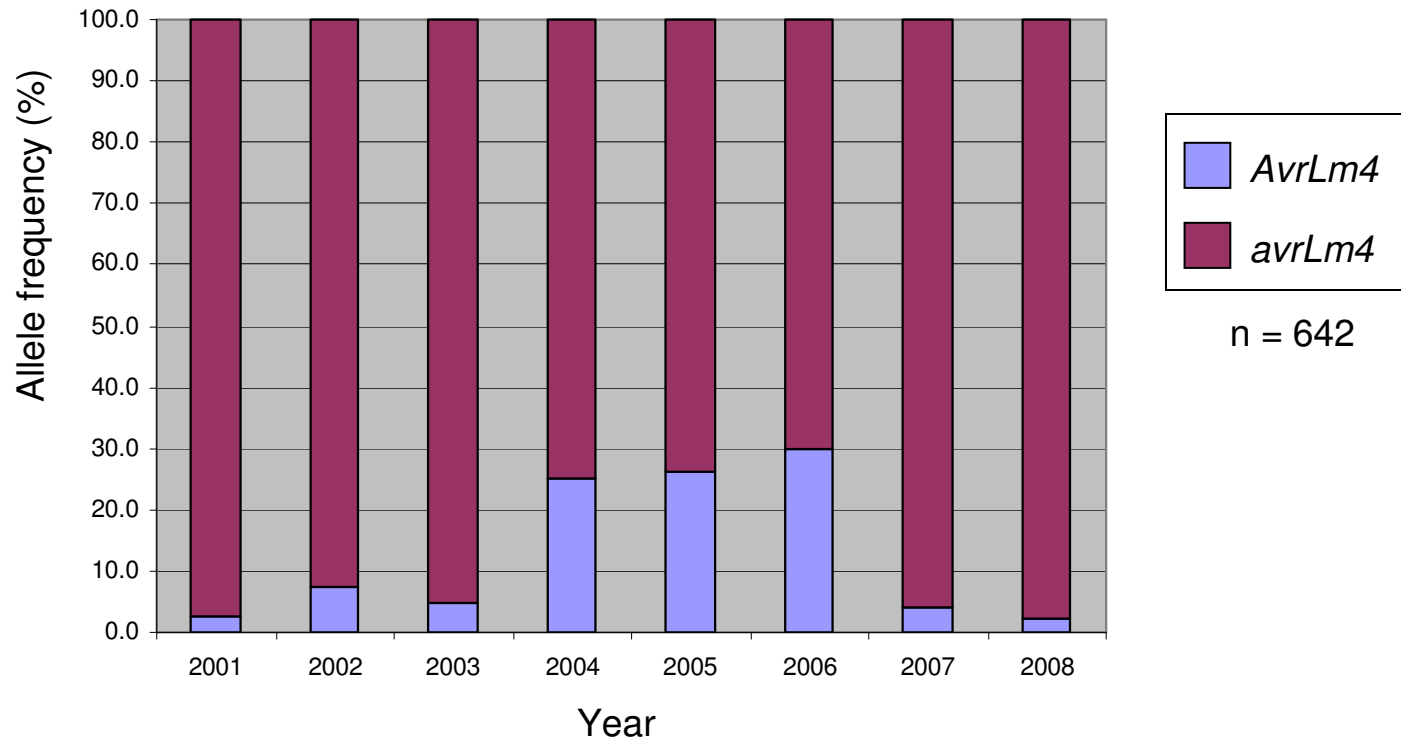
15 isolates tested

Monitoring avirulence allele frequencies in Australian blackleg isolates

- Isolates (877) cultured from stubble
 - collected at NVT trial sites (Vic, NSW and SA).
 - with different sources of resistance
 - Polygenic (eg. var. Beacon) - 782 isolates (2001 – 2008)
 - Sylvestris (eg. var. Surpass400) - 95 isolates (2004 – 2008)
- Tested with molecular markers for *AvrLm1*, *AvrLm6*, ***AvrLm4*** and mating-type

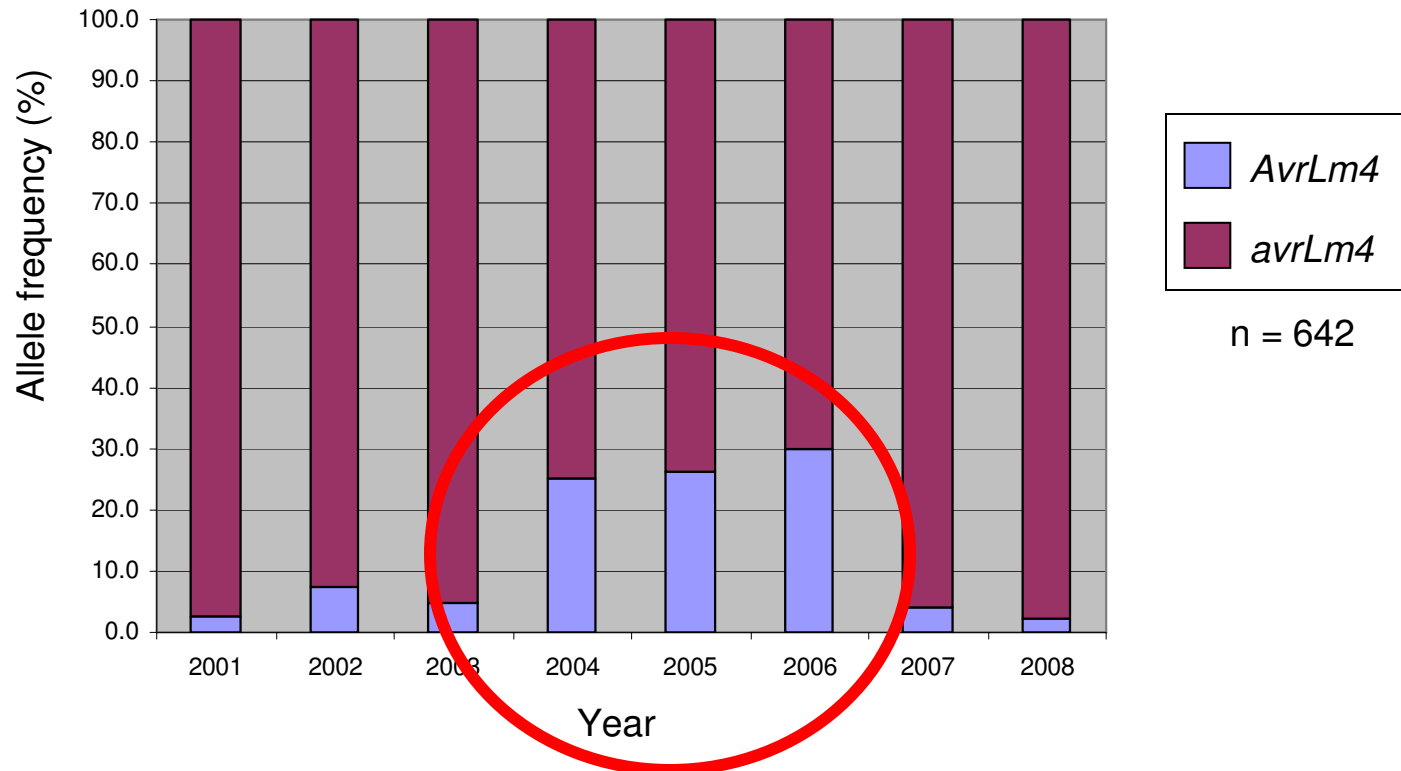


AvrLm4 allele frequencies (2001-2008)



- High frequency of isolates virulent towards *Rlm4*
 - Consistent with the presence of *Rlm4* in numerous Australian polygenic varieties including Beacon

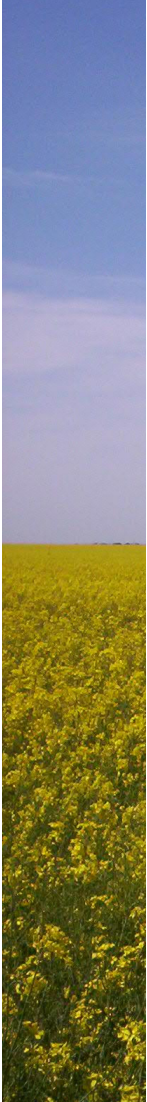
AvrLm4 allele frequencies (2001-2008)



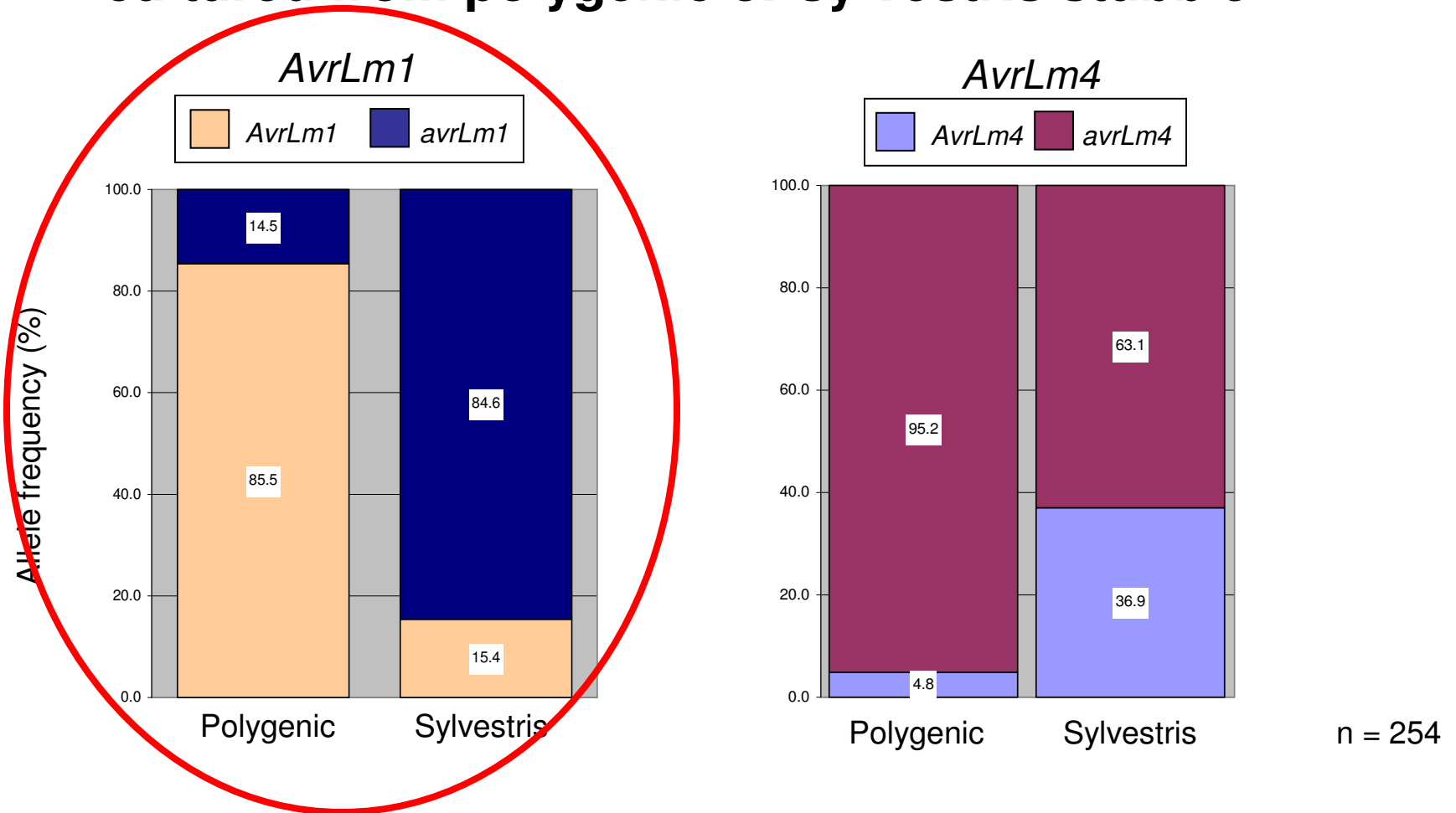
- High frequency of isolates virulent towards *Rlm4*, particularly prior to or after 2004-2006

***AvrLm1* and *AvrLm4* allele frequencies in blackleg isolates cultured from different stubble sources**

- 2004 - 2006 isolates (254)
- Isolates classified on basis of stubble source (polygenic or sylvestris)
 - Both classes of isolates collected at all sites
- Determined *AvrLm1* and *AvrLm4* allele frequencies



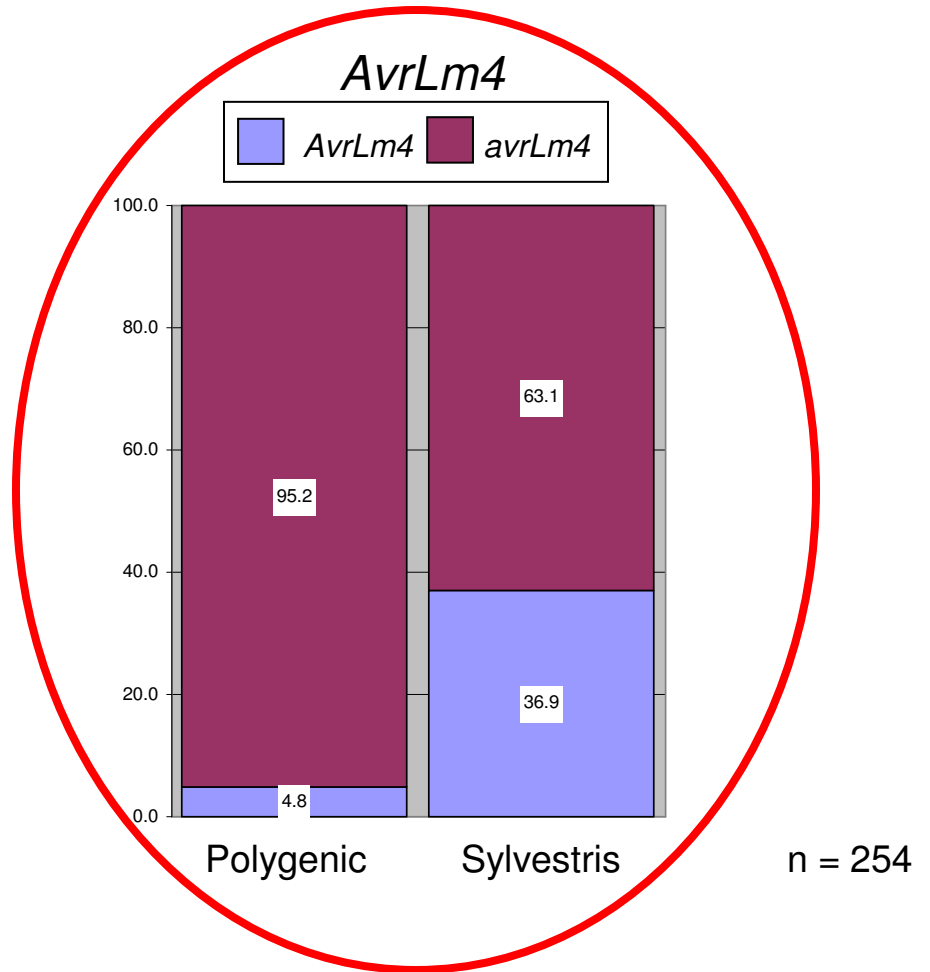
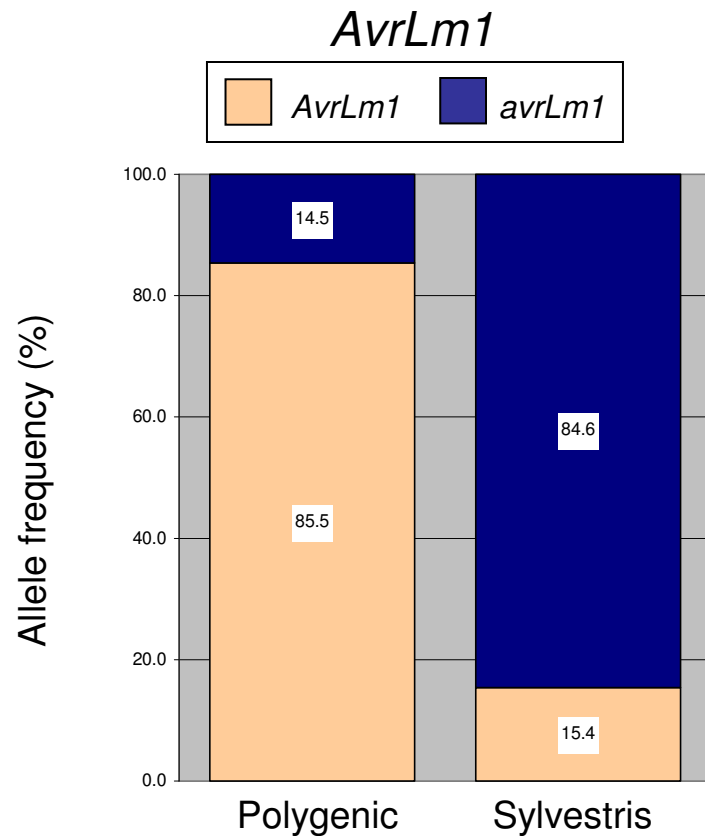
AvrLm1 and *AvrLm4* allele frequencies in isolates cultured from polygenic or sylvestris stubble



Populations from sylvestris-derived stubble have a higher frequency of isolates virulent towards *Rlm1*, than those from polygenic stubble

- Consistent with *Rlm1* in sylvestris-derived varieties

AvrLm1 and *AvrLm4* allele frequencies in isolates cultured from polygenic or sylvestris stubble



Isolates virulent towards *Rlm4* are lower frequency when cultured from sylvestris stubble compared to polygenic stubble

- Consistent with *Rlm4* in polygenic, not sylvestris varieties



Conclusions

- Highlights the dynamic nature of populations of blackleg isolates
- The frequency of isolates virulent towards *Rlm4* is extremely high in all years (2001 – 2008).
- Consistent with *Rlm4* being present in previous and current Australian varieties
 - Glasshouse tests show *Rlm4* is present in older Australian varieties including Karoo, Maluka and Oscar, which provide sources of polygenic resistance in current varieties.

Implications

- *Rlm4* has been maintained as a resistance source in current polygenic varieties



Conclusions/Implications - II

- Populations cultured from sylvestris stubble have lower frequency of isolates virulent towards *Rlm4*
 - Polygenic varieties that contain *Rlm4* may be more resistant in regions where sylvestris varieties were previously grown
- The frequencies of isolates virulent towards particular resistance genes are consistent with the resistance genes in the varieties they were cultured from
 - Consistent with our previous findings for *AvrLm1* and *AvrLm6*
- Avirulence allele frequencies change quickly over time (within one year)
 - Supports the use of a ‘rotation of resistance’ management strategy



Acknowledgements



**Thierry Rouxel
Mylene Balesdent**



Steve Marcroft



Phil Salisbury



Australian Government
Grains Research and Development Corporation



Grains Research &
Development Corporation