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ORIGINAL PAPER

Incidence and severity of blackleg caused by *Leptosphaeria* spp. in juncea canola (*Brassica juncea* L.) in Australia

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Abstract Juncea canola (*Brassica juncea* L.) is being developed throughout the worlds canola growing countries as a drought tolerant, shatter resistant and highly blackleg resistant option to canola (*Brassica napus* L.). Juncea canola was grown commercially in Australia for the first time in 2007. This study determined the incidence and severity of blackleg infection in juncea canola prior to commercial release throughout south-eastern Australia in 2006 and 2007, and then again 5 years after commercialisation (2010–2013) to determine if blackleg severity had increased. Blackleg was found at all 127 sites surveyed throughout Victoria, New South Wales, South Australia and Western Australia. The severity of blackleg infection differed among sites and among the juncea canola cultivars and breeding lines suggesting that differences in resistance may be present. This is the first report that *L. maculans* isolates virulent on *B. juncea* are already widespread throughout the Australian canola growing regions and contradicts the widespread opinion that *B. juncea* is immune to blackleg. This also demonstrates that blackleg infection was already occurring in juncea canola prior to commercialisation of this crop in Australia and that disease management strategies similar to those used in canola cultivation will need to be implemented.

Keywords *Leptosphaeria maculans* · *Leptosphaeria biglobosa* · Blackleg · Juncea canola · Survey

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Introduction

Canola (*Brassica napus* L.) production ranks third behind wheat and barley in Australia. The area sown to canola has increased from 1.4 million ha in 2009 to 2.4 million ha in 2013 (http://www.australianoilseeds.com). High canola prices compared to cereals are driving the increase in cultivation with prices reaching 550–600 AUD/tonne. Low rainfall regions offer further growth to the Australian canola industry, however current canola cultivars are not reliable in the low rainfall zone.

Brassica juncea L. is known to have increased heat and drought tolerance compared to *B. napus* as well as other advantages such as early vigour and increased shatter tolerance (Oram et al. 2005). For these reasons *B. juncea* has been bred to meet canola quality oil and meal standards. The improved crop has been termed juncea canola, and was commercialised in Australia in 2007 (Burton et al. 2008). Prior to the commercialisation of juncea canola only small areas of *B. juncea* for condiment use were grown in Australia, however not typically in the regions where juncea canola is now being grown (Van de Wouw et al. 2008, 2009). Juncea canola has the potential to become a significant break crop for

low rainfall areas of Australia such as the Mallee and areas of northern New South Wales, parts of Queensland and the Geraldton area of Western Australia (Oram et al. 2005). In 2012/2013 up to 10,000 ha of juncea canola was sown in Australia (Burton pers. comm.).

In addition to the above mentioned agronomic traits research has shown *B. juncea* has a greater level of resistance to the fungal disease blackleg than *B. napus* (Kirk and Oram 1978, Marcroft et al. 2002). Blackleg disease is the most significant disease of canola (*Brassica napus* L.) in Australia (Murray and Brennan 2012) with the ability to cause widespread plant death, with yield losses as high as 80 % (Colton and Potter 1999). A species complex of two Dothideomycete species, *Leptosphaeria maculans* (Desm.) Ces. et de Not and *L. biglobosa*, is responsible for blackleg disease in Australia (Mendes-Piereira et al. 2003). The *L. maculans* species has been divided into two subclades, but only one subclade 'brassicae' is present in Australia (Mendes-Piereira et al. 2003). *Leptosphaeria biglobosa* has been separated into six subclades, with isolates from three of these subclades reported in Australia (Plummer et al. 1994; Van de Wouw et al. 2008; Vincenot et al. 2008). *Leptosphaeria* isolates cultured from *B. juncea* stubble collected in northern New South Wales were *L. biglobosa* 'canadensis', however isolates cultured from *B. juncea* stubble collected in Victoria were *L. maculans* (Van de Wouw et al. 2008).

Blackleg in canola is managed using cultural and chemical practices, such as maintaining a minimum 500 m buffer between the last season's residues and the current crop, fungicide seed dressings and foliar applications, and host plant resistance. In *B. napus* there are at least 18 qualitative resistance genes (*Rlm1, Rlm2, Rlm3, Rlm4, Rlm5, Rlm6, Rlm7, Rlm8, Rlm9, Rlm10, Rlm11, RlmS, LepR1, LepR2, LepR3, LepR4, BLMR1* and *BLMR2*) conferring resistance to *L. maculans* (Balesdent et al. 2013; Delourme et al. 2006; Eber et al. 2011; Long et al. 2011; Van de Wouw et al. 2009; Yu et al. 2005, 2008). *Rlm5* and *Rlm6* are reported to be present in *B. juncea* (Chèvre et al. 1997; Barret et al. 1998; Balesdent et al. 2002, 2005), as well as a recessive resistance gene (*LMJR2*) (Saal et al. 2004). The presence of these resistance genes in the commercialised juncea canola cultivars is unknown.

In canola qualitative resistance is effective when first released, however, it has not proven effective in the long-term, being quickly overcome by the pathogen (Sprague et al. 2006). Similar situations have occurred in France (Rouxel et al. 2003; Brun et al. 2000). The large amount of sexual crossing and the ability of sexual spores to travel long distances gives this pathogen the ability to overcome resistance (Howlett 2004; Sprague et al. 2006). A new qualitative resistance for commercial *B. napus* cultivars was introgressed from *B. rapa* ssp. *sylvestris* and released in Australia in 2000. This resistance displayed a hypersensitive response when inoculated with *L. maculans* isolates (Li and Sivasithamparam 2003), but became ineffective only 3 years after the first cultivar was released (Sprague et al. 2006), which is similar to what happened in France where a single dominant resistance gene, *Rlm1*, became ineffective in only 3 years when it was exposed to its own inoculum (Brun et al. 2000).

As juncea canola is a new crop in Australia it is unknown how severe blackleg infection will be, if blackleg infection will increase over time as more juncea canola is grown and if current juncea canola resistance will be overcome as has occurred in canola. It is also uncertain if the same management practices will be required for juncea canola cultivation as for canola.

This study determined the level of blackleg disease present in juncea canola cultivars at their commercialisation to set a benchmark for comparison by future studies. At this early stage of juncea canola cultivation it is also important to know whether virulent *Leptosphaeria* populations are already present within the growing regions and if differences in resistance are evident within the juncea canola cultivars.

Materials and methods

Regional blackleg survey sites

In 2006, 2007, 2010, 2011, 2012 and 2013 a number of sites were surveyed throughout Victoria, South Australia, New South Wales and Western Australian (Fig. 1, Table 1). At each location standard regional agronomic practices for growing canola and juncea canola were used. The trial sites were provided by the Victorian Department of Environment and Primary Industries, New South Wales Department of Primary Industries, the South Australian Research and Development Institute, Department of Agriculture and Food, Western Australia and the National Variety Trials. These sites are used to assess new breeding lines and commercial cultivars for their agronomic potential and are located throughout the canola growing regions of south eastern and western Australia.

At each site, each breeding line and cultivar (Table 2) was replicated in three plots, generally eight metres long and six rows wide. During 2006 nine breeding lines and cultivars (six *B. juncea* and three *B. napus*) were sampled at each site. During 2007 five breeding lines and cultivars (three *B. juncea* and two *B. napus*) were sampled at each site. During 2010–2013 two breeding lines and cultivars (one *B. juncea* and one *B. napus*) were sampled. The *B. juncea* breeding lines were selected based on their blackleg resistance response from previous blackleg nurseries. The *B. napus* cultivars were chosen as a representative of the current commercial canola cultivars.

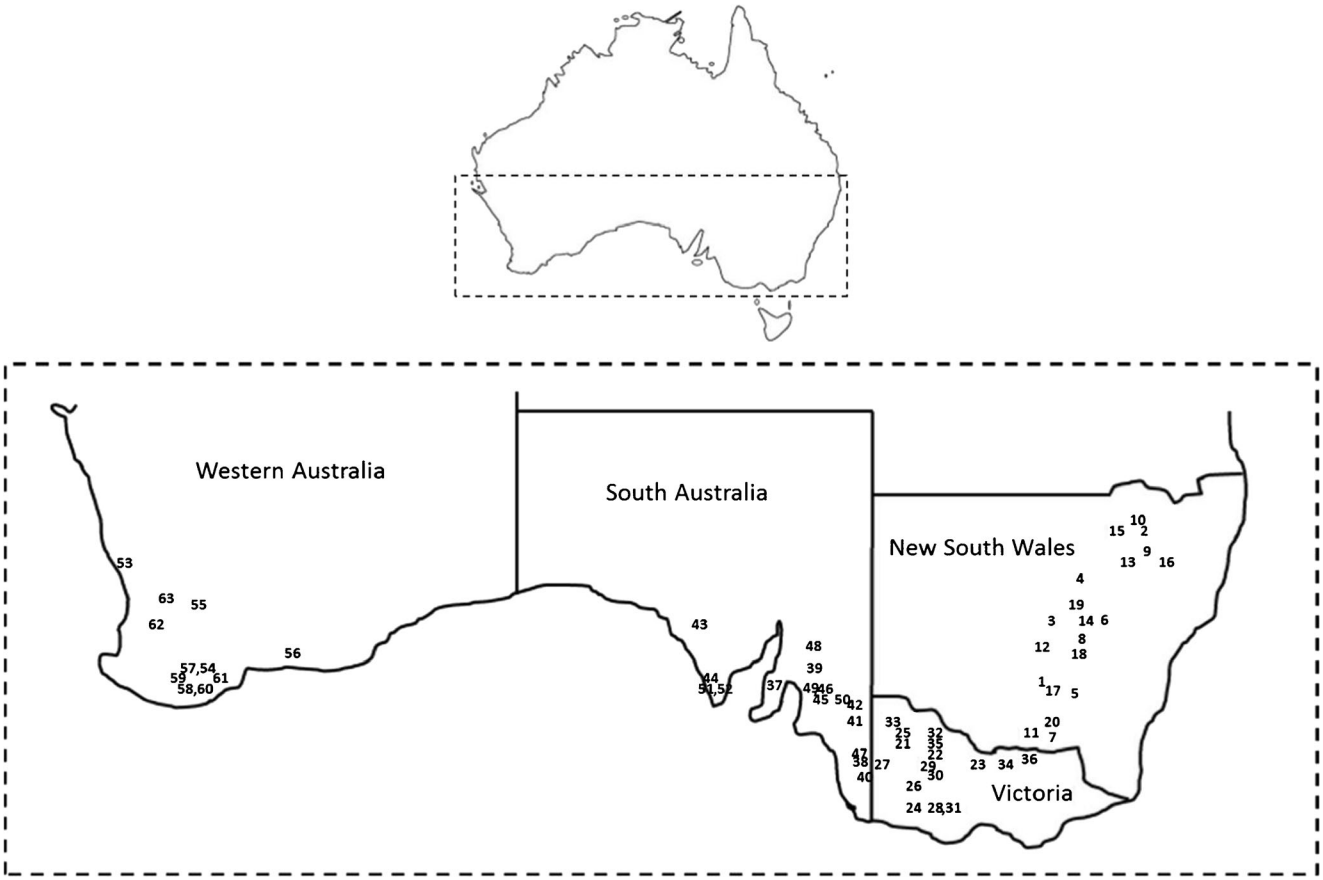

Fig. 1 Locations of survey sites across Australia for all years. *Numbers* represent individual field sites (see Table 1 for details)

Blackleg severity assessment

Each cultivar / breeding line was sampled by selecting the second row into the experimental plot, 1 m from the end. Twenty consecutive plants (60 plants in total from the 3 reps) were pulled from the soil, the plants were cut at the crown and the cross sectional area was visually assessed for blackleg by the percentage of internal infection (0–100 %) (Marcroft et al. 2004). Dead plants were recorded as 100 % internal infection.

Leptosphaeria isolate collection

Single spore isolates of *Leptosphaeria* spp. were collected from the scored stubble at a minimum of three locations each year (stems were over summered in Dimboola, Victoria) (Elliott et al. 2011) and the spores grown on V8 agar to determine that symptoms scored in the field were due to blackleg infection. The pycnidiospores produced in culture were then used to inoculate *B. juncea* and *B. napus* at a concentration of 10 million spores / ml. Tween 20 was added to the spore solution and it was sprayed onto 4 week old cotyledons using a Preval® Power Unit. Plants were placed in high humidity for 48 h and the subsequent symptoms were identified as those caused by *Leptosphaeria* spp.

Statistical analysis

Disease severity data were analysed using analysis of variance (ANOVA) tests. Log (base 10) transformations were used prior to analysis in order to normalize the data. Least significant difference tests were used to compare differences between cultivars within sites. All analyses were performed using MINITAB® Release 14.13.

Results

Blackleg disease was present at all sites surveyed in all years. Disease severity was low in 2006 and 2007 due to drought conditions (Table 1). Mean internal infection levels in the juncea canola breeding lines and cultivars were below 9, 15 and 23 % in New South Wales, Victoria and South Australia, respectively (Table 3). Mean internal infection levels in the *B. napus* cultivars were below 6, 10

Table 1 Location of surveyed sites, mean annual rainfall range and the years each site was surveyed for blackleg disease

Map number	Town	State	Region	Years surveyed (growing season rainfall mm*)
1	Beckom	NSW	NSW Central West	2011 (196), 2012 (156), 2013 (254)
2	Bellata	NSW	NSW North West Slopes and Plains	2006 (252), 2013 (175)
3	Condobolin	NSW	NSW Central West	2007 (163)
4	Coonamble	NSW	NSW North West Slopes and Plains	2006 (174), 2007 (187)
5	Cootamundra	NSW	NSW South West Slopes	2012 (271), 2013 (262)
6	Cudal	NSW	NSW Central West Slopes	2012 (240), 2013 (285)
7	Gerogery	NSW	NSW Riverina	2010 (493), 2011 (273), 2012 (267) 2013 (355)
8	Grenfell	NSW	NSW Central West Slopes	2010 (484), 2011 (252), 2012 (244) 2013 (319)
9	Gunnedah	NSW	NSW North West Slopes and Plains	2007 (284)
10	Gurley	NSW	NSW North West Slopes and Plains	2007 (226)
11	Lockhart	NSW	NSW Riverina	2012 (136), 2013 (290)
12	Merriwagga	NSW	NSW Lower Western	2006 (135)
13	Mullaley	NSW	NSW North West Slopes and Plains	2013 (174)
14	Parkes	NSW	NSW Central West Slopes	2013 (356)
15	Rowena	NSW	NSW North West Slopes and Plains	2006 (169)
16	Tamworth	NSW	NSW North West Slopes and Plains	2006 (188), 2007 (303)
17	Temora	NSW	NSW South West Slopes	2011 (201)
18	Thuddungra	NSW	NSW South West Slopes	2006 (134)
19	Trangie	NSW	NSW Central West Slopes	2006 (107)
20	Wagga Wagga	NSW	NSW South West Slopes	2006 (153), 2007 (195), 2010 (471), 2011 (197), 2012 (160), 2013 (251)
21	Beulah	Vic	Vic Mallee	2007 (169)
22	Charlton	Vic	Vic Mallee	2012 (175), 2013 (247)
23	Diggora	Vic	Vic North central	2010 (408), 2011 (224), 2012 (173) 2013 (231)
24	Hamilton	Vic	Vic Western District	2012 (441), 2013 (462)
25	Hopetoun	Vic	Vic Mallee	2007 (163)
26	Horsham	Vic	Vic Wimmera	2006 (160), 2007 (253), 2011 (186)
27	Kaniva	Vic	Vic Wimmera	2012 (282), 2013 (390)
28	Lake Bolac	Vic	Vic Western District	2010 (427)
29	Minyip	Vic	Vic Wimmera	2010 (349), 2011 (200), 2012 (256) 2013 (335)
30	Rupanyup	Vic	Vic Wimmera	2012 (256)
31	Streatham	Vic	Vic Western District	2011 (260), 2012 (321), 2013 (401)
32	Ultima	Vic	Vic Mallee	2007 (162)
33	Walpeup	Vic	Vic Mallee	2006 (95), 2007 (212)
34	Wunghnu	Vic	Vic North East	2012 (211), 2013 (263)
35	Wycheproof	Vic	Vic Mallee	2007 (148)
36	Yarrawonga	Vic	Vic North East	2012 (206), 2013 (222)
37	Arthurton	SA	SA Yorke Peninsula	2010 (389), 2011 (320), 2012 (314) 2013 (476)
38	Bordertown	SA	SA South East	2010 (365), 2011 (264), 2012 (304) 2013 (418)
39	Clare	SA	SA Mid North	2011 (314)
40	Frances	SA	SA South East	2006 (170), 2012 (329)
41	Lameroo	SA	SA Mallee	2007 (178)
42	Loxton	SA	SA Mallee	2007 (136)
43	Minnipa	SA	SA Upper Eyre Peninsula	2007 (91)
44	Mt Hope	SA	SA Lower Eyre Peninsula	2010 (440), 2011 (336), 2012 (394) 2013 (417)
45	Riverton	SA	SA Mid North	2010 (441), 2011 (292), 2012 (252) 2013 (429)
46	Rosedale	SA	SA Mid North	2007 (309)
47	Sherwood	SA	SA South East	2007 (303)
48	Spalding	SA	SA Upper Mid North	2012 (207), 2013 (343)
49	Turretfield	SA	SA Mid North	2012 (288), 2013 (397)

Table 1 (continued)

Map number	Town	State	Region	Years surveyed (growing season rainfall mm*)
50	Waikerie	SA	SA Mallee	2007 (123)
51	Wangary	SA	SA Lower Eyre Peninsula	2011 (428), 2012 (408)
52	Wanilla	SA	SA Lower Eyre Peninsula	2010 (520)
53	Badgingarra	WA	WA North Central	2012 (337), 2013 (446)
54	Broomehill	WA	WA Great Southern	2010 (160)
55	Corrigin	WA	WA Central	2012 (150), 2013 (306)
56	Gibson	WA	WA South Coast	2010 (404), 2012 (344), 2013 (451)
57	Katanning	WA	WA South West	2012 (273), 2013 (338)
58	Kendenup	WA	WA South West	2012 (344), 2013 (401)
59	Kojonup	WA	WA Great Southern	2010 (235), 2011 (465), 2012 (350) 2013 (437)
60	Mt Barker	WA	WA South West	2011 (535)
61	South Stirling	WA	WA South Coast	2012 (457), 2013 (556)
62	Williams	WA	WA South West	2010 (215), 2011 (383), 2012 (297) 2013 (415)
63	York	WA	WA Central	2011 (374)

*Growing season rainfall is April – October inclusive, information has been collected from the nearest Bureau of Meteorology weather station

Table 2 Canola and juncea canola cultivars and breeding lines surveyed

Line	History	Species	Year surveyed
Dune	Commercialised 2007	*B. juncea*	2006
			2007
JC05002	Advanced breeding line	*B. juncea*	2006
			2007
JC05006	Advanced breeding line	*B. juncea*	2006
			2007
JC05007	Advanced breeding line	*B. juncea*	2006
JC05016	Advanced breeding line	*B. juncea*	2006
JC05034	Advanced breeding line	*B. juncea*	2006
JC06006	Advanced breeding line	*B. juncea*	2006
JBOT800407	Advanced breeding line	*B. juncea*	2011
			2012
			2013
Oasis CL	Commercialised 2008	*B. juncea*	2010
SARDI515	Advanced breeding line	*B. juncea**	2007
SARDI631	Advanced breeding line	*B. juncea**	2007
AG Outback	Commercialised 2001	*B. napus*	2006
			2007
AV Opal	Commercialised 2006	*B. napus*	2006
AV Garnet	Commercialised 2007	*B. napus*	2010
			2011
Crusher TT	Commercialised 2011	*B. napus*	2013
ATR Marlin	Commercialised 2007	*B. napus*	2012
Tarcoola	Commercialised 2007	*B. napus*	2006
			2007
AV Jade	Commercialised 2006	*B. napus*	2006
AG Spectrum	Commercialised 2004	*B. napus*	2006

* denotes breeding lines that are not canola quality

and 12 % in New South Wales, Victoria and South Australia, respectively. In 2010–2013 drought conditions had ceased and mean internal infection levels of *B. napus* were higher, with mean internal infection levels ranging from 9 to 59 %. No plant death due to blackleg was evident at any of the sites in 2006 and 2007, however plant death was evident in 2010, 2011, 2012 and 2013 (data not shown).

Significant differences in mean internal infection severity between the juncea canola breeding lines and cultivars within some sites in 2006 and 2007 are shown in Table 4. When the internal infection levels of *B. juncea* breeding lines and cultivars were compared to *B. napus* cultivars at the same location, nine sites in 2006 displayed significantly higher infection levels in the *B. juncea* breeding lines and cultivars (Fig. 2). In 2007, eight locations had significantly higher infection levels in the *B. juncea* breeding lines and cultivars than in the *B. napus*, four of which were locations known to or likely to have *L. biglobosa* existing there. Four sites had significantly lower infection levels in the *B. juncea* breeding lines and cultivars compared to the *B. napus* cultivars (Fig. 3). In each of 2010 and 2011 all sites except one had lower mean internal infection severity

Table 3 Mean internal infection levels of *Brassica juncea* and *B. napus* over all sites Letters denote significant differences at, $p<0.05$, across breeding lines and cultivars for each location (significance displayed horizontally only)

Year	*B.juncea*	*B.napus*
2006	10.7a	5.4b
2007	6.9a	7.8b
2010	4.7a	19.3b
2011	8.1a	30.9b
2012	13.9a	38.5b
2013	9.9a	27.9b

Table 4 The mean internal infection severity (percentage) caused by either *L. maculans* or *L. biglobosa* for all canola cultivars and juncea canola breeding lines and cultivars at each site surveyed in 2006 and 2007

Site	Year	Canola cultivars					Juncea canola cultivars									Site mean
		AG-Outback	AG-Spectrum	AV-Jade	AV-Opal	Tarcoola	JC0-5002	JC0-5006	JC0-5007	JC0-5016	JC0-5034	JC0-6006	Dune	SARDI-515*	SARDI-631*	
Bellata#	2006	9a				8a	6a	9a	16a	11a	12a		6a			10
Beulah	2007	11b				13c	12bc	2a					2a			8
Condobolin#	2007	6b				3a	5b	8c					4bc			5
Coonamble#	2006	5a			6ab	6abc	10bcd	16e	12de	19e	11cde		12de			11
	2007	0a				0a	7b	5b					9b			4
Frances	2006		8ab	9a			30c	16bc					21bc			17
Gunnedah#	2007	0a				0a	0a	1b					0a			0
Gurley#	2007	10b				3a	5b	15c					16c			10
Hopetoun	2007	4a				5a	5a	5a					4a			5
Horsham	2006	5a		4a	4a	3a	18b	16b	10b	10b	25b		21b			12
	2007	10a				7a	13b	13ab					25b			13
Lameroo	2007	21a				11a								11a	7a	13
Loxton	2007	5a				7a								4a	2a	5
Merriwagga	2006	5a			6ab	3a	6abc	4abc	8c	8bc	14bc		6abc			7
Minnipa	2007	3a				17cd	8bc	7b					8cd	12de	16e	10
Rosedale	2007	23c				13b								7a	6a	12
Rowena^	2006	5a			6a	8a	11a	7a	7a	6a	7a		8a			7
Sherwood	2007					7a							2a	6a	5a	5
Tamworth^	2006	7a		4a	4a	4a	9ab	8ab				20c	18bc			9
	2007	0a				3a	0a	1a					1a			1
Thuddungra	2006	2a			3a	4a	4a	5a	5a	6a	3a		4a			4
Trangie	2006	7ab			6a	7abc	10abcd	11bcd	15d	13cd	10abcd		11abcd			10
Ultima	2007	26c				15b	6a	12b					6a			13
Wagga Wagga	2006	2a			5ab	3ab	5b	5b	4ab	2ab	6ab		1a			4
	2007	1a				6ab	30c	4b					4ab			9
Waikerie	2007	11a				4a								3a	3a	5
Walpeup	2006	4ab		5a	7abc	8abc	9abc	12c	11c	23bc	7abc		11c			10
	2007	7c				6b	7ab	4a					2ab			5
Wycheproof	2007	3ab				8b	12d	1a					5c			6

Letters denote significant differences at, $p<0.05$, across varieties for each location (significance displayed horizontally only)

^Denotes sites where *L. biglobosa* has been found previously

# Denotes sites where *L. biglobosa* is likely to exist

* Denotes *B. juncea* lines that are not canola quality

in the *B. juncea* compared to the *B. napus* (Figs. 4 and 5). In 2012, 21 sites had lower mean internal infection severity in the *B. juncea* breeding line compared to the *B. napus* and one site, Lockhart NSW, had higher mean internal infection severity in the *B. juncea* breeding line compared to the *B. napus* (Fig. 6). Ten sites had no significant difference between the internal infection severities of the two species. In 2013, 24 sites had lower mean internal infection severity in the *B. juncea* breeding line compared to the *B. napus* and one site, Charlton (Victoria), had higher mean internal infection severity in the *B. juncea* breeding line compared to the *B. napus* (Fig. 7). Seven sites had no significant difference between the internal infection severities of the two species.

Discussion

This is the first extensive survey of blackleg disease in juncea canola in its potential growing regions of south-

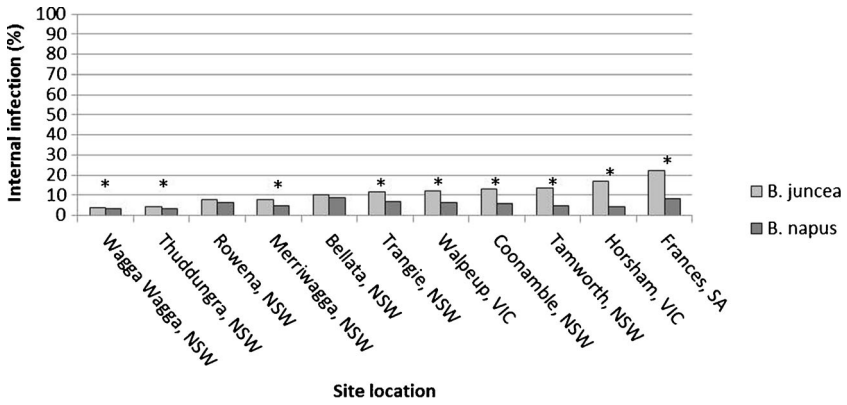

Fig. 2 Mean internal infection levels, caused by *L. maculans* and *L. biglobosa,* of *B. juncea* and *B. napus* lines and cultivars at sites in New South Wales, Victoria and South Australia, in 2006, in order of increasing disease severity in the juncea canola lines and cultivars (*denotes $p<0.05$)

eastern Australia and is also one of the few examples of a "pre-commercialization" assessment of disease potential for any crop. Our study showed that although the Australian juncea canola industry is yet to be established, blackleg infection was found at low levels at all sites that were surveyed during 2006 and 2007. Previous work showed that *L. maculans* isolates capable of infecting and causing internal infection in *B. juncea* exist in Australia (Ballinger and Salisbury 1996), however, this is the first study to show that blackleg, capable of causing disease in *B. juncea*, is already widespread throughout the Australian canola growing regions on juncea canola, even before the crop is widely grown.

In the pre-commercialisation survey (2006–2007), the internal infection levels in the *B. juncea* breeding lines and cultivars were low, however the severity of internal infection was also very low in the *B. napus* plots. This may be a consequence of low rainfall in the surveyed areas during 2006 and 2007. The mean internal infection severities were low during 2006 and 2007, and it was probable that the disease would not have resulted in yield loss as internal infection levels greater than 50 % are required to significantly lower yield per plant in canola (Marcroft et al. 2004). Although *B. juncea* is considered more blackleg resistant than *B. napus* (Kirk and Oram 1978; Chèvre et al. 1997; Purwantara et al. 1998), at nine sites in 2006 and eight in 2007 the mean level of infection in the juncea canola breeding lines and cultivars was higher than the *B. napus* cultivars at the same site.

The results from the 2010–13 survey show that although disease severity was increased overall, the disease severity was consistently lower in the *B. juncea* cultivar compared to the *B. napus*. This suggests that although disease severity was higher in the *B. juncea* cultivars at some sites in the earlier survey, when conditions are conducive to greater disease the severity did not rise in the *B. juncea* cultivars to the same extent as it did in the *B. napus* cultivars. One possible explanation for this is the virulence profile of the *Leptosphaeria* population. The resistance genes of the juncea canola cultivars are currently unknown, however they are assumed to be different to those of the *B. napus* cultivars (Marcroft et al. 2012). Due to this, there is potentially a lower frequency of isolates that can attack the juncea canola cultivars compared to the *B. napus* cultivars, as the fungus has not previously been exposed to large areas of this resistance. This is positive news for the emerging juncea canola industry, however does not mean that disease severity will not rise in the future.

Shifts in the *Leptosphaeria* population structure occur in response to the resistance genes deployed in commercial cultivars (Sprague et al. 2006; Brun et al. 2000). As acreage of juncea canola increases so too will the selection pressure

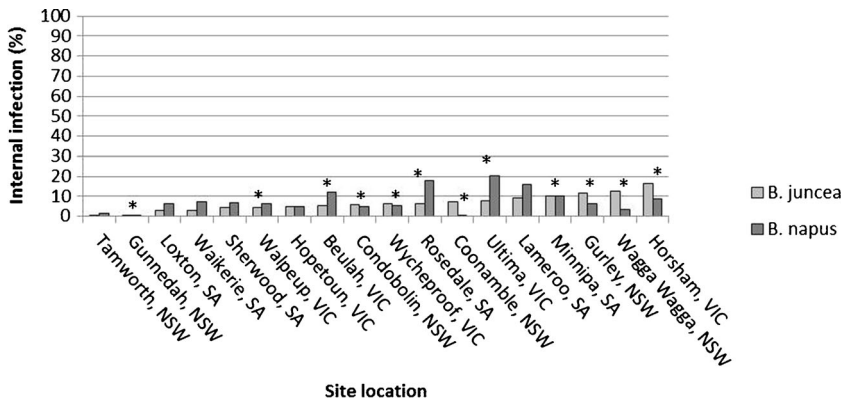

Fig. 3 Mean internal infection levels, caused by *L. maculans* and *L. biglobosa,* of *B. juncea* and *B. napus* lines and cultivars at sites in New South Wales, Victoria and South Australia, in 2007 in order of increasing disease severity in the juncea canola lines and cultivars (* denotes $p<0.05$)

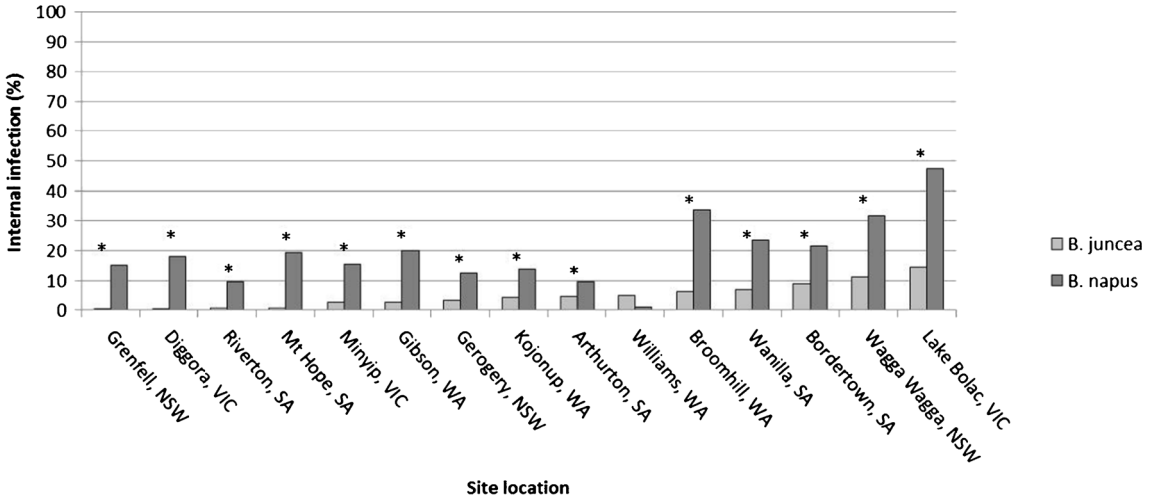

Fig. 4 Mean internal infection levels, caused by *L. maculans* and *L. biglobosa,* of *B. juncea* and *B. napus* cultivars at sites in New South Wales, Victoria, South Australia and Western Australia in 2010, in order of increasing disease severity in the juncea canola cultivar (*denotes $p<0.05$)

placed on the pathogen, resulting in a greater number of virulent isolates over time. The resistance genes present in juncea canola can be overcome in the same way that *B. napus* genes can be, as shown in field trials in France where *Rlm6* resistance has been overcome (Brun et al. 2010).

The difference in mean internal infection severity between juncea canola breeding lines and cultivars within sites may reflect differences in resistance. In *B. napus* there are both qualitative and quantitative genes that play a part in the plants total resistance. Marcroft et al. (2012) showed that *B. napus* cultivars with the same complement of qualitative resistance genes could display different reactions due to their quantitative resistance.

Three genes, *Rlm5*, *Rlm6* and *LMJR2* have been identified in *B. juncea* (Chèvre et al. 1997; Barret et al. 1998; Balesdent et al. 2002, 2005; Saal et al. 2004). There may also be additional currently unidentified genes present in *B. juncea* that could confer resistance to *L. maculans*. There is also the possibility of genes being introgressed from *B. napus* during crossing for desirable oil quality and agronomic traits. Alternative combinations of these resistance genes are possible within the juncea canola cultivars tested and would explain the differences in internal infection severity seen. In addition, nothing is currently known about quantitative plant resistance in *B. juncea* and the role it may play. Screening of cultivars using a variety of *Leptosphaeria* isolates would need to be

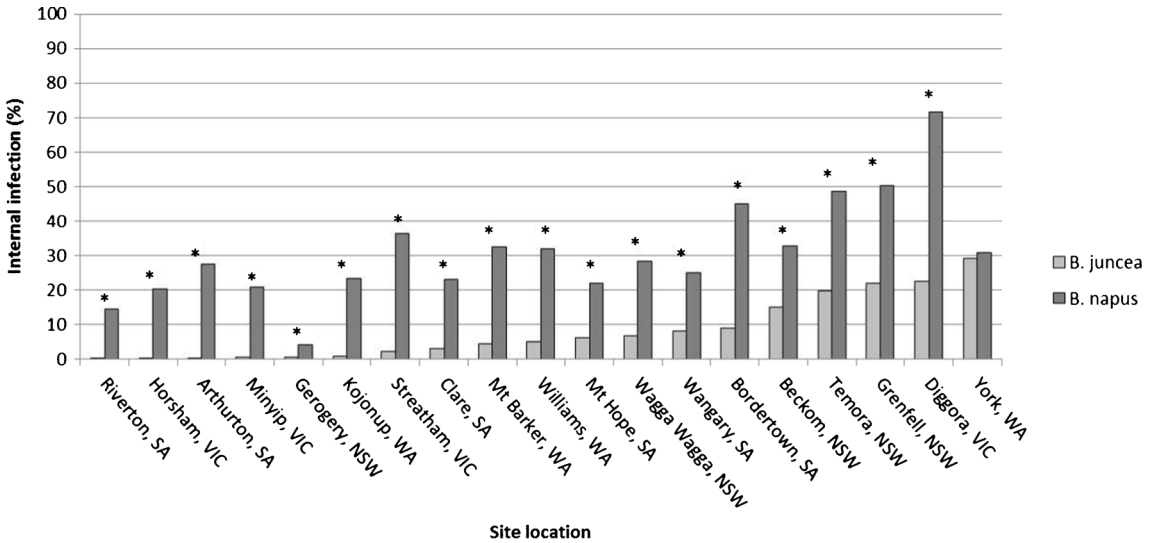

Fig. 5 Mean internal infection levels, caused by *L. maculans* and *L. biglobosa,* of *B. juncea* and *B. napus* lines at sites in New South Wales, Victoria, South Australia and Western Australia in 2011 in order of increasing disease severity in the juncea canola line (* denotes $p<0.05$)

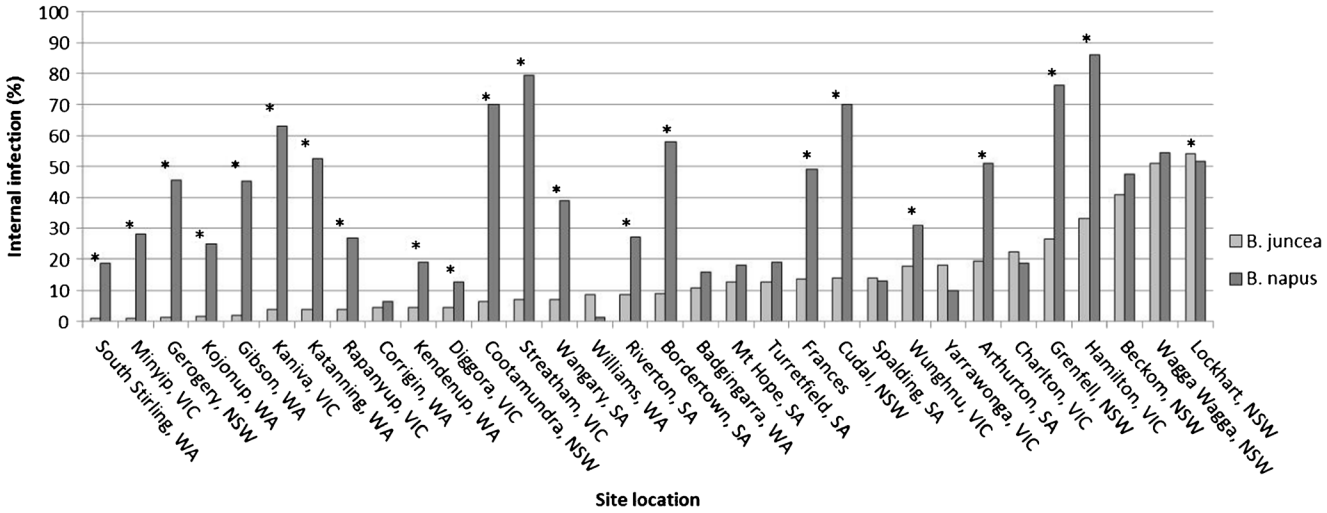

Fig. 6 Mean internal infection levels, caused by *L. maculans* and *L. biglobosa,* of *B. juncea* and *B. napus* lines and cultivars at sites in New South Wales, Victoria, South Australia and Western Australia in 2012 in order of increasing disease severity in the juncea canola line (* denotes $p<0.05$)

done to more clearly identify whether differences occur. Differences in resistance sources within juncea canola cultivars would be a positive finding for future breeding of juncea canola in Australia.

In order to maintain the blackleg resistance in *B. juncea*, and reduce the likelihood of *L. maculans* overcoming resistance genes, it is essential that the management techniques similar to those used in *B. napus* production be adopted for juncea canola production. This includes separating crops from the previous years stubble (Marcroft et al. 2004), and the application of a fungicide (seed dressing) if past disease levels deem it necessary. Breeding for resistance to blackleg and monitoring of disease severity in juncea canola should continue to be a high priority within the Australian canola industry. By monitoring disease severity growers can be warned if high disease severity, and hence yield losses are expected to occur.

As the primary locations for juncea canola production will be in low rainfall regions, the disease pressure, and therefore the selection pressure placed onto *L. maculans*, is likely to be low. Although this study has shown that blackleg isolates capable of attacking juncea canola are widespread, wise management of the resistance should allow juncea canola to be widely grown in low rainfall environments.

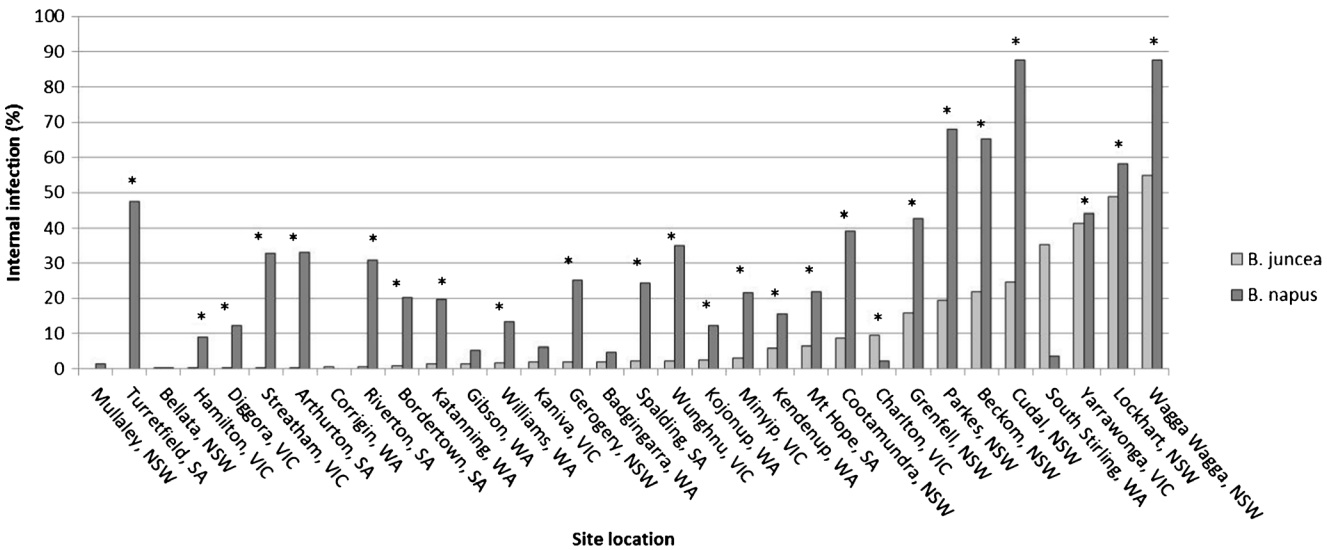

Fig. 7 Mean internal infection levels, caused by *L. maculans* and *L. biglobosa,* of *B. juncea* and *B. napus* lines and cultivars at sites in New South Wales, Victoria, South Australia and Western Australia in 2013 in order of increasing disease severity in the juncea canola line (* denotes $p<0.05$)

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References

Balesdent MH, Attard A, Kühn ML, Rouxel T (2002) New avirulence genes in the phytopathogenic fungus *Leptosphaeria maculans*. Phytopathology 92:1122–1133

Balesdent MH, Barbetti MJ, Li H, Sivasithamparam K, Gout L, Rouxel T (2005) Analysis of *Leptosphaeria maculans* race structure in a world-wide collection of isolates. Phytopathology 95:1061–1071

Balesdent MH, Fudal I, Ollivier B, Bally P, Grandaubert J, Eber F, Chèvre A, Leflon M, Rouxel T (2013) The dispensable chromosome of *Leptosphaeria maculans* shelters an effector gene conferring avirulence towards *Brassica rapa*. New Phytol 198:887–898

Ballinger DJ, Salisbury PA (1996) Seedling and adult plant evaluation of race variability in *Leptosphaeria maculans* on *Brassica* species in Australia. Aust J Exp Agric 36:485–488

Barret P, Guérif J, Reynoird JP, Delourme R, Eber F, Renard M, Chèvre AM (1998) Selection of stable Brassica napus-B. juncea recombinant lines resistant to blackleg (Leptosphaeria maculans) 2: a 'to and fro' strategy to localise and characterise interspecific introgressions on B. napus genome. Theor Appl Genet 96:1097–1103

Brun H, Levivier S, Somda I, Ruer D, Renard M, Chèvre AM (2000) A field method for evaluating the potential durability of new resistance sources: application to the *Leptosphaeria maculans-Brassica napus* pathosystem. Phytopathology 90:961–966

Brun H, Chèvre AM, Fitt BDL, Powers S, Besnard AL, Ermel M, Huteau V, Marquer B, Eber F, Renard M, Andrivon D (2010) Quantitative resistance increases the durability of qualitative resistance to *Leptosphaeria maculans* in *Brassica napus*. New Phytol 185:285–299

Burton W, Salisbury P, Potts D (2003) The potential of canola quality *Brassica juncea* as an oilseed crop for Australia. In '13th Australian Research Assembly on Brassicas'. Tamworth, New South Wales, Australia pp. 62–64

Burton WA, Flood RF, Norton RM, Field B, Potts DA, Robertson MJ, Salisbury PA (2008) Identification of variability in phenological responses in canola-quality *Brassica juncea* for utilisation in Australian breeding programs. Aust J Agric Res 59:874–881

Chèvre AM, Barret P, Eber F, Dupuy P, Brun H, Tanguy X, Renard M (1997) Selection of stable *Brassica napus-B. juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*). 1. Identification of molecular markers, chromosomal and genomic origin of the introgression. Theor Appl Genet 95:1104–1111

Colton B, Potter TD (1999) History. In 'In: Canola in Australia: the first thirty years'. (Eds PA Salisbury, TD Potter, M G, AG Green). (Published for the 10th International Rapeseed Congress, Canberra, Australia

Delourme R, Chèvre AM, Brun H, Rouxel T, Balesdent MH, Dias JS, Salisbury P, Renard M, Rimmer SR (2006) Major gene and polygenic resistance to *Leptosphaeria maculans* in oilseed rape (*Brassica napus*). Eur J Plant Pathol 114:41–52

Eber F, Lourgant K, Brun H, Lode M, Huteau V, Coriton O, Alix K, Balesdent MH, Chèvre AM (2011) Analysis of *Brassica nigra* chromosomes allows identification of a new effective *Leptosphaeria maculans* resistance gene introgressed in *Brassica napus*. 13th International rapeseed congress, Prague 5–9 June 2011

Elliott VL, Marcroft SJ, Norton RM, Salisbury PA (2011) Reaction of *Brassica juncea* to Australian isolates of *Leptosphaeria maculans* and *Leptosphaeria biglobosa* 'canadensis'. Can J Plant Pathol 33: 38–48

Howlett BJ (2004) Current knowledge of the interaction between *Brassica napus* and *Leptosphaeria maculans*. Can J Plant Pathol 26:245–252

Kirk JTO, Oram RN (1978) Mustards as possible oil and protein crops for Australia. J Aust Inst Agric Sci 44:143–156

Li H, Sivasithamparam K (2003) Breakdown of a Brassica rapa subsp. sylvestris single dominant blackleg resistance gene in B. napus rapeseed by Leptosphaeria maculans field isolates in Australia. Plant Dis 87:752

Long Y, Wang Z, Sun Z, Fernando DWG, McVetty PBE, Li G (2011) Identification of two blackleg resistance genes and fine mapping of one of these two genes in a *Brassica napus* cultivar 'Surpass 400'. Theor Appl Genet 122:1223–1231

Marcroft SJ, Purwantara A, Salisbury PA, Potter TD, Wratten N, Khangura R, Barbetti MJ, Howlett BJ (2002) Reaction of a range of Brassica species under Australian conditions to the fungus, *Leptosphaeria maculans*, the casual agent of blackleg. Aust J Exp Agric 42:587–594

Marcroft SJ, Sprague SJ, Pymer SJ, Salisbury PA, Howlett BJ (2004) Crop isolation, not extended rotation length, reduces blackleg (*Leptosphaeria maculans*) severity of canola (*Brassica napus*) in south-eastern Australia. Aust J Exp Agric 44:601–606

Marcroft SJ, Elliott VL, Cozijnsen AJ, Salisbury PA, Howlett BJ, Van de Wouw AP (2012) Identifying resistance genes to *Leptosphaeria maculans* in Australian *Brassica napus* cultivars based on reactions to isolates with known avirulence genotypes. Crop Pasture Sci 63: 338–350

Mendes-Piereira E, Balesdent MH, Brun H, Rouxel T (2003) Molecular phylogeny of the *Leptosphaeria maculans-L. biglobosa* species complex. Mycol Res 107:1287–1304

Murray GM, Brennan JP (2012) The current and potential costs from diseases of oilseed crops in Australia. Grains Research and Development Corporation, Australia

Oram RN, Kirk JTO, Veness PE, Hurlstone CJ, Edlington JP, Halsall DM (2005) Breeding Indian mustard [*Brassica juncea* (L.) Czern.] for cold-pressed, edible oil production — a review. Aust J Agric Res 56: 581–596

Plummer KM, Dunse K, Howlett BJ (1994) Non-aggressive strains of the blackleg fungus, *Leptosphaeria maculans*, are present in Australia and can be distinguished from aggressive strains by molecular analysis. Aust J Bot 42:1–8

Purwantara A, Salisbury PA, Burton WA, Howlett BJ (1998) Reaction of *Brassica juncea* (Indian mustard) lines to Australian isolates of *Leptosphaeria maculans* under glasshouse and field conditions. Eur J Plant Pathol 104:895–902

Rouxel T, Penaud A, Pinochet X, Brun H, Goutl D, Schmit J, Balesdent MH (2003) A 10-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the Rlm1 resistance gene of oilseed rape. Eur J Plant Pathol 104:871–881

Saal B, Brun H, Glais I, Struss D (2004) Identification of a *Brassica juncea* derived recessive gene conferring resistance to *Leptosphaeria maculans* in oilseed rape. Plant Breed 123:505–511

Sprague SJ, Marcroft SJ, Hayden HL, Howlett BJ (2006) Major gene resistance to blackleg in *Brassica napus* overcome within three years of commercial production in southeastern Australia. Plant Dis 90:190–198

Van de Wouw AP, Thomas VL, Cozijnsen AJ, Marcroft SJ, Salisbury PA, Howlett BJ (2008) Identification of *Leptosphaeria biglobosa* '*canadensis*' on *Brassica juncea* stubble from northern New South Wales, Australia. Australas Plant Dis Notes 3:124–128

Van de Wouw AP, Marcroft SJ, Barbetti MJ, Li H, Salisbury PA, Gout L, Rouxel T, Howlett BJ, Balesdent MH (2009) Dual control of

avirulence in *Leptosphaeria maculans* towards a *Brassica napus* cultivar with 'sylvestris-derived' resistance suggests involvement on two resistance genes. Plant Pathol 58:305–313

Vincenot L, Balesdent MH, Li H, Barbetti MJ, Sivasithamparam K, Gout L, Rouxel T (2008) Occurrence of a new subclade of *Leptosphaeria biglobosa* in Western Australia. Phytopathology 98:321–329

Yu F, Lydiate DJ, Rimmer SR (2005) Identification of two novel genes for blackleg resistance in *Brassica napus*. Theor Appl Genet 110:969–979

Yu F, Lydiate DJ, Rimmer SR (2008) Identification and mapping of a third blackleg resistance locus in *Brassica napus* derived from B. *rapa* subsp. sylvestris. Genome 51:64–72