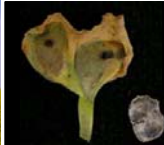


Issues in deploying seedling and adult plant resistance


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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

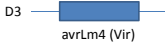
- 12 differential isolates
- Screen them against cultivars and determine the presence and absence of resistance genes

	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)

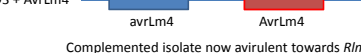
D5



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
Tornado	R	R	I	I	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
Summit	S	I	S	S	R	R	R	R	R	R	R	R	R	S	I	S	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
Sapphire	S	S	S	R	R	I	S	S	S	R	R	R	S	I	S	R	R	R	R	R
Skipton	S	S	S	S	S	S	S	S	S	S	I	S	S	I	R	R	R	R	I	I
BLN3347	S	S	S	S	S	S	S	S	S	I	S	S	S	S	I	R	R	R	R	R
Karoo	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	I	S
Emblem	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	I	S	S	R
Wesroona	S	S	S	S	I	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	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.