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Epidemiology of root rot caused by *Leptosphaeria maculans* in *Brassica napus* crops

Susan J. Sprague · Barbara J. Howlett · John A. Kirkegaard

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Abstract The infection of above-ground tissues of *Brassica napus* by *Leptosphaeria maculans* is well understood. However, root infection (root rot) under field conditions, the development of root rot over time and its relationship to other disease symptoms caused by *L. maculans* has not been described. A survey of *B. napus* crops was conducted in Australia to investigate the incidence and severity of root rot. Additionally, the pathway of root infection was examined in field experiments. Root rot was present in 95% of the 127 crops surveyed. The severity and incidence of root rot was significantly correlated with that of crown canker; however, the strength of this relationship was dependent on the season. Root rot symptoms appeared before flowering and increased in severity during flowering and at maturity, a pattern similar to crown canker suggesting that the infection of the root is an extension of the crown canker phase of the *L. maculans* lifecycle. All isolates of *L. maculans* tested in glasshouse experiments caused root rot and crown canker in *B. napus* and *Brassica juncea*. In the field, the main pathway of root infection is via invasion of cotyledons or leaves by airborne ascospores, rather than from inoculum in the soil. Root rot was present in crops in fields that had never been sown to *B. napus* previously, in plants grown in fumigated fields, and in glasshouse-grown plants inoculated in the hypocotyl with *L. maculans*.

Keywords Blackleg · Canola · Oilseed rape · Phoma stem canker · Rapeseed · Root infection

S. J. Sprague · B. J. Howlett
School of Botany, The University of Melbourne, Parkville, VIC 3010, Australia

S. J. Sprague (✉) · J. A. Kirkegaard
CSIRO Plant Industry,
GPO Box 1600, Canberra, ACT 2602, Australia
e-mail: Susan.Sprague@csiro.au

Introduction

Leptosphaeria maculans (anamorph *Phoma lingam*) (Phoma, blackleg) causes a variety of symptoms on oilseed rape (*Brassica napus*) including spots on the leaves and pods, stem lesions and crown cankers (West et al. 2001). While symptoms in oilseed rape roots have also been associated with *L. maculans,* the importance of root disease (hereafter called root rot) and its relationship to crown canker is not understood. *Leptosphaeria maculans* is the most important fungal pathogen in the main regions of the world growing *B. napus* (canola, oilseed rape) including Australia, Europe and North America (Fitt et al. 2006). Crown cankers are considered the most devastating disease symptom as they inhibit the uptake of water and nutrients, thereby limiting crop yield (Gugel and Petrie 1992). Crown cankers develop following the systemic

growth of the fungus within the plant from infection of above-ground tissues, usually the cotyledons or leaves (Hammond et al. 1985).

While *L. maculans* is widely recognised as a foliar pathogen it has also been reported in roots of *B. napus* (Hornig, 1985; Brun and Jacques, 1991; Sosnowski et al. 2001; Evans et al. 2003; Sprague et al. 2007) as well as other brassicaceous species (Henderson 1918; Gibbs 1938; Gugel et al. 1990). Although these studies recognise that *L. maculans* invades roots, there has been some conjecture surrounding the pathway of infection. Cabbage seedlings grown in soil sieved to remove infected cabbage residues did not develop disease (Henderson 1918). In contrast, disease developed in swedes (Gibbs 1938), cabbages (Henderson 1918) or oilseed rape plants (Sosnowski et al. 2001) when roots were either dipped in suspensions containing inoculum or seedlings were transplanted into soil containing diseased residues (Henderson 1918). In each of the above studies, roots were damaged due to either intentional wounding or during transplantation of seedlings.

Cytological studies with an isolate of *L. maculans* tagged with a reporter gene, green fluorescent protein, showed infection of *B. napus* roots when inoculum was applied to both above-ground tissues or directly to intact roots (Sprague et al. 2007). Hyphae from inoculum applied above-ground grew within all tissues of the stem and hypocotyl and at the onset of flowering proliferated into the roots within xylem vessels where they were mainly contained. This study indicated that root infection can result from both above- and below-ground sources of inoculum applied artificially. This is consistent with findings of Li et al. (2007) who reported collapse of the stem and/or hypocotyl tissue in *B. napus* seedlings grown in soil inoculated with pycnidiospores or ascospores; however, root tissue was not assessed. The source of root infection under field conditions is unknown. Such sources could include crop residues incorporated during cultivation, ascospores or pycnidiospores on the soil surface incorporated at sowing (Li et al. 2007), or infected roots remaining in biopores from previous crops (Cresswell and Kirkegaard 1995). Thus it remains unclear how the overall contribution of root rot fits into disease caused by *L. maculans* and its effect on plant function. In the UK, Evans et al. (2003) reported that root rot occurred as a result of saprophytic colonisation of senescing plants and was associated with crown canker. In contrast, in Australia Sosnowski et al. (2001) reported that root rot could occur in plants that did not have crown canker. These contrasting findings may indicate that root infection is caused by different infection pathways, resistance mechanisms in the host or environmental conditions.

The aims of the study described in this paper are to (i) determine the prevalence of root rot in commercial oilseed rape crops in southeastern Australia, (ii) improve the understanding of the development of root rot symptoms and how it relates to crown canker, (iii) investigate the likely pathway by which *L. maculans* infects oilseed rape roots under natural conditions of inoculation in the field, and (iv) determine the ability of different isolates of *L. maculans* to cause root rot.

Materials and methods

Survey of commercial oilseed rape crops

To determine the incidence and severity of root rot in commercial oilseed rape crops, fields in northern Victoria and southern New South Wales (NSW), Australia, were sampled during three growing seasons (2003 to 2005). Fields were selected to include a wide range of cropping practices and cultivars, including fields in which oilseed rape had never been grown. Sixteen *B. napus* cultivars with *L. maculans* Resistance Ratings (RR) ranging from 5.5 to 9.0 were sown in the fields surveyed. The RR system (www.canolaaustralia.com) is used to rank cultivars for crown canker resistance on a scale of 1.0 (highly susceptible) to 9.0 (highly resistant) based on the percentage of plants that survive to maturity. The cultivars surveyed had either polygenic resistance (44C11, 45C75, 46C76, Beacon, Grace, Oscar, Pinnacle, Rainbow, Rivette, Sapphire, Spectrum, Stubby and Tornado) or major gene resistance derived from *Brassica rapa* subspecies *sylvestris* (Hyola 60, Surpass 603CL and Surpass 501TT). In 2003, this major gene resistance was overcome at a number of discrete locations in southeastern Australia resulting in up to 90% yield loss (Sprague et al. 2006a, b). Cultivars with this resistance source

were not widely grown in subsequent years in southeastern Australia due to fears that *L. maculans* isolates capable of overcoming this resistance source would become widespread.

In 2003, 26 fields were sampled during flowering and again at maturity prior to swathing, while a further 41 fields were sampled only at maturity. In 2004 and 2005, 43 and 17 fields, respectively, were sampled at maturity. Of the 127 fields, 11 had never grown a crop of *B. napus* previously. Within each field, plants were randomly sampled from five locations at least 20 m apart (total of 20 to 60 plants). Plants were pulled from the ground and assessed for the incidence and severity of crown canker at the base of the stem and in the tap root. Crown canker severity was assessed by cutting plants at the crown transversely with a pair of secateurs and visually quantifying the area (0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100%) of the internal stem surface that was blackened, as described by Marcroft et al. (2004). The severed tap root portion of the plant was then cut longitudinally and the severity scored. Blackening of the root tissue was often observed in the central xylem tissues with the outer pith tissues appearing undamaged. Root rot severity was assessed with the following scale: S0 (no infection), S1 (minor infection extending <1 cm longitudinally in xylem tissue or covering <10% of the cut surface), S2 (infection >1 cm long or covering <50% of the cut surface), S3 (moderate infection with blackening on ~50% of the cut surface), S4 (infection with blackening covering between 50 and 80% of the cut surface) and S5 (severe infection with blackening on >80% of the cut surface or extending the entire length of the xylem) (Fig. 1).

The roots of plants collected during the survey of commercial crops in 2003 were assessed for the presence of pathogens. Approximately 20 sections (~5 mm^2) of both healthy and diseased tissue were collected from tap roots and lateral roots with sections taken from the edges of necrotic tissue in diseased plants. Sections were taken across a range of cultivars and each section was taken from a different plant. Each section was surface-sterilised by washing in 70% ethanol followed by a rinse in sterile distilled water (SDW) supplemented with 50 mg l^{-1} chloramphenicol. Sections were then soaked for 1.5 min in bleach solution (1:4 commercial bleach) followed by two rinses in SDW, supplemented with chloramphenicol. Tissue sections were dried on sterile filter paper and placed onto 1/4 strength potato dextrose agar (PDA) supplemented with chloramphenicol (50 mg l^{-1}) and streptomycin (100 mg l^{-1}). Plates were incubated at 20°C under fluorescent light on a 12 h cycle for 5 to 14 days. Cultures were identified as *L. maculans* by the pink pycnidial ooze containing conidia and the morphology of the sporulating pycnidia (Punithalingam and Holliday 1972).

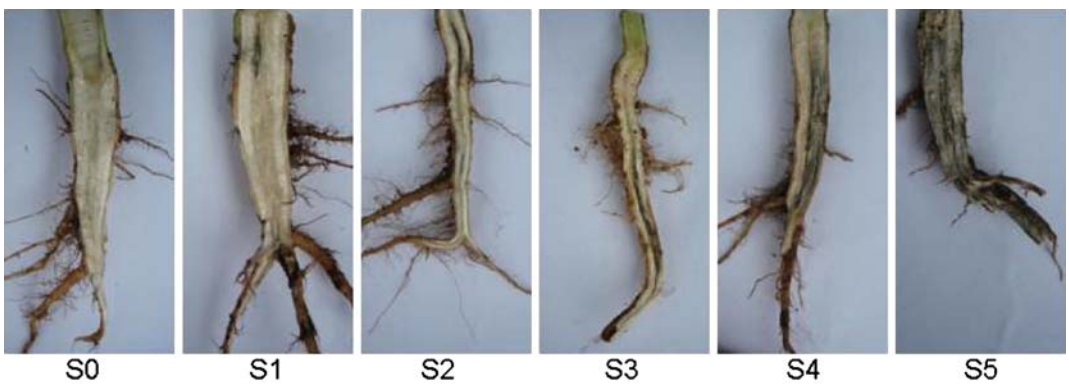


Fig. 1 *Brassica napus* roots with different levels of root rot severity: S0 (no infection), S1 (minor infection extending <1 cm longitudinally in xylem tissue or covering <10% of the cut surface), S2 (infection >1 cm long or covering <50% of the cut surface), S3 (moderate infection with blackening on ~50% of the cut surface), S4 (infection with blackening covering between 50 and 80% of the cut surface) and S5 (severe infection with blackening on >80% of the cut surface or extending the entire length of the xylem)

Assessment of root rot development in the field

The time-course of root rot symptom expression under natural inoculation conditions was assessed in a field experiment established at Galong (650 mm annual rainfall) in NSW in 2004. In June, seed of cv. Grace (RR 6.5) was sown in three replicate plots (8 m×2 m). Plants were assessed for root rot and crown canker severity at stem elongation, first flower, full flower, pod fill and maturity. A random sample of 20 plants from each plot (total 60 plants) at each time of assessment was scored for root rot and crown canker severity as described above.

Assessment of root rot in fumigated field plots

Field experiments using fumigation treatments were established in 2003 at Wallendbeen (650 mm annual rainfall) and Ardlethan (450 mm annual rainfall) to determine the role of soilborne inoculum in the infection of *B. napus* roots. The experiments were a random block design with three blocks containing randomised combinations of cultivar and soil treatment (fumigated or control) in plots 8 m× 2 m. Soil was fumigated to a depth of 25 cm using methyl bromide (50 g m^{-2}) injected into plots under plastic and left for three days. After removal of the plastic, plots remained undisturbed for 2 weeks before sowing. Cultivars Hyola 60 (RR 9.0) and Rainbow (RR 6.5) were sown at a rate of 3.5 kg ha^{-1} in June. As plants were beginning to emerge at Wallendbeen (16 days after sowing and 27 days after fumigation), soil cores (0 to 10 cm) were collected from fumigated and control plots. Ten soil cores taken at random from each plot were combined and a 500 g sub-sample was oven-dried at 40°C for 72 h and sent to the South Australian Research and Development Institute Root Disease Testing Service, where the quantity of *L. maculans* DNA present was determined as described by Sosnowski et al. (2006).

Growth and disease assessments were carried out at the early seedling stage at Wallendbeen, and at plant maturity at both Wallendbeen and Ardlethan. At the cotyledon stage, 15 seedlings were carefully removed at random from each plot, washed and assessed for symptoms of root disease. The plant density (plants m^{-2}) when plants were at the two to four-leaf stage, was recorded for each plot by counting the number of plants along an 8 m row. Fifty seedlings from each plot were assessed for the incidence of leaf lesions. In addition, due to differences in seedling development between the fumigated and control plots, hypocotyl length, leaf area and above-ground biomass were measured for 12 plants taken at random from each plot at the two to four-leaf stage. At plant maturity, 20 individual plants from each plot at both sites were scored for incidence and severity of internal infection at the base of the stem and in the roots, as described previously.

Inoculation of Brassica cultivars with *L. maculans* isolates

A pot experiment was conducted in the glasshouse to determine whether *L. maculans* isolates varied in their ability to cause root rot, and whether the type of host tissue (leaves, stubble and root tissue) from which isolates were cultured affected this ability. *Leptosphaeria maculans* isolates were collected from diseased root, leaf and stem tissue from various locations in Australia (Table 1). Each isolate (32) was grown on 10% Campbell's V8 juice agar and pycnidiospores were collected after 14 days by gentle agitation with a small volume of SDW and spore concentration was adjusted to 10^7 spores ml^{-1}.

Pots (150 mm diam×300 mm deep) of pasteurised potting medium (compost containing recycled soil, leaf mulch, vermiculite, peat moss, river loam, perlite, and river sand with additions of lime and blood and bone; steam-pasteurised at 70°C for 45 min) were sown with seed of three Brassica cultivars: *B. napus* cv. Q2 (RR 2.5, a highly susceptible Canadian line), *B. napus* cv. Sapphire (RR 7.5) and *B. juncea* line Zem 1 (Marcroft et al. 2002). *Brassica juncea* was used as this species is more resistant to *L. maculans* than *B. napus* (Ballinger and Salisbury 1996; Purwantara et al. 1998; Marcroft et al. 2002). Plants were grown in a glasshouse (20/15°C±2°C day/night) and were inoculated 5 weeks after sowing when they were at the four-leaf growth stage. Each plant was inoculated by stabbing the stem at the junction between the stem and the cotyledons with a sterile toothpick dipped in pycnidiospore suspension, or SDW (Sosnowski et al. 2001). This method was used in an attempt to avoid any inherent genetic resistance of the cultivars that might inhibit growth of

Table 1 Isolates of *L. maculans* collected from different tissues of *B. napus* at several locations across southeastern Australia

Isolate	Tissue	Source	Year isolated	Location	Collector
IBCN18	Stubble	Ascospores	1986	Millicent, SA	P. Salisbury
167/00b	Roots	Hyphae	2000	Bendigo, VIC	M. Sosnowski
208/00	Roots	Hyphae	2000	Bordertown, SA	M. Sosnowski
211/00	Roots	Hyphae	2000	Edillilie, SA	M. Sosnowski
212/00	Roots	Hyphae	2000	Wanilla, SA	M. Sosnowski
222/00	Roots	Hyphae	2000	Wonwondah, SA	M. Sosnowski
6/01	Roots	Hyphae	2000	n/a, NSW	M. Sosnowski
Lm 16	Stubble	Ascospores	2001	Wonwondah, VIC	H. Hayden
Lm 21	Stubble	Ascospores	2001	Wonwondah, VIC	H. Hayden
Lm 30	Stubble	Ascospores	2001	Wonwondah, VIC	H. Hayden
Lm 59	Leaf	Pycnidiospores	2001	Wonwondah, VIC	H. Hayden
Lm 66	Leaf	Pycnidiospores	2001	Wonwondah, VIC	H. Hayden
Lm 79	Leaf	Pycnidiospores	2001	Wonwondah, VIC	H. Hayden
Lm 84	Leaf	Pycnidiospores	2001	Wonwondah, VIC	H. Hayden
Lm 103	Leaf	Pycnidiospores	2001	Wonwondah, VIC	H. Hayden
Lm 111	Leaf	Pycnidiospores	2001	Wonwondah, VIC	H. Hayden
Lm 716	Stubble	Ascospores	2003	Lockhart, NSW	H. Hayden
Lm 718	Stubble	Ascospores	2003	Lockhart, NSW	H. Hayden
Lm 720	Stubble	Ascospores	2003	Lockhart, NSW	H. Hayden
Lm 732	Stubble	Ascospores	2003	Lockhart, NSW	H. Hayden
Lm737	Stubble	Ascospores	2003	Lockhart, NSW	H. Hayden
Lm750	Stubble	Ascospores	2003	Grenfell, NSW	H. Hayden
Lm 757	Stubble	Ascospores	2003	Grenfell, NSW	H. Hayden
Lm 1/04b	Roots	Pycnidiospores	2003	Lockhart, NSW	S. Sprague
Lm 2/04a	Roots	Pycnidiospores	2003	Ardlethan, NSW	S. Sprague
Lm 2/04b	Roots	Pycnidiospores	2003	Ardlethan, NSW	S. Sprague
Lm 3/04a	Roots	Pycnidiospores	2003	Dirnaseer, NSW	S. Sprague
Lm 3/04b	Roots	Pycnidiospores	2003	Dirnaseer, NSW	S. Sprague
Lm 4/04b	Roots	Pycnidiospores	2003	Wallendbeen, NSW	S. Sprague
Lm 5/04a	Roots	Pycnidiospores	2003	Galong, NSW	S. Sprague
Lm 5/04b	Roots	Pycnidiospores	2003	Galong, NSW	S. Sprague
Lm 5/04c	Roots	Pycnidiospores	2003	Galong, NSW	S. Sprague

n/a = unknown

the fungus from the leaf into the stem and hypocotyl. Pots were covered for three days with clear plastic to maintain high humidity. Two pots containing three plants of each cultivar were inoculated with each isolate (total of six plants/cultivar/isolate). Plants were fertilised and watered as required. At maturity, each plant was individually assessed for the severity of root rot. Plants were washed to remove the potting medium then cut transversely at the base of the stem. The root was cut longitudinally and root rot severity was scored on a scale of S0 to S5 as described previously.

Statistical analyses

All analyses were performed using Genstat version 10 (Payne et al. 2007). Root rot severity data were analysed using ordinal regression. The results from this analysis are presented as the modal root rot severity score which is the root rot score with the highest associated probability for a particular treatment. Correlation analyses were performed on selected data. Correlations between root rot and crown canker severity were analysed using the gamma statistic for ordinal data.

Results

Incidence and severity of root rot in commercial oilseed rape crops

In 2003, 86% of fields surveyed had mature plants with root rot while in 2004 and 2005 all fields had infected plants. While root rot severity was generally low in each year, some individual fields had high levels of root rot (Fig. 2a–c). The average incidence of root rot was 42% in 2003, 49% in 2004 and 72% in 2005 (Fig. 2d–f). In each year, *L. maculans* was consistently isolated from segments of diseased root tissue, as well as from root tissue that did not appear diseased (data not shown). Eleven of the 127 fields surveyed that had never been sown to oilseed rape had plants with root rot; the incidence ranged from 5 to 80%.

In general, cultivars with a RR <8.0 had a higher incidence of root rot and plants with severe root rot (S4–S5) had a higher incidence than those cultivars with a RR≥8.0 (Table 2). The three cultivars with RR ≥8.0 all had major gene resistance. Of the 18 fields sown to cultivars with major gene resistance in 2003, six had >20% of plants with crown canker indicating that the resistance may have been overcome in these fields. Two fields had plants with an average crown canker severity of 13 and 17%, while all other fields had between 0 and 6%. Three of the fields with >20% of plants with crown cankers had >20% of plants with root rot; however, the incidence of severe root rot was <2% in these fields.

Development of root rot in the field and the association with crown canker

In the survey of commerical crops, root rot increased in incidence and severity from flowering to swathing in 2003. The percentage of fields that had plants with root rot increased from 81% at flowering to 100% at swathing. The average incidence of root rot per field increased from 14% to 65%, while the incidence of severe root rot increasd from 0 to 20%, indicating that disease symptoms appear prior to flowering and become more severe as the plant matures. The level of infection in the stem followed a similar pattern (data not shown).

In the time-course study of the development of disease symptoms caused by *L. maculans*, the progression of root rot and crown canker during plant development followed a similar pattern. Disease severity was low at stem elongation, then increased slowly during flowering and rapidly as the plant matured (Fig. 3). Disease incidence of both crown canker and root rot followed a similar pattern with a rapid increase at maturity (Fig. 4). Disease pressure was high at Galong with 60% of plants with one or more leaf lesions at the cotyledon to one-leaf stage and 81% at the two to four-leaf stage.

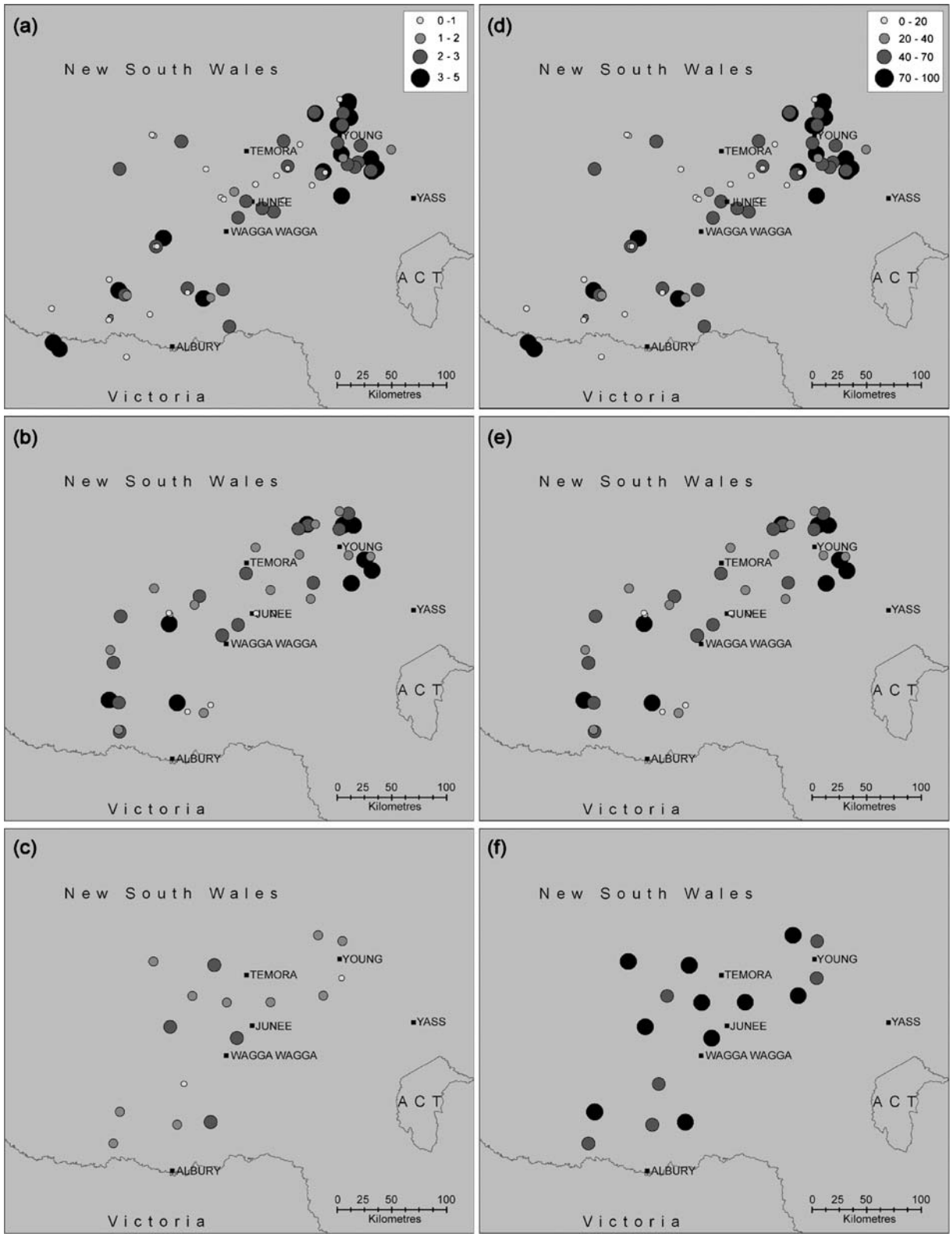
Necrotic tissue associated with root rot was most often observed in the inner tissues of *B. napus* roots but its location along the root was variable. The inner tissues of *B. napus* roots are principally composed of secondary xylem and xylem parenchyma (McCully et al. 2008). In some plants, root rot appeared as an extension of crown canker with necrotic tissue extending from the crown downwards into the tap root. However, root rot was also observed in the lower portions of the taproot and/or primary laterals, separated from disease in the crown by apparently healthy root tissue. Only root rot in the taproot was scored in this study. On occasion, root rot was associated with lesions on the root surface.

There was a significant positive relationship between the severity and incidence of crown canker and root rot in the fields surveyed in all years (Fig. 5a–c, d–f). The strength of the relationship varied between years, indicating that environmental factors play a role in determining disease development and its expression.

Pathway of root infection in the field

Leptosphaeria maculans was below detectable levels in soil collected from fumigated plots at Wallendbeen, but untreated plots had *L. maculans*-specific DNA ranging from below detectable levels to 1203 pg g^{-1} soil with a mean of 313 pg g^{-1} soil. The incidence of leaf lesions on seedlings in the fumigated and untreated plots was similar with a mean of 36%; <1% of plants of cv. Hyola 60 had lesions compared with cv. Rainbow, which had an average of 71% of

Fig. 2 Sites of *B. napus* crops surveyed for root rot severity in 2003 (**a**), 2004 (**b**) and 2005 (**c**) and incidence in 2003 (**d**), 2004 (**e**) and 2005 (**f**). The number of fields sampled was 67 in 2003, 43 in 2004 and 17 in 2005


(a)
0 - 1
1 - 2
2 - 3
3 - 5
New South Wales
YOUNG
TEMORA
JUNEE
YASS
WAGGA WAGGA
A C T
ALBURY
Victoria
0 25 50 100
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(d)
0 - 20
20 - 40
40 - 70
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New South Wales
YOUNG
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Table 2 Effect of *L. maculans* RR on the incidence of root rot and the incidence of plants with severe root rot (root rot score S4 or S5) in commercial crops of *B. napus* surveyed in southern New South Wales and northern Victoria between 2003 and 2005

RR	Average plants infected/field (%)			Average plants with severe root rot/field (%)		
	2003	2004	2005	2003	2004	2005
5.0			89 (2)			21
5.5	37±17 (6)	47 (2)	78 (1)	16±8	3	12
6.0	56±6 (11)	52±7 (16)	66±4 (8)	18±6	8±2	6±2
6.5	61±4 (31)	48±8 (12)	74±10 (4)	15±3	9±3	16±6
7.0		58±7 (12)	73 (2)		11±4	18
7.5		32 (1)			3	
8.0[a]	14±6 (14)			2±1		
8.5[a]	7 (2)			0.2		
9.0[a]	11 (2)			0.1		

[a] Cultivars with major gene resistance to *L. maculans* derived from *B. rapa* subsp. *sylvestris*. These cultivars were not grown in 2004 and 2005.

Values are mean ± SE. Values in parentheses indicate number of fields sampled.

plants infected. At maturity, very low levels of root rot and crown canker were observed in cv. Hyola 60 compared to cv. Rainbow (data not shown), and hence only the data collected for cv. Rainbow were used to analyse differences between fumigation treatments. The incidence of severe root rot (S4–S5) was greater at Wallendbeen (45%) compared to Ardlethan (20%) but was similar in both fumigated and untreated plots at both sites, indicating that under field conditions soil inoculum does not play a major role in the development of root rot. There was no effect of fumigation on crown canker severity (data not shown).

Variation in the ability of *L. maculans* isolates to cause root rot

All isolates of *L. maculans* tested in glasshouse experiments produced root rot symptoms in each of the *B. napus* cultivars and *B. juncea*, while control plants inoculated with SDW had no symptoms (Table 3). There were significant differences ($P < 0.001$) in the ability of isolates to cause root rot symptoms. Despite these differences, a similar mean level of root rot severity was caused by isolates collected from leaves, roots or stubble (data not shown).

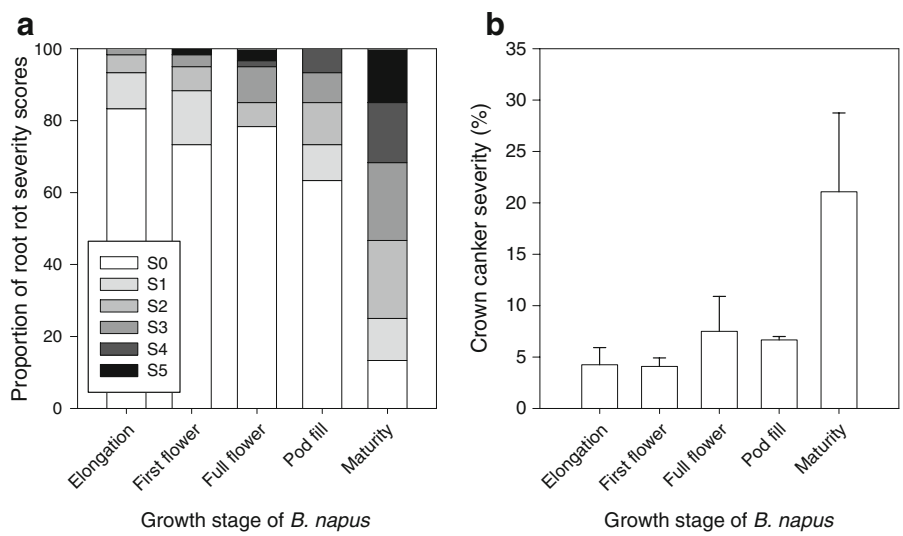

Fig. 3 Changes in proportion of root rot severity scores (**a**) and crown canker severity (**b**) in *B. napus* cv. Grace at several plant growth stages at Galong, New South Wales in 2004. Crown canker severity values are mean ± SE. n=60

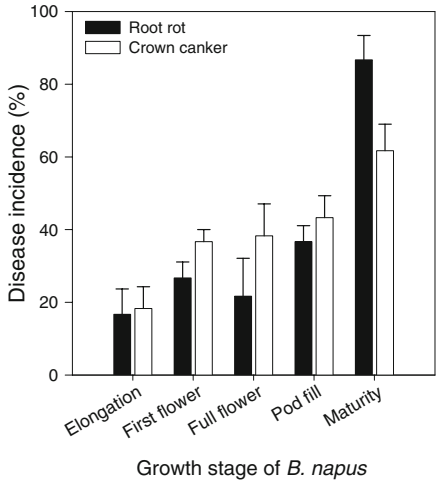

Fig. 4 Incidence of root rot and crown canker in *B. napus* cv. Grace at several plant growth stages at Galong, New South Wales in 2004. Values are mean ± SE. $n=3$

There were significant differences in the severity of root rot between the three cultivars examined but the severity of root rot did not vary between different isolate and cultivar combinations (Table 3). For each isolate, the severity of root rot was generally highest in *B. napus* cv. Q2, moderate in *B. napus* cv. Sapphire and lowest in *B. juncea*. There was a significant positive relationship between root rot and crown canker severity in each of the three cultivars inoculated (Fig. 6). The relationship in *B. juncea* differed from that in the *B. napus* cultivars whereby the increase in crown canker severity with increased root rot severity in *B. juncea* was less than in the *B. napus* cultivars. Only five of the almost 200 inoculated plants of *B. juncea* had crown canker >20% and three of these plants were inoculated with IBCN18.

Discussion

Root rot of oilseed rape occurred in all years and was widespread across the area where the survey and field experiments were conducted in south-eastern Australia. Disease levels were generally low, probably associated with the relatively late maturation of pseudothecia resulting from dry summers and late autumn rain. The finding that root rot was widespread in commercial crops is consistent with previous surveys conducted in Australia (Hind-Lanoiselet et al. 2003) and in the UK (Evans et al. 2003) indicating that root rot is part of the *L. maculans* lifecycle in all areas where this pathogen infects *B. napus*. *Leptosphaeria biglobosa* as has also been identified in roots of *B. napus* from plants collected in England and France (West et al. 2002b). Although necrosis of root tissue has been associated with premature senescence of *B. napus* (Hornig 1985; Brun and Jacques 1991; Hind-Lanoiselet et al. 2003), premature senescence was rarely observed in this survey, even in plants with severe root rot, suggesting that these symptoms may be caused by a pathogen other than *L. maculans,* or specific environmental conditions.

Root rot occurred in fields that had never previously been sown to oilseed rape and in field experiments in which inoculum was eliminated by fumigation confirming that the root rot occurs via foliar infection under natural conditions of inoculation. This pathway of infection was also confirmed by the pot experiments with pasteurised potting medium where spore suspension applied to stems resulted in the development of root rot symptoms similar to those observed in the field. This is consistent with our previous findings, whereby infection of roots occurred from a foliar source of inoculum (Sprague et al. 2007). This conclusion does not discount the possibility that *L. maculans* may opportunisitically infect oilseed rape through the soil. Potential sources of inoculum include infected stubble, which can remain intact for a number of years (Marcroft et al. 2004), infected root residues, which remain intact in biopores in the soil (Cresswell and Kirkegaard 1995; Watt et al. 2005) and airborne spores, which land on the soil or are released from crop residues (Li et al. 2007). Indeed, Sprague et al. (2007) demonstrated that under laboratory conditions, *L. maculans* was able to infect intact roots of oilseed rape. In the field, *L. maculans* may infect roots from soil inoculum opportunistically, but the overwhelming source of inoculum appears to be airborne ascospores.

The incidence and severity of root rot differed between cultivars with polygenic and major gene resistance. Three of the 15 oilseed rape cultivars assessed in the survey had major gene resistance conferred by *B. rapa* subspecies *sylvestris*. In the majority of fields sown to these cultivars, the level of root rot was extremely low while the levels were much higher in cultivars with polygenic resistance. This finding is consistent with results from the fumigation experiments where cv. Hyola 60 (major

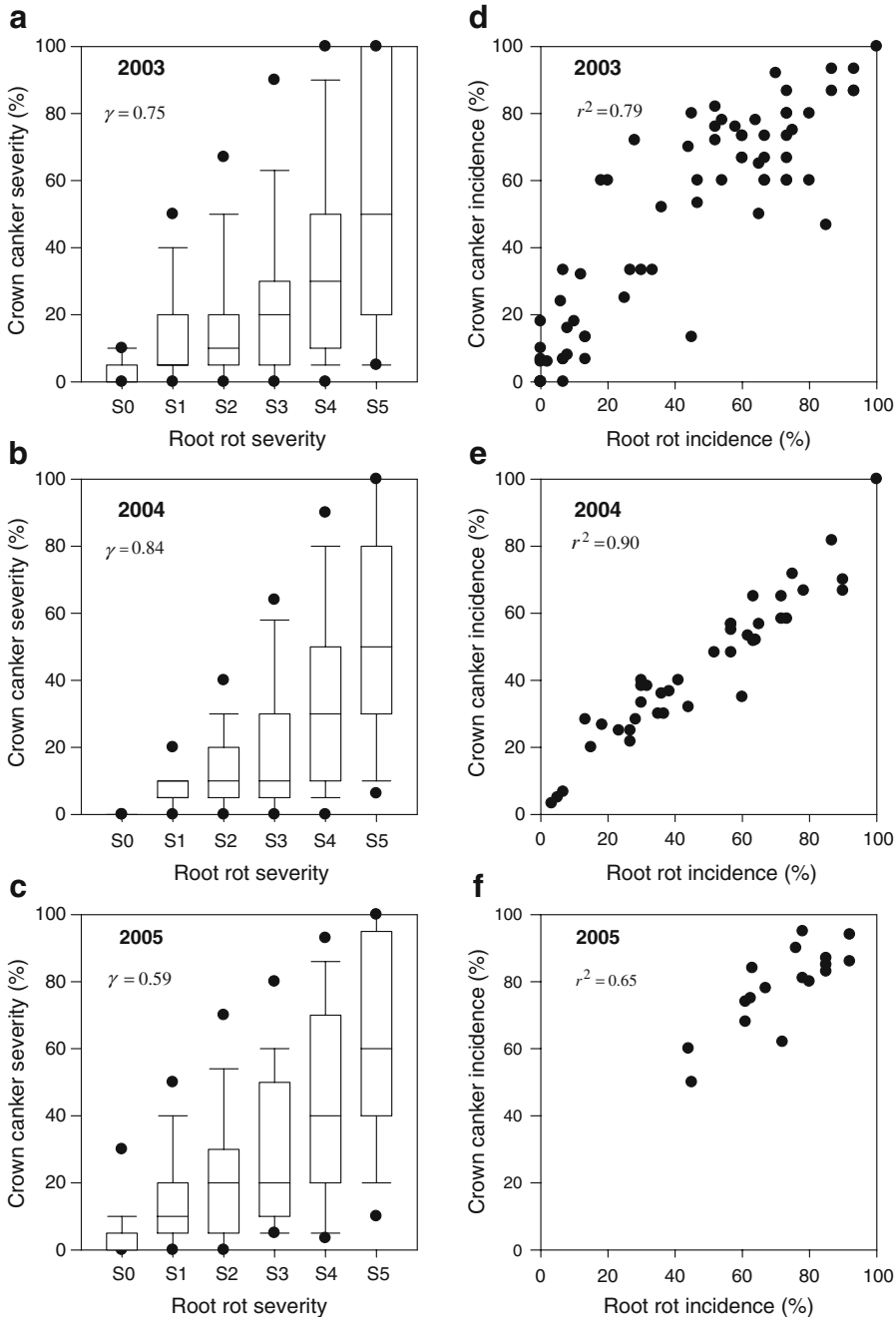

Fig. 5 Relationship between the severity of root rot and crown canker in 2003 (**a**), 2004 (**b**) and 2005 (**c**), and the incidence of root rot and crown canker in 2003 (**d**), 2004 (**e**) and 2005 (**f**) in commercial crops of *B. napus* surveyed in southern New South Wales and northern Victoria. The whiskers on plots **a**–**c** represent the 10th and 90th percentiles with outliers represented by the black circles

gene resistance) had little disease compared to cv. Rainbow (polygenic resistance). However, an increased incidence of crown canker was observed in some fields with cultivars containing the major gene, indicating that this resistance had been overcome at these locations. Root rot severity tended to be greater in these fields than in those fields with low levels of crown canker. Given that the presence of the major gene from *B. rapa* subspecies *sylvestris* inhibits spore germination and fungal penetration on the leaf surface (Li et al. 2004; Sosnowski et al. 2004), this result supports our interpretation of the field and pot experiments that fungal invasion of roots is initiated by foliar infection and not via soilborne inoculum.

Although there was a trend of lower root rot severity and incidence in Australian *B. napus* cultivars with a

Table 3 The modal root rot severity score for isolates of *L. maculans* inoculated on *B. napus* cvs Q2 and Sapphire, and *B. juncea* cv. Zem1 (n=6)

Isolate	Root rot severity		
	Q2	Sapphire	Zem1
Lm 021	5	3	1
Lm 1/04b	5	3	1
Lm 103	5	3	1
Lm 111	3	2	1
Lm 16	5	3	1
Lm 167/00b	5	3	1
Lm 2/04a	3	2	1
Lm 2/04b	3	2	1
Lm 208/00	5	5	3
Lm 211/00	3	2	1
Lm 212/00	3	2	1
Lm 222/00	5	3	2
Lm 3/04a	5	5	3
Lm 3/04b	3	2	1
Lm 30	3	2	1
Lm 4/04b	3	2	1
Lm 5/04a	5	3	2
Lm 5/04b	5	3	1
Lm 5/04c	5	3	1
Lm 59	5	5	2
Lm 6/01	2	1	1
Lm 66	5	5	2
Lm 716	5	5	2
Lm 718	5	5	3
Lm 720	5	3	1
Lm 732	5	3	1
Lm 737	5	3	2
Lm 750	5	3	1
Lm 757	3	2	1
Lm 79	5	5	3
Lm 84	5	3	1
IBCN 18	3	2	1

The main effect of isolate and cultivar were highly significant ($P < 0.001$) but there was no significant interaction.

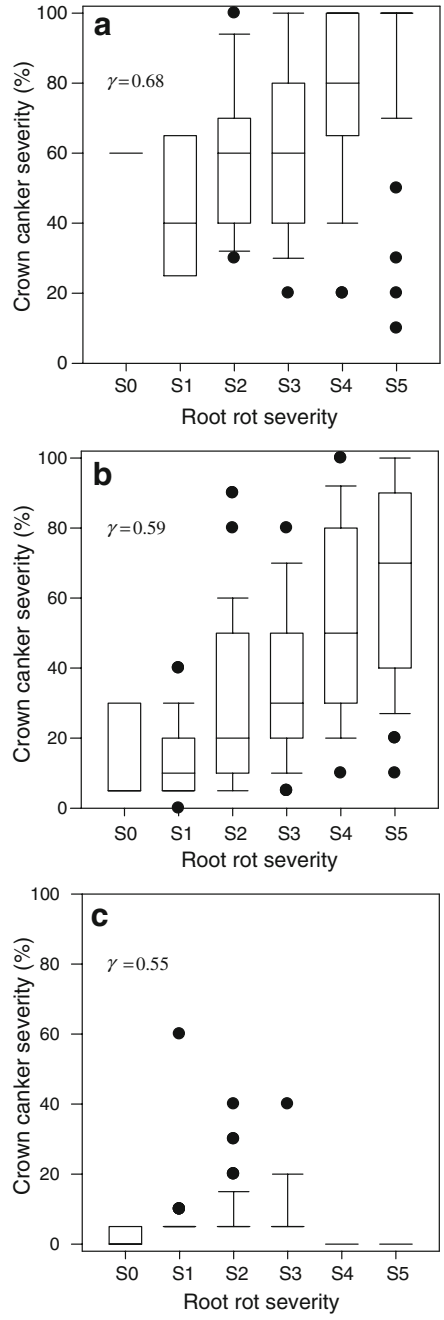

Fig. 6 Relationship between root rot and crown canker severity in *B. napus* cultivars Q2 (**a**) and Sapphire (**b**), and *B. juncea* cv. Zem1 (**c**) inoculated with individual isolates of *L. maculans*. The whiskers on plots **a**–**c** represent the 10th and 90th percentiles with outliers represented by the black circles

higher RR compared with lower-rated cultivars in commercial crops, it is difficult to draw conclusions on the relationship between RR and severity of root rot since inoculum load and environmental conditions vary widely between fields. However, in the pot experiment, root rot was more severe in cv. Q2, which is highly susceptible to crown canker compared with cv. Sapphire, which is highly resistant. The finding that crown canker and root rot severity are correlated in *B. napus* suggests that selection for resistance to canker may

also be effective against root rot, given that the main pathway of infection is from above-ground tissues. In contrast to *B. napus*, root rot in *B. juncea* inoculated in the glasshouse was present even when crown canker symptoms were negligible. This is consistent with findings of Keri (1991) in Canada who showed that many lines of *B. juncea* inoculated with individual isolates did not have symptoms in the stem but the roots were discoloured. Currently in breeding programmes for both *B. napus* and *B. juncea* in Australia, lines are screened for low levels of crown canker, but root symptoms are not assessed. This finding has particular relevance given the first commercial cultivar of canola-quality *B. juncea* was released in Australia in 2007. In addition, accessions of Brassica species such as *B. carinata* and *B. nigra* have been screened in Australia for crown canker resistance to identify novel types of resistance to *L. maculans* which can be introgressed into *B. napus* (Marcroft et al. 2002). While it appears that crown canker and root rot are correlated in Australian *B. napus* cultivars, further experiments testing resistance to root rot in *B. napus* cultivars from Europe and Canada, *B. juncea* and other Brassica species in the field are required to confirm this relationship in other host species.

The severity of root rot was low in plants until maturity. This observation is supported by Evans et al. (2003) who reported little infection of roots at stem extension or flowering in the UK and the cytological study of Sprague et al. (2007) where *L. maculans* was observed growing into roots at the onset of flowering. Sprague et al. (2007) reported discolouration of tissue ahead of the advancing infection front in the root which may explain the presence of symptoms in roots prior to flowering. Yield loss associated with crown canker severity >50% has been demonstrated (West et al. 2002a; Marcroft et al. 2004); however, the loss of function and reduced flow in xylem vessels due to root infection may also contribute to yield loss.

All isolates of *L. maculans* tested in this study were able to infect oilseed rape roots when pycnidiospores were applied directly into the crown, irrespective of the type of tissue from which isolates were collected. Although only a relatively small number of isolates (32) were tested, this finding in combination with the widespread nature of symptoms in commercial crops, suggests that most, and probably all, isolates have the ability to cause root rot. Although Australian isolates of *L. maculans* are generally more virulent than those in Europe or Canada (Balesdent et al. 2005), there was significant variability in the severity of root rot caused by different isolates. This finding is consistent with other studies that have shown variability in the pathogenicity of Australian *L. maculans* isolates on leaves at the seedling stage (Ballinger and Salisbury 1996) and on stems at the adult plant stage (Pang and Halloran 1995; Ballinger and Salisbury 1996).

In conclusion, root rot caused by *L. maculans* is widespread in commercial *B. napus* crops in southeastern Australia, and probably worldwide where *L. maculans* is found. It most likely arises following infection of above-ground tissues by airborne ascospores in the field as an extension of the crown canker phase of the disease. While our findings suggest that *B. napus* cultivars resistant to crown canker may also have lower levels of root rot, a wider range of cultivars and Brassica species needs to be assessed. The development of root rot in the absence of crown canker in *B. juncea* requires further study, particularly in regions such as Australia where cultivars with canola-quality seed will potentially be cultivated over large areas. Given the close relationship between crown canker and root rot, further *B. napus* studies are required to separate the effects of crown canker and root rot on yield.

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