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Short communication

Brassica napus plants infected by *Leptosphaeria maculans* after the third to fifth leaf growth stage in south-eastern Australia do not develop blackleg stem canker

S.J. Marcroft[1,2], M.R. Sosnowski[3,4], E.S. Scott[3], M.D. Ramsey[4,5], P.A. Salisbury[1,6], and B.J. Howlett[7]
[1]*Department of Primary Industries, 3401, Horsham, Vic., Australia (Phone: +61-3-5381-2294; E-mail: marcroft@bigpond.net.au);* [2]*Grains Innovation Park, Private Bag 260, 3401, Horsham, Vic., Australia;* [3]*Discipline of Plant and Pest Science, The University of Adelaide, Waite Campus, 5064, Glen Osmond, SA, Australia;* [4]*South Australian Research and Development Institute (SARDI), GPO Box 397, 5001, SA, Australia;* [5]*Animal and Plant Control Commission, GPO Box 1671, 5001, SA, Australia;* [6]*Institute of Land and Food Resources, The University of Melbourne, 3010, Vic., Australia;* [7]*School of Botany, The University of Melbourne, 3010, Vic., Australia*

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Abstract

Blackleg (Phoma stem canker) caused by *Leptosphaeria maculans* is the most damaging disease of *Brassica napus* (canola, rapeseed, colza) worldwide and is controlled by sowing blackleg resistant cultivars and crop management strategies that reduce exposure to inoculum and fungicide application. In experiments in south-eastern Australia, canola cultivars inoculated after the three to five leaf growth stage did not develop stem canker. Although mature canola plants are known to be less susceptible to blackleg than seedlings, this highlights for the first time the specific importance of protecting seedlings up to the three to five leaf growth stage in Australia. This would typically correspond to a period of four to six weeks after emergence. Canola plants are likely to be significantly less vulnerable to infection after this growth stage. However, this timing may vary due to the influence of environmental conditions.

The primary source of *Leptosphaeria maculans* inoculum is ascospores, which are discharged from pseudothecia on canola (*Brassica napus*) stubble during and after rainfall (Howlett, 2004). In Australia, the initial ascospore discharge occurs with the onset of autumn/winter rains (April–June), which normally coincides with canola sowing time (McGee, 1977). Recent work has shown that the seasonal commencement of ascospore showers in Australia can be predicted and, in some seasons, canola crops can be sown prior to the commencement of ascospore discharge (Salam et al., 2003). Although blackleg lesions can form on all parts of the plant, studies in Australia (Sosnowski et al., 2004), France (Poisson and Pérès, 1999), Germany (Badawy et al., 1992) and the UK (Hammond and Lewis, 1986) have shown that infection on seedlings leads to most severe stem cankers. However, the growth stage(s) at infection in south-eastern Australia, after which plants no longer develop stem canker is unknown.

An experiment was conducted in the glasshouse in 2001 to identify the oldest growth stage of canola plants (cv. Hyola 42) at which leaf inoculation with *L. maculans* would lead to canker development. Hyola 42 has a rating of 2.5 on the Australian Blackleg Resistance Ratings (BRR) where one is susceptible and nine is highly resistant (Blackleg Resistance Ratings, 2004). Plants at five different growth stages (GS 1, GS 2.3, GS 2.5, GS 3.1 and GS 4.1; Harper and Berkenkamp, 1975), were sprayed with conidia spores (10^6 conidia ml^{-1}) until runoff with the virulent *L. maculans* isolate 66/97 (Sosnowski et al., 2001). Plants were

assessed for the presence or absence of leaf lesions, external stem lesions and rotting crown tissue (cankers).

The incidence of leaf lesions 14 days after inoculation did not vary significantly regardless of the growth stage of plants when inoculated (Table 1). However, the incidence of external stem lesions 28–36 days after inoculation was significantly greater on plants inoculated at GS 1 (cotyledon), GS 2.3 (three-leaf) and GS 2.5 (five-leaf) growth stages compared to plants inoculated at GS 3.1 (inflorescence-visible) or GS 4.1 (flowering) growth stages (Figure 1). External stem lesions were visible on 61–89% of plants inoculated between GS 1 and GS 2.5, and on 14–39% of plants inoculated at GS 3.1 and GS 4.1.

LSD $P < 0.05$ (14 days after inoculation) = 20.8; (21 days after inoculation) = 29.2; (28 days after inoculation) = 28.3; (36 days after inoculation) = 28.8.

Growth stages are GS 1 (cotyledon), GS 2.3 (three leaf), GS 2.5 (five leaf), GS 3.1 (inflorescence visible) and GS 4.1 (flowering) as according to Harper and Berkenkamp (1975).

Forty-nine days after inoculation, cankers had developed on most plants inoculated at GS 1 and GS 2.3. A third of plants inoculated at GS 2.5 had developed cankers, but few cankers were recorded on plants inoculated at GS 3.1 or GS 4.1 (Table 1).

A field experiment to investigate the interaction amongst cultivars with a range of blackleg resistance and their growth stage when exposed to natural inoculum was conducted during 2002. Australian canola cultivars with a range of polygenic blackleg resistance (Karoo (BRR 3.5), Dunkeld (BRR 6.0) and AV-Sapphire (BRR 8.0)) were grown in pots to particular growth stages (Table 2) and then moved into a commercial field at Wonwondah, Victoria that contained blackleg-infested canola stubble from the previous season's crop. For each growth stage for each cultivar, 15 plants were examined. The number of leaf lesions resulting from natural infection by *L. maculans* was similar on all plants regardless of cultivar or growth stage. For all three cultivars screened, internal infection (scored as 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% visible internal infection of the cross section at the crown) was most severe on plants that were infected at GS 2.1 and GS 2.3 (Table 2).

The incidence and severity of internal infection at the crown of the plant was influenced by the growth stage of the plant when infection occurred. None of the cultivars developed significant stem infection when plants were inoculated after the three-leaf growth stage. Only plants inoculated at or prior to the three-leaf growth stage developed severe stem canker or died. However, plants infected at later growth stages developed small amounts of internal infection at the crown. The lower level of disease in older plants is probably due to the size of the plant, as the fungus takes longer to grow from the leaf to the crown in larger (mature) plants than in smaller (young) plants.

Although the field experiment was undertaken at only one site in south-eastern Australia, this study highlights that blackleg management must focus on protecting seedlings up to 6 weeks after germination. Seedlings can be protected by breeding specifically for blackleg resistance effective at the seedling stage (Rouxel et al., 2003), sowing before the commencement of ascospore showers (Salam et al., 2003) sowing in situations that avoid high densities of inoculum (Marcroft

Table 1. Effect of growth stage of *Brassica napus* cv. Hyola 42 on incidence of leaf lesions and stem canker caused by *Leptosphaeria maculans* in a glasshouse experiment

Growth stage at inoculation	14 days after inoculation		49 days after inoculation	
	Growth stage at assessment	Leaf lesion incidence (%)	Growth stage at assessment	Canker incidence (%)
Cotyledon (GS 1)	Three-leaf (GS 2.3)	73a	Flowering complete (GS 4.4)	83a
Three-leaf (GS 2.3)	Inflorescence visible (GS 3.1)	100a	Seeds in lower pods translucent (GS 5.1)	86a
Five-leaf (GS 2.5)	Flowering (GS 4.1)	100a	Seeds in lower pods green (GS 5.2)	33b
Inflorescence visible (GS 3.1)	Lower pods filling (GS 4.3)	86a	Seeds in lower pods brown (GS 5.4)	3c
Flowering (GS 4.1)	Flowering complete (GS 4.4)	98a	Seeds in lower pods brown (GS 5.4)	0c

Values within a column with the same letter are not significantly different ($P = 0.05$).

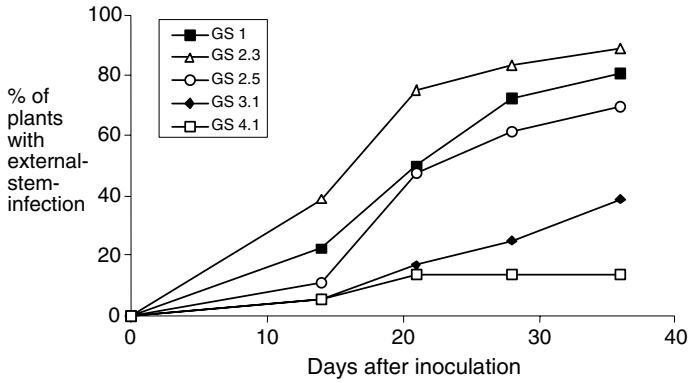

Figure 1. Effect of the growth stage of *Brassica napus* at time of inoculation with *Leptosphaeria maculans* on the incidence of external stem lesions in a glasshouse experiment. For each time point, 12 plants were inoculated.

Table 2. Blackleg disease severities expressed as internal infection and mortality after natural infection with *Leptosphaeria maculans* at different growth stages of *Brassica napus*

Cultivar (Blackleg resistance rating)	Average percentage internal infection of the crown and mortality (percentage, in parenthesis)				
	One-leaf GS 2.1	Three-leaf GS 2.3	Five-leaf GS 2.5	Seven-leaf GS 2.7	Inflorescence visible GS 3.1
Karoo (3.5)	89a (64a)a	80a (36b)	37b (0c)	21c (0c)	11d (0c)
Dunkeld (6)	55a (20a)	38b (7b)	22c (0c)	10d (0c)	5d (0c)
AV-Sapphire (8)	12a (0a)	8ab (0a)	5bc (0a)	4bc (0a)	1c (0a)

Values within a row with the same letter (a, b, c or d) are not significantly different ($P = 0.05$) Data was $\log_{10}$ transformed before analyses

[a]Values in parenthesis are mortality percentage.

et al., 2004) and by protecting seedlings with fungicides (Khangura and Barbetti, 2002).

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