Early-flowering canola – what is the blackleg risk?

Susie Sprague[1], John Graham[1], Rohan Brill[2] and Colin McMaster[2]

[1] CSIRO Agriculture and Food, Box 1700, Canberra, ACT 2601, www.csiro.au, Susan.Sprague@csiro.au
[2]NSW DPI, Wagga Wagga/Orange, NSW

Abstract
Blackleg, caused by the fungus *Leptosphaeria maculans*, is the most important disease limiting canola production in Australia and worldwide. Development of crown canker via asymptomatic growth from infected leaves results in significant yield loss. However, *L. maculans* can infect all parts of the plant, including flowers, siliques, peduncles, branches and upper stem, collectively termed upper canopy infection. Earlier seeding and warmer winters are speeding up phenology resulting in crops flowering during the mid to late-winter period when conditions are conducive for infection. Field experiments were conducted at two sites in southern NSW in 2016 to investigate the development of upper canopy infection and to determine its effect on grain yield. Early flowering during June and July increased blackleg symptoms on all upper canopy canola components. The predominant symptom was infected pods which reduced grain yield by up to 26% (1 t/ha) in the earliest flowering crops. Infection levels were lower in later flowering treatments and did not cause yield loss when flowering commenced after mid-August. Gains in yield potential by flowering in late-July/early August predicted by simulation analyses and validated in recent research trials must take into account risks of biotic constraints such as blackleg.

Keywords
Leptosphaeria maculans, phoma, flowering date, sowing time, yield loss.

Introduction
Leptosphaeria maculans is ubiquitous in all canola-growing regions of Australia and is estimated to result in an average 15% yield loss across Australia annually (Murray and Brennan 2012). This estimate of yield loss is due to development of cankers at the crown of the plant limiting uptake of water and nutrients. Crown cankers develop from leaf infections whereby the fungus grows asymptomatically within the plant to the crown. Crown canker is controlled by cultural methods (distance from previous years' stubble that releases airborne spores), fungicides and genetic resistance. Due to its genetic make-up and life traits, *L. maculans* has a very high evolutionary potential (MacDonald and Linde 2002). Genetic resistance has been overcome in as little as three years in Australia (Sprague et al. 2006) and tolerance to the fungicide fluquinconazole, which is routinely applied as a seed-dressing, has also been widely detected (A. Van de Wouw pers. comm.).

All above- and below-ground plant parts of *Brassica napus* are susceptible to infection by *L. maculans* (West et al. 2001; Sprague et al. 2007). Upper canopy infection is the term used throughout this paper for all symptoms caused by *L. maculans* on flowers, pods (siliques), branches and upper stem in crops that have undergone stem elongation. Although crown canker has been the primary cause of yield loss, observations across canola-growing regions of Australia since 2010 indicate that upper canopy blackleg infection is causing significant levels of yield loss equivalent to or greater than crown canker at some sites (Marcroft et al. 2016). Anecdotally, upper canopy infection is related to the earlier commencement of flowering in commercial crops during a period in which spore release coincides with cool, moist conditions conducive for infection. Data around the impact of these symptoms on crop growth and ultimately yield is lacking. The aim of the experiments presented in this paper was to investigate the role of flowering time in development of upper canopy infection and determine any associated yield penalty by the application of fungicides.

Methods
Cultivar and experimental design
Field trials were established in locations with a history of blackleg at Wagga Wagga and Canowindra, NSW, with cultivar 44Y89CL (Blackleg Resistance Group BC, Blackleg Resistance Rating R-MR) at three sowing dates in 2016 (Table 1). An Untreated crop (no fungicides applied) was compared to a "Full control" treatment (fungicide applied to seed, fertiliser, foliar application during early stem elongation and 2 sprays during flowering) to ascertain the level of disease in crops starting to flower at different times and to determine any associated yield penalty by control with fungicides. Due to the unusually wet seasonal

conditions, the fungicide regime applied did not fully control disease development particularly in the earliest flowering crops, and therefore the estimates of disease loss may be greater. The trials were a split plot design with 3 replicate blocks and 3 sowing times. Treatment plots (12 m x 1.5 m, 6 rows at 25 cm spacing) were randomised within each sowing time. The three sowing times were used to generate a range of flowering times with the fungicide treatments imposed to determine the level of yield loss associated with upper canopy blackleg infection.

Table 1. Sowing date, growing season rainfall (Apr-Oct), timing of fungicide applications, start and end flowering and maturity dates for cv. 44Y89CL at Wagga Wagga and Canowindra, NSW in 2016.

Site (in-crop rainfall)	Sowing date	Fungicide application dates during flowering	Start flowering	End flowering	Maturity
Wagga Wagga (625 mm)	30 Mar	27 Jun, 28 Jul	22 Jun	2 Sep	12 Oct
	13 Apr	28 Jul, 23 Aug	17 Jul	15 Sep	21 Oct
	2 May	23 Aug, 20 Sep	18 Aug	27 Sep	7 Nov
Canowindra (628 mm)	1 Apr	11 Jul, 1 Aug	10 Jul	15 Sep	18 Oct
	14 Apr	2 Aug, 23 Aug	1 Aug	20 Sep	25 Oct
	28 Apr	23 Aug, 13 Sep	15 Aug	1 Oct	7 Nov

Disease assessments

Infection of pods, branches and upper main stems, as well as premature pod loss, was assessed at crop maturity by scoring 20 individual plants/plot. Each plant was given a separate score for each of the symptoms using a 0 to 4 scale, whereby 0 = no disease symptom, 0.5 = small amount of symptom present, 1 = <10% pods or tissue area affected, 2 = 10-29% pods or tissue area affected, 3 = 30-49% pods or tissue area affected, 4 = ≥50% pods or tissue area affected.

Yield assessments

Hand cuts (2 x 1m^2) were taken from each plot at a similar stage to when commercial crops would be windrowed, approximately 50% seed colour change. Samples were air-dried prior to threshing with a mechanical thresher. Grain yield was calculated from oven-dried (48h at 70ºC) weights.

Statistical analysis

Data from each site was analysed separately by ANOVA using appropriate models in Genstat v.16 to determine main effects of treatment (sowing time and fungicide application) and interactions. Fisher's protected l.s.d. (P<0.05) was used to determine differences between treatment means. Disease data was analysed using ordinal regression with data presented as means.

Results

Crops started flowering across a wide window at both sites from the different sowing dates. At Wagga Wagga, crops at the earliest sowing time started flowering on 22 June and approximately monthly thereafter for later sowing times (Table 1). At Canowindra, crops started flowering approximately 2 weeks later than at Wagga Wagga for the first 2 sowing times but flowering times were similar for sowing 3 (mid-August).

Wagga Wagga had a higher level of all upper canopy infection types (pod, branch and main stem) than Canowindra, probably reflecting the greater intensification of canola production in the local region with an associated higher inoculum load (Tables 2 and 3). At both sites, all symptoms were highest at the earliest flowering time and declined significantly with later flowering (P<0.001). At both sites, main stem and branch infection was relatively low (<1.4) with pod infection the predominant symptom type. Extremely high levels of pod infection were present in the Untreated at the earliest flowering time (mean score 3.6 across both sites), and declined with later flowering although was still present in crops flowering in mid-August.

At Wagga Wagga, yield loss associated with upper canopy infection was 1 t/ha (26%) in the earliest flowering crop (Table 2). The high level of yield loss was predominantly due to ~25% of pods (score of 1.8) lost prematurely prior to harvest in addition to yield reductions in affected pods retained on the plant. As pod infection was not fully controlled by 2 applications of fungicide, the yield loss was likely even greater. In the mid-July flowering crop, pod infection resulted in a 0.29 t/ha yield loss (this excludes the 0.29 t/ha yield advantage provided by the application of fungicide to seed and fertiliser – data not presented). The mid-

August flowering crop had moderate levels of pod infection (2.0) but control with fungicides did not provide a yield increase.

At Canowindra, pod infection was also the primary symptom affecting yield with a 0.38 t/ha (10%) reduction in the crop flowering in mid-July (Table 3). Although a 0.86 t/ha yield penalty was recorded in the early August flowering crop, there was also a high level of Sclerotinia stem rot in this treatment. As at Wagga Wagga, the mid-August flowering crop had the lowest level of pod infection (0.8) with no yield benefit provided by the application of fungicides.

Table 2. Symptoms of upper canopy blackleg infection and grain yield in crops flowering on different dates for cv. 44Y89CL at Wagga Wagga, NSW in 2016. The 'Untreated' is compared to a 'Full control' at each flowering time whereby disease was controlled by fungicide applications to provide an estimate of yield loss associated with blackleg infection. *Indicates significantly different from Untreated treatment for data within each sowing date.

Start of flowering date	Treatment (Sowing date)	Pod infection (0-4 scale)	Premature pod loss (0-4 scale)	Main stem infection (0-4)	Branch infection (0-4)	Grain yield (t/ha)
22 Jun	*30 Mar*					
	Untreated	3.9	1.8	1.4	1.4	2.88
	Full control	3.5	0.7*	0.9*	0.6*	3.87*
17 Jul	*13 Apr*					
	Untreated	2.8	0.2	0.3	0.2	2.99
	Full control	1.6*	0.0*	0.1*	0.0*	3.57*^
18 Aug	*2 May*					
	Untreated	2.0	0.1	0.3	0.4	2.91
	Full control	1.2*	0.1	0.2	0.2*	3.01

^There was a 0.29 t/ha yield advantage by the addition of fungicides to seed and fertiliser alone (data not presented).

Table 3. Symptoms of upper canopy blackleg infection and grain yield in crops flowering on different dates for cv. 44Y89CL at Canowindra, NSW in 2016. The 'Untreated' is compared to a 'Full control' at each flowering time whereby disease was controlled by fungicide applications to provide an estimate of yield loss associated with blackleg infection. *Indicates significantly different from Untreated for data within each sowing date.

Start of flowering date	Treatment (Sowing date)	Pod infection (0-4 scale)	Premature pod loss (0-4 scale)	Main stem infection (0-4)	Branch infection (0-4)	Grain yield (t/ha)
10 Jul	*1 Apr*					
	Nil	3.3	0.6	0.6	0.4	3.24
	Full fungicide	2.5*	0.1*	0.0*	0.0*	3.66*
1 Aug	*14 Apr*					
	Nil	2.0	0.1	0.1	0.1	2.93
	Full fungicide	1.2*	0.1	0.0*	0.0	3.79*^
15 Aug	*28 Apr*					
	Nil	0.8	0.1	0.1	0.0	3.24
	Full fungicide	0.8	0.1	0.0	0.0	3.22

^Yield effect cannot solely be attributed to control of pod infection due to high levels of Sclerotinia stem rot.

Discussion

Upper canopy infection is the collective term for symptoms resulting from infection by *L. maculans* on a wide range of plant parts in canola crops that have undergone stem elongation. Our research shows that crops flowering early in the winter period (June/July) due to earlier seeding or mild winter conditions are at greater risk of upper canopy infection than those flowering later (August). Symptoms of upper canopy infection generally occur together and the impact on yield development of each individual symptom is therefore difficult to elucidate. However, low levels of branch and main stem symptoms were present in the 2016 season and as such provided the opportunity to ascertain yield loss primarily associated with infection of pods. The earliest flowering crop at both sites had the greatest yield loss due to pod infection and was associated with highest levels of premature pod loss due to blackleg infection. Premature loss of infected pods tends to occur close to harvest after flowering has finished, with the plant unable to compensate by producing more flowers or set more seeds/pod.

Earlier planting times for canola in south-eastern Australia can provide increased grain yield, oil and water-use efficiency (Kirkegaard et al. 2016). To achieve these yield gains, it is critical to commence flowering in the optimum window to avoid the impact of frost, heat and water stress (Lilley 2016). Simulation analyses in APSIM applying a frost/heat index can account for these abiotic factors in predicting potential yield (Lilley et al. 2015; Lilley 2016), however, this study clearly shows that biotic stresses such as disease also require consideration if yield improvements gained through earlier flowering times are to be realised. These studies reveal the need to build and integrate disease models into APSIM to improve yield predictions from different sowing date x genotype scenarios.

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