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Gene-for-Gene resistance is expressed in cotyledons, leaves and pods, but not during late stages of stem colonisation in the *Leptosphaeria maculans*-*Brassica napus* pathosystem

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Abstract

The blackleg fungus, *Leptosphaeria maculans*, interacts with canola (*Brassica napus*) in a gene-for-gene manner. These major resistance genes are well characterised in the seedling stage of development, but not in other plant organs. Cotyledons, leaves, pods and stems of plants of two cultivars of *B. napus*, each harbouring a different major resistance gene (*Rlm1* and *Rlm4*) were inoculated with two individual *L. maculans* isolates with different alleles of the corresponding avirulence genes (*AvrLm1*, *avrLm4* and *avrLm1*, *AvrLm4*) and the disease phenotype in terms of lesion development was determined. Major gene resistance was expressed in cotyledons, all leaves and during pod set, but not in the stems of the adult plant. This is the first time major gene resistance has been shown to be effective in *B. napus* pods.

Keywords: Seedling resistance, major gene resistance, stem cankers, quantitative resistance, pod infection

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26 **Introduction**

27 *Leptosphaeria maculans*, which causes blackleg disease, is the major fungal pathogen of *Brassica*
28 *napus* (canola, oilseed rape) worldwide (Fitt et al., 2006; Sprague et al., 2006). The primary inoculum
29 is ascospores (sexual spores) released from sexual fruiting bodies on infested canola stubble, which
30 generally occurs during rainfall that coincides with the sowing period. Windborne ascospores land on
31 cotyledons and leaves, germinate and invade the plant via the stomatal aperture. However, in some
32 regions, such as Canada, asexual spores have also been proposed as a source of inoculum (Ghanbarnia
33 et al., 2009; Ghanbarnia et al., 2011). The fungus grows down the petiole, and invades and kills cells
34 of the stem cortex, resulting in a blackened canker that may completely girdle the base of the stem;
35 hence the name 'blackleg' (Chen and Howlett, 1996; Hammond and Lewis, 1987; Hammond et al.,
36 1985). Additionally the fungus can grow into the root where the cortex becomes blackened and rotten
37 (Sprague et al., 2007). Pod infections can occur when rainfall events coincide with pod development,
38 resulting in contaminated seed (West et al., 2001).

39 Control of blackleg disease is through management strategies such as crop rotation (Marcroft et al.,
40 2004), fungicide use (West et al., 2002) and stubble avoidance (Fitt et al., 2006; Marcroft et al., 2004),
41 as well as breeding for resistance (Delourme et al., 2006; Hayward et al., 2012). Two types of
42 resistance to blackleg disease are reported; minor gene (quantitative) and major gene (qualitative)
43 resistance (Delourme et al., 2006). Minor gene resistance, also referred to as adult plant resistance or
44 quantitative resistance, is assumed to involve numerous resistance genes, each contributing a small
45 additive effect. This type of resistance is generally poorly understood, and is thought to be expressed
46 in the stems of the adult plants (Delourme et al., 2006; Huang et al., 2009; Stuthmann et al., 2007).
47 Minor gene resistance has been reported to increase the durability of major gene resistance. In a
48 recurrent selection experiment, near-isogenic cultivars that had the major resistance gene *Rlm6* in the
49 presence or absence of minor gene resistance, were sown into blackleg-infested stubble of the a *Rlm6*
50 cultivar, each year for five years. The major gene resistance conferred by *Rlm6* was overcome within
51 three years of recurring selection in the absence of minor gene resistance. However, it was still
52 effective after five years when in combination with minor gene resistance (Brun et al., 2010;
53 Delourme et al., 2014). In the past, minor gene resistance has been considered isolate-non-specific,
54 i.e. the resistance is partially effective on all isolates (Stuthmann et al., 2007). However, recently
55 Marcroft et al., (2012b) suggested that minor-gene resistance can be isolate-specific. A set of isolates
56 all virulent towards the major resistance gene *Rlm4*, were each individually inoculated onto
57 cotyledons of cultivars harbouring *Rlm4*. These isolates were all fully virulent on cotyledons. The
58 infected plants were then grown through to maturity and assessed for disease severity at the crown. If
59 minor-gene resistance was partially effective against all isolates, it would be expected that all isolates
60 would cause a similar level of disease. However, the set of isolates caused a range of disease severity

61 levels from fully susceptible to fully resistant suggesting that minor gene resistance can be isolate-
62 specific, and that different resistance genotypes can be discriminated.

63 Major gene resistance in the *B. napus*-*L. maculans* interaction occurs in a race-specific ‘gene-for-
64 gene’ manner whereby a resistance gene in the host has a corresponding avirulence gene in the fungus
65 (Balesdent et al., 2005; Flor, 1955). Seventeen resistance genes (*Rlm1-11*, *RlmS*, *LepR1-4*, *BLMR1*,
66 *BLMR2*) have been reported (Balesdent et al., 2013; Delourme et al., 2006; Long et al., 2011; Van de
67 Wouw et al., 2009; Yu et al., 2005; Yu et al., 2008). Two of these major resistance genes, *LepR3* and
68 *Rlm2*, have been cloned (Larkan et al., 2015; Larkan et al., 2013). In *L. maculans*, ten avirulence
69 genes have been mapped with six cloned (*AvrLm1*, *AvrLm2*, *AvrLm4-7*, *AvrLm6*, *AvrLmJ1*, *AvrLmI1*)
70 (Balesdent et al., 2005; Balesdent et al., 2013; Fudal et al., 2007; Ghanbarnia et al., 2012; Ghanbarnia
71 et al., 2015; Gout et al., 2006; Parlange et al., 2009; Van de Wouw et al., 2014a).

72 Previous studies on expression of major gene resistance in *B. napus* have given conflicting results.
73 For example, in studies carried out to map major gene resistance in *B. napus* cultivar Major, Ferreira
74 et al. (1995) inoculated cotyledons and stems of double haploid lines generated from a cross with cv.
75 Major and found that the major gene resistance was expressed in both these organs and mapped to a
76 single locus. However, other studies have suggested that there is no relationship in the *L. maculans* -
77 *B. napus* interaction between seedling and adult resistance based on field observations and glasshouse
78 inoculations (Ballinger and Salisbury, 1996). Ballinger and Salisbury (1996) analysed 12 *B. napus*
79 lines by inoculating seedlings by spraying with conidia and assessing the amount of necrosis on
80 cotyledons and the degree of stem cankering at maturity. No correlation between seedling resistance
81 and adult plant resistance was observed. Finding a correlation between seedling resistance tested in a
82 glasshouse and adult plant resistance assessed through field observations will depend on a number of
83 factors including the genotypes of the isolates used in glasshouse experiments, the frequency of
84 avirulence alleles in the field populations and the type of resistance present in the cultivars being
85 tested.

86 In some regions of Australia, canola is grown as a dual purpose crop whereby the plants are grazed
87 by sheep or cattle and then allowed to flower and set seed for harvest later in the season (Kirkegaard
88 et al., 2008; Sprague et al., 2014). Such plants have wounded/damaged stem tissue that is exposed to
89 airborne ascospore inocula released from stubble after rain events and thus it is important to be able to
90 predict in which organs and growth stages major gene resistance is expressed. In this paper we
91 investigate the sites of expression of major gene resistance in the *B. napus*-*L. maculans* interaction in
92 organs (cotyledons, leaves and pods) that the fungus infects during the Australian canola-growing
93 season. We also assess infection in mature stems when minor plant resistance is assumed to be
94 effective. We also discuss implications of our findings on canola breeding and blackleg disease
95 management strategies.

97 **Materials and Methods**

98 ***Leptosphaeria maculans* isolates:** *Leptosphaeria maculans* isolates D13 and D14 were used to screen
99 for expression of resistance conferred by the major resistance genes *Rlm1* and *Rlm4* in several organs
100 of *B. napus*. Isolate D13 (genotype *avrLm1*, *AvrLm4*) is virulent towards *Rlm1* but avirulent towards
101 *Rlm4* in cotyledons, and isolate D14 (genotype *AvrLm1*, *avrLm4*) is avirulent towards *Rlm1* and
102 virulent towards *Rlm4* in cotyledons (Marcroft et al., 2012b).

103
104 ***Brassica napus* cultivars:** Cultivar AV-Garnet harbours resistance gene *Rlm1* and CB-Telfer harbours
105 resistance gene *Rlm4* (Marcroft et al., 2012b). Cultivar Westar, which has no resistance genes, was
106 used as a susceptible control. All plants were grown in a polyhouse through the normal Australian
107 canola-growing season (July through November) with natural lighting and no temperature control.

108
109 **Inoculation of *B. napus* with *L. maculans*:** Experiments were established to assess whether major
110 gene resistance was expressed at the cotyledon, leaf, pod and stem growth stages. These stages are
111 expressed in terms of the phenological appearance and also on the BBCH (BASF, Bayer, Ciba-Geigy
112 and Hoechst) decimal codes for growth stages scale for *B. napus* (Weber and Bleiholder, 1990).
113 Conidial spore suspensions were made to a final concentration of 5×10^6 spores/ml with 0.5% Tween
114 20 (BDH Chemicals, UK) for both isolate D13 and D14. All organ types were wounded using a 25
115 gauge needle and then inoculated with 10 μ l of the spore suspension. At each growth stage tested,
116 cotyledons (BBCH 10) of cultivars Westar, AV-Garnet and CB Telfer were inoculated as a positive
117 control for conidia viability.

118 To assess expression of resistance in leaves, 12 plants of each cultivar were grown to the four leaf
119 stage (BBCH 15) and then all four leaves of each plant were inoculated. Each side of the main vein
120 was wounded; isolate D13 was always inoculated on the left hand side while isolate D14 was always
121 inoculated on the right hand side when looking at the adaxial side of the leaf. Disease severity data
122 were recorded for each of the first four leaves. To assess whether resistance was also expressed in
123 older leaves, 12 plants of each cultivar were grown and when plants were between the 4-8 leaf stages
124 (BBCH 15-19), all leaves were inoculated with each isolate. Disease severity data were collected
125 without recording leaf number, as differences in maturity between plants made it too difficult to
126 assign growth stages to the leaves.

127 Expression of resistance during the pod set growth stage was assessed in four plants of each
128 cultivar. When plants were at the pod set growth stage (BBCH 72-78), pods were wounded with a 25
129 gauge needle three times, at sites spread evenly across the length of the pod. Each pod was inoculated
130 with a single isolate, with 10 μ l of the spore suspension placed on each wound. For each plant, an
131 entire branch of pods was inoculated with a single isolate. Pod number for each branch varied from
132 between 6 - 23. Total numbers of pods evaluated for each interaction were: 42 for the AV-Garnet

/D13 interaction, 54 pods for the AV-Garnet /D14 interaction, 63 pods for the CB Telfer/D13 interaction and 64 for the CB Telfer/D14 64 interaction.

To assess stem infections, 12 plants were grown until flowering/pod set (BBCH 65-78) and stems were wounded at the axial of the oldest two leaves and inoculated with 10 µl of spore suspension of an individual isolate.

No plants were used to assess expression of resistance in multiple organs; separate plants were used.

Disease assessment: Disease severity was assessed on cotyledons at 17 days post inoculation (dpi) on a scale from 0 (no darkening around wounds, as in water only controls) to 9 (large gray-green lesions with profuse sporulation) (Koch et al., 1991). On cotyledons, a median score of ≤ 3 is considered avirulent whilst a median score of ≥ 5 is considered virulent. For leaves, a 0 – 4 scale was used at 21 dpi; 0 indicated no lesions, 1 indicated lesions < 1 mm in length, 2 indicated lesions 1-5 mm in length, 3 indicated lesions 5-10 mm in length and 4 indicated lesions > 10 mm in length. Median scores ≤ 1.0 were considered an avirulent reaction by the isolate while scores >1.0 were considered virulent. For pods, isolates that formed lesions > 2 mm diameter at 21 dpi were considered virulent, while those forming lesions < 2 mm diameter were considered avirulent. Data were presented in two ways, average percentage pod infection and average percentage pod lesion development. Percentage pod infection refers to the percentage of pods that had at least one virulent reaction (lesion > 2mm diameter) from the three inoculation sites. Percentage pod lesion development refers to the percentage of lesions that developed from the total number of inoculation sites; this parameter was measured as although each pod was wounded three times, not all infection sites resulted in lesions. The percentage of pod infection and pod lesion development were determined for each of the four individual plants inoculated for each cultivar and the average overall values presented. Disease severity in stems was assessed as previously described (Marcroft et al., 2012a). Briefly, plants were cut at the crown and the percentage of internal infection (blackening) was visually assessed at eight weeks post inoculation.

All data collected from leaves, stems and pods were analysed using a one-way Analysis of Variance using GenStat (16th Edition). For each organ type, the appropriate parameter was compared between isolates on a single cultivar. All p-values less than 0.05 were considered significant.

Results

Gene-for-gene resistance is expressed in cotyledons and leaves

Cotyledons of cultivars AV-Garnet, CB Telfer and Westar were inoculated with isolates D13 and D14 to confirm the gene for gene interactions at the seedling stage between avirulence genes *AvrLm1* and *AvrLm4*, and major resistance genes *Rlm1* and *Rlm4*, respectively (Fig. 1). As expected, isolate D13

(genotype *avrLm1*, *AvrLm4*) was virulent towards AV-Garnet (*Rlm1*) (median disease severity score >5) and avirulent towards CB Telfer (*Rlm4*) (median disease severity score <3). Conversely, isolate D14 (genotype *AvrLm1*, *avrLm4*) was avirulent towards AV-Garnet (*Rlm1*) and virulent towards CB Telfer (*Rlm4*). Both isolates were virulent towards the susceptible control, Westar (no resistance genes).

The first four leaves (BBCH15) were inoculated and disease severity determined. Isolate D13 had an average disease severity score >1 on each leaf of AV-Garnet and was thus considered virulent. This isolate had an average disease severity score <1.0 on each leaf of CB Telfer, and thus was considered avirulent. Isolate D14 was virulent on CB Telfer and avirulent on AV-Garnet (Fig. 2). The average disease scores were statistically analysed between isolate D13 and D14 for each cultivar. All values were significantly different, ranging from $0.0001 < p < 0.02$ (Table 1). Average disease severity scores were lower on leaves 3 and 4 than on leaves 1 and 2. On the younger, waxier leaves (leaves 3 and 4), fewer lesions developed than on older leaves (1 and 2) as the conidial suspensions often dispersed quickly. Furthermore leaves of AV-Garnet were much waxier than those of CB Telfer. Regardless of these limitations, virulent reactions were only ever observed in the D13/AV-Garnet and D14/CB Telfer interactions.

After the four leaf growth stage, the two cultivars grew at different rates making recording of leaf age difficult. All leaves present at the 4-8 leaf growth stages were inoculated with the two isolates. Disease severity scores were measured and the average for the pooled data determined (Fig. 3 and Table 1). As seen for cotyledons and leaves 1-4, isolate D13 was virulent (disease severity score >1) on AV-Garnet and avirulent on CB Telfer (disease severity score <1) while the converse was seen for isolate D14. Average disease severity scores were significantly different between isolate D13 and D14 on both AV-Garnet ($p < 0.0001$) and CB Telfer ($p < 0.0001$) (Table 1).

‘Gene-for-gene’ resistance is expressed during pod set

Pods of AV-Garnet and CB Telfer were inoculated with either isolate D13 or D14 (Fig. 4 and Table 1). At least one lesion (percentage pod infection) developed on 49% of the AV Garnet pods and on only 16% of the CB Telfer pods when inoculated with isolate D13. Conversely, the percentage of pod infection for CB Telfer inoculated with isolate D14 was 81% and only 17% for AV-Garnet (Fig. 4). These values were significantly different for both AV-Garnet ($p < 0.0001$) and CB Telfer ($p < 0.0001$) (Table 1). The percentage of lesion development was 23% on AV-Garnet and only 6% on CB Telfer for isolate D13. For isolate D14, the percentage of lesion development was only 6% on AV Garnet and 39% on CB Telfer. These values were significantly different for both AV-Garnet ($p = 0.0029$) and CB Telfer ($p < 0.0001$). Furthermore, isolate D13 colonised the interior of pods of AV-Garnet (Fig. 4a) and isolate D14 colonised the interior of pods of CB Telfer (Fig. 4d). Although some virulent lesions were observed on some AV-Garnet pods inoculated with D14 (Fig. 4b) and some CB Telfer pods inoculated with D13 (Fig. 4c), these lesions were always small and restricted (between 2

and 5 mm diameter) and neither mycelia nor contaminated seeds were observed within the pods. A second experiment was carried out in which four plants per cultivar and a maximum of 25 pods were assessed. Results were consistent between the two experiments (data not shown).

Gene-for-gene resistance is not expressed during stem colonisation

Stems of AV-Garnet and CB Telfer were inoculated with either isolate D13 or D14 and eight weeks later disease severity was assessed (Fig. 5 and Table 1). Both isolates D13 and D14 colonised stems of CB Telfer but neither isolate colonised stems of AV-Garnet. No significant differences were detected ($p=0.736$ for AV-Garnet and $p=0.574$ for CB Telfer, see Table 1). A second experiment was carried out where four plants per cultivar were assessed. Results were consistent between the two experiments (data not shown). These data suggest that the gene-for-gene interaction is not effective during colonisation of the stem.

Discussion

In the current study, we show that major gene resistances conferred by *Rlm1* and *Rlm4* genes in the *L. maculans* - *B. napus* interaction appear to be expressed in cotyledons, leaves and pods, but not during late stages of stem colonisation. As mentioned earlier, conflicting results exist regarding the expression of major gene resistance in *B. napus* (Ballinger and Salisbury, 1996; Ferreira et al., 1995). Many of these earlier experiments were carried out with isolates and/or cultivars where the virulence and/or resistance genotypes were poorly understood. For example, Ferreira et al. (1995) used cv. Major to look at resistance in both seedlings and adult plants; however, it was unknown whether this cultivar harboured a single or multiple resistance genes. Knowledge of the identity of the resistance genes in a cultivar is essential for investigating the expression of resistance so that appropriate isolates can be selected to screen for resistant and susceptible reactions. Since these early studies, molecular methods for determining the genotypes of both host and pathogen have become readily available (Balesdent et al., 2005; Marcroft et al., 2012b; Rouxel et al., 2003). In the current study we have used two well characterised isolates that differ in their ability to infect cultivars with *Rlm1* or *Rlm4* major gene resistance, which has allowed us to make conclusions about the expression of major gene resistance in different plant organs of canola.

In other plant-pathogen interactions major gene resistance is often referred to as seedling-resistance as it is usually assessed at an early stage of development (Ellis et al., 2014; Stuthmann et al., 2007). However, in several plant-pathogen interactions, major gene resistance is expressed throughout the life of the plant: for example, *Puccinia* spp. and wheat (Ellis et al., 2014), *Pyrenophora teres* f. *teres* and barley, and *Cochliobolus sativus* and barley (Steffenson et al., 1996) and *Xanthomonas oryzae* pv. *oryzae* and rice (Century et al., 1999). In some cases a resistance gene

may only be effective at the adult stage and not the seedling stage. For instance, *Lr34*, an adult plant resistance gene of wheat conferring major (but not complete) resistance to several fungal pathogens has been cloned (Krattinger et al., 2009). In tomatoes with the major resistance *Cf-9B* gene, which confers resistance towards the tomato wilt fungus *Cladosporium fulvum*, the resistant reaction becomes more effective as the plant matures (Panter et al., 2002).

Our findings suggest that the term 'seedling resistance', which is often used to describe major-gene resistance, is inappropriate in the *L. maculans*-*B. napus* interaction; we suggest that the term 'major gene resistance' should be used instead. Breeding programmes in Australia select for blackleg resistance by screening canola lines in blackleg nurseries, which are field sites where canola is sown directly into the stubble of the previous year's crop to create high disease pressure. Recently breeders have also begun to use the ascospore shower method whereby seedlings are exposed to showers of ascospores released from blackleg-infested canola stubble in shadehouses or glasshouses, and the degree of stem colonisation determined at plant maturity (Marcroft et al., 2012b). A drawback of this approach is that if the lines being screened have effective major gene resistance, fungal growth will be arrested at the seedling stage, and therefore differences in minor gene resistance will not be detected. Thus plants will be resistant at maturity, regardless of the expression of minor gene resistance in the stems (Delourme et al., 2004; Van de Wouw et al., 2014b).

Differential sets of isolates have been exploited to identify complements of major resistance genes in *B. napus* seedlings (Balesdent et al., 2005; Marcroft et al., 2012b; Rouxel et al., 2003). Such differential sets for assessing minor gene resistance in stems would be extremely valuable to assist in breeding for blackleg resistance. However, if the previously developed methods of inoculating cotyledons and growing through to maturity are used, each differential set of isolates would need to overcome the major gene resistance in the cotyledons of the cultivars being assessed. An example of this was reported by Marcroft et al., (2012b) where isolates that were virulent towards *Rlm4* were inoculated onto cotyledons of *Rlm4* cultivars, grown to maturity and assessed for disease to assess different complements of minor gene resistance in stems of cultivars that all express *Rlm4*. If major gene resistance can be bypassed by inoculation directly into the stem of adult plants, a single set of differential isolates can potentially be developed and used to screen minor gene resistance in the stem, regardless of the presence of major gene resistance. This hypothesis relies on the assumption that differences in minor gene resistance can be clearly discriminated between cultivars by individual isolates.

In the current study AV-Garnet was resistant to both isolates while CB Telfer was susceptible to both during stem colonisation. These results suggest that AV-Garnet may have a higher level of minor gene resistance than CB Telfer does. This result is consistent with the Australian blackleg ratings of these two cultivars whereby AV-Garnet was released as a moderately resistant cultivar whilst CB Telfer as a moderately susceptible cultivar (2012 Blackleg Management Guide www.grdc.com.au/uploads/documents/GRDC-FS-BlacklegManagementGuide-Revised.pdf).

Another possibility is that temperature may be influencing the expression of the major resistance genes and therefore the interaction between the isolates and the relevant cultivars. The expression of major resistance gene *Rlm6* is influenced by temperature (Huang et al., 2006). It is unknown whether expression of *Rlm1* or *Rlm4* is affected by temperature. Although temperature was not recorded in the current study, cotyledons and stems were inoculated at the same time and under the same conditions. The expected isolate/cultivar reactions were observed, suggesting temperature was not a factor in this experiment.

An implication of our findings is that for dual purpose crops, plants with effective major gene resistance that have been grazed, may be susceptible to blackleg isolates infecting the wounded stems, regardless of the avirulence genotype of the isolates in the population.

It is unknown why the gene-for-gene interaction is not expressed in the late stage of stem colonisation during the *L. maculans*-*B. napus* interaction. One possibility is that the resistance genes, avirulence genes or both are not expressed at this time. The *LepR3* and *Rlm2* resistance genes and the corresponding avirulence genes, *AvrLm1* and *AvrLm2*, have been cloned (Ghanbarnia et al., 2015; Gout et al., 2006; Larkan et al., 2015; Larkan et al., 2013). This provides an opportunity to design experiments aimed at determining expression of these partner genes in organs throughout the plant, which may shed light into the organ specificity of these gene-for-gene interactions.

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494 **Table 1.** A summary of data collected from all organ types and cultivar-isolate interactions.

495

Organ type	Parameter tested	Cultivar	Isolate	Average	# of replicates	p-value ^a
True leaf 1	Average disease severity (%)	Garnet	D13	2.25	12	0.0011
			D14	0.75		
		Telfer	D13	0.5	12	<0.0001
			D14	3.3		
True leaf 2	Average disease severity (%)	Garnet	D13	2.33	12	0.02
			D14	0.58		
		Telfer	D13	0.66	12	<0.0001
			D14	2.58		
True leaf 3	Average disease severity (%)	Garnet	D13	1.9	12	0.01
			D14	0.41		
		Telfer	D13	0.25	12	0.013
			D14	1.25		
True leaf 4	Average disease severity (%)	Garnet	D13	1.16	12	0.015
			D14	0.166		
		Telfer	D13	0	12	0.016
			D14	1.08		
True leaves 4-8	Average disease severity (%)	Garnet	D13	1.1	76	<0.0001
			D14	0.09		
		Telfer	D13	0.17	76	<0.0001
			D14	1.46		
Pod	Pod infection (%)	Garnet	D13	49.3	4	0.00044
			D14	17.0		

		Telfer	D13	16.7	4	0.0006
			D14	81.2		
Pod	Pod lesion development (%)	Garnet	D13	23.2	4	0.0029
			D14	5.7		
		Telfer	D13	5.6	4	<0.0001
			D14	39.2		
Stem infection	Internal infection (%)	Garnet	D13	10.6	8	0.736
			D14	9.4		
		Telfer	D13	50.0	8	0.574
			D14	53.8		

^a Data were analysed using a one-way Analysis of Variance using GenStat (16th Edition). For each organ type, the appropriate parameter was compared between isolates on a single cultivar. All p-values less than 0.05 were considered significant.

Figure legends:

Fig. 1: Cotyledons of *Brassica napus* cultivars inoculated with *Leptosphaeria maculans* isolates 17 days post-inoculation. Isolate D13 is virulent on AV-Garnet (a) and avirulent on CB Telfer (c). Isolate D14 is avirulent on AV-Garnet (b) and virulent on CB Telfer (d).

Fig. 2: The second leaf of *Brassica napus* cultivars AV-Garnet and CB Telfer inoculated with *Leptosphaeria maculans* isolates, D13 (left) and D14 (right) at 21 days post inoculation. (a and b) Isolate D13 is virulent on AV-Garnet while D14 is avirulent. (c and d) Isolate D14 is virulent on CB Telfer while D13 is avirulent. Arrows indicate inoculation sites on each leaf. (e) Average disease scores for each of the first four leaves of each cultivar. An average disease score of >1.0 is considered susceptible (dotted line). Bars represent standard error from 48 leaves representing 12 different plants. Statistical differences determined by Analysis of Variance (** p<0.01 ***p<0.001).

Fig. 3: Average disease scores on *Brassica napus* leaves from the 4-8 leaf growth stage of cultivars AV-Garnet and CB Telfer inoculated with *Leptosphaeria maculans* isolates D13 and D14 at 21 days post inoculation. Isolate D13 gave higher disease scores on leaves of AV-Garnet at the 4-8 leaf growth stages than isolate D14 is. Conversely, isolate D14 is significantly more virulent on leaves of CB Telfer than isolate D13 is. Average disease scores were determined from 72 leaves at 4-8 leaf

growth stages representing 12 different plants. Bars represent the standard error. Statistical differences determined by Analysis of Variance (** $p < 0.01$ *** $p < 0.001$).

Fig. 4: Pods of *Brassica napus* inoculated with *Leptosphaeria maculans* isolates D13 and D14. Pods of AV-Garnet (a) display a virulent reaction when inoculated with isolate D13 but pods of CB Telfer (c) display an avirulent reaction 21 days post inoculation. Conversely, pods of AV-Garnet (b) display an avirulent reaction when inoculated with isolate D14 but CB Telfer (d) displays a virulent reaction. Arrow indicates contaminated seed within the pod from a virulent reaction. Within each panel, the upper picture shows the exterior of the pod whilst the lower picture shows the interior. (e) The average percentage of infected pods (at least one virulent lesion per pod) and the average percentage of pod lesion development (percentage of lesions developed from total number of infection sites) of cultivars AV-Garnet and CB Telfer after inoculation with either isolate D13 or D14. Isolate D13 infected 49.3% of the AV-Garnet pods and only 16.7% of the CB Telfer pods. Isolate D14 infected 17% of the AV-Garnet pods and 81.3% of the CB Telfer pods. Error bars represent the standard error from four plants and a minimum of 42 pods assessed. Each pod was wounded three times. Statistical differences determined by Analysis of Variance (** $p < 0.01$ *** $p < 0.001$).

Fig. 5: Stems of *Brassica napus* inoculated with *Leptosphaeria maculans* isolates D13 and D14 at eight weeks post inoculation. Cultivar CB Telfer is extensively colonised by both isolates D13 (a) and D14 (b) while AV-Garnet shows little internal infection by either D13 (c) or D14 (d). Although more infection was observed in CB-Telfer, there were no significant differences in disease severity between the two isolates on either cultivar ($p = 0.736$ for AV-Garnet and $p = 0.574$ for CB Telfer). (e) Percentage of internal infection of cultivars AV-Garnet and CB Telfer after inoculation with either isolate D13 or D14. Bars represent standard error from data from one stem segment from each of 12 plants.

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