Lesion and sclerotia development in four pulse species when inoculated with different isolates of *Sclerotinia sclerotiorum*

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Abstract
The fungal pathogen *Sclerotinia sclerotiorum* has the potential to affect pulse crops as well as canola. Pulse crops are important break crops in cereal cropping systems, but rotations may need to be managed when canola is included in the rotation, as sclerotia, the hard melanised survival structures of *S. sclerotiorum*, can last up to seven years in soil. This research sought to determine the susceptibility to, and severity of, Sclerotinia stem rot in narrow-leafed lupin (*Lupinus angustifolius*), faba bean (*Vicia faba*), chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*).

Three different isolates of *S. sclerotiorum* were inoculated onto plants and lesion length, plant height, pod count, survival and sclerotia count recorded. Lupins were the most susceptible, followed by lentil and then chickpea, with the greatest number of sclerotia recorded. There was a significant difference between species and between isolates. Faba beans were the most tolerant and no sclerotia formed within faba bean stems. Isolate CU10.12 was least virulent, causing the smallest yield penalty (pod count), the shortest lesions, no sclerotia, and no plant deaths. Isolate CU8.20 was the most virulent in all these measures. The isolate of *S. sclerotiorum* as well as the pulse in the rotation is therefore important when determining potential disease severity and future inoculum contribution when including pulses in the rotation.

Introduction
Sclerotinia sclerotiorum (Lib.) de Bary (Sclerotinia) is a necrotrophic phytopathogenic fungus with a broad range of host plant species and one of the causal species of the disease Sclerotinia stem rot (Boland & Hall, 1994). Symptoms of Sclerotinia stem rot include leaf wilt or drop, watery soft rot, or dry lesions on stems, branches and stalks. Sclerotia, the hard melanised survival bodies of *S. sclerotiorum*, persist in the soil to produce apothecia in subsequent growing seasons, which disseminate ascospores into the air. These ascospores settle on senescent parts of a host plant, germinate, then spread and infect healthy parts of the plant with mycelium. Favourable conditions will result in sclerotia forming from the mycelium. Following death of the plant, these sclerotia can remain viable in the soil for up to seven years (Kora et al. 2008). Due to the wide variety of dicot plant hosts that are susceptible to this disease it can be difficult to control in an agricultural setting. It has been reported to occur in 408 plant species (Boland & Hall, 1994). Sclerotinia costs the Australian canola industry up to $10.1 million per year (Murray & Brennan, 2012), and crop rotations, which function as break crops, are an important cultural practice to manage recurrence of the disease. However, broad leaf break crops including pulses are used to help manage cereal diseases, reduce the drain on plant available nitrogen in the soil, improve microbe diversity in the soil and improve soil structure (Kirkegaard et al. 2008). Most reports of Sclerotinia have focused on its impact on canola (*Brassica napus*) and lupins, with level of incidence and severity in Faba bean not well documented. To date, this disease has not been the cause of great economic losses in lentil crops, however it remains a risk, particularly if this crop is selected in rotation with canola, and in wet seasons. Sclerotinia occurs infrequently in chickpeas but can cause significant losses (Grains Research and Development Corporation, 2019).

The aim of this experiment was to test the susceptibility to and severity of *Sclerotinia sclerotiorum* - across four pulse crop species, *Lens culinaris* (lentil), *Cicer arietinum* (chickpea), *Vicia faba* (faba bean), *Lupinus angustifolius* (narrow-leafed lupin).

Materials and Methods
The trial of the four pulse species (lentil cv. Hurricane XT, chickpea cv. Genesis 836, faba bean cv. Rana and lupin cv. Barlock) was sown at the field trials area at Curtin University, Bentley, Western Australia, on 1st of June 2018. Five rows of 10 m of each species were sown, with a 30 cm row spacing, and 60 cm between each species. In 2017 the area had been sown to canola and inoculated with *S. sclerotiorum*. Agar plugs of three *S. sclerotiorum* isolates collected from *Brassica napus* hosts (GM17P 3.2 from Greenough, WA

(Michael et al. 2019), CU10.12 from Geraldton, WA and CU8.20 from South Stirling, WA (Denton-Giles et al. 2018) were generated by surface sterilising five F2 sclerotia of each isolate in bleach (White King premium bleach; 4% v/v sodium hypochlorite) for 4 mins, 4 mins in 4% ethanol, 4 mins in sterile Milli-Q water. Sclerotia were then cut in half and placed in 90mm Petri-dishes containing ½ potato dextrose agar (PDA) supplemented with neomycin (50µg ml-1), streptomycin (100 µg ml-1), and ampicillin (100 µg ml-1) and incubated at 20°C in the dark until actively growing mycelium was visible (3-5 days). Each isolate was subcultured once onto PDA by extracting an agar plug from the margins of the mycelial growth.

On 23rd August 2018, the trial was divided into three blocks of 3 m along its length across all species, with 0.5 m guard area at each end. For ease of management, four plants in each block were infected with one of the three Sclerotinia isolates. Thus, there were four replicates per species, per isolate. An agar plug (5 mm diameter) was taken from the actively growing mycelial growth in the agar plates of one of the isolates and placed on a length of parafilm. The parafilm was wrapped around the stem of the plant above the second leaf. The average diameter of the stems at the inoculation point was 3 mm, 5 mm, 15 mm and 8 mm for lentil, chickpea, faba bean and lupin respectively. A separate, random selection of four plants per species, per isolate were flagged with streamers, for which control measurements would be taken. Plant growth scores were recorded weekly with reference to the respective GRDC Western Grow Notes (Grains Research and Development Corporation, 2019), and plant height recorded fortnightly on the same plants in each plot, until inoculation. Prior to inoculation, on 23rd of August plant growth scores for each species were uniform across the three blocks, and were recorded as V8, R4, 203 and 4.0 for lentil, chickpea, faba bean and lupin respectively. A two-way fully factorial ANOVA ($n=60$, $\alpha=0.05$) on plant height measured on this date showed there was no significant difference between plants of the same species or between blocks within a species ($F=1.89$; d.f.=8,45; $p>0.05$). The trial was irrigated following inoculation to increase the humidity within the trial.

On 4th October, 126 days after sowing (DAS), Sclerotinia infected plants were removed from the soil to record plant height (cm), number of pods, plant survival and lesion length (mm). The length of stem affected by the infection were cut from the rest of the plant and dried for a week, before counting the number and length of sclerotia and total weight of sclerotia per plant.

A field pea (*Pisum sativum* L.) crop was also included in this trial but was overcome with symptoms believed to be consistent with Ascochyta blight and could not be investigated in the context of this study. No symptoms suspected to be caused by Ascochyta blight or any fungal pathogen other than *S. sclerotiorum* were observed on the other crop species. The appearance of the lesions only occurred where the *S. sclerotiorum* mycelia plugs had been placed on the inoculated plants in the trials and was consistent with infection by *S. sclerotiorum*.

Results

No plant deaths were recorded in lupins or faba beans following inoculation with any isolate of *S. sclerotiorum*, and in chickpeas and lentils, no plant deaths were recorded following inoculation with isolate CU10.12. Inoculation with isolates CU8.20 and GN17P3.2 resulted in up to 50% plant death in chickpeas and lentils respectively.

Lesion length data did not meet the assumptions of normality. A square root transformation met the assumption of normality, but not homogeneity of variance. A two-way fully factorial ANOVA ($n=48$, $\alpha=0.05$) on the transformed data indicated that there was no significant difference between pulse species. The mean lesion length of lentil (51.83 mm) was not significantly greater than that of chickpea, lupin or faba bean (means of 35.83, 42.83 and 44.17 mm respectively). However, there was a significant difference ($F=14.464$; df=2,36; $p<0.001$, Figure 1) between *S. sclerotiorum* isolates with regards to lesion length. The lesion length (mm) of plants inoculated with isolate CU10.12 were significantly shorter than those inoculated with isolates CU8.20 or GN17P3.2 (means of 18.63, 58.50 and 42.83mm respectively). Chickpea and lentil displayed the largest lesion lengths with GN17P3.2, whereas faba bean and lupin had the largest lesion length with isolate CU8.20.

Lupins had the largest total number of sclerotia across the whole experiment (14), ranging from zero to six in an individual plant, followed by lentil (6) and chickpea (5), both with a range of zero to two sclerotia per plant. No sclerotia developed within the lesions on the faba beans. Despite having the largest sclerotia count,

there were no plant deaths in the lupins, nor in the faba beans. Chickpea and lentil each suffered three plant deaths out of 12 inoculated plants. A Fisher's exact test for count data (n=15, α=0.05) indicated that there was a significant association (p=0.038, Figure 2) between *S. sclerotiorum* isolate and plant species with regards to number of sclerotia within a lesion. For all plant species, no sclerotia were recorded in lesions of isolate CU10.12. Isolate CU8.20 resulted in the greatest number of sclerotia (14), followed by GN17P3.2 (11 sclerotia). For chickpea and lentil, infection by isolate CU8.20 resulted in fewer sclerotia (1 and 2 respectively), compared to infection by isolate GN17P3.2 (4 sclerotia each). For lupin, infection by isolate CU8.20 resulted in far greater sclerotia numbers (11) compared to GN17P3.2 (3 sclerotia). For isolate CU8.20, the weight of sclerotia for each plant species collectively was 0.01g for lentil, 0.02g for chickpea and 0.19g for lupin. The mean length for individual sclerotes of this isolate was 3.5mm, 7mm and 5mm for lentil, chickpea and lupin respectively. For isolate GN17P3.2, the weight of sclerotia for each plant species collectively was 0.01g for lentil, 0.03g for chickpea and 0.08g for lupin. The mean length for individual sclerotes of this isolate was 3mm, 6mm and 3.25mm for lentil, chickpea and lupin respectively.

A chi square test (n=96; α=0.05) found an association between the number of pods produced for each plant type and the isolate with which they were infected ($\chi 2$=75.12; df=9; p<0.001). Infection by isolate CU8.20 resulted in the lowest mean number of pods per plant, followed by GN17P3.2, then CU10.12 (mean of 2, 5 and 6 pods per plant respectively). However, in chickpeas, infection by either CU8.20 or GN17P3.2 resulted in no pods and in lupins more pods were produced per plant following infection by isolates CU10.12 and GN17P3.2 than in the control plants.

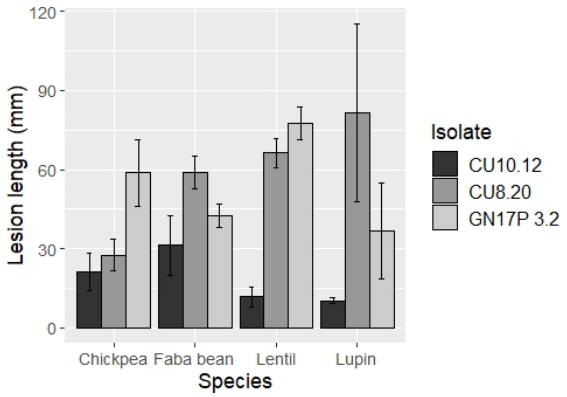

Figure 1. Mean lesion length (mm) ±standard errors of n=4 replicates 126 DAS of faba bean, chickpea, lentil and lupin inoculated with *S. sclerotiorum* isolates CU10.12, CU8.20 and GN17P3.2

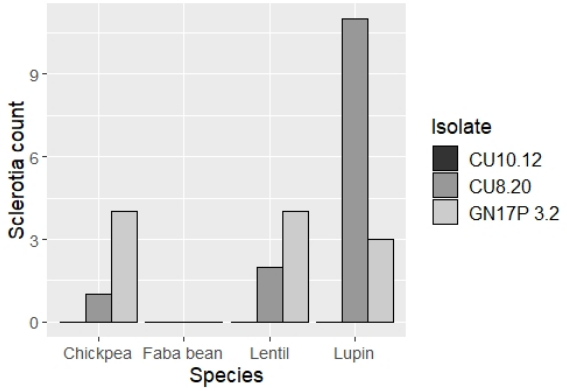

Figure 2. Total number of sclerotia 133 DAS in chickpea, faba bean, lentil and lupin inoculated with *S. sclerotiorum* isolates CU10.12, CU8.20 and GN17P3.2

Discussion

All the pulse species tested were found to be susceptible to infection by *Sclerotinia sclerotiorum*, which is supported by Boland and Hall (1994). Faba bean was the least susceptible plant species, having no sclerotia produced within the stem and no plant deaths. Although lupins also had no plant deaths following inoculation with *S sclerotiorum*, the greatest number of sclerotia were produced within this species. Bearing in mind that chickpea had the most plant deaths but the smallest lesion length, and lupins the inverse relationship, it is considered that plant death may be influenced by stem diameter. Depending on its susceptibility to infection in the field, lupin would be a poor choice for Sclerotinia management in a paddock, due to the large number of sclerotia produced, particularly when infected with isolate CU8.20. Lentil and chickpea would also contribute to an increase in the number of sclerotia in the soil if used as a break crop, depending on the isolate.

Isolate CU10.12 was found to be the least virulent isolate in all the tested pulse species, inflicting a shorter lesion length, few or no sclerotia and no plant deaths. Isolate CU8.20 by comparison was the most virulent overall. These results mirror the source of the CU8.20 and CU10.12 isolates, with Denton-Giles et al. (2018) also finding CU8.20 to be more aggressive than CU10.12. It is interesting that chickpea and lentil however were more susceptible to isolate GN17P3.2 than to CU8.20. Therefore, it can be concluded that it does matter which isolate is dominant in your crop, and the host species being grown as to the impact on that crop, sclerotia development and subsequently the impact on the productivity of future crops that host the disease.

However, Michael et al. (2019) have shown that isolate diversity can be high within a paddock, with several different isolates present at the same location. Also, none of the species we tested was completely resistant to any of the *Sclerotinia sclerotiorum* isolates tested. It is therefore important for growers to be aware of the potential susceptibility of pulse crops to Sclerotinia stem rot when planning their paddock rotations.

The stems of the faba bean were still quite green at harvest, especially compared to the other species. Given the size of the lesions it is possible that with a longer natural progression of plant maturity and drying out sclerotia may form in this species as well. Current on-going research does suggest that faba bean is less susceptible to Sclerotinia stem rot (Khangura et al. 2018), although it does not state whether this is in relation to lesion development and subsequent plant health or sclerotia formation within infected stems.

As evidenced by the figures, there was a large variation in the results for all measures, even between replicates of the same species-isolate interaction. To improve the detail in this study it is recommended to inoculate a greater number of plants with each isolate and to test several different varieties of each species. We recommend expansion of this research to study a diverse range of faba bean cultivars to identify candidates that vary in their susceptibility to Sclerotinia. Producers dealing with Sclerotinia stem rot infection within a paddock should consider paddock rotation history, disease incidence in previous years and crop sensitivity to minimise the disease within a paddock.

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