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Using a systems approach to investigate the efficacy of a disease rating system for Sclerotinia stem rot in canola

Sarita Jane Bennett, Ashmita Rijal Lamichhane and Pippa J Michael

Centre for Crop and Disease Management (CCDM), Curtin University, Perth, WA, 6102, Email: www.ccdm.com.au, sarita.bennett@curtin.edu.au

Abstract
Sclerotinia stem rot (SSR), caused by the necrotrophic fungus *Sclerotinia sclerotiorum*, is a major, but unpredictable disease of canola in Australia. However, there is no disease rating system for current canola varieties. Over the last four years the most common varieties of canola grown in Western Australia were assessed in the field using natural disease occurrence, and in glasshouse experiments using manual inoculations, to determine their susceptibility to SSR. The results highlighted the complexity and unpredictable nature of SSR with infection levels and varietal response varying depending on seasonal conditions and time of infection, despite limited levels of genetic resistance to SSR in current varieties. It is suggested that a disease rating system should not be based purely on in-season plant infection, but should also include potential for future infections through the contribution of sclerotia from infected plants to the system.

Keywords
Sclerotinia sclerotiorum, *Brassica napus*, sclerotia, infection risk

Introduction
Sclerotinia stem rot (SSR) caused by the fungal pathogen *Sclerotinia sclerotiorum* is a serious disease of canola (*Brassica napus*). It was first reported in Western Australia (WA) in 2008 and has since spread across the Australian grainbelt. It has a wide host range occurring in other broadleaf crops in the rotation, including all pulse species. SSR is reported in canola crops in most years, but significant infection events are sporadic and hard to predict due to the life cycle of the pathogen and the specific environmental conditions required for the disease to develop. Infection by *S. sclerotiorum* can occur through carpogenic or myceliogenic germination of sclerotia, the hard melanized structures that remain in the soil over summer. Following pre-conditioning over summer (Michael et al. 2021), in carpogenic germination, sclerotia germinate during the winter growing season producing apothecia from which ascospores are released. Sclerotia germination and ascospore release is typically timed to occur as canola flowers (Michael et al. 2020). Ascopores land on canola petals, which on petal drop, collect in the leaf axes of canola, and under conducive wet and humid conditions, leads to infection.

Recommendations on whether to spray for SSR are based on the testing of petals for the presence of *S. sclerotiorum*, the use of the SclerotiniaCM App (https://www.agric.wa.gov.au/apps/sclerotiniacm-sclerotinia-management-app), and other infection-prediction models, such as SkleroPro, developed in Germany (Koch et al. 2007). However, there is no Sclerotinia disease rating system, such as that published (GRDC 2020) for blackleg (*Leptosphaeria maculans*). The aim of the paper is to investigate the efficacy of a Sclerotinia disease resistance rating system for canola varieties in Australia.

Methods
Infection response following inoculation
Trials were conducted in the hoophouse at Curtin University in 2019 and 2020. A total of 10 varieties and 17 varieties were included in 2019 and 2020 respectively. In 2019, the 10 varieties were sown into single pots, as a five-block randomised block design, and inoculated with one of four isolates of *S. sclerotiorum* that are widespread across the WA grainbelt (Michael et al. 2020). Of these CU8.20 and CU8.24 have been shown to be highly aggressive, whereas CU11.7 and CU11.19 are less aggressive (Denton-Giles et al. 2018). Canola stems were inoculated with *S. sclerotiorum* at 30%flowering using the method described in Denton-Giles et al. (2018) with two inoculation treatments (on the stem, and in the leaf axis). Inoculation in the leaf axis was to mimic natural infection in the

field. In 2020, the same method and design was used for the trial, with varieties inoculated with the same four isolates, plus a control inoculation with no isolate, to be able to determine yield penalty per plant. Only stem inoculation was used. The plants were watered as required.

Following inoculation, lesion length was recorded every seven days up to 28 days, and lesion length over time was used to calculate Area Under the Disease Progression Step (AUDPS; Simko & Piepho 2012). At plant maturity, seed yield, and sclerotia number and weight per plant were recorded.

Natural infection response

Field trials were conducted over four years from 2017 - 2020 across the Western Australian grainbelt to determine the susceptibility of released canola varieties to natural SSR infection. Field trials were set out using small plots of 10 m x 1.6 - 1.8 m, as a 3-block randomised trial, with a +/- fungicide application of Prosaro® at 400 ml/ha at 30% flowering by variety. A total of 13 varieties were evaluated over the four years in genotype by environment by management (G x E x M) trials, with the open-pollinated (OP) variety ATR Bonito and the hybrid variety Hyola 449TT included in all 25 trials across four years, and the OP variety ATR Mako and hybrid variety InVigor T4510 included in 18 trials from 2018-2020 (Table 1).

Potential infection risk was determined by randomly collecting 12 petals from each unsprayed plot in Replicate 1, at 30% flowering, from all trials in 2018-2020. The petals were placed onto potato dextrose agar (PDA) (four petals per 9 mm plate) and were incubated in the dark at 20°C. After two days visible signs of actively growing mycelium were present if *S. sclerotiorum* ascospores were present on the petal. Disease incidence was recorded at leaf drop, but before the canola stems turned brown, by randomly counting the number of diseased stems in the first five plants along a randomly placed 50 cm ruler x 10 per plot. Plot yield was recorded at harvest.

Analysis of results was conducted using Genstat v.20 (VSN International Ltd, UK) and RStudio v.1.2 (2009-2019 RStudio, Inc.). Data were plotted using the ggplot2 R-package. Following tests for normality and homogeneity of variance, data were transformed where required.

Results

Infection response following inoculation

In 2019, isolate CU 8.20 was significantly ($P<0.05$) different to the other three isolates following Tukey's post hoc test, with a greater lesion area at 28 days post inoculation, a higher AUDPS and higher sclerotia weight (Table 1 & Figure 1). Although there were significant differences between canola varieties following ANOVA in all the measured variables, the same varieties were not consistently affected in terms of either lesion length or sclerotia production. Yield differences were related to canola type rather than Sclerotinia infection. Inoculation in the leaf axis led to significantly longer lesions and greater sclerotia production than stem inoculation, but the response was the same in all varieties (data not presented).

Table 1. Summary of analysis of variance on variables scored following inoculation of *S. sclerotiorum* of 4 commonly found isolates (CU8.20, CU8.24, CU11.7, and CU11.19) on 10 (2019) and 17 (2020) varieties of canola grown in Western Australia. Variance ratio presented. * = P<0.05.

		Lesion area (28 d)	AUDPS	Sclerotia no.	Total sclerotia wt	Av. Sclerotia wt	Yield
2019	Variety (9 df)	7.34*	10.60*	4.22*	2.61*	2.99*	6.68*
	Isolate (3 df)	9.29*	7.90*	ns	3.63*	11.07*	ns
	Variety x isolate	ns	1.63*	0.024*	ns	3.38*	1.57*
2020	Variety (16 df)	8.89*	13.80*	9.29*	6.13*	7.04*	11.23*
	Isolate (3 df)	19.45*	21.45*	17.34*	15.05*	7.24*	4.03*
	Variety x isolate	0.94	1.17	1.49*	1.38	1.14	1.66*

In 2020, a control treatment was included to determine the extent of yield loss following inoculation with the different isolates on a per plant basis. There was a significant (P<0.05) difference between all isolates and the control, apart from CU 11.7 (Data not shown). The isolates leading to the greatest yield loss were CU 8.20, CU 8.24 and CU 11.19. Following inoculation all other variables were significantly different between varieties and isolates with the greatest number and weight of sclerotes produced following inoculation with CU8.24. However, the results recorded in 2019 and 2020 are different, in relation to the most aggressive isolate and variety type, particularly in the response of the triazine tolerant (TT) and Roundup Ready (RR) hybrid varieties in AUSPS (Figure 1).

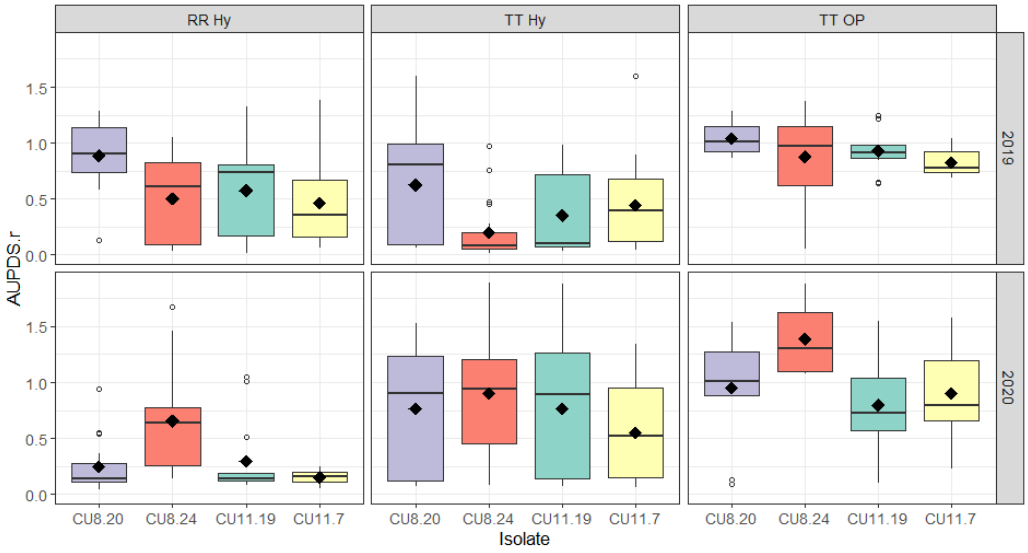

Figure 1. Area under the disease progression step (AUDPS) of canola varieties, shown as canola type inoculated with four WA *S. sclerotiorum* isolates in 2019 and 2020. RR Hy – Roundup Ready® hybrid varieties, TT Hy – Triazine tolerant hybrid varieties, TT OP – Triazine tolerant open-pollinated varieties. Box plots show 25 and 75% percentiles, Solid line – median value, solid diamond – mean value.

Natural infection response

Disease incidence across the 25 trials varied from 0% to an average of 20% across a trial. An ANOVA across canola varieties by trial sites by years found that the greatest differences were between years (P<0.05), and then between trial sites (P<0.05) rather than between varieties. All sites contained plots with no disease. Greater levels of disease were recorded in 2020, leading to a higher average disease incidence in those varieties that were only grown in 2020. All varieties included in the trials in 2019 had a minimum disease incidence of 0%. Average yield (t/ha) was higher in the recently released varieties that were only included in 2020, but there was no significant difference in yield between treated and untreated plots per variety (Figure 2).

Petal testing found that Sclerotinia ascospores were present across all sites at 30% flowering, with sites recording 55 to 100% Sclerotinia ascospore presence. There was a significant difference between sites within a year (P<0.05), but no significant difference between years. A regression analysis to explain disease incidence by petal infection was also not significant, showing that low levels of petal infection explains low levels of SSR, but at high levels of petal infection, SSR infection ranges from 0 to the maximum recorded in each year.

Discussion and Conclusion

Developing a varietal disease resistance rating system for SSR in canola relies on knowledge of the response of all available canola varieties to SSR to all *S. sclerotiorum* isolates, plus only addresses infection in-season and not the potential contribution for future infection. The research presented highlights a number of issues in relation to the ability to achieve this, as discussed below.

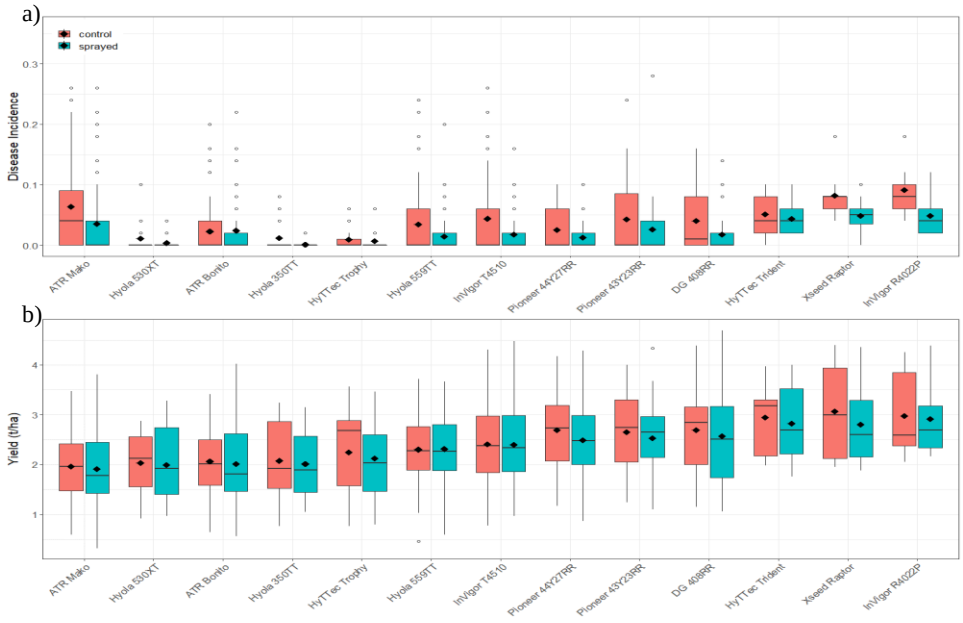

Figure 2. a) Sclerotinia stem rot disease incidence, and b) final yield of canola varieties across the 25 trials conducted from 2017-2020. Control – unsprayed, sprayed – at 30% flowering. Not all varieties were included in all trials.

Firstly, significant diversity in *S. sclerotiorum* isolates has been identified in WA alone (Michael et al. 2020; 2021). This study examined canola varietal response to only four isolates, with significant differences identified between isolates, and also in their response under different environmental conditions each year. Secondly, SSR infection is sporadic, patchy and does not occur at high levels in all years, despite the presence of *S. sclerotiorum* ascospores on more than 55% of petals tested at 30% flowering. Field testing for resistance to SSR is therefore challenging and expensive as infection levels in many years are not high enough to accurately determine resistance, as well as not occurring evenly across the trial site. Finally, there is currently little genetic resistance to SSR in Australian canola varieties with all varieties susceptible to infection (Denton-Giles et al. 2018). This results in little separation of varieties, with environmental conditions more important than canola variety in determining potential for infection, as has been shown in hoophouse trials and the field in this study.

It is therefore suggested that it is not currently possible to develop a Sclerotinia stem rot (SSR) disease rating system for canola due to the lack of genetic resistance in current varieties, the diversity of *S. sclerotiorum* isolates and the difficulties in field testing due to the sporadic nature of the disease.

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