Sclerotia contamination of canola and lupin grain by the fungal pathogen Sclerotinia in the Western Australian grainbelt

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Abstract
Sclerotia of *S. sclerotiorum*, a widespread necrotrophic plant pathogen causing Sclerotinia stem rot (SSR) disease in canola and pulses, is the source of inoculum between growing seasons and can survive for many years in the soil. It has been suggested that mature sclerotia could spread wide distances between farms and regions through collection with the grain during the harvesting process, as shown in weed seeds. This study determined the disease incidence, number of sclerotia present in harvested grain and yield of 312 small scale field plots across 10 sites between 2017, 2018 and 2020. Furthermore, harvested grain samples were collected from 14 canola and two lupin fields in 2020. Results found that management, variety and site had a significant impact on levels of disease incidence and sclerotia contamination within the small-scale field plots. Over 80% of the 16 farmer samples had sclerotia contamination, with lupin samples having a higher average contamination than canola samples.

Keywords
Sclerotinia sclerotiorum, dispersal, disease management, *Brassica napus*, *Lupinus angustifolius*

Introduction

Sclerotinia stem rot (SSR), caused by the necrotrophic plant pathogen *Sclerotinia sclerotiorum*, is a major disease of canola and pulses in Australia. Management of SSR is under pressure as crop rotations narrow and inoculum levels rise due to an escalation of canola plantings in Australia and long-term persistence of inoculum (called sclerotia) in the soil up to 8 years (Derbyshire and Denton-Giles 2016). After a period of conditioning (typically hot-dry temperatures experienced over summer), mature sclerotia can germinate either carpogenically (producing airborne ascospores from apothecia) or myceliogenically (by production of hyphae direct from sclerotia in or on the soil) (Michael et al, 2021). Most epidemics caused by SSR are attributed to infection by air-borne ascospores, which usually spread within a 40-60 m radius of the sclerotia source, but have also been documented to spread up to several kilometers via air currents (Michael et al, 2020). The dissemination of genetic material of *S. sclerotiorum* beyond this range is poorly understood, although it has been suggested that mature sclerotia could be spread via human-mediated transfer between farms and regions, through collection with the grain during the harvesting process (Michael et al, 2021), as happens with weed seeds (Michael et al, 2010).

The objective of this study was to determine the level of sclerotia contamination in the harvested grain of canola within the Western Australian grain-belt. Two approaches were taken; firstly, using grain sampled from small-scale G (genotype) x E (environment) x M (management) canola field trials, and secondly by samples taken directly from commercial fields.

Methods

Small-plot field trials

To investigate the effect of G x E x M on susceptibility of canola to natural SSR infection, ten small-scale field plot experiments were established in 2017, 2018 and 2020 in the grain-belt of Western Australia (Figure 1). Each site was sown with a minimum of two commercial canola varieties (Table 1) at recommend field rates, with two fungicide treatments applied (control and 400mL/ha Prosaro® fungicide applied at 30% flowering). Plots (10 m x 1.6-1.8 m) were arranged in a randomised 3 block design and maintained agronomically according to recommended current farmer practice. Disease incidence (DI) per plot was determined prior to harvest but before stems had changed colour, which enabled ease of assessment of characteristic SSR stem lesion bleaching. Briefly, 10 measurements of

five stems within a 50 cm range were scored for the presence of SSR lesions. Plots were harvested during November/December using small-scale field harvester equipment, grain yields recorded and ~2 kg grain subsamples were retained to determine sclerotia contamination per kilogram. Sclerotia was separated from grain using a 300 mm diameter sieve with 2 mm woven wire.

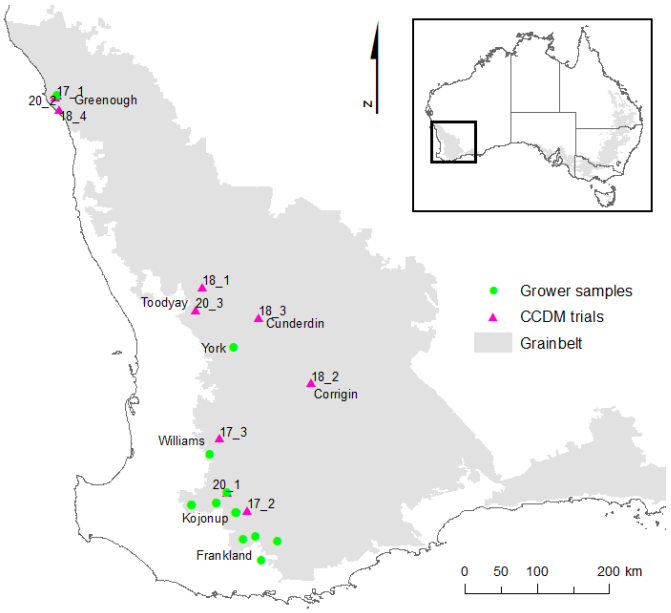

Figure 1. Map showing locations of 10 small-plot field trials (▲) sown over a three-year period (2017-18, 2020) and 16 grower grain samples (●) collected after harvest in 2020.

Table 1. Summary of field sites showing canola varieties sown at each site (TT = Triazine tolerant, GT = Glyphosate tolerant, OP = Open pollinated, Hy = Hybrid)

			Sites										
			2017			2018			2020				
Variety	**Type**	**Maturity**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	∑
ATR Bonito	TT OP	early-mid	■	■	■	■	■	■	■	■	■	■	10
ATR Mako	TT OP	early-mid				■	■	■	■	■	■	■	7
DG 408RR	RR Hy	early-mid							■	■	■	■	3
Hyola 559TT	TT Hy	mid	■	■	■	■	■	■	■	■	■	■	10
HyTTec Trident	TT Hy	early							■	■	■	■	3
InVigor R4022P	TF Hy	early-mid							■	■	■	■	3
InVigor T4510	TT Hy	early-mid				■	■	■	■	■	■	■	7
Pioneer 43Y23RR	RR Hy	early							■	■	■	■	3
Pioneer 44Y27RR	RR Hy	early-mid							■	■	■	■	3
Xseed Raptor	TF Hy	early-mid							■	■	■	■	3
			2	2	2	4	4	4	4	10	10	10	

Large-scale farmer samples

Sixteen grain samples (2-3 kg) were collected following harvest in 2020 from the WA grain-belt (Figure 1). Two of the 16 samples were Jurien lupins, the remainder were canola (4 open-pollinated, 10 hybrid varieties). Sclerotia was separated from grain using a 300 mm diameter sieve with 2 mm woven wire.

Analysis

Data were analysed in Genstat v20 (VSN International Ltd, UK) using an analysis of variance (ANOVA), with transformation applied when required. Data were plotted as boxplots using the R-package ggplot2 and maps generated with ArcMap Version 10.4 (Esri Inc.). Each boxplot visualizes

the mean, median, two hinges (25th and 75th percentiles), two whiskers (largest value no further than 1.5 × interquartile range), and all outliers.

Results

Fungicide application had a significant impact (P<0.001) on disease incidence and sclerotia contamination, with plots that were sprayed showing lower levels of disease (5.1%) and sclerotia contamination (0.6 sclerotia/kg) than unsprayed plots (8.4% DI, 1.5 sclerotia/kg) (Figure 2). Unsprayed plots were more likely to have sclerotia contamination (53%) than sprayed plots (47%). There was a very weak correlation (0.36, P<0.001) between disease incidence and sclerotia contamination in unsprayed plots from field trial samples (n=156), and an even lower correlation (0.32, P<0.001) for all field plots (n=312). There was no significant effect of management on yield (t/ha).

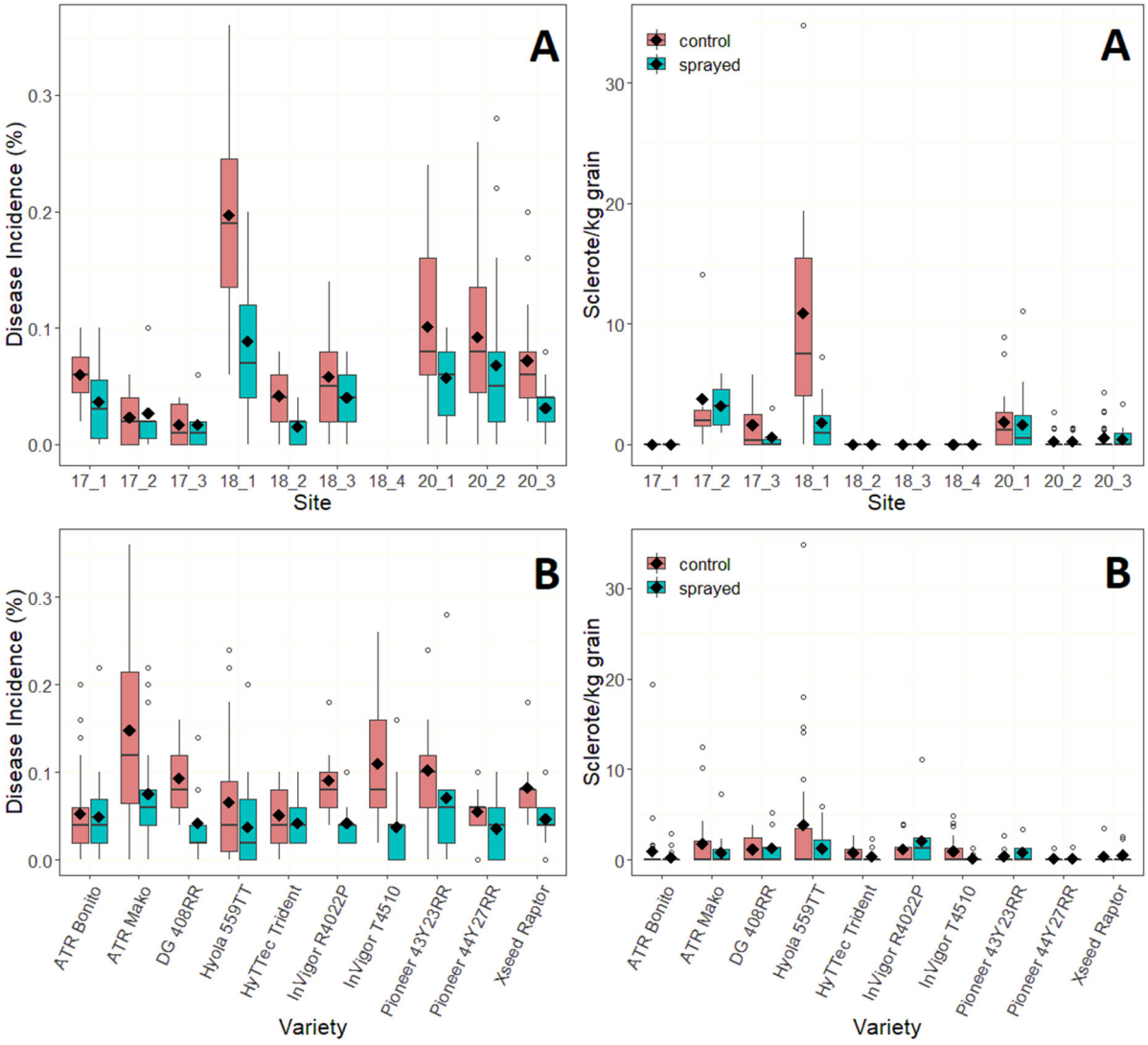

Figure 2. Boxplots showing disease incidence (% of infected plants) and number of sclerotia per 1 kg grain of canola sown in the WA grain-belt. Data is grouped by site (A) and variety (B). Two management treatments were applied; no fungicide (control) and fungicide at 30% flowering (sprayed).

Site and variety also had a significant effect on DI and contamination levels (Figure 2) with site 18_1 having the highest levels of disease and sclerotia contamination. However, although Mako had the highest average disease level, it did not have the highest sclerotia contamination.

The majority of the 16 farmer samples (81%) had sclerotia contamination, with sclerotia numbers ranging from 1-271 sclerotia/kg of grain. Lupins had the highest average number of sclerotia present (215 and 271 sclerotia/kg) (Table 2), followed by canola with an average of 7.6 sclerotia/kg (range of 1-28 sclerotia/kg). Canola samples from the Frankland region had the highest contamination, followed by Geraldton, Kojonup and York (which did not have any contamination).

Table 2. Average sclerotia contamination in grains sampled from large-scale farms

	Canola		Lupins	
Location	**Number of samples**	**Sclerotia per 1 kg grain[1]**	**Number of samples**	**Sclerotia per 1 kg grain[1]**
Frankland	6	15.1 ± 3.8		
Geraldton	1	10.8		
Kojonup	5	0.9 ± 0.2	2	243.3 ± 28
York	2	0.0 ± 0.0		
	14	7.6 ± 2.5	2	243.3 ± 2

[1]Mean ± SE

Conclusion

Results from this study demonstrate that fungicide application at 30% flowering can significantly reduce SSR disease incidence and sclerotia contaminates in harvested canola grain. However, whilst different host genotypes were shown to have varying levels of susceptibility to infection by the pathogen, those varieties with higher disease levels did not necessarily lead to higher sclerotia contamination in harvested grain.

This study has confirmed the presence of sclerotia in harvested grain within the WA grain-belt and highlights the potential spread of *S. sclerotiorum* inoculum through the transfer of contaminated grain by human-mediated processes such as machinery or seed transfer. Furthermore, with the large majority of Western Australian canola exported to international markets and stringent receival standards of canola grain, the presence of foreign contaminants including fungal sclerotia can have a significant impact on grain quality and overall profitability. This impact can be mitigated by cleaning seed with high levels of sclerotia contaminates, and is particularly important in years when SSR infection levels are high. Management of SSR in the field is also important as this has the potential to reduce inoculum levels prior to harvest, and thus the risk of sclerotia contaminants in harvested grain, as well as minimising inoculum carryover into the following growing season.

References

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