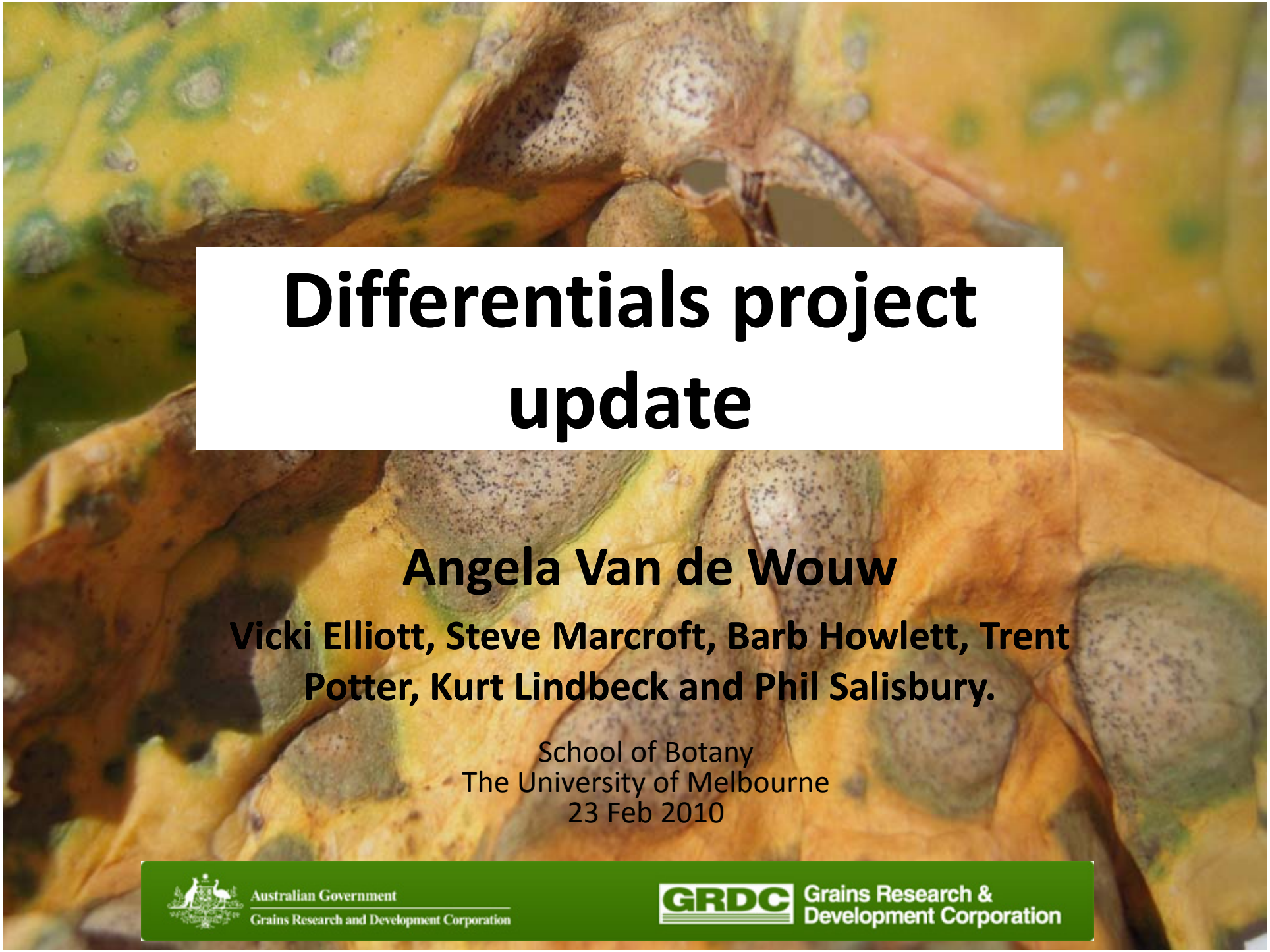

Differentials project update

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The University of Melbourne

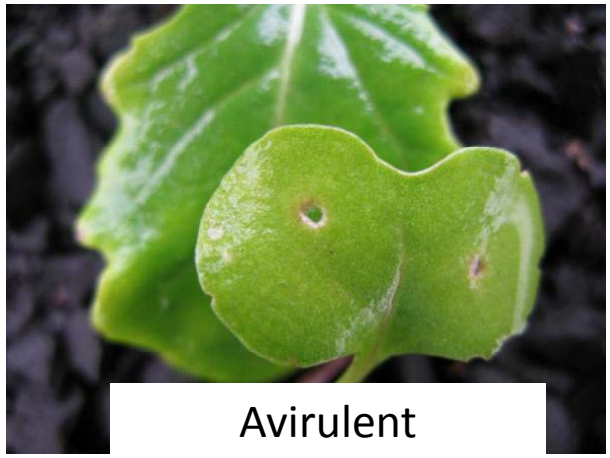
23 Feb 2010

Australian Government
Grains Research and Development Corporation

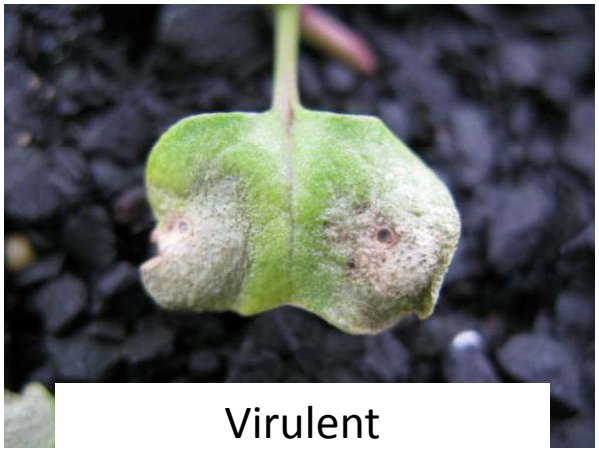
GRDC Grains Research & Development Corporation

Brassica - L. maculans interaction

- Plant contains resistance genes
- Corresponding avirulence (*Avr*) gene in the fungus
- When the avirulence gene is mutated an isolate becomes virulent



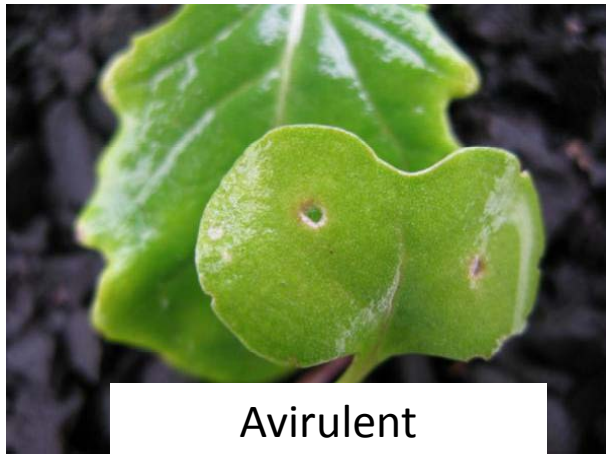
Avirulent
(resistant reaction)



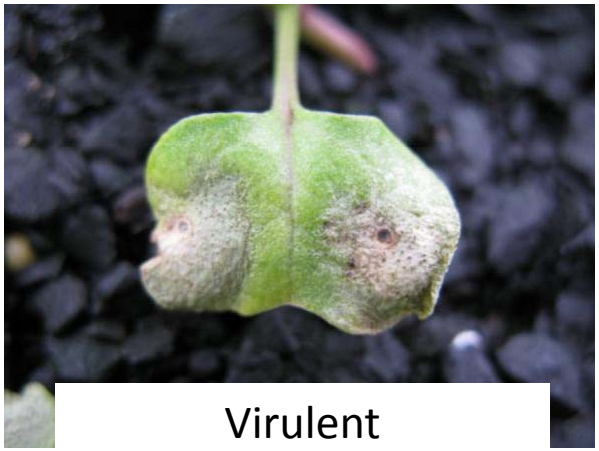
Virulent
(susceptible reaction)

Background – major gene resistance

- Do not know suite of resistance genes in Australian varieties
- The French routinely characterize R genes in breeding lines and commercial varieties
- They use a differential set of isolates that can discriminate *Rlm1 – Rlm9* and *LepR3*.



Avirulent
(resistant reaction)



Virulent
(susceptible reaction)

Background – adult plant resistance

- Adult plant (minor gene) resistance is also important in controlling blackleg

Cotyledon rating	Maturity Rating	Percentage
R	R	88
R	S	9
R	I	3
S	S	47
S	R	42
S	I	11

- Quantitative resistance increases the durability of qualitative resistance to *Leptosphaeria maculans* in *Brassica napus*

  Brun *et al.,* 2010. New Phytologist

  - *Rlm6* crossed into a susceptible background and a background with minor gene resistance. Varieties sown into *B. napus* stubble

  - *Rlm6* rendered ineffective within 3 years in susceptible background but still effective after 5 years with minor gene resistance

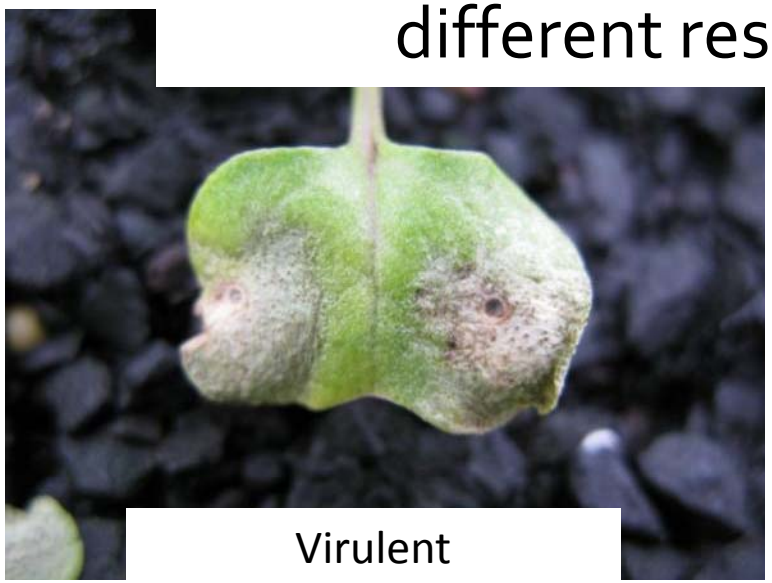
Aims of the project

- Identify Australian blackleg isolates that can be used
  - to screen for known or unknown major gene resistance
  - to screen for adult plant resistance genes.
- Use these isolates to characterise resistance genes in Australian cultivars and lines
  - Group cultivars and lines with similar sources of resistance

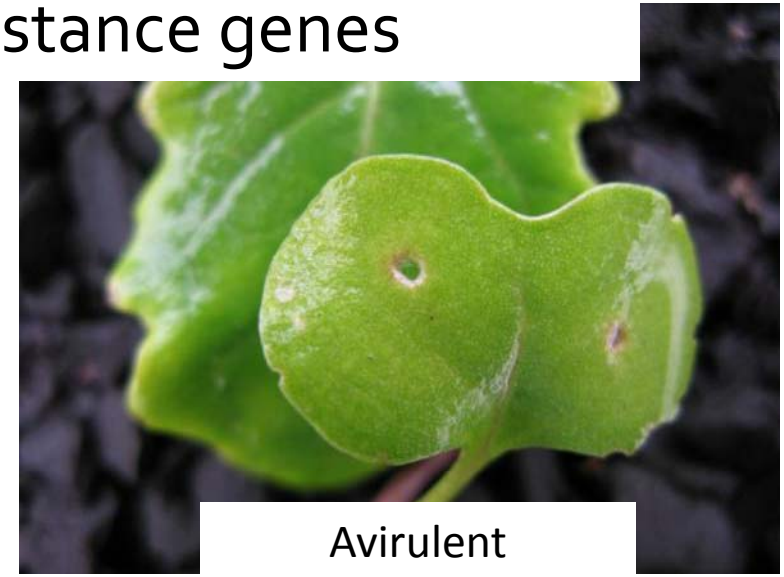
Cotyledon pathogenicity screens

- Identify major gene resistance (*Rlm1-Rlm10*, *LepR1-4*)

Same isolate screened on varieties with different resistance genes



Virulent
(susceptible reaction)



Avirulent
(resistant reaction)

Identify different sources of adult plant resistance

Determining major-gene resistance using differential *L. maculans* isolates

- 33 varieties (30 *B. napus* and 3 *B. juncea*) sent to French
  - Screen with isolates of known *Avr* genotype
  - Identify major-gene resistance
    - *Rlm1-Rlm9* and *RlmS* (sylvestris-derived resistance)
    - Tells us which of these genes are present and which are absent
  - DOES NOT identify adult plant resistance

R genotypes of varieties

Variety	R genotype
Wesbrook	No R genes
AV Garnet	*Rlm1, Rlm9*
Caiman	*Rlm7, Rlm9 (Rlm4)*
Ripper	*Rlm2, Rlm4*
46C76	*Rlm3*
Oscar	*Rlm3*
Taparoo	*Rlm3*
Q2	*Rlm3, Rlm9*
Tl1Pinnacle	*Rlm3, Rlm9*
AG-Emblem	*Rlm4*
ATR Summit	*Rlm4*
BLN3347	*Rlm4*
Dunkeld	*Rlm4*
Karoo	*Rlm4*
Narendra	*Rlm4*
Thunder TT	*Rlm4*
Wesroona	*Rlm4*

Variety	R genotype
ATR Barra	*Rlm4, Rlm9*
AV-sapphire4	*Rlm4, Rlm9*
AV-sapphire1	*Rlm4, Rlm9*
AV-sapphire2	*Rlm4, Rlm9*
Skipton	*Rlm4, Rlm9*
Tarcoola	*Rlm4, Rlm9*
Tornado TT	*Rlm4, Rlm9*
46Y78	*RlmS?*
Surpass 400	*Rlm1, RlmS*
ATR Marlin	*RlmS?*
Av Jade	Potentially new R gene?
Dune	Potentially new R gene?
Hyola50	Potentially new R gene?
Oasis CL	Potentially new R gene?
Rocket CL	Potentially new R gene?
Sahara CL	Potentially new R gene?

R genotypes of varieties – *Rlm3*

Variety	R genotype
Wesbrook	No R genes
AV Garnet	*Rlm1, Rlm9*
Caiman	*Rlm7, Rlm9 (Rlm4)*
Ripper	*Rlm2, Rlm4*
46C76	*Rlm3*
Oscar	*Rlm3*
Taparoo	*Rlm3*
Q2	*Rlm3, Rlm9*
Tl1Pinnacle	*Rlm3, Rlm9*
AG-Emblem	*Rlm4*
ATR Summit	*Rlm4*
BLN3347	*Rlm4*
Dunkeld	*Rlm4*
Karoo	*Rlm4*
Narendra	*Rlm4*
Thunder TT	*Rlm4*
Wesroona	*Rlm4*

Variety	R genotype
ATR Barra	*Rlm4, Rlm9*
AV-sapphire4	*Rlm4, Rlm9*
AV-sapphire1	*Rlm4, Rlm9*
AV-sapphire2	*Rlm4, Rlm9*
Skipton	*Rlm4, Rlm9*
Tarcoola	*Rlm4, Rlm9*
Tornado TT	*Rlm4, Rlm9*
46Y78	*RlmS?*
Surpass 400	*Rlm1, RlmS*
ATR Marlin	*RlmS?*
Av Jade	Potentially new R gene?
Dune	Potentially new R gene?
Hyola50	Potentially new R gene?
Oasis CL	Potentially new R gene?
Rocket CL	Potentially new R gene?
Sahara CL	Potentially new R gene?

R genotypes of varieties – *Rlm9*

Variety	R genotype
Wesbrook	No R genes
AV Garnet	*Rlm1, Rlm9*
Caiman	*Rlm7, Rlm9 (Rlm4)*
Ripper	*Rlm2, Rlm4*
46C76	*Rlm3*
Oscar	*Rlm3*
Taparoo	*Rlm3*
Q2	*Rlm3, Rlm9*
Tl1Pinnacle	*Rlm3, Rlm9*
AG-Emblem	*Rlm4*
ATR Summit	*Rlm4*
BLN3347	*Rlm4*
Dunkeld	*Rlm4*
Karoo	*Rlm4*
Narendra	*Rlm4*
Thunder TT	*Rlm4*
Wesroona	*Rlm4*

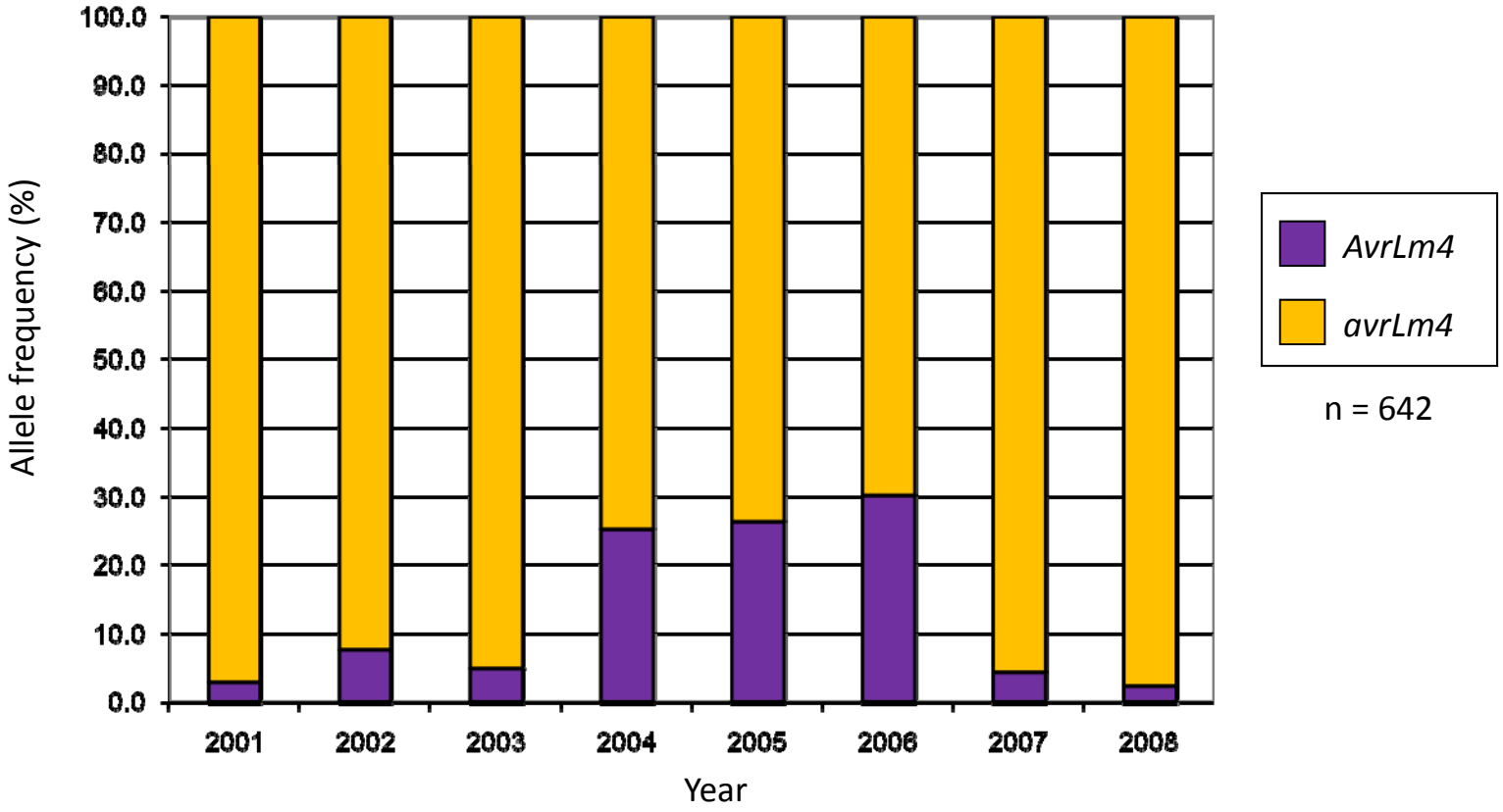
Variety	R genotype
ATR Barra	*Rlm4, Rlm9*
AV-sapphire4	*Rlm4, Rlm9*
AV-sapphire1	*Rlm4, Rlm9*
AV-sapphire2	*Rlm4, Rlm9*
Skipton	*Rlm4, Rlm9*
Tarcoola	*Rlm4, Rlm9*
Tornado TT	*Rlm4, Rlm9*
46Y78	*RlmS?*
Surpass 400	*Rlm1, RlmS*
ATR Marlin	*RlmS?*
Av Jade	Potentially new R gene?
Dune	Potentially new R gene?
Hyola50	Potentially new R gene?
Oasis CL	Potentially new R gene?
Rocket CL	Potentially new R gene?
Sahara CL	Potentially new R gene?

R genotypes of varieties – *Rlm4*

Variety	R genotype
Wesbrook	No R genes
AV Garnet	*Rlm1, Rlm9*
Caiman	*Rlm7, Rlm9 (Rlm4)*
Ripper	*Rlm2, Rlm4*
46C76	*Rlm3*
Oscar	*Rlm3*
Taparoo	*Rlm3*
Q2	*Rlm3, Rlm9*
Tl1Pinnacle	*Rlm3, Rlm9*
AG-Emblem	*Rlm4*
ATR Summit	*Rlm4*
BLN3347	*Rlm4*
Dunkeld	*Rlm4*
Karoo	*Rlm4*
Narendra	*Rlm4*
Thunder TT	*Rlm4*
Wesroona	*Rlm4*

Variety	R genotype
ATR Barra	*Rlm4, Rlm9*
AV-sapphire4	*Rlm4, Rlm9*
AV-sapphire1	*Rlm4, Rlm9*
AV-sapphire2	*Rlm4, Rlm9*
Skipton	*Rlm4, Rlm9*
Tarcoola	*Rlm4, Rlm9*
Tornado TT	*Rlm4, Rlm9*
46Y78	*RlmS?*
Surpass 400	*Rlm1, RlmS*
ATR Marlin	*RlmS?*
Av Jade	Potentially new R gene?
Dune	Potentially new R gene?
Hyola50	Potentially new R gene?
Oasis CL	Potentially new R gene?
Rocket CL	Potentially new R gene?
Sahara CL	Potentially new R gene?

AvrLm4 allele frequencies (2001 -2008)



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

R genotypes of varieties – new R genes?

Variety	R genotype
Wesbrook	No R genes
AV Garnet	*Rlm1, Rlm9*
Caiman	*Rlm7, Rlm9 (Rlm4)*
Ripper	*Rlm2, Rlm4*
46C76	*Rlm3*
Oscar	*Rlm3*
Taparoo	*Rlm3*
Q2	*Rlm3, Rlm9*
Tl1Pinnacle	*Rlm3, Rlm9*
AG-Emblem	*Rlm4*
ATR Summit	*Rlm4*
BLN3347	*Rlm4*
Dunkeld	*Rlm4*
Karoo	*Rlm4*
Narendra	*Rlm4*
Thunder TT	*Rlm4*
Wesroona	*Rlm4*

Variety	R genotype
ATR Barra	*Rlm4, Rlm9*
AV-sapphire4	*Rlm4, Rlm9*
AV-sapphire1	*Rlm4, Rlm9*
AV-sapphire2	*Rlm4, Rlm9*
Skipton	*Rlm4, Rlm9*
Tarcoola	*Rlm4, Rlm9*
Tornado TT	*Rlm4, Rlm9*
46Y78	*RlmS?*
Surpass 400	*Rlm1, RlmS*
ATR Marlin	*RlmS?*
Av Jade	Potentially new R gene?
Dune	Potentially new R gene?
Hyola50	Potentially new R gene?
Oasis CL	Potentially new R gene?
Rocket CL	Potentially new R gene?
Sahara CL	Potentially new R gene?

Developing a set of Australian differential isolates

	IBCN13	IBCN15	IBCN16	IBCN17	IBCN18	IBCN75	IBCN76	PHW1223
AvrLm1	-	-	-	-	+	+	+	-
AvrLm2	+	-	-	-	+	-	-	-
AvrLm3	-	-	-	-	-	-	+	-
AvrLm4	-	-	-	+	+	-	-	-
AvrLm5	+	+	+	+	-	+	+	+
AvrLm6	+	+	-	+	-	+	+	+
AvrLm7	-	-	-	+	+	-	-	-
AvrLm8	-	+	-	+	-	+	+	+
AvrLm9	+	-	-	-	-	-	-	+
AvrLmS	nd	nd	nd	nd	nd	nd	nd	+

- Need to identify isolates that can attack 'potentially new R genes' and *LepR1-LepR4*
- Currently screening isolates on a range of the varieties with known R genes

Identifying adult plant resistance

- We want to characterise minor gene resistance
  - Screen varieties with single isolates that can differentiate classes of minor gene resistance, if present
- All 33 varieties were sown at Horsham across four different stubble sources
- Recorded lesion score at the first leaf stage and adult plant survival

Rlm4 varieties have different minor gene resistance

Cultivar	R gene	45Y77		ATR-Beacon		AV-Garnet		Dune	
		Lesion	Adult	Lesion	Adult	Lesion	Adult	Lesion	Adult
BLN3347	*Rlm4*	S	R	S	R	S	R	S	I
Emblem	*Rlm4*	S	I	S	R	S	R	S	I
Summitt	*Rlm4*	S	R	S	I	S	R	S	R
Dunkeld	*Rlm4*	S	I	S	I	S	R	S	I
Karoo	*Rlm4*	S	S	S	S	S	S	S	S
Narendra	*Rlm4*	S	S	S	S	S	S	S	S
Tarcoola	*Rlm4*	S	R	S	R	S	R	S	I
ThunderTT	*Rlm4*	S	R	S	R	S	R	S	R
Wesroona	*Rlm4*	S	S	S	S	S	I	S	S

- All varieties susceptible at the first leaf stage of development

Rlm4 varieties have different minor gene resistance

Cultivar	R gene	45Y77		ATR-Beacon		AV-Garnet		Dune	
		Lesion	Adult	Lesion	Adult	Lesion	Adult	Lesion	Adult
BLN3347	*Rlm4*	S	R	S	R	S	R	S	I
Emblem	*Rlm4*	S	I	S	R	S	R	S	I
Summitt	*Rlm4*	S	R	S	I	S	R	S	R
Dunkeld	*Rlm4*	S	I	S	I	S	R	S	I
Karoo	*Rlm4*	S	S	S	S	S	S	S	S
Narendra	*Rlm4*	S	S	S	S	S	S	S	S
Tarcoola	*Rlm4*	S	R	S	R	S	R	S	I
ThunderTT	*Rlm4*	S	R	S	R	S	R	S	R
Wesroona	*Rlm4*	S	S	S	S	S	I	S	S

- All varieties susceptible at the first leaf stage of development
- Not all varieties susceptible at the adult stage

Rlm4 varieties have different minor gene resistance

Cultivar	R gene	45Y77		ATR-Beacon		AV-Garnet		Dune	
		Lesion	Adult	Lesion	Adult	Lesion	Adult	Lesion	Adult
BLN3347	*Rlm4*	S	R	S	R	S	R	S	I
Emblem	*Rlm4*	S	I	S	R	S	R	S	I
Summitt	*Rlm4*	S	R	S	I	S	R	S	R
Dunkeld	*Rlm4*	S	I	S	I	S	R	S	I
Karoo	*Rlm4*	S	S	S	S	S	S	S	S
Narendra	*Rlm4*	S	S	S	S	S	S	S	S
Tarcoola	*Rlm4*	S	R	S	R	S	R	S	I
ThunderTT	*Rlm4*	S	R	S	R	S	R	S	R
Wesroona	*Rlm4*	S	S	S	S	S	I	S	S

- All varieties susceptible at the first leaf stage of development
- Not all varieties susceptible at the adult stage
- Minor gene resistance does not behave the same on all stubble sources
    - potential to rotate major and minor R gene resistance

The year ahead

- Identify isolates that discriminate additional major R genes
- Screen varieties with single isolates that will discriminate minor R genes
  - Already identified potential isolates
  - Screen all cultivars with only *Rlm4* resistance with 20 single isolates
- Begin to characterise varieties into resistance groups

Acknowledgements

Dr Thierry Rouxel and Dr Mylene Balesdent

INRA

marcroft GRAINS PATHOLOGY

Victoria The Place To Be

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