Rotation of blackleg resistance sources to minimise disease severity in canola (*Brassica napus*)

Stephen J. Marcroft[1], Angela P. Van de Wouw[2], Vicki Elliott[1], Philip A. Salisbury[3], Trent D. Potter[4] and Barbara J. Howlett[2]

[1]Marcroft Grains Pathology P/L, Grains Innovation Park, Horsham, VIC 3400, Australia;
[2]School of Botany, The University of Melbourne, VIC 3010, Australia;
[3]Melbourne School of Land and Environment, The University of Melbourne, VIC 3010, Australia;
[4] SARDI Innovative Food and Plants, PO Box 618, Naracoorte, SA 5271, Australia
Email: steve@grainspathology.com.au

ABSTRACT

The blackleg fungus *Leptosphaeria maculans* has a high propensity to overcome resistance in *Brassica napus* (canola) cultivars. In 2003 such a breakdown was observed in regions including Eyre Peninsula and Bordertown, South Australia where cultivars with resistance derived from *B. rapa* ssp. *sylvestris* (sylvestris-derived resistance) had been grown extensively (Sprague *et al*. 2006). This paper describes field and pot experiments that show durability of resistance genes can be prolonged by rotation of different sources of blackleg resistance. When canola cultivars with different resistance sources were sown sequentially for three years on top of stubble from the previous year's crop at two field sites, disease severity decreased. In contrast, they had high disease severity when grown on stubble with the same resistance. Also small plot trials in regions were monitored at sites where sylvestris-derived resistance had been overcome in 2003, after which time cultivars with this resistance were no longer sown commercially. Disease severity of plants with sylvestris-derived resistance in the plot trials decreased from 2003 to 2008, reflecting a decrease in selection pressure for virulence in the fungal populations towards sylvestris-derived resistance.

INTRODUCTION

Blackleg (phoma stem canker) caused by *Leptosphaeria maculans* Desm. (Ces & de Not) is the major disease of *Brassica napus* L. (canola or oilseed rape) worldwide causing significant crop losses in Europe, North America and Australia (Fitt *et al*., 2006, Howlett 2004). *Leptosphaeria maculans* is a very successful pathogen as it sexually reproduces on canola stubble producing vast quantities of genetically diverse wind-borne ascospores that are dispersed considerable distances (Howlett 2004). Nearly all ascospores originate from stubble from the previous year's crop with older stubble accounting for few spores (Marcroft *et al*., 2003). Separating crops from stubble from the previous year by at least 500m minimises disease (Marcroft *et al*., 2004), and this strategy is now widely deployed by Australian growers.

Two types of blackleg resistance are deployed in canola. These are major gene resistance, which is usually effective at the point of entry into the plant (leaf or cotyledon) and polygenic resistance (quantitative), which allows initial infection, but slows growth of the pathogen within the plant (Delourme *et al*., 2006). As yet no resistance genes have been characterised in any Brassica species, and for most Australian cultivars little is known about the number or nature of resistance genes present. Pathogenicity tests with a defined set of *L. maculans* isolates have shown that some Australian cultivars are heterogeneous with different proportions of resistance genes (Balesdent *et al*. 2002; 2006). The effectiveness of blackleg resistance in these cultivars has decayed slowly over the years resulting in further yield losses (Delourme *et al*., 2006, Cowling 2008, Elliott PhD thesis 2010). In contrast, a rapid decline in effectiveness of resistance derived from *B. rapa* ssp. *sylvestris* (sylvestris-derived resistance) caused severe yield losses in regions where this source of resistance was widely sown at the Eyre Peninsula South Australia, from 2000 to 2003. Initially these cultivars had no lesions or cankering but in 2003, severe yield losses were recorded (Sprague *et al*. 2006). This decline in effectiveness of resistance was due to an increase in frequency of virulent isolates in fungal populations under selection pressure from this resistance source. A less dramatic breakdown in resistance occurred in France where over a 10 year period the resistance gene, *Rlm1* became ineffective (Rouxel *et al*., 2003, Sprague *et al*., 2006).

Few sylvestris-derived cultivars have been sown commercially in Australia since 2003, although they have been sown in disease nurseries and in Australian National Variety Testing (NVT) yield evaluation sites. At these NVT sites, canola cultivars (cv. ATR-Beacon with 'polygenic' resistance, and cv. Surpass 501TT with sylvestris-derived resistance) have been monitored each year (2004 to 2008) for blackleg severity. Analysis of these data provides an opportunity to examine whether cultivars with sylvestris-derived resistance can regain effective resistance against blackleg.

In this manuscript we describe field and pot experiments aimed at determining whether deployment of canola cultivars with different combinations of blackleg resistance genes can increase the durability of resistance sources in canola cultivars and thus reduce yield loss. We show that in isolates cultured from cultivars with different resistance sources the frequency of virulent isolates changes significantly and that these isolates differ in their ability to cause disease on corresponding resistance genes.

MATERIALS AND METHODS

Brassica cultivars

Brassica cultivars used in this study were selected for their different sources of blackleg resistance and are termed either "sylvestris" indicating that their blackleg resistance is derived from *B. sylvestris* or termed "non-sylvestris" where their resistance was derived from sources other than sylvestris. (Table1).

Table 1. Resistance genes in cultivars used in this study.

Cultivar	Resistance genes	Resistance category	References
Surpass400	*Rlm1, RlmS*	Sylvestris-derived	Van de Wouw *et al.* 2009
Surpass501TT	*Rlm1, RlmS*	Sylvestris-derived	Marcroft and Van de Wouw pers. comm
Surpass603CL	*Rlm1, RlmS*	Sylvestris-derived	Marcroft and Van de Wouw pers. comm
45Y77CL	*RlmS*	Sylvestris-derived	Marcroft and Van de Wouw pers. comm
ATR-Beacon	*Rlm3, Rlm4*	Non-sylvestris	Marcroft and Van de Wouw pers. comm
AV-Garnet	*Rlm1, Rlm9*	Non-sylvestris	Marcroft and Van de Wouw pers. comm
Columbus	*Rlm1, Rlm3*	Non-sylvestris	Rouxel *et al.* 2003
Dune	Unknown	Juncea	

Disease assessment

Plant mortality was assessed by either one of two methods either by; counting the number of dead plants that had visible stem canker, or by counting the number of seedlings to emerge and then counting the number of plants alive at plant maturity. Internal infection was determined by pulling plants from the soil and then severed at the crown with a pair of secateurs to score internal infection severity. Internal infection was determined by visually inspecting the cross section of the crown and then estimating the percent of the crown discoloured by *L. maculans* infection. The plants were scored as 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% internal infection (Marcroft *et al.* 2004).

Field survey of blackleg severity in cultivars with polygenic or sylvestris-derived resistance

In 2004, the level of internal infection caused b y *L. maculans* was assessed in cultivars with sylvestris-derived resistance (cv. Surpass 501TT) grown in National Variety Trial (NVT) yield sites (cultivars sown in small plots, approx. 1 m by 10 m) in southern Australia (data not shown). Seven sites were identified with high levels of blackleg disease in cultivars with sylvestris-resistance. From 2004-2009, both cultivars Surpass501TT (sylvestris) and ATR-Beacon (non-sylvestris) were sown at the seven NVT sites and monitored for blackleg severity. The sites differed between paddocks each year but were always sown in the same region (usually the same farm).

Sixty plants from plots of both cvs.Surpass501TT and ATR-Beacon were assessed for blackleg. The plants were taken from the same row in each plot and were sequentially removed. All three replicates were sampled.

Rotation of different blackleg resistance sources

The theory to rotate resistance sources was preliminary tested by placing plants of sylvestris resistance in pots on sylvestris and then non-sylvestris stubble to measure the effect of stubble type on disease severity. In 2003, a commercial crop at Bordertown, South Australia of Surpass603CL (sylvestris resistance) with severe blackleg symptoms was identified. In 2004, pots (20 cm diameter) were sown in a shadehouse with cultivars containing sylvestris resistance. When the plants were at the one leaf growth stage, five pots (20 plants) were placed into the Surpass603CL (sylvestris) stubble, plant mortality caused by blackleg in these pots was 100%. Also in 2004, a one hectare plot of ATR-Beacon (non-sylvestris) was sown into the same cv.Surpass603CL stubble paddock at Bordertown.

The experiment was repeated in 2005, with the pots being placed on the ATR-Beacon (non-sylvestris) stubble (site still had some Surpass603CL stubble from the 2003 commercial crop). The sylvestris plant mortality in 2005 fell to 39%. This preliminary result of reduced disease severity led to three sites being established to determine blackleg severity when four different sources of resistance were rotated.

Rotation of resistance experiments were established at two sites, in 2007 (Nurcong and Kalkee, Victoria) and at one site in 2008 (Horsham, Victoria). Each of the three sites were sown to four 0.5ha plots. Each plot was sown to either 45Y77CL (sylvestris resistance), and each of the non-sylvestris resistant cultivars ATR-Beacon, AV-Garnet or Dune (*B. juncea*). The sites were isolated by at least 2km from any other canola stubble and each 0.5ha plot was isolated by at least 200m from each other.

In both 2008 (at Nurcong and Kalkee) and 2009 (Horsham) rows of each cultivar (45Y77CL, ATR-Beacon, AV-Garnet and Dune) were sown into each stubble type. In addition, plants containing all the sources of resistance were propagated in pots and placed onto the stubble at Nurcong. Plants were assessed for mortality by counting all plants to emerge and then the number of surviving plants at crop maturity. The sites were assessed for other diseases and pests to ensure that plant mortality was due to blackleg.

RESULTS

Field survey (2004-2009) of blackleg severity in cultivars with sylvestris resistance

The percentage of internal infection in cvs.Surpass501TT (sylvestris resistance) and ATR-Beacon (non-sylvestris) was determined in the same 7 regions for six years. At these sites, cultivars with sylvestris-derived resistance were grown in large acreage up until 2004. Between 2004 and 2007, very few cultivars with sylvestris-derived resistance were sown. From 2008 onwards, the acreage of cultivars with sylvestris-derived resistance began to increase again (see Figure 1).

To compare blackleg severity between the sylvestris and the non-sylvestris cultivars, the internal infection score of the non- sylvestris cultivar was subtracted from the internal infection score of the sylvestris cultivar (Table 2). For instance, in 2004 at Arthurton, the cultivar with sylvestris-derived resistance had 78% internal infection and the cultivars with polygenic resistance had 25%. The score for the Arthurton site was therefore 53 (78 minus 25) (Table 2). In 2004 most of the sites had a disease severity score of between 30 and 50. By 2005 the difference in scores at all sites was much lower. By 2006 five sites had less disease in the cultivars with sylvestris-derived resistance than in those with non-sylvestris resistance. The levels of disease in 2007 and 2008 were similar in both sources of resistance. However, in 2009 the disease level in the sylvestris resistance cultivar across all sites was significantly higher compared to the non-sylvestris cultivar. This increase in disease coincides with large scale plantings of new cultivars containing the RlmS sylvestris resistance in 2008.

Table 2. Internal infection (%) comparison between cultivars with sylvestris-derived resistance and cultivars with non-sylvestris resistance in high rainfall sites in South Australia.

	Year[A]					
NVT Sites	2004	2005	2006	2007	2008	2009
Arthurton	53	8*	-22*	11*	-2	50*
Frances	2	24	-16*	-29	2*	n.a.
Keith	51	46	-11*	6	n.a.	n.a.
Minlaton	50	3*	-6	6	0	29*
Mt Hope	34	2*	1	10	4	38*
Riverton	42	17*	-12*	1	-3	42*
Yeelanna	51	30	29	11*	-3	14

[A] Minus values indicate when a cultivar with non-sylvestris resistance has more disease than a cultivar with sylvestris-derived resistance.
* Indicates a significant (P=0.05) change in internal infection comparison either from the previous year or the first year (2004).

When the data is averaged across all seven high rainfall sites a clear decrease in the level of disease within the cultivars with sylvestris-derived resistance is seen from 2004 to 2008 and then a increase in 2009 (Fig. 1). This correlates with the initial decrease and then recovery of acreage of sylvestris derived cultivars in the field.

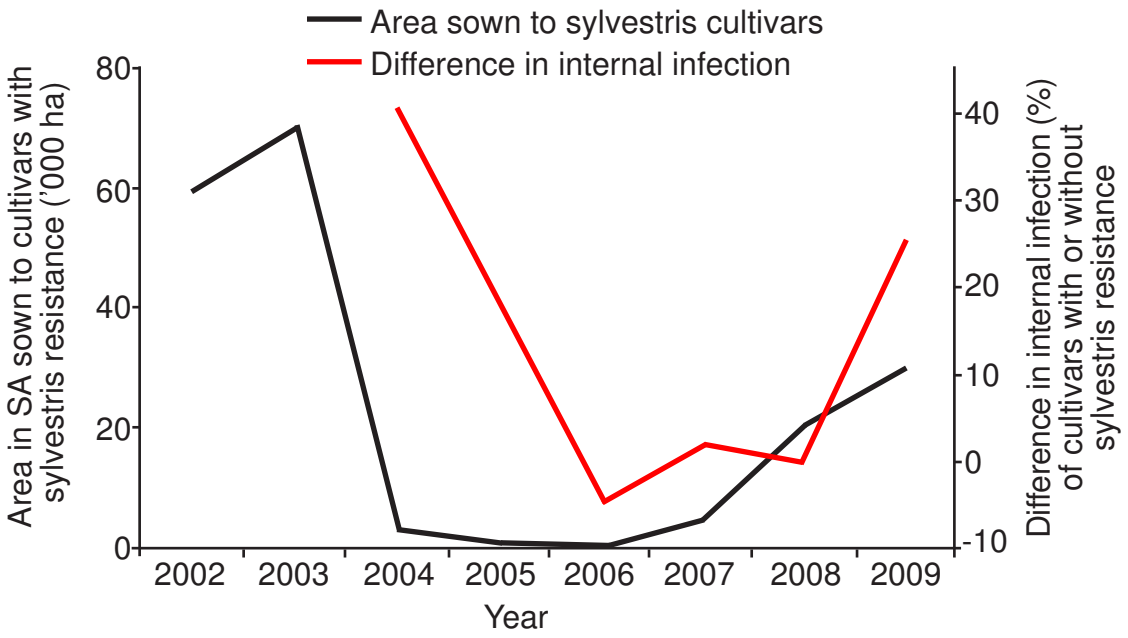

Fig. 1. Average difference in internal infection (%) comparison between cultivars with sylvestris-derived resistance and cultivars with non-sylvestris resistance. Area sown to cultivars containing sylvestris resistance in South Australia,

Internal infection (%) indicates the extra disease in the sylvestris compared to non-sylvestris cultivar.

Rotation of different blackleg resistance sources

This experiment found that blackleg severity was influenced by which stubble each cultivar was sown into. When sources of resistance: 45Y77CL (*RlmS*); AV-Garnet (*Rlm1*, *Rlm9*); ATR-Beacon (*Rlm3*, *Rlm4*) and Dune (resistance unknown) were sown into their own stubble, they had either the same or more disease than when they were sown into stubble from a different cultivar. In no cases did a cultivar that was sown into its own stubble have less disease (Fig. 2).

At the Nurcong site, five cultivars (45Y77CL, AV-Garnet, ATR-Beacon, Surpass501TT (*Rlm1*, *RlmS*) and Dune) were sown in pots and placed onto the four different stubble types. For all four *B. napus* cultivars, less internal infection was observed in cultivars that were sown in to stubble of a different resistance source compared to cultivars sown into the same resistance source (Fig. 2). These differences were statistically different for three out of the four cultivars. For the *B. juncea* cultivar, Dune, no significant difference was observed and the levels of internal infection were less than 5%.

At all three field sites, 45Y77CL, ATR-Beacon and AV-Garnet were sown into stubble of the same and other cultivars. The average plant survival of each cultivar when grown on either its own stubble or stubble of the other cultivars was determined for each site. For all cultivars, greater survival was observed when sown into stubble with a different resistance source. This change in survival was significantly different in more than 50% of the time. Never did we see a decrease in survival when a cultivar was grown on a different stubble source compared to when it is grown on its own stubble source.

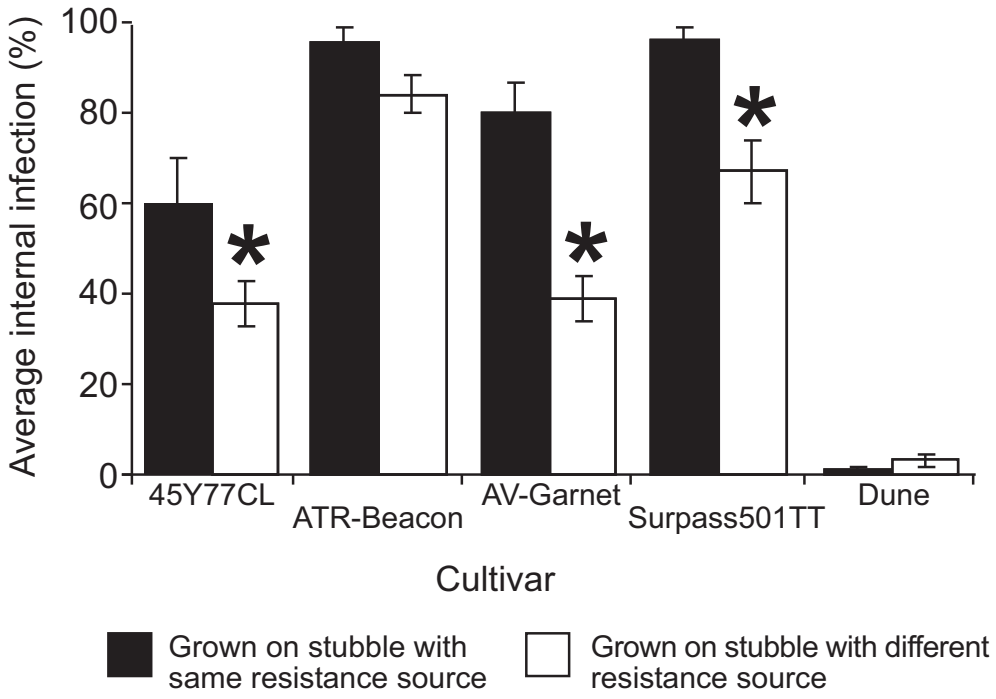

Fig. 2. Average internal infection of cultivars in pots placed on either stubble containing the same resistance source or stubble containing a different resistance source.

DISCUSSION

Some plant pathogen interactions show a 'boom and bust' disease cycle as reviewed recently by McDonald (2010). Such a cycle for *Leptosphaeria maculans* and *Brassica napus* was demonstrated by the breakdown of resistance in the Eyre Peninsula in 2003, and also the results of this study. Prior to 2000 all farmers on Eyre Peninsula sowed cultivars with 'polygenic' resistance, which resulted in some yield loss from blackleg. When cultivars with sylvestris

resistance were released their uptake was extremely high. Canola crops showed virtual immunity to blackleg and consequently yields increased. However, during 2003 yield losses were up to 90%. Polygenic cultivars that had previously had many blackleg symptoms showed significantly less cankering in field trials, reflecting the change in virulence in the populations. Cultivars with sylvestris-derived resistance were withdrawn from sale and farmers switched back to sowing cultivars with polygenic resistance. We have shown that by 2007 in field trials in this region, the sylvestris-derived cultivars had as low levels of disease severity as polygenic cultivars, reflecting that isolates with virulence towards sylvestris-derived resistance had significantly decreased in frequency.

Our data from plot trials and pot experiments at Horsham and Bordertown are also consistent with the 'boom and bust' nature of the disease. At Bordertown where sylvestris-derived resistance had broken down in 2003, such cultivars suffered high disease severity during 2004 and 2006. Unsurprisingly, cv Columbus, which like Surpass 400 has *Rlm1,* (Van de Wouw et al. 2009) showed much higher levels of disease in 2004 and 2006 at Bordertown compared to Horsham. By 2006 disease severity was low and similar at both sites. The congruency between plot trials and pot experiments means that pot experiments – placing pots of plants on stubble- are a very useful way of readily assessing the frequency of virulences towards particular resistance genes in a fungal population derived from stubble.

Recently Brun *et al*., (2010) have shown that quantitative resistance can contribute significantly to durable resistance. These authors showed resistance conferred by *Rlm6* in a canola background that included quantitative resistance was still effective after three years, but in isogenic lines with *Rlm6* that lacked quantitative resistance were severely diseased after only three years. Thus ensuring that cultivars retain quantitative resistance should be an important aim of breeding programs.

Our data provide guidelines for Australian farmers who sow canola. Firstly farmers should grow cultivars with different sources of resistance every two years to minimise blackleg severity. These cultivars should be sown by more than 500 m apart. To achieve this aim, growers need to be informed of the resistance genes in each commercial cultivar and to have access to a range of cultivars with different resistance sources.