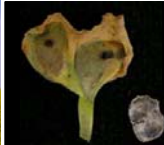


## Issues in deploying seedling and adult plant resistance


Angela Van de Wouw,  
The University of Melbourne  
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### Managing Blackleg Disease


- Two types of resistance
  - Seedling and adult



No resistance  
= disease




Major gene  
(seedling) resistance  
= no disease




Minor gene (adult  
plant) resistance  
= some disease

### Seedling resistance

- Resistance gene in the plant which corresponds to an avirulence gene in the fungus
- Expressed at the seedling stage
- First resistance gene cloned from *B. napus* in 2012
- All or nothing response
- We can use isolates to screen for known resistance genes (*Rlm1-Rlm9, LepR1-4*)











Susceptible  
(virulent allele)



Resistant  
(avirulent allele)

### Determining the presence of resistance genes using single isolates

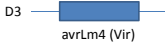
- 12 differential isolates
- Screen them against cultivars and determine the presence and absence of resistance genes

	Westar	Garnet (Rlm1)	Telfer (Rlm4)	Mustang (Rlm4)
D3				
D5				

### Confirming the presence of resistance genes in 'unknown' cultivars


- AvrLm1 and AvrLm4 have been cloned
- Transform virulent isolates with the Avirulence allele
- Can use these isolates to determine the presence of Rlm1 and Rlm4 in cultivars with unknown resistance

D3



avrLm4 (Vir)

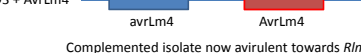
D5



AvrLm4 (Avr)

























Transform isolate D3 with AvrLm4 (Avr)

D3 + AvrLm4



avrLm4      AvrLm4



Complemented isolate now avirulent towards Rlm4.

	Westar	Garnet (Rlm1)	Telfer (Rlm4)	Mustang (Rlm4)	Agamax (Rlm1, Rlm4)	VT525 (Rlm1, Rlm4)
D5						
D3						
D3+AvrLm1						
D3+AvrLm4						

**Conclusions:** Mustang must have *Rlm4* but does not have *Rlm1*;  
Agamax and VT525 must have both *Rlm1* and *Rlm4*

### Adult plant resistance

- Multiple minor genes contributing to resistance. Expressed at the adult plant stage and poorly understood
- Assumed that adult plant resistance confers partial resistance to all isolates
- Can we characterise adult plant resistance?
  - For screening adult plant resistance we first need to overcome seedling resistance
- Identified 20 isolates that can overcome *Rlm4* seedling resistance.
  - These were inoculated onto 13 *Rlm4* containing cultivars

### Adult plant resistance screen for *Rlm4* cultivars

Cultivars	Isolates																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Thunder	R	I	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Tornado	R	R	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Barra	S	S	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Summit	S	I	S	S	R	R	R	R	R	R	R	R	R	S	I	S	R	R	R	R	R
Tarcoola	S	S	S	S	R	R	R	I	I	S	R	I	S	R	R	R	R	R	R	I	R
Sapphire	S	S	S	R	R	I	S	S	S	R	R	R	S	I	S	R	R	R	R	R	R
Skipiton	S	S	S	S	S	S	S	S	S	S	I	S	S	I	R	R	R	R	I	I	R
BLN3347	S	S	S	S	S	S	S	S	S	I	S	S	S	S	I	R	R	R	R	R	R
Karoo	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	R	S	S	I	S
Emblem	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	R
Dunkeld	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	R
Wesroona	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R
Narendra	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R

S = susceptible reaction, R = resistant reaction, I = intermediate reaction

### Problems with screening for adult plant resistance



- Clear differences in adult plant resistance were detected in *Rlm4* harbouring cultivars.
  - These isolates could be used for screening for adult plant resistance in *Rlm4* only cultivars
- Problem: A set of isolates would need to be identified for all R genes and R gene combinations
- Now using the tub screen (ascospore showers) as a means to characterise adult plant resistance

### Challenges for breeders - I

- If seedling resistance is effective whilst selecting in the field, adult plant resistance will not be selected for
- If seedling resistance is ineffective (overcome) whilst selecting in the field, adult plant resistance will be selected for
- This is evident in cultivars such as Tornado and Thunder
  - These were originally considered to have no major resistance genes (termed polygenic cultivars).
  - Since shown to have *Rlm4* resistance gene
  - Suggests *Rlm4* was probably ineffective in the field during selection
  - As a result these cultivars have great adult plant resistance (resistant against all 13 isolates we screened and showed great stability in the field)

### Challenges for breeders - II

- We have confirmed that
  - some cultivars have stacked resistance genes
  - that unknown cultivars such as GT-Mustang contain more than one resistance gene
- This causes issues for rotation of resistance genes
  - GT-Mustang contains two resistance genes – one is a novel resistance gene, the second is *Rlm4*

### Stacking resistance genes causes problems for rotation

Cultivar	Seedling genotype	Exposed to Group B stubble	Exposed to Group F stubble
ATRCOBBLER	<i>Rlm4</i> , <i>Rlm9</i>	100	99
CBTELFER	<i>Rlm4</i>	99	100
GTVIPER	Unknown (Group F)	37	96

- Rlm4* cultivars can not be sown after Group F cultivars.
- Group F can be sown after *Rlm4* cultivars
- Need to include GT-Mustang in Group B to maintain rotation

### Challenges for breeders - III

- McDonald et al suggest that resistance genes should be used in isolation for pathogens such as blackleg
- Our data supports McDonald et al suggesting rotation of resistance genes is more effective than stacking
- Issues for industry is that breeders probably don't know that genes are stacked until cultivar R screened prior to release

### Blackleg Resistance Groups

- Previously we have shown that rotation of cultivars with different resistance genes reduces the risk of disease (Marcroft et al Plant Pathology 2012)
- Aim: Release resistance information to farmers so that they can manage resistance in the field
- Blackleg Resistance groups released for the first time last year in the Blackleg Management Guide
- Blackleg resistance groups are determined using information on both seedling and adult plant resistance

### Determining seedling resistance

- All cultivars are screened with 12 differential isolates to characterise seedling resistance genes
- Compare patterns of avirulent and virulent reactions to determine presence/absence of resistance genes (Marcroft et al Crop and Pasture Science 2012)
- We now also have the transformed isolates to determine the presence/absence of Rlm1 and Rlm4 in 'unknown' cultivars

### Determining adult plant resistance

- All cultivars are screened via the ascospore shower (tub screen) method (Marcroft et al Crop and Pasture Science 2012)
- Cultivars are exposed to stubbles representing seven different resistance sources
- Plants are grown to maturity and levels of internal infection assessed
- Cultivars rated as susceptible (>75% internal infection) or resistant (<75% internal infection) – extremely high disease pressure

### Determining Blackleg Resistance group

- If the cultivar has a characterised seedling resistance gene (e.g. Rlm1) it is placed into a corresponding group. In addition, if a cultivar also shows >75% internal infection on a stubble source from another rotation group then it is also placed into that group.
- If the cultivar has an unknown seedling resistance genotype, it is grouped on differences in adult plant resistance as determined from the tub screen. In this case, if a cultivar has >75% internal infection on a specific stubble source it is placed in that group.
- B. juncea cultivars will be placed into a Juncea group, regardless of its adult internal infection score.

### Blackleg Resistance Groups

Group A – Rlm1 and sylvestris resistance  
 Group B – Rlm4  
 Group C\* – Rlm2, Rlm3, Rlm9, none  
 Group D\* – Unknown (Hyola50) seedling resistance  
 Group E\* – Unknown (Thumper) seedling resistance  
 Group F\* – Unknown (Mustang) seedling resistance  
 Group G – Juncea resistance

\*Notes

- Group C cultivars have any combination of Rlm2, 3, 9 or no R genes.

**When releasing the resistance groups, only cultivars with MR rating or above will be given a resistance group.**