Epidemiological Aspects of Blackleg Disease of Canola in South-Eastern Australia

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ABSTRACT

The recent increase in area sown to canola in Australia has resulted in large acreages of canola (*Brassica napus*) stubble. This stubble harbours ascospores, which are the primary inoculum of blackleg, caused by the fungus (*Leptosphaeria maculans*). Fields containing 6 to 42-month old stubble were evaluated for the number of ascospores released in three rainfall (high, medium and low) sites in Victoria, south eastern Australia. Ascospores were discharged from stubble of all ages and at all sites, however, more than 97% of the total ascospore load was released from the previous season's canola stubble, with minimal amounts from older stubble. The critical factor in determining amount of ascospore inoculum was the physical amount of stubble persisting. In all three sites stubble broke-down quickly with only small amounts left after 2 years. This finding was supported by studies in New South Wales (also in south eastern Australia) which found that canola crops grown every second year had the same incidence of blackleg infection compared to that in crops grown in fieldsafter a three year break from canola. The distance between current canola crops and stubble from the previous season was critical to infection severity; crops within 100 m of last years stubble had a significant incidence of stem cankering. Our research suggests that long crop rotations are not as important in reducing disease levels as physical isolation of crops from stubble of the previous season. Consequently we now recommend that grain growers in south eastern Australia sow canola crops at least 100 m from canola stubble from the previous year's crop with distances of at least 500 m if possible.

INTRODUCTION

Brassica napus (canola) is an important crop in Australia, both in terms of export income and as a component of sustainable rotations. The area under cultivation in Australia increased from 100,000 ha in 1990 to 1.1 million ha in 2001. Blackleg, caused by the fungus *Leptosphaeria maculans* is the most important disease of canola in Australia with widespread epidemics destroying the fledgling industry in the early 1970s (Howlett *et al*. 1999). Canola stubble contains ascospores, the primary inoculum of blackleg. The breeding and deployment of blackleg-resistant canola cultivars enabled the industry to be re-established from the mid 1980s. However, resistance in most cultivars grown in Australia is incomplete and high disease pressure can result in substantial yield loss or even crop failure (Khangura and Barbetti, 2001). Thus, effective crop management practices are crucial for farmers to minimise yield loss caused by blackleg. In Australia there have been few published reports of epidemiology including timing of ascospore release and stubble longevity (for review see West *et al*. 2001).

Currently, farmers are advised to grow canola no more frequently than 1 in 4 years within the same field to allow sufficient time for all infected canola stubble to breakdown. However, due to the high profitability of canola and rotational benefits to cereal crops (controlling root diseases), the area sown to canola has increased significantly. In some districts in New South Wales, it is now sown in short rotations (canola / wheat / canola), often adjacent to canola stubble from the previous year. In this paper we report the quantification of survival and dissemination of ascospores of *L. maculans* from pseudothecia in canola stubble of different ages and in different environments in south eastern Australia. Additionally we describe the relationship between inoculum source and plant infection by analysis of blackleg infection patterns in canola crops from different rotation periods (1in 2 years; and 1 in 4 years) and at different distances from 6-month old canola stubble.

Materials and Methods

Rate of stubble breakdown

During 2000 and 2001, fields of canola stubble in Victoria with known cropping histories in three rainfall sites (mm) (high, Lake Bolac; medium, Wonwondah; and low, Birchip) and mean maximum temperature were selected. Canola stubble was collected from the soil surface during June of both years when the canola crops were at the open cotyledon growth stage. This timing of stubble collection coincided with the timing of ascospore release and hence natural blackleg infection of canola. Stubble was collected from 40 quadrats (0.1 m^2) evenly spaced along a "W" transect. The stubble was then cut, washed with water, oven-dried at 70 ^{0}C for

48 h and then finally weighed. From these data, the amount of stubble (kg/ha) was estimated.

Pattern of ascospore discharge from pseudothecia on stubble of different ages and sites

To measure the number of ascospores discharged from canola stubble, pieces of stubble collected from the fields described above were cut at the crown and then 6 cm up the stem and placed in a Burkard ascospore liberation tunnel. Twenty five pieces were subjected to 100% humidity in a dew chamber for 24 h, immersed in water for 1 min and then placed into chambers in the liberator (ie. 175 stems for each field) at 20oC. The ascospores discharged from the pseudothecia over a 4 h period were trapped onto microscope slides and then counted using a compound microscope (x 400 magnification). Ascospores of*L. maculans* were the predominant species discharged and were readily recognised by their shape and characteristic septa.

Severity of blackleg in crops grown in 1 in 4 years or 1 in 2 years rotation and at varying distances from canola stubble

The effect of rotation length on blackleg infection was determined at Junee, NSW, a medium rainfall site, whilst the relationship between severity of infection to the distance from the inoculum source was determined at Wonwondah. During 2000 and 2001, the level of cross-section internal infection of the stem caused by *L. maculans* was assessed on 100 plants at maturity (just prior to windrowing) at each sample point. Plants were chosen by walking a "W" transect. Each plant was were pulled out of the ground, and the stem was cut at the crown and assessed visually for the percent cross-section internal infection (Ballinger and Salisbury 1996). In another experimentAt Wonwondah a straight line transect was used to marked 0, 25, 50, 100, 200, 300, 400, 500, 600, 800 and 1000 metres from 6-month old stubble in the same canola crop. Three replications of 50 plants each were scored for cross-section internal infection at each distance with 2 transects in different fields.

Statistical analyses

All data were transformed using Log (base 10) transformation to achieve normality prior to using analysis of variance techniques. Differences amongst sites, and stubble age and leaf lesion densities were determined using REML, the algorithm in GENSTAT for unbalanced data sets (Payne *et al*. 1995). Analyses of mean spore release per hectare were completed using ANOVA after Log (base 10) transformation of the data for each site individually.).

RESULTS

Rate of stubble breakdown

In the three sites in Victoria, stubble broke down very quickly between 6 and 18 months after the original canola crop was harvested. Eighteen months after the original canola crop was harvested On average 87% of the original 6-month old stubble was no longer on the soil surface 12 months later (18 months after the original canola crop was harvested) (Table 1). The amount of stubble varied considerably between individual fields with stubble of the same age due to environmental conditions and stubble management practices employed. In a separate experiment at Wonwondah, the amount of stubble present 2 months after a canola crop had been harvested (before any management had occurred) was estimated. Four months later, the amount of stubble had declined by 81% after the field had been harrowed, pre-drilled with urea and sown.

Release of ascospores from stubble of varying ages and in different environments

The number of ascospores discharged from pseudothecia on stubble of all ages (6 to 42-month old) collected from all three Victorian sites was counted. Most ascospores were released from 6-month old stubble at the high rainfall site and by 30 months very few were discharged, probably because few were left in the pseudothecia. The potential for ascospore discharge per hectare from each field was calculated by multiplying the weight of stubble (kg/ha) by the number of ascospores discharged by that stubble (ascospores discharged / kg of stubble) (Table 2). At the low rainfall site, the difference in ascospore discharge between 6 and 18-month old stubble fields was large, but not significant due to high variability between individual fields. Lower variability at the medium and high rainfall sites resulted in significant differences in ascospore discharge from 6-month old stubble fields compared to older stubble fields. At the low and high rainfall sites, over 99% of all discharged ascospores originated from 6-month old stubble, while at the medium rainfall site, the value was 95% (Table 3).

Effect of rotation length on severity of blackleg infection

Significant differences in blackleg infection were observed between long (1 in 4) and short (1 in 2) rotations at Junee, NSW (Table 4). The average cross-section internal infection during 2001 was significantly higher in the long compared to the short rotation. There were no significant differences between rotation frequency for plants with >80% cross-section internal infection. If the rotation length influenced infection severity, the plants from the short rotation (1 in 2) should have had more severe infection than the plants from the long rotation (1 in 4). As this did not occur, rotation length is not considered important in blackleg severity.

Relationship between the severity of blackleg infection on canola crops and the proximity of canola stubble.
The distance required to significantly reduce the severity of blackleg infection was determined using straight line transects immediately adjacent to 6-month old canola stubble to 1000 m away. Both the median and >80% cross-section internal infection decreased significantly in the first 50 m but did not then significantly decrease for the next 400 m. Between 500 and 1000 m there was no significant reduction in disease severity (Figure 1).

DISCUSSION

Our findings show that canola stubble breaks down quickly during the first season after the original canola crop is grown with only a small proportion of stubble remaining on the soil surface for up to four years. Different environmental conditions did not greatly influence the rate of stubble break down but did influence the number of ascospores discharged. The presence of higher amounts of 6-month old stubble at the low rainfall Victorian site was probably due to greater adoption of conservation tillage techniques than at the other sites. However, stubble at the low rainfall site degraded at the same rate as that in the higher rainfall sites and did not result in a higher discharge of ascospores per hectare.

Our findings results suggest that if conditions are favourable for ascospore discharge (high rainfall), a high proportion of all available ascospores will be discharged within a few months. Conversely, in low rainfall conditions, levels of ascospore discharge may be low but pseudothecia can still discharge ascospores during future rainfall events. This is consistent with results of Baird *et al*. (1999) who found that in the US *L. maculans* can be isolated from stubble for at least 3 years provided that the stubble remains in good condition. These authors found that the fungus cannot be isolated from severely degraded stubble. Of the variables we analysed, the most important factor in determining numbers of discharged ascospores was the physical amount of stubble available to harbour pseudothecia. Since most canola stubble breaks down rapidly, stubble from the most recent crop accounts for the majority of ascospores discharged. These observationsThis finding is are consistent with those of McGee (1977) who found that in 1972 in Victoria, 90% of stubble had disappeared by the second season after the canola crop. Only In our study about only 20% of stubble survived from the time of harvest to 6 months later, even when the stubble was not deliberately destroyed. Consequently 80% was either buried during sowing or broke down naturally before it ever had the chance to cause blackleg infection.

Although the critical threshold of ascospore numbers required to cause significant levels of cankering is unknown, the large variability in the number of ascospores discharged from fields of 6-month old stubble may explain the variable amount of disease observed in commercial crops in south eastern Australia. If conditions are highly favourable for ascospore discharge and subsequent infection, it is probable

that cultivars with incomplete resistance will experience considerable yield loss. Our research demonstrates that all canola crops within a canola growing district are likely to have blackleg infection regardless of the rotation used or distance to 6-month old stubble, the only difference between crops will be the severity of infection. All plants within the study area were infected with blackleg but the plants adjacent to 6-month old stubble also had the most severe stem canker. This suggests that blackleg resistant canola plants are able to restrict *L. maculans* infection if limited numbers of ascospores land on them. We would advise farmers in south eastern Australia to wherever possible spread their canola crops across their farm to avoid sowing a new crop adjacent to last season's canola stubble, rather than only being concerned with in-field rotation. The finding that disease severity decreases significantly in crops up to 100 m from 6-month old stubble, then remains constant from 100 to 400 m demonstrates that farmers can use a small isolation zone (100 m) to significantly reduce the amount of blackleg infection in their crops.

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FIGURE LEGENDS AND TABLES

Table 1. Effect of stubble age on the rate and variability of breakdown of canola stubble.

Stubble age (months)	Mean stubble weight for three rainfall sites (kg/ha)	Amount of stubble broken down (%)	Range of stubble weight from individual fields (kg/ha)
6	1100[a]	0.0	300 – 2580
18	140[b]	87	30 – 290
30	40[c]	96	15 – 90
42	30[d]	97	0 – 80

Data are an average from all sites (low, medium and high annual rainfall) in Victoria during 2000 and 2001. Values with the same suffix vertically (a, b, c or d) are not significantly different (*P=0.05*).

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Table 2. Number of *Leptosphaeria maculans* ascospores discharged per hectare from canola stubble of different ages at three sites in Victoria.

Site	Stubble age (months)	Ascospores released per hectare (millions)	Range of numbers of ascospores released per hectare (millions)		
Low rainfall	6	261	0.35	-	778
(Birchip)	18	1.9[ab]	1.13	-	3.4
	30	0.55[b]	0.035	-	1
	42	0.122[b]	0.02	-	0.32
Medium rainfall	6	116[a]	0.45	-	407
(Wonwondah)	18	3.5[b]	0.034	-	14

	30	2.5 [b]	0.03	-	5.6
	42	0.27[c]	0.01	-	0.05
High rainfall	6	507	90	-	890
(Lake Bolac)	18	1.46	0.01	-	6
	30	0.012	0.008	-	0.20
	42	n.a.		-	n.a.

Values with the same suffix (a or b) are not significantly different (*P=0.05*).

n.a., not available; stubble was not collected from these paddocks

Table 3. Percentage of *Leptosphaeria maculans* ascospores discharged from pseudothecia on canola stubble of different ages from different three sites in Victoria.

Site	Leptosphaeria maculans aAscospores discharged (%)			
months	6	18	30	42
Low rainfall	99.0	0.7	0.2	0.1
Medium rainfall	95.1	2.9	2.0	0.0
High rainfall	99.7	0.3	0.0	*

* stubble was not collected from these fields.

Table 4. Effect of rotation length on severity of blackleg disease of canola.

Year	Rotation length	Median cross section internal infectionCSII	Percentage of plants with >80% CSII
2000	Long	32.0a	19.0a
2000	Short	28.7a	13.3a
2001	Long	31.6a	16.3a
2001	Short	22.2b	10.0a

Rotation length was long (1 canola crop in 4 years) or short (1 in 2 years). During 2000 and 2001, blackleg severity was assessed at Junee, NSW as median cross section internal infection (CSII) of stems and percentage of plants with >80% internal infection of stems. Values followed by the same letter are not significantly different (P=0.05); n.a., not available.

Figure 1 Relationship between blackleg disease severity expressed as cross section internal-infection (CSII) and distances of canola crop to 6-month old canola stubble.