Classifying cultivars into different resistance groups

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Staying Ahead of BlackLeg (SABL) monitoring & managing host & pathogen

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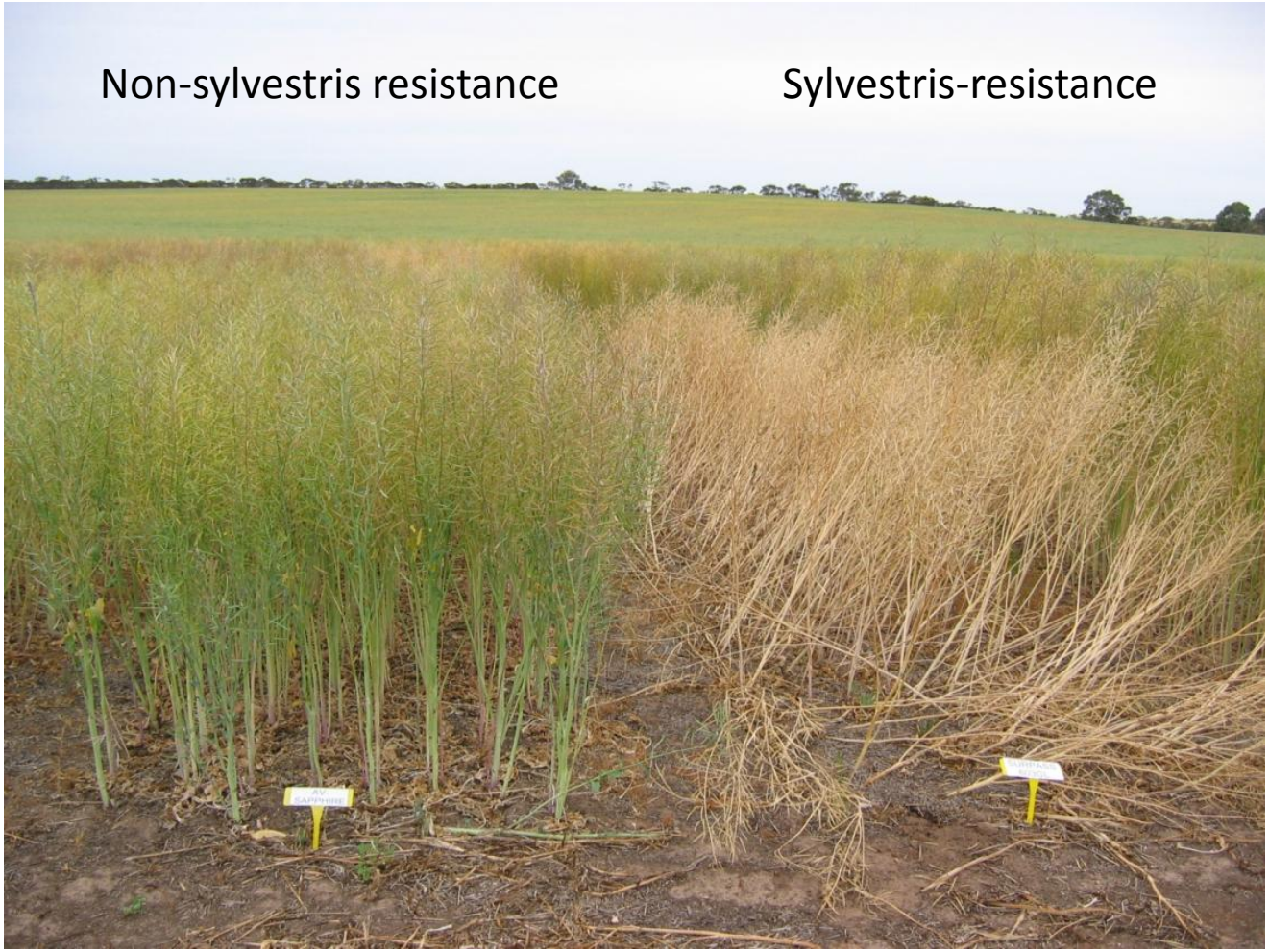
Differentials and Staying Ahead of BlackLeg projects

- Characterising seedling and adult plant resistance
- Classifying cultivars into resistance groups
- Monitoring effectiveness of resistance in the field
- Release of rotation groups to industry

Can we control blackleg by rotating cultivars that contain different resistance genes?

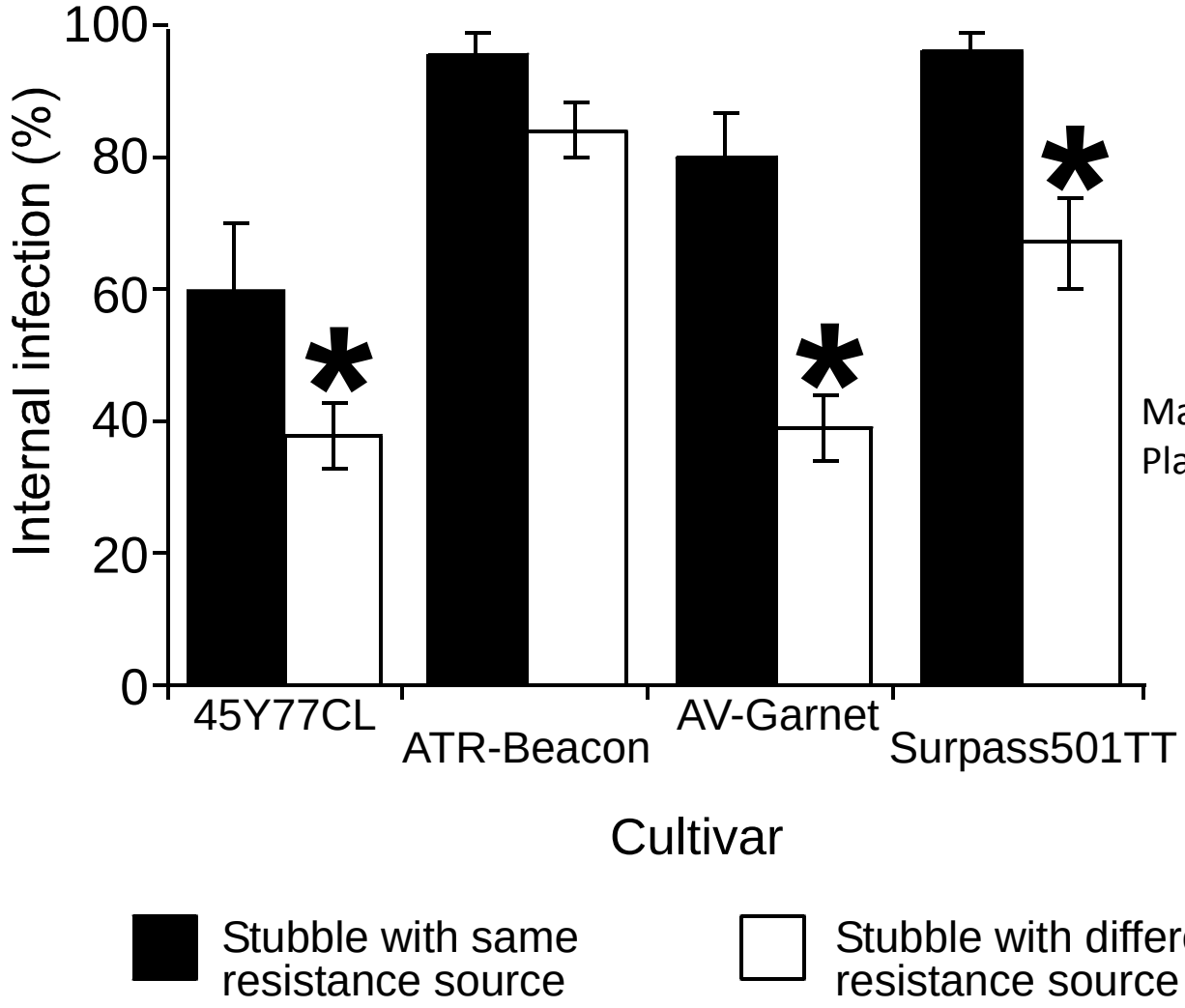


Rotation of cultivars with different resistance genes

2007

2008

Cultivars sown into stubble of a different cultivar have less blackleg compared to being sown in their own stubble



Marcroft *et al*. 2012 Plant Pathology

Cultivars exposed to stubble of a different cultivar have less blackleg disease compared to being exposed to their own stubble

AV-Garnet on AV-Garnet stubble

AV-Garnet on ATR-Cobbler stubble

Field data supported by glasshouse experiments (ascospore showers)

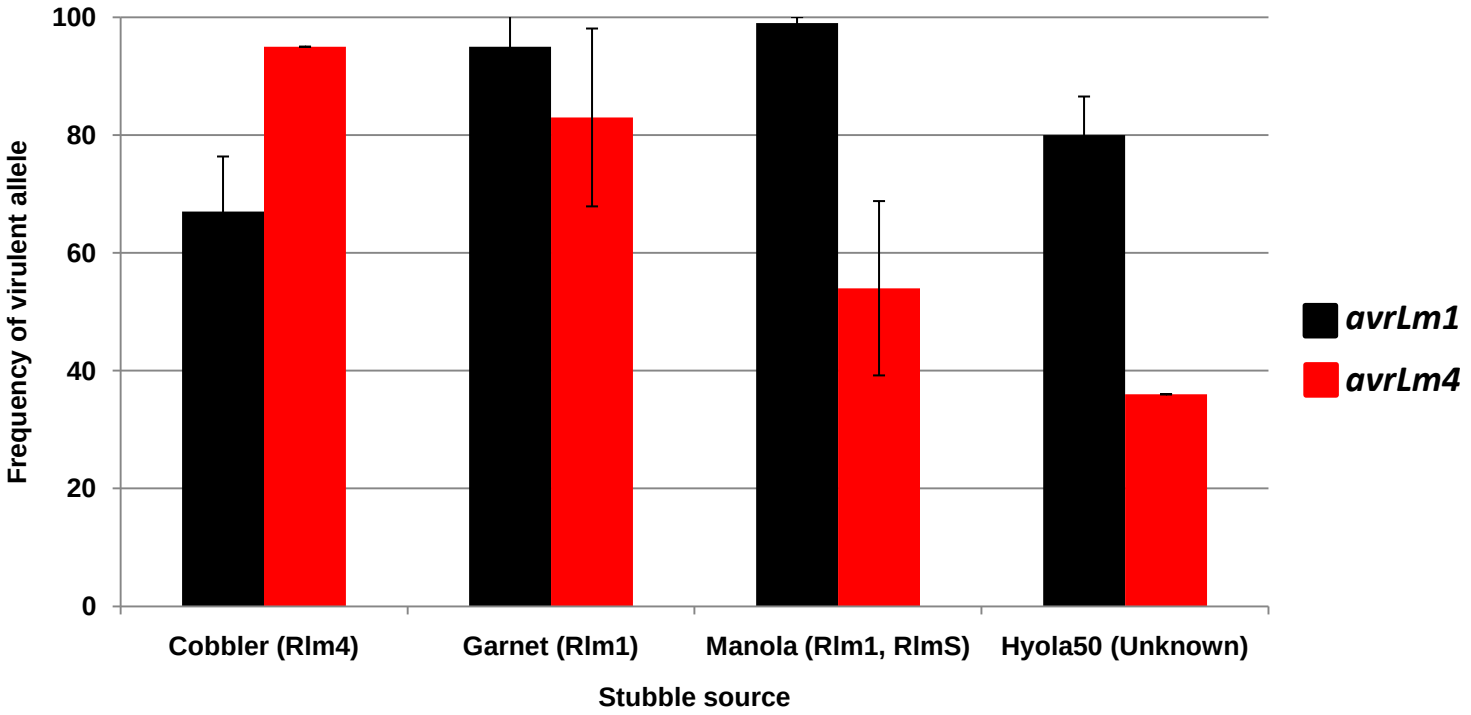
Hyola 50 on Hyola 50 stubble

Hyola 50 on ATR-Cobbler stubble

ATR-Cobbler on ATR-Cobbler stubble

ATR-Cobbler on Hyola 50 stubble

Frequency of virulent isolates is dependent on the presence of the resistance gene



The frequency of isolates virulent towards *Rlm4* is highest in populations collected from cultivars with *Rlm4* (Cobbler). Frequency lower in populations collected from cultivars lacking *Rlm4* (Manola)

Managing blackleg resistance

- Population is extremely dynamic
- Can’t beat blackleg but we are developing management strategies to stay ahead of it (SABL)
- Rotation of cultivars with different resistance genes minimises blackleg disease

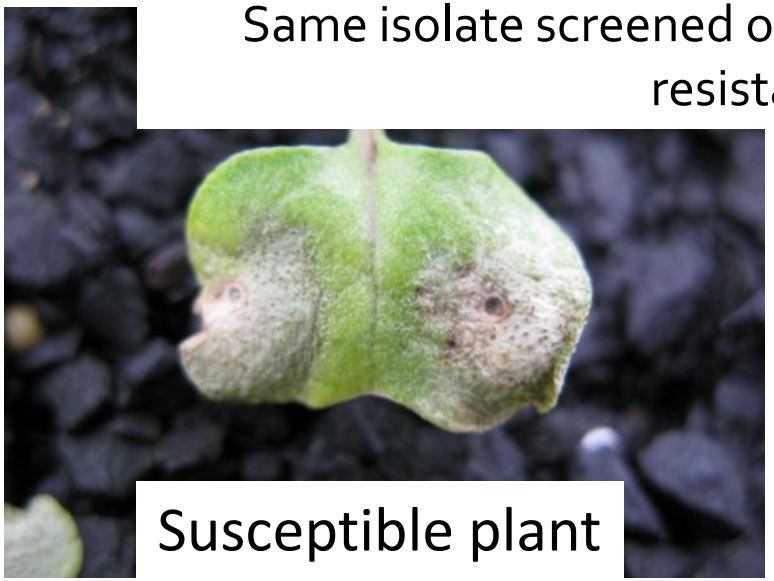
Staying Ahead of BlackLeg

- **Aim:** to increase durability of resistance and deliver a national disease management plan for farmers:
  - Monitor disease severity & fungal populations for changes in virulence
  - Determine if rotating cultivars with different resistance gene complements minimises disease
  - Deliver management plan to growers
- **Requirement:** knowledge of complement of seedling and adult resistance genes in Australian breeding lines and cultivars (UM34)
  - Develop a set of differential blackleg isolates
  - Use differential isolates to characterise seedling resistance
  - Screen for adult plant resistance
  - Deliver data to breeders

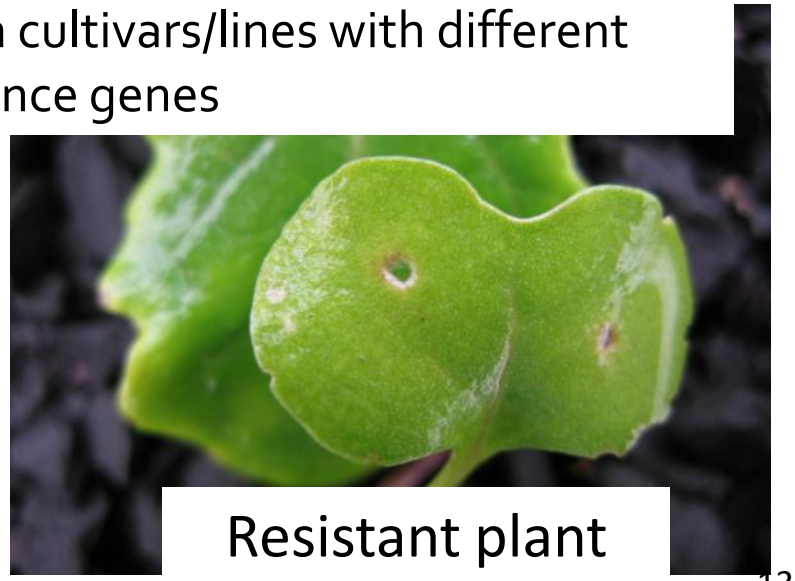
Seedling resistance

- Identify major gene resistance (*Rlm1-Rlm9*, *RlmS*)
- Similar system used routinely in France
- Identified 12 Australian isolates for characterising seedling resistance genes

Same isolate screened on cultivars/lines with different resistance genes



Susceptible plant (virulent isolate)



Resistant plant (avirulent isolate)

Differential isolates

Isolate	*Rlm1*	*Rlm2*	*Rlm3*	*Rlm4*	*Rlm5*	*Rlm6*	*Rlm7*	*Rlm8*	*Rlm9*	*RlmS*
D1	Vir	Avr	Vir	Vir	Avr	Avr	Vir	Vir	Avr	Avr
D2	Vir	Vir	Vir	Vir	Avr	Avr	Vir	Avr	Vir	Avr
D3	Vir	Vir	Vir	Vir	Avr	Vir	Vir	Vir	Vir	Vir
D4	Vir	Vir	Vir	Avr	Avr	Avr	Avr	Avr	Vir	Avr
D5	Avr	Avr	Vir	Avr	Vir	Vir	Avr	Vir	Vir	Avr
D6	Avr	Vir	Vir	Vir	Avr	Avr	Vir	Avr	Vir	Avr
D7	Avr	Vir	Avr	Vir	Avr	Avr	Vir	Avr	Vir	Avr
D8	Vir	Vir	Vir	Vir	Avr	Vir	Avr	nd	Vir	Vir
D9	Vir	Vir	Vir	Vir	Avr	Avr	Avr	nd	Vir	Vir
D10	Vir	Vir	Vir	Vir	Avr	Avr	Vir	Avr	Avr	Avr
D13	Vir	Vir	Vir	Avr	nd	Avr	Avr	nd	Vir	Vir
D14	Avr	Vir	Vir	Vir	Avr	Vir	Avr	nd	Vir	Avr

Resistance genotype is inferred by the phenotypic reaction of individual isolates inoculated on to cotyledons of cultivars

Genotyping seedling resistance

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D13	D14
ThunderTT	6.9	7.1	8.4	1.2	1.4	7.5	8.9	6.8	6.7	7.9	1.3	9.0

Lesions scored on size and necrosis (0-9 scale)
Average lesion scores <3.5 are avirulent, >5.0 are virulent

Compare lesion scores with avirulence genotypes

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D13	D14
ThunderTT	6.9	7.1	8.4	1.2	1.4	7.5	8.9	6.8	6.7	7.9	1.3	9.0

Isolate	*Avr Lm1*	*Avr Lm2*	*Avr Lm3*	*Avr Lm4*	*Avr Lm5*	*Avr Lm6*	*Avr Lm7*	*Avr Lm8*	*Avr Lm9*	*Avr LmS*
D1	Vir	Avr	Vir	Vir	Avr	Avr	Vir	Vir	Avr	Avr
D2	Vir	Vir	Vir	Vir	Avr	Avr	Vir	Avr	Vir	Avr
D3	Vir	Vir	Vir	Vir	Avr	Vir	Vir	Vir	Vir	Vir
D4	Vir	Vir	Vir	Avr	Avr	Avr	Avr	Avr	Vir	Avr
D5	Avr	Avr	Vir	Avr	Vir	Vir	Avr	Vir	Vir	Avr
D6	Avr	Vir	Vir	Vir	Avr	Avr	Vir	Avr	Vir	Avr
D7	Avr	Vir	Avr	Vir	Avr	Avr	Vir	Avr	Vir	Avr
D8	Vir	Vir	Vir	Vir	Avr	Vir	Avr	nd	Vir	Vir
D9	Vir	Vir	Vir	Vir	Avr	Avr	Avr	nd	Vir	Vir
D10	Vir	Vir	Vir	Vir	Avr	Avr	Vir	Avr	Avr	Avr
D13	Vir	Vir	Vir	Avr	nd	Avr	Avr	nd	Vir	Vir
D14	Avr	Vir	Vir	Vir	Avr	Vir	Avr	nd	Vir	Avr

Cultivar = *Rlm4* resistance

Seedling resistance genes

Cultivar	Resistance genes
AV-Garnet	*Rlm1, Rlm9*
ATR-Cobbler	*Rlm4, Rlm9*
CB-Telfer	*Rlm4*
Surpass501TT	*Rlm1, RlmS*
45Y77	*Rlm1, RlmS*
Hyola50	Unknown/New
XceedOasisCL	Unknown/New

Adult plant resistance

- Expressed in the adult plant stage
- Contribution of multiple minor genes
- Unlike seedling resistance genes, we can not identify the specific genes
- We have developed a system for comparing cultivars and identifying overall differences in adult plant resistance

Screening for adult plant resistance using ascospore showers (tub screen)

AV-Garnet on AV-Garnet stubble

AV-Garnet on ATR-Cobbler stubble

Differences in adult plant resistance

Cultivar	Seedling R gene	Stubble source					
		AVGARNET (Rlm1, Rlm9)	MONOLA76 TT (Rlm1, RlmS)	ATRCOBBLER (Rlm4, Rlm9)	CBJARDEE HT (Rlm2)	HYOLA50 (Unknown)	OASISCL (Juncea)
AVGARNET	Rlm1, Rlm9	94	73	80	34	71	80
ATRCOBBLER	Rlm4, Rlm9	89	100	80	95	66	59
SURPASS501TT	Rlm1, RlmS	60	100	79	100	78	59
XCEEDOASISCL	Juncea	18	0	4	11	78	84
CB Telfer	Rlm4	54	85	90	75	61	71
HYOLA50	Unknown	11	0	50	35	95	23
GTMUSTANG	Unknown	28	21	35	41	6	41

Rules for classifying cultivars into resistance groups

1. Cultivars with same seedling resistance are grouped together
2. If a cultivar also shows >75% internal infection on a stubble source from another rotation group then it will also be placed in that group
3. If the cultivar is in all four *B. napus* groups tested so far (A, B, C and D), it will have no rotational benefit.
4. If the cultivar has an unknown seedling resistance, but behaves differently to Hyola50 (Group D unknown) it is assigned to group E or F.
5. *B. juncea* cultivars will be placed into the Juncea group G.

The rotation groups

Finalised R groups

Group A –Rlm1; or Rlm1 and RlmS

Group B –Rlm4

Group C* – Rlm2, Rlm3, Rlm9,

Group D* – Unknown seedling resistance

Group E* – Unknown seedling resistance

Group F* – Unknown seedling resistance

Group G – Juncea resistance

All groups also include differences in adult plant resistance

No rotational benefit – No rotational differences detected to date

Not tested – No seedling data available to date

*Notes

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

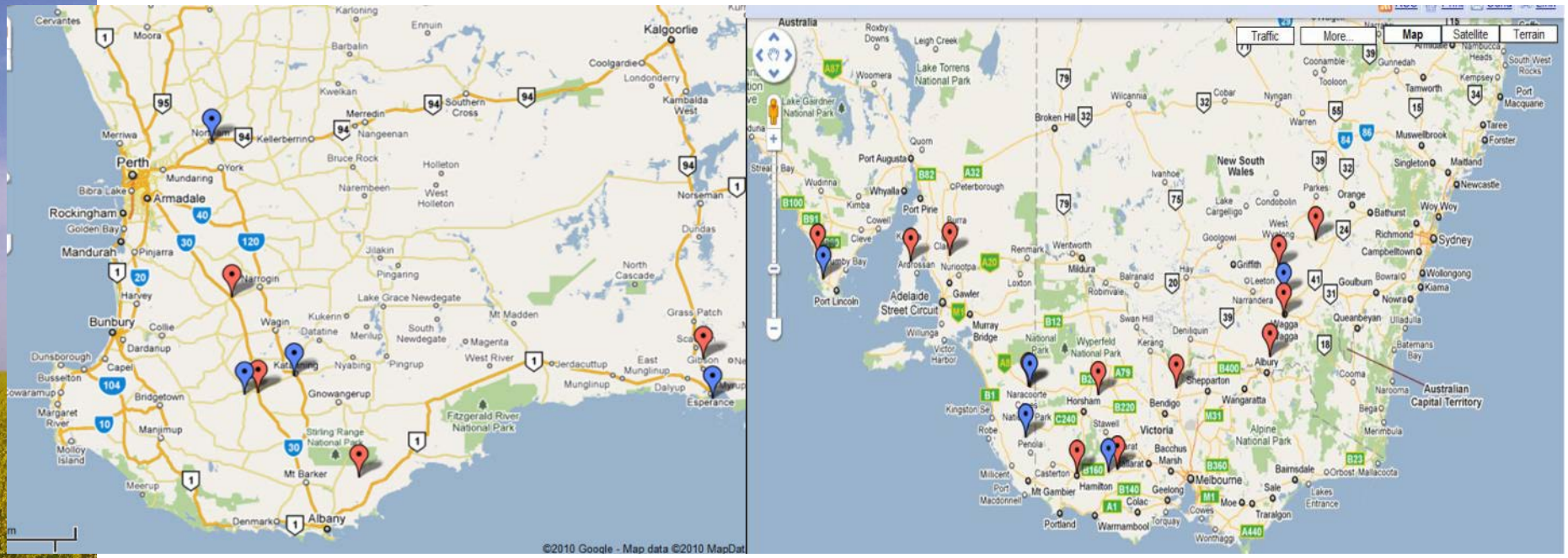
Group D cultivars behave similar to Hyola50.

Group E** cultivars behave differently to Hyola50 – PacSeeds germplasm

Group F** cultivars behave differently to Hyola50 – NuSeed germplasm

Blackleg Resistance Group monitoring – Staying Ahead of BlackLeg

16 GRDC & 4 Pacific Seeds funded sites



2010/2011 – 5 resistance groups represented (6 different cultivars sown)
2012 – 7 resistance groups to be represented

Cultivars destructively sampled and assessed for internal infection

Cultivars grown in triplicate plots

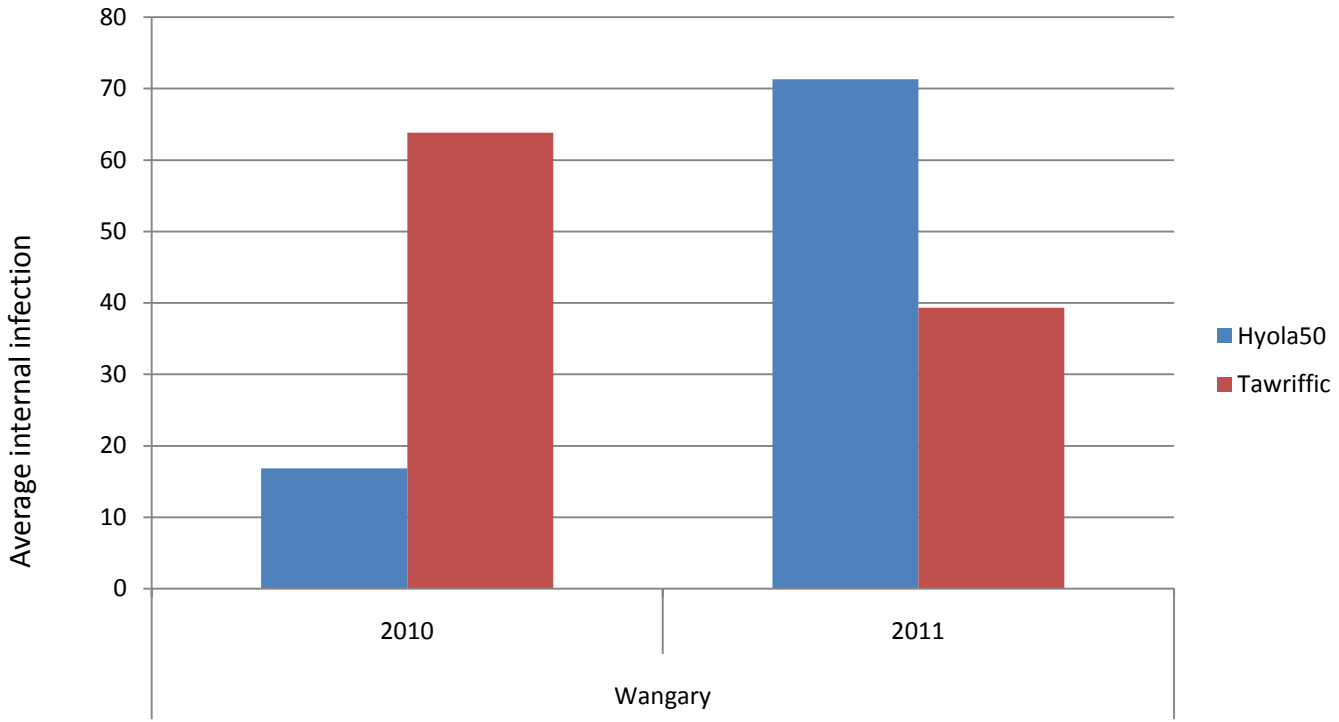
20 plants assessed per plot
(60 plants in total)

Monitoring Resistance groups in the field

Row Labels	R genes	Arth	BT	Clare	Minyip	Mt Hope	RivT	Wangary
AVGARNET	1,9	26	45	22	21	21	14	25
SURPASS501TT	S	50	53	65	68	22	29	55
CBTELFER	4	25	22	43	22	60	15	34
ATRSTINGRAY	3, 9	3	11	4	3	10	2	6
JUNCEATT	Juncea	0	8	3	1	6	0	8
HYOLA50	?	8	0	4	0	16	2	71
TAWRIFFICTT	?	23	40	37	43	37	22	39
Site average		**19**	**26**	**26**	**22**	**24**	**12**	**34**

Average internal infection determined from 60 plants

Disease severity in Hyola50 and Tawriffic at Wangary in 2010 and 2011



- Hyola50 is grown widely on the lower EP (Wangary).
- In the adjacent NVT trial, Hyola50 + fungicide a few plants died
- A warning to farmers on the lower EP has been released

Managing your risk of blackleg in canola cultivar HYOLA® 50 on the Eyre Peninsula

In 2011, in a blackleg monitoring trial site on the lower Eyre Peninsula (SA), the canola **cultivar Hyola® 50 had much higher levels of blackleg** than expected. Specifically, blackleg severity in cv. Hyola®50 increased from **16% to 71% internal infection** at Wangary from 2010 to 2011 (Figure 1). This is the same region where *sylvestris* resistance was overcome in 2003.

Hyola®50 has been grown extensively on the lower Eyre Peninsula, therefore **Hyola® 50 may have even higher disease severity in 2012** if its resistance is overcome by the blackleg fungus. In other sites that were surveyed across South Australia, New South Wales, Western Australia and Victoria, the level of disease in Hyola®50 is still low (less than 25% internal infection), (refer Table 1).

Figure 1. Blackleg severity in cv. Hyola® 50 at Wangary

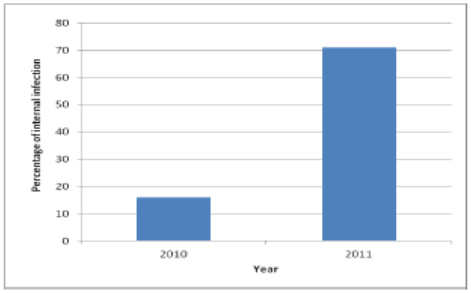


Figure 2. Dead Hyola® 50 plant at Wangary in the 2011 blackleg monitoring site.

Table 1. Blackleg severity of Hyola® 50 at monitoring sites in eastern Australia during 2011. Typically yield loss will occur in plants with more than 30% internal infection, with severe yield loss occurring in plants with more than 50% internal infection.

NSW monitoring sites	Percentage internal infection	SA monitoring sites	Percentage internal infection	VIC monitoring sites	Percentage internal infection
Beckom	1	Arthurton	8	Diggora	3
Gerogery	1	Bordertown	0	Horsham	2
Grenfell	1	Clare	4	Minyip	0
Temora	0	Riverton	2	Streatham	0
Wagga Wagga	6	Mt Hope –Eyre Pen.	16		
		Wangary –Eyre Pen.	71	WA monitoring site York	23

Acknowledgements

Funding for surveys, trials and associated research is provided by the Grains Research and Development Corporation (GRDC). The Lower Eyre Agricultural Development Association (LEADA) for providing the Wangary trial site.

Recommendations for growers on the lower Eyre Peninsula

Consult the 'Blackleg Risk Assessor' (www.grdc.com.au) for advice on all blackleg control practices.

1. *Choose a canola cultivar different to Hyola®50 with high levels of blackleg resistance for the Eyre Peninsula (use only the current year's ratings), blackleg ratings are available at www.grdc.com.au.*
2. *Separate this year's canola crop from last year's canola stubble by a minimum of 500m.*
3. *Do not sow Hyola®50 within 500 m of its own stubble from a 2011 or 2010 crop (older stubble does not release enough spores to warrant concern).*
4. *Ensure that canola seed has been treated with fluquinconazole or fertiliser amended with flutriafol as an insurance against blackleg.*
5. *Monitor blackleg severity within the 2012 crop to assist with decision making in 2013.*

Selecting an alternative cultivar to Hyola®50.

ALL Cultivars listed below have tested as more susceptible to the EP Hyola®50 stubble

HYOLA® 751TT	Pacific seeds	XCEED™ OASIS CL	Viterra
HYOLA® 444TT	Pacific seeds	ATR GEM	Nuseed
HYOLA® 433	Pacific seeds		

THESE ARE THE ONLY CULTIVARS THAT HAVE HIGHER BLACKLEG RATINGS BUT MAY BE MORE SUSCEPTIBLE TO HYOLA®50 STUBBLE ON LOWER EP IN SOUTH AUSTRALIA.

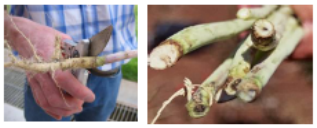
How does blackleg overcome resistance?

Unlike cereal rusts in Australia where all individual fungal spores are genetically identical, blackleg is a sexually reproducing fungal pathogen. The spores that attack crops each year are a result of sexual recombination occurring on canola stubble over the summer and autumn.

Blackleg spores are genetically different to each other, so there will always be individual spores that can attack each resistance gene in canola. If a blackleg spore is able to attack, it will colonise the canola plant and then reproduce on the stubble, releasing more spores in subsequent years capable of overcoming that resistance gene or genes. When a particular canola cultivar with specific resistance genes is sown, selection for blackleg spores this cultivar occurs . The number of virulent spores initially is low so that it takes a number of years before they increase to a frequency that can overcome resistance and cause yield loss. This situation can be compared to the overuse of one herbicide group and the subsequent selection of herbicide-resistant ryegrass plants.

Different cultivars can differ in stability of their resistance; some cultivars lose resistance quickly while other cultivars have not lost resistance to date. In cultivars with polygenic resistance the loss of resistance is normally gradual – termed an 'erosion of resistance'. Where resistance has been eroded a 'Reduced Resistance' warning has been placed on the blackleg rating of the particular cultivar.

Figure 3. Seedling death (highlighted blackleg pinched hypocotyl). If diseased roots are observed the likely cause is 'Damping-off fungi'.



Figures 4 & 5. Plants with various levels of internal infection. Cut 50 random plants at the crown after windrowing. An average of 30% or more internal infection indicates yield loss.

Figure 6. Check for stem cankers at plant maturity.

2012 Monitoring site details

- Will be sown along side NVT trials in (hopefully) 8 sites per state (NSW, Vic, SA, WA).
- No fungicide use.
- Will contain one cultivar from each R group.
- Only R group names will be displayed to growers- no cultivar names will be used.
- Seed companies will be encouraged to have monitoring sites in their yield and blackleg trial sites and to contribute data.
- Seed companies will not know which cultivar represents the R group.

2012 release of data from resistance group monitoring

- Results of monitoring sites will be displayed on NVT-Online website by Christmas each year.
- Growers will have monitoring site data and their own paddock data.
- They will only have R group data – not cultivar data.
- If they identify a problem the grower / advisor can then consult the Blackleg Ratings (with R groups) to determine which cultivar is best for their situation.
- **Warnings will not be released**

Suggested release to growers

- Resistance groups for each cultivar named on the blackleg ratings.

Variety	Blackleg Resistance Rating Bare Seed		Site number	Blackleg Resistance Rating +Fluquinconazole	Site number		Blackleg Resistance Groups	Type
CONVENTIONAL VARIETIES								
Hyola® 50	R		36	R-MR	8	P	D	
Hyola® 433	R-MR		17	R-MR	8	P	D	
SARDI 515M	R-MR		20	MR-MS	8	P	G	INDUSTRIAL MUSTARD
Victory®V3001	MR	R	20	R	8	P	A	HIGH STABILITY OIL
AV-Garnet	MR	R	38	MR	8	P	A,B,C,G	
CB™ Agamax	MR-MS	P	8				No rotational benefit	

Supporting information

BLACKLEG RESISTANCE GROUPS

All cultivars have been placed into groups based on their resistance complement (see Blackleg Ratings). Blackleg will most likely overcome resistance if cultivars with the same resistance complement are sown in close proximity for three years or more. By rotating resistance groups growers can avoid resistance breakdown and reduce disease severity.

HOW TO USE BLACKLEG RESISTANCE GROUPS

Step 1. Identify your risk of blackleg

- Use the Blackleg Risk Assessor (next page) to identify if you are in a high risk region and monitor your crops to determine is blackleg is severe.
- If blackleg is not severe in your crop continue with current management techniques as listed in the Blackleg Risk Assessor.
- If you have high blackleg severity and have used the same cultivar for 3 years or more move to steps 2 and 3.

Step 2. Identify the Resistance Group of your current cultivar from the Blackleg Ratings.

Step 3. Select cultivar from different Resistance Group. If your current cultivar belongs to multiple groups do not choose a cultivar from any of these groups.

Blackleg Resistance Group monitoring

Representative cultivars from all Blackleg Resistance Groups are sown in trial sites in all canola producing regions across Australia and monitored for blackleg severity. These data provide regional information on effectiveness of each blackleg Resistance Group. These monitoring data are available on the NVT-online website.

One document with Ratings, Risk Assessor and R Groups

- Blackleg ratings to become one A3 – 4 page publication which has the Blackleg Ratings, Resistance Group explanation, and Blackleg Risk Assessor.
- *– need new document for spring 2012, then updated in March 2013 with new ratings and R groups.*

Release of rotation groups to industry

- Blackleg rotation groups have already been provided to seed companies
- Groups will be provided to growers in spring 2012 after consultation with seed companies, farming system groups and advisors.
- 2012 we will
  - Group all new cultivars/advanced breeding lines for seedling and adult plant resistance
  - Monitor disease severity in cultivars representing all resistance groups across Australia