Inoculum potential of *Sclerotinia sclerotiorum* sclerotia depends on isolate and host plant

Andrew Taylor[1], Emma Coventry[1], Claire Handy[1], Jon S. West[2], Caroline S. Young[3] and John P. Clarkson[1]

[1] Warwick Crop Centre, School of Life Sciences, University of Warwick, Wellesbourne, Campus, Wellesbourne, Warwick CV35 9EF, UK.

[2] Rothamsted Research, Harpenden, AL5 2JQ. UK

[3] ADAS, Drayton Farm, Alcester Road, Stratford upon Avon CV37 9RQ, UK

Abstract

The soil-borne fungus *Sclerotinia sclerotiorum* infects many important crop plants. Central to the success of this pathogen is the production of sclerotia, which enables survival in soil and constitutes the primary inoculum. This study aimed to determine how crop plant type and *S. sclerotiorum* isolate affects sclerotial production and germination and hence inoculum potential. Three *S. sclerotiorum* isolates (L6, L17, L44) were used to inoculate plants of bean, carrot, lettuce, oilseed rape (OSR) and potato and the number and weight of sclerotia per plant quantified. Carpogenic germination of sclerotia collected from different hosts was also assessed for L6. Production of sclerotia was dependent on both crop plant type and *S. sclerotiorum* isolate with OSR and lettuce supporting the greatest number (42-122) and weight (1.6-3.0 g) of sclerotia per plant. The largest sclerotia were produced on oilseed rape (33-

66 mg). The three *S. sclerotiorum* isolates exhibited a consistent pattern of sclerotial production irrespective of crop type; L6 produced large numbers of small sclerotia while L44 produced smaller numbers of large sclerotia with L17 intermediate. Germination rate and percentage was greatest for larger sclerotia (4-6.7mm) and also varied between host plants. Combining sclerotial production data and typical field crop densities suggested that infected carrot and OSR could produce the greatest number (3944 m^{-2}) and weight (73 g m^{-2}) of *S. sclerotiorum* sclerotia respectively, suggesting these crops potentially contribute a greater increase in inoculum. This information, once further validated in field trials, could be used to inform future crop rotation decisions.

Introduction

The cosmopolitan necrotrophic fungus *Sclerotinia sclerotiorum* (Lib) de Bary infects more than 400 species of plants throughout the world (Boland & Hall, 1994) leading to severe economic losses in a wide range of crops, including beans, carrot, lettuce, oilseed rape (OSR) and potatoes. In bean (*Phaseolus vulgaris* L., both dry and green bean), the pathogen causes white mould disease, leading to losses of up to 100% under favourable conditions (Schwartz & Singh, 2013) with the fungus infecting stems, branches, leaves, flowers, pods and seeds at every stage of crop development (Viteri *et al.*, 2015). In carrot, *S. sclerotiorum* has been reported in over twenty carrot producing countries (Kora et al., 2003) and affects both foliage and roots causing substantial canopy die-back and subsequent crown rot (Jensen *et al.*, 2008). This can also then lead to post-harvest epidemics in storage (Foster *et al.*, 2008). *S. sclerotiorum* also causes lettuce ‘drop’ where plants quickly wilt in the field due to

infection of the stem base and lower leaf axils followed by rapid rotting of the tissue (Young *et al.*, 2004, Clarkson *et al.*, 2014). As in other crops, the disease can be very damaging with up to 50% losses reported in UK lettuce (Young et al., 2004). In OSR, *S. sclerotiorum* causes a stem rot and results in substantial yield losses in all major growing areas such as Australia, Canada, UK and USA (Derbyshire & Denton Giles, 2016). In this case, flower petals are initially infected which fall onto leaves or leaf axils resulting in development of the disease in stems (Derbyshire & Denton Giles, 2016). Finally, *S. sclerotiorum* can also be a significant disease in potato where it causes a stem rot, infecting the lower parts of the stems towards the end of the growing season, leading to reduced yields (Ojaghian *et al.*, 2012). As for OSR, stem lesions are associated with infected flowers dropping onto stems (Atallah & Johnson, 2004).

Central to the success of *S. sclerotiorum* as a plant pathogen is the production of numerous sclerotia on infected plants. In addition to representing a huge reproductive potential and providing the primary inoculum for subsequent epidemics, these sclerotia enable the fungus to survive overwinter in soil (Adams & Ayers, 1979). *S. sclerotiorum* sclerotia can then germinate carpogenically or myceliogenically, depending on environmental conditions with the former resulting in the production of apothecia and airborne ascospores. This often represents the principal source of inoculum and hence allows infection of above-ground plant parts (Bolton *et al.*, 2006). Carpogenic germination of *S. sclerotiorum* occurs only in sclerotia that are brought close to the soil surface and, in temperate climates, a period of cold conditioning is also required to break dormancy (Phillips, 1987, Clarkson *et al.*, 2007). Ascospores are then released from mature apothecia under a wide range of conditions with up to $7.6x10^5$ spores released over a 20-day period. (Clarkson *et al.*, 2003).

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The density of *S. sclerotiorum* sclerotia in the soil is clearly an important factor in disease epidemics although very few studies have attempted to quantify populations and relate this to disease incidence. Surveys in lettuce fields in the USA with a history of *Sclerotinia* disease have reported densities ranging from 0.08-2.9 sclerotia / 100 g soil in in Yuma, Arizona (Chitrampalam & Pryor, 2013), while an average of 0.06 / 100 cm^3 of soil was reported in the San Joaquin Valley, California (Hao & Subbarao, 2005). Other work has demonstrated a positive correlation between the density of *S. sclerotiorum* sclerotia used to inoculate beds of lettuce (2-100 sclerotia m^{-2}) and disease incidence at harvest (5-71%) although the authors indicated that in the desert production area under investigation, mycelial germination of sclerotia was the predominant mode of infection as conditions were not conducive to carpogenic germination and development of apothecia (Chitrampalam *et al.*, 2010). In the many lettuce growing areas where conditions do allow production of apothecia, small numbers of *S. sclerotiorum* sclerotia can still lead to high levels of disease incidence (Hao & Subbarao, 2005). This is because the inoculum potential of sclerotia germinating carpogenically is much higher than for those germinating to produce mycelium due to the large number of airborne ascospores released which dramatically increase the range of infection from a single sclerotium. Work in other crops such as oilseed rape, soybean and sunflower has also demonstrated strong relationships between either sclerotial density or apothecial production and Sclerotinia disease incidence (Gugel, 1986, Holley & Nelson, 1986, Lehner *et al.*, 2017)

The number of *S. sclerotiorum* sclerotia that are returned to the soil not only depends on the number of plants infected but also on the host type but very little information exists on sclerotial production on different crops. One study reported variation in the

number and weight of *S. sclerotiorum* sclerotia produced by different *S. sclerotiorum* isolates on carrot, celery and selected cultivars of cabbage (Leiner & Winton, 2006). However, this work was carried out using detached plant material, rather than a live host. Other researchers also demonstrated that, *in vitro*, different isolates of both *S. sclerotiorum* and *S. trifoliorum* produced different numbers and weights of sclerotia under the same conditions (Akram *et al.*, 2005, Li *et al.*, 2008, Vleugels *et al.*, 2013). Furthermore, it has been shown that larger *S. sclerotiorum* sclerotia tend to produce more apothecia than smaller ones and are also more likely to germinate (Ben-Yephet *et al.*, 1993, Dillard *et al.*, 1995, Hao *et al.*, 2003, Warmington & Clarkson, 2016). However, these studies used sclerotia produced on a single crop type (lettuce; (Ben-Yephet *et al.*, 1993), or on detached potato tubers (Hao *et al.*, 2003) or produced artificially *in vitro* (Dillard *et al.*, 1995, Warmington & Clarkson, 2016). Hence, both crop type and *S. sclerotiorum* isolate potentially have a significant impact on the build-up of inoculum in the field through affecting the number and size of sclerotia produced.

The aim of this study was to quantify sclerotial production by different *S. sclerotiorum* isolates on five different crop hosts using whole plants and hence estimate the risk of inoculum build-up associated with each. In addition, the effect of sclerotial size on the rate and final level of germination as well as the number of apothecia produced was also investigated.

Materials and Methods

S. sclerotiorum isolates and production of sclerotia on agar

The 17 *S. sclerotiorum* isolates used in this study were obtained from wild buttercup (*Ranunculus acris*, eight isolates), carrot (*Daucus carota*, one isolate), OSR (*B*. *napus*, one isolate), lettuce (*Lactuca sativa*, four isolates), pea (*Pisum sativum*, two isolates) and celery (*Apium graveolens*, one isolate). A single isolate of *S. subarctica* (isolate HE1 from wild buttercup) was also included for comparison; this related pathogen often produces larger sclerotia than *S. sclerotiorum* isolates (Clarkson *et al.*, 2010). Isolates represented different microsatellite haplotypes (Table 1) assigned through a previous study investigating the population structure of *S. sclerotiorum* and *S. subarctica* in the UK (Clarkson *et al.*, 2017); Table 1). In addition, the relative aggressiveness of the same 18 isolates had also been evaluated previously on three brassica hosts (Taylor et al., 2015). Stocks of each *S. sclerotiorum* isolate were maintained as sclerotia stored at 5°C and new cultures initiated by bisecting a sclerotium, placing it face down on potato dextrose agar (PDA) and incubating at 20°C for 4 days to produce actively growing cultures for use in experiments. To assess the production of sclerotia by the different *S. sclerotiorum* isolates on agar, 5mm agar plugs from actively growing cultures were placed at the centre of PDA plates and incubated at 20°C for four weeks (five replicate plates per isolate). Sclerotia were then picked off and the number and weight of sclerotia per plate recorded. Data were analysed using a REML analysis in Genstat version 18 (VSN International). As all data were close to the normal distribution, correlations with aggressiveness were examined using Pearson correlation coefficients (Genstat).

147 Table 1: *Sclerotinia* isolates used in this study.

Isolate	**Host**	**Location**	**Microsatellite haplotype***
S. sclerotiorum			
C28	Carrot	Nottinghamshire	390
CE11	Celery	Norfolk	1
DG4	Buttercup	Warwickshire	391
EV9	Buttercup	Powys	28
HE33	Buttercup	Herefordshire	138
L17	Lettuce	Sussex	1
L44	Lettuce	Sussex	63
L5	Lettuce	Sussex	2
L6	Lettuce	Sussex	3
O92	Oilseed rape	Herefordshire	182
P2	Pea	Herefordshire	31
P7	Pea	Herefordshire	1
R12	Buttercup	Warwickshire	21
R17	Buttercup	Warwickshire	199
R19	Buttercup	Warwickshire	72
R28	Buttercup	Warwickshire	6
R30	Buttercup	Warwickshire	206
S. subarctica			
HE1	Buttercup	Herefordshire	1

148 *Clarkson et al., (2017)

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150 **Number of *S. sclerotiorum* sclerotia produced on different crop plants**

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152 Three *S. sclerotiorum* isolates (L6, L17 and L44) were selected for determining the
153 number of sclerotia produced on five different crop plants based on differences in
154 sclerotial production *in vitro*. Actively growing cultures of each isolate were produced
155 as described previously and 5 x 5 mm agar plugs from the leading edge used to
156 inoculate sterile wheat grain placed in Petri dishes. Following incubation at 20°C for
157 3 days, colonised wheat grain was then used as inoculum for each crop; oilseed rape
158 (cv. Temple, three experiments of 10 replicate plants), lettuce (cv. Montel, eight

experiments of 10 replicate plants), dwarf bean (cv. Tendergreen, six experiments of 10 replicate plants), carrot (cv. Nairobi, four experiments of 10 replicate plants) and potato (cv. Estima, three experiments of 10 replicate plants). Plants were inoculated at flowering with the exception of lettuce (30 cm diameter plant) and carrot (4-5 fully opened leaves). For bean and potato inoculations, stems were first cut in two different places with a scalpel and 4-5 wheat grains placed into each wound. Lettuce, carrot and oilseed rape plants were inoculated by placing 10 wheat grains into different axils next to the main stem (2-3 grains per axil). A high level of humidity was maintained to encourage infection and disease development for all inoculated plants using automatic misting sprays in a controlled temperature glasshouse (lettuce, bean and carrot) set at a constant 15°C with supplementary lighting as required to achieve a 16 h photoperiod. Potato and OSR experiments were carried out in polytunnels with overhead irrigation. After plants were fully colonised and necrotic, watering was halted and the tissue allowed to die back and dry out completely before sclerotia were collected, weighed and counted. For OSR, the main stem was split open and sclerotia from inside the stem added to the count. Data were analysed using REML in Genstat. As all data was close to a normal distribution, correlations with sclerotial production on agar were examined using Pearson correlation coefficients (Genstat).

Effect of crop type and size of sclerotia on carpogenic germination

To investigate the effect of size of *S. sclerotiorum* sclerotia on carpogenic germination, isolate L6 was selected due to its ability to consistently produce apothecia in previous work (Taylor *et al.*, 2017). Sclerotia of this isolate collected from plants across multiple experiments were pooled according to the crop type on which they were produced and

passed through sieves of increasing diameter to divide them into four size classes; <2 mm, 2-4mm, 4-6.7 mm and >6.7 mm. Sclerotia were then buried at 1 cm depth in compost (150 g, John Innes No. 1, J Arthur Bowers, UK) placed in 600 ml plastic boxes (Malsar Kest Ltd, UK) and water content adjusted to 30% w/v. There were 30 sclerotia per box, and three replicate boxes per crop/size combination. The sclerotia were initially conditioned by placing the boxes at 5°C for 40 days to promote carpogenic germination (Clarkson et al., 2014) after which they were placed at 15°C and the number of germinating sclerotia and number of apothecia produced for each sclerotium recorded over a 12-week period. For sclerotia in the size class 4-6.7 mm collected from carrot and bean plants, only one box of sclerotia was set up due to the very low numbers obtained in this size class. Sclerotia in the size class >6.7mm were only obtained from oilseed rape and in this case, due to their size, only 16 sclerotia were placed in each box (three replicate boxes). The germination of *S. sclerotiorum* sclerotia produced on the crop plants was compared with those produced on sterilised wheat grain *in vitro*, as used routinely for production of sclerotia in several previous studies (Mylchreest & Wheeler, 1987, Liu & Paul, 2007, Warmington & Clarkson, 2016). As before, three replicate boxes (30 sclerotia per box) were set up for each size class with the exception of those >6.7mm where a single box was set up due to low numbers of sclerotia of this size being produced on wheat grain. The number of sclerotia germinating and the number of apothecia per sclerotium was again recorded over a 12-week period and the time taken to reach one third (33%) germination calculated.

Results

Production of sclerotia on agar

Significant differences in the number of sclerotia produced by the 18 *S. sclerotiorum* and *S. subarctica* isolates were observed, with mean number ranging from 17 (*S. subarctica* isolate HE1) to 44 (*S. sclerotiorum* isolate L6) per PDA plate ($P<0.001$, Fig. 1a). Significant differences were also observed between isolates for the mean weight of sclerotia produced per plate ($P<0.001$, Fig. 1b) which ranged from 116 mg (*S. sclerotiorum* L44) to 306 mg (*S. sclerotiorum* EV9) and also the mean weight per sclerotium ($P<0.001$, Fig. 1c) which ranged from 5.0 mg (*S. sclerotiorum* L6) to 16.9 mg (*S. subarctica* HE1). There were no significant correlations between aggressiveness on three brassica types (*B. oleracea*, *B. napus*, *B. rapa*) assessed previously (Taylor *et al.*, 2015) and the number of sclerotia ($r=0.42$, $P=0.083$), weight of sclerotia ($r=-0.23$, $P=0.35$) or weight per sclerotium ($r=-0.46$, $P=0.055$). Based on these results, three *S. sclerotiorum* isolates (all from lettuce) were selected for further experiments on plants based on variation in number of sclerotia produced per plate (L6, L17 and L44 producing 44, 36 and 29 sclerotia per plate respectively).

Number of *S. sclerotiorum* sclerotia produced on different crop plants

All plants inoculated with *S. sclerotiorum* resulted in infection, complete colonisation and plant death for all crop types with the exception of potato where in contrast to L6, isolates L17 and L44 failed to completely colonise plants. Significant differences were observed in the mean number of sclerotia produced on the different host plants

(P<0.001, Fig. 2a), with the largest number produced on lettuce (122 sclerotia per plant for isolate L6) and the smallest number produced on bean (7 sclerotia per plant for isolate L44). Significant differences between the mean weight of sclerotia per plant (P<0.001, Fig. 2b) were also observed ranging from 0.21 g on bean (L44) to 3.09 g on OSR (L6). The mean weight per sclerotium produced by each isolate was generally consistent across the different crops (10-28 mg) with the exception of OSR where significantly heavier sclerotia (33-60 mg) were produced for all three isolates (P<0.001; Fig. 2c). OSR also supported the highest proportion of larger sclerotia; for example, for *S. sclerotiorum* isolate L6, 17% were in the size category 4-6.7 mm compared to 3.2-7.4% in the other crop types (Table 2).

Significant differences in the mean number of sclerotia produced were also observed between the *S. sclerotiorum* isolates when averaged across the different crop types with isolates L6, L17 and L44 producing 75, 54 and 32 sclerotia per plant respectively (P < 0.001, LSD = 6.87; Fig 2a). However, isolate L44 also produced significantly heavier sclerotia (P<0.001; Fig 2b) across crop type (27 mg) compared to L17 (19 mg) and L6 (16 mg). This therefore resulted in there being no significant differences overall between isolates for the mean total weight of sclerotia produced per plant (P=0.16). Isolate L6 produced a greater mean number and weight of sclerotia on potato compared to the other isolates as this was the only isolate to completely colonise plants. Significant crop x isolate interactions were observed for mean number of sclerotia (P<0.001), mean weight (P=0.004) of sclerotia and mean weight per sclerotium (P<0.001) suggesting that isolates behave differently depending on host. However, crop type was the main driving factor for the variation in these data.

Across the three isolates, the mean number of sclerotia produced on agar was correlated with the number produced on crops (r=0.49, P=0.031). However, there was no correlation between results on agar and on plants for mean total weight of sclerotia (r=0.11, P=0.35), or for the mean weight per sclerotium (r=0.073, P=0.40).

Effect of crop type and size of sclerotia on carpogenic germination

Percentage germination was significantly greater for larger sclerotia of *S. sclerotiorum* L6 produced on crop plants (P<0.001; Table 2) with those >6.7mm having a mean germination of 95.8% compared with those <2 mm having a mean germination of 62.4% across the different crop types. This was consistent irrespective of crop type (P=0.68 for crop x size interaction). This trend was also observed for the sclerotia produced on wheat grain where the largest sclerotia (>6.7 mm) resulted in 100% germination compared with 78.9% for the smallest sclerotia (<2 mm). Larger sclerotia of *S. sclerotiorum* isolate L6 also produced significantly more apothecia than smaller ones (P<0.001, Table 2) irrespective of crop type (P=0.70 for crop x size interaction). The largest sclerotia (>6.7 mm) produced on OSR yielded an average of 11.9 apothecia per sclerotium compared to 1.1 apothecia per sclerotium for sclerotia <2 mm, 1.6 apothecia per sclerotium for sclerotia 2-4 mm and 2.5 apothecia per sclerotium for sclerotia 4-6.7 mm (across all crop types). The same trend was again observed for *S. sclerotiorum* sclerotia produced on wheat grain (P<0.001; Table 2) where mean numbers of apothecia ranged from 1.6 (<2 mm) to 15.4 (> 6.7mm).

Larger sclerotia of *S. sclerotiorum* isolate L6 also germinated significantly faster ($P<0.001$; Table 2) with a time to 33% germination of 22 days in the largest sclerotia (>6.7 mm) compared to 60 days in the smallest (< 2 mm). Again, this was consistent across the crop types ($P=0.16$ for interaction). Whilst there were significant differences ($P=0.003$) in time to 33% germination in the sclerotia artificially produced on wheat grain, the time for 33% germination for the largest sclerotia (mean 22 days) was not significantly different from the smallest (mean 24 days) with the mid-range sized sclerotia germinating more rapidly (14-16 days).

Crop type significantly affected the number of apothecia produced ($P<0.001$; Table 2) and this was particularly evident for OSR where sclerotia generally produced more apothecia; e.g. for the size class 4.0-6.7 mm, sclerotia from OSR produced 3.2 apothecia compared to 2.0, 1.9, 2.6 and 1.7 for potato, bean, lettuce and carrot respectively. Crop type also had a significant effect on final percentage germination ($P<0.001$), with those from OSR exhibiting greater germination; e.g. for size class 2-4 mm sclerotia from OSR resulted in 80.0% germination compared to 59.6, 66.7, 71.1 and 45.6% for potato, bean, lettuce and carrot respectively. Finally, crop type also had a significant effect on germination rate (time to 33% germination, $P<0.001$; Table 2). Sclerotia from bean were generally the fastest to germinate; e.g. in the size class 2-4mm the time to 33% was 30 days compared to 42, 41, 35 and 69 days for potato, oilseed rape, lettuce and carrot respectively. Sclerotia from carrot were all particularly slow to germinate (53-71 days).

Estimation of sclerotial production for different crops in the field

Using field planting densities of 3.3, 8, 28, 40 and 150 plants m^{-2} for potato, lettuce, OSR, bean and carrot respectively, the number of *S. sclerotiorum* sclerotia that could ~~be~~ potentially be returned to the soil for each crop was calculated for each isolate (assuming complete plant colonisation). This resulted in the greatest potential number of sclerotia being returned for carrot (2666-4794 m^{-2}; mean 3944 m^{-2} across isolates) compared to the other crops with the fewest sclerotial returns from potato (50-298 m^{-2}; mean 145 m^{-2} across isolates (Fig. 3a). When weight of sclerotia produced by the different crops was considered, OSR (56.3-86.4 $g^{-1}m^{-2}$, mean 73.3 $g^{-1}m^{-2}$ across isolates) and carrot (35.2-82.6 $g^{-1}m^{-2}$, 60.5 $g^{-1}m^{-2}$ across isolates) returned the greatest value (Fig. 3b).

Table 2. Germination and number of apothecia produced by different sizes of *S. sclerotiorum* isolate L6 sclerotia produced on different crop plants.

Crop Plant	Size class (mm)	Percentage of sclerotia in size class	Percentage germination	Time to 33% germination (days)	Number of apothecia per sclerotium
Potato	< 2.0	41.7	73.3	51.7	1.1
	2.0-4.0	53.6	59.6	42.3	1.3
	4.0-6.7	4.5	84.4	33.0	2.0
	> 6.7	0.2	-	-	-
	LSD		*14.7**	*13.4***	*0.46****
Bean	< 2.0	43.6	50.0	57.7	1.0
	2.0-4.0	50.2	66.7	29.7	1.6
	4.0-6.7	5.1	60.0	25.0	1.9
	> 6.7	1.1	-	-	-
	LSD		*14.7**	*13.4***	*0.48****
OSR	< 2.0	25.0	64.4	56.3	1.1
	2.0-4.0	52.7	80.0	41.3	2.3
	4.0-6.7	16.6	83.3	32.0	3.2
	> 6.7	5.7	95.8	22.3	11.9
	LSD		*14.7**	*13.4***	*0.98 (1.10)*
Lettuce	< 2.0	37.2	59.2	62.3	1.1
	2.0-4.0	59.1	71.1	35.3	1.5
	4.0-6.7	3.2	86.7	26.0	2.6
	> 6.7	0.5	-	-	-
	LSD		*14.7*	*13.4*	*0.47*
Carrot	< 2.0	42.8	43.3	71.3	1.2
	2.0-4.0	48.6	45.6	69.0	1.5
	4.0-6.7	7.4	46.7	53.0	1.7
	> 6.7	1.1	-	-	-
	LSD		*14.7**	*13.4***	*0.57*****
Mean (across all crop types)	< 2.0	38.1	*62.4a*	*59.9a*	*1.1a*
	2.0-4.0	52.9	*68.4a*	*43.5b*	*1.6a*
	4.0-6.7	7.4	*84.0b*	*31.6c*	*2.5b*
	> 6.7	1.7	*95.8b*	*22.3c*	*11.9c*
Wheat grain	< 2.0	46.5	*78.9a*	*23.7a*	*1.6a*
	2.0-4.0	48.5	*88.9b*	*16.0b*	*3.9b*
	4.0-6.7	4.8	*97.8c*	*14.0b*	*4.9c*
	> 6.7	0.3	*100c*	*22.0a*	*15.4d*

Letters denote significant differences following REML analysis.

* when comparing any treatment to the 4.0-6.7 size class where the LSD was 20.1.

**19.2

***0.66

****0.79 NEEDS MORE CLARITY

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Discussion

The number of sclerotia in the soil increases when successive susceptible crops become infected by *S. sclerotiorum* and this is a growing problem in many cropping systems (Mueller *et al.*, 2002). The situation is exacerbated by short rotations and pressure on land use as *S. sclerotiorum* sclerotia are generally thought to survive for 4-5 years in soil under natural conditions (Adams & Ayers, 1979) although this period can vary depending on factors such as depth, temperature and moisture (Adams, 1975, Mitchell & Wheeler, 1990, Duncan *et al.*, 2006, Ćosić *et al.*, 2012). However, few researchers have attempted to investigate and quantify the effect of crop type and *S. sclerotiorum* isolate on the production of sclerotia and hence understand how these factors might influence subsequent inoculum pressure.

This is the first study to investigate differences in sclerotial production on whole plants rather than on detached tissue which was the approach used in a previous study by Leiner and Winton (2006). Here, a greater number of sclerotia were produced on carrot pieces compared to celery and these were also larger, with weights ranging from 38-90 mg compared to 14-18 mg (Leiner & Winton, 2006). In the same work, the weight of sclerotia produced by two different *S. sclerotiorum* isolates on three different iceberg lettuce varieties ranged from 14-26 mg. By comparison, an almost identical weight range for *S. sclerotiorum* sclerotia produced on lettuce (13-28 mg) was observed in this study, but much smaller sclerotia were produced on carrot (12-14 mg) with the largest sclerotia produced on oilseed rape (33-60 mg). Leiner & Winton (2006) also estimated that 250-500 sclerotia might be produced on a single lettuce plant depending on *S. sclerotiorum* isolate which is substantially more sclerotia than

observed on the infected butterhead lettuce in this work (55-122 per plant). However, this might be explained by iceberg lettuce forming a dense compact head similar to a cabbage which would have a much greater amount of biomass and hence sustain a greater level of sclerotial production than the open, leafy butterhead variety of lettuce used here.

Commented [JW3]: It might be type rather than variety???? i.e the type is iceberg or butterhead but I think there are different varieties of iceberg or butterhead

The *S. sclerotiorum* isolates tested in this study exhibited variation in the number and weight of sclerotia produced both on PDA and on plants. This is consistent with previous reports where sclerotial production was assessed on agar media (Akram *et al.*, 2005, Li *et al.*, 2008, Vleugels *et al.*, 2013). Interestingly, the three *S. sclerotiorum* isolates each showed a consistent pattern of sclerotial production irrespective of crop type; L6 produced large numbers of small sclerotia while L44 produced small numbers of large sclerotia with L17 intermediate between the two. Leiner & Winton (2006) also observed that one of the two *S. sclerotiorum* isolates they examined consistently produced more sclerotia on cabbage, carrot, celery and lettuce tissue. As well as being more numerous, the sclerotia from this isolate were also larger. This in contrast to our results where all three isolates produced a comparable total biomass of sclerotia either by producing a large number of small sclerotia (L6) or a small number of large sclerotia (L44). These different findings may be due to different experimental approaches but merits further investigation with a greater range of isolates from different locations. The production of large sclerotia by some *S. sclerotiorum* isolates may also confer a survival advantage as they have been shown to persist for longer periods in the soil (Harvey *et al.*, 1995).

As indicated previously, variation in sclerotial production between different *S. sclerotiorum* isolates has generally only been investigated previously on agar media. In this study, only a weak correlation was observed between the number of sclerotia produced on PDA *in vitro* and on plants (r=0.49) and there was no correlation between the results on plants and agar for either total weight of sclerotia or mean weight per sclerotium. This highlights the importance of using whole plants to properly assess sclerotial production by different *S. sclerotiorum* isolates. There was also no significant correlation between either weight of sclerotia per plant or weight per sclerotium with pathogenicity on brassica (Taylor et al., 2015). This is in agreement with previous work for *S. sclerotiorum* isolates from sunflower (Li *et al.*, 2008) but in contrast to work on *S. trifoliorum* from red clover where a negative correlation was observed between the number of sclerotia produced and aggressiveness (Vleugels *et al.*, 2013).

It was also clear from the results that as sclerotial size increased from small (< 2.0 mm, mean weight 4.6mg) to medium (2.0-4.0 mm, mean weight 18.4 mg) and large (4.0-6.7 mm, mean weight 43.3mg), percentage germination and number of apothecia produced also increased. This observation was generally consistent across all crop types with the larger sclerotia produced on OSR (> 6.7 mm) resulting in a mean of 12 apothecia per sclerotium. In the few similar studies that have been reported, Ben-Yephet et al., 1993 collected sclerotia from infected lettuce in the field and showed that small sclerotia (1-7 mg) resulted in 11-21% germination compared to 29-31% germination for larger sclerotia (7-40 mg). Dillard et al. (1995) produced sclerotia on cornmeal/vermiculite and following a cold conditioning treatment, observed germination ranging from 76% for small sclerotia (1.68-3.36 mm) to 98% for medium (3.36-4.75 mm) and large (>4.75 mm) sclerotia with mean numbers of apothecia per

sclerotium of 1.5, 3.2 and 7.8 respectively, which closely matches the results presented here. Similarly, using sclerotia produced on autoclaved potato pieces, Hao et al. (2003) recorded 30% germination for small sclerotia (< 1mm) and 100% for large sclerotia (>4.75 mm) and also reported a significant correlation between size of sclerotia and the number of stipes produced per apothecium. However, none of these previous studies tested *S. sclerotiorum* sclerotia produced on a range of crop plants. Furthermore, the results presented here also indicated that larger sclerotia germinate more quickly which, to our knowledge, has not been previously reported. The reason for the rapid, high levels of carpogenic germination as well as the production of multiple apothecia associated with larger sclerotia of *S. sclerotiorum* may be due to the accumulation of greater nutrient reserves which are utilised during germination (Willetts & Bullock, 1992). However, little is known about how the composition or quantity of different compounds found in sclerotia such as glycogen, protein, polyphosphate and lipid affects survival or germination (Willetts & Bullock, 1992). The number and weight of *S. sclerotiorum* sclerotia produced on agar has been demonstrated previously to increase linearly with sucrose concentration but subsequent production of apothecia for the larger sclerotia was slower or completely inhibited (Budge & Whipps, 1991). Although this conflicts with the results from this and other reports, no conditioning treatment of the sclerotia was employed which the authors suggest may be required by larger sclerotia if nutrient depletion during the survival phase of the pathogen plays a part in triggering carpogenic germination. It was also observed in this study that *S. sclerotiorum* sclerotia produced on oilseed rape and those produced artificially on autoclaved wheat grain germinated more rapidly and resulted in a higher level of germination. This could again relate to the nutrient status

of the sclerotia and highlights the need to understand how different artificial and natural substrates affect both formation and germination of *S. sclerotiorum* sclerotia.

The data generated for the number of *S. sclerotiorum* sclerotia produced on infected plants in this study allowed estimation of the inoculum potential for different crops. Although infection of OSR and lettuce resulted in greater numbers of sclerotia per plant than the other crops, carrot was identified as potentially supporting the greatest returns of sclerotia to the soil when planting density in the field was taken into account, producing ~~up~~ between 2666 and 4794 sclerotia m^{-2} compared with 1026-2754 sclerotia m^{-2} in OSR. However, based on the weight of sclerotia produced per plant and crop density, both OSR and carrot constitute the greatest returns with 86 g and 83 g sclerotia m^{-2} respectively. These are estimated figures and also assume that all plants are completely colonised by *S. sclerotiorum* which is unlikely in a field situation. Nonetheless these estimates appear to be consistent with some preliminary field data where sclerotia on the soil surface were counted in quadrats for crops with high levels of Sclerotinia disease. Here, up to 3000 sclerotia m^{-2} were recorded for a carrot crop compared to up to 900 m^{-2} for OSR and 195 m^{-2} for potato (C. Young, ADAS Drayton, personal communication). Data from a published field survey in the UK suggested that pea, potato and sunflower crops resulted in high returns of sclerotia to the soil of up to six sclerotia kg^{-1} soil with 92 sclerotia m^{-2} being recorded on the soil surface of an infected pea crop (Archer *et al.*, 1992). These crops were therefore considered a major threat to subsequent OSR plantings. However, often subsequent tillage operations will redistribute sclerotia left on the soil surface throughout the soil profile and many will be killed by microorganisms or adverse environmental conditions (Alexander & Stewart, 1994, Ćosić *et al.*, 2012). Only a small proportion of sclerotia that remain

close to the soil surface can subsequently germinate (Wu & Subbarao, 2008) and these are difficult to quantify. As stated in the introduction, just a few germinating *S. sclerotiorum* sclerotia are required to initiate disease due to the large numbers of ascospores produced by apothecia and as few as two apothecia per m^{-2} were shown to lead to 11% disease incidence in soybean (Mueller *et al.*, 2002).

In summary, sclerotial production and germination by *S. sclerotiorum* is dependent on isolate, host plant and size of sclerotia. Both carrot and OSR potentially return more sclerotia by weight or number to the soil compared to the other plants tested here and hence potentially pose a bigger disease risk for subsequent susceptible crops. However, further work is required to extensively quantify sclerotial production in the field in order to validate this. The information can then be used to inform crop rotation strategies as part of an integrated control programme to manage *Sclerotinia* disease.

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622 **Figure legends**

623

624 Figure 1 a) number per plant b), total weight per plant and c) individual weight for
625 sclerotia produced by 18 *S. sclerotiorum* isolates on PDA. Error bars represent the
626 LSD (5% level).

627

628 Figure 2 a) number per plant b), total weight per plant and c) individual weight for
629 sclerotia produced by three *S. sclerotiorum* isolates on five different crop plants. Error
630 bars represent the LSD (5% level). + indicates incomplete colonisation.

631

632 Figure 3 a) estimated number and b) weight of sclerotia produced on different crop
633 plants in the field. Error bars represent the LSD (5% level).

Fig 1

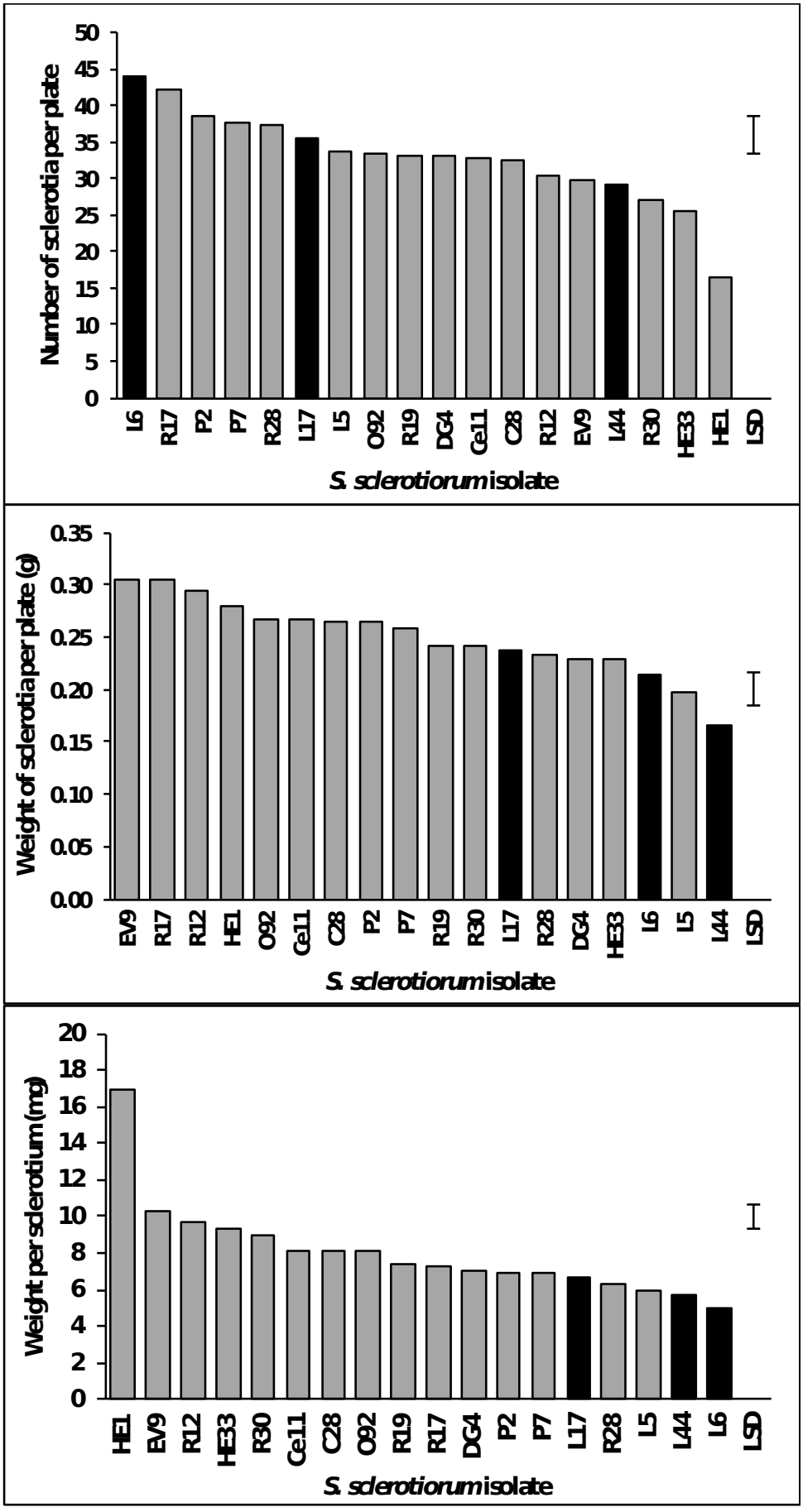

Number of sclerotia per plate
50
45
40
35
30
25
20
15
10
5
0
L6 R17 P2 P7 R28 L17 L5 O92 R19 DG4 Ce11 C28 R12 EV9 L44 R30 HE33 HE1 LSD
S. sclerotiorum isolate
Weight of sclerotia per plate (g)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
EV9 R17 R12 HE1 O92 Ce11 C28 P2 P7 R19 R30 L17 R28 DG4 HE33 L6 L5 L44 LSD
S. sclerotiorum isolate
Weight per sclerotium (mg)
20
18
16
14
12
10
8
6
4
2
0
HE1 EV9 R12 HE33 R30 Ce11 C28 O92 R19 R17 DG4 P2 P7 L17 R28 L5 L44 L6 LSD
S. sclerotiorum isolate

Fig. 2

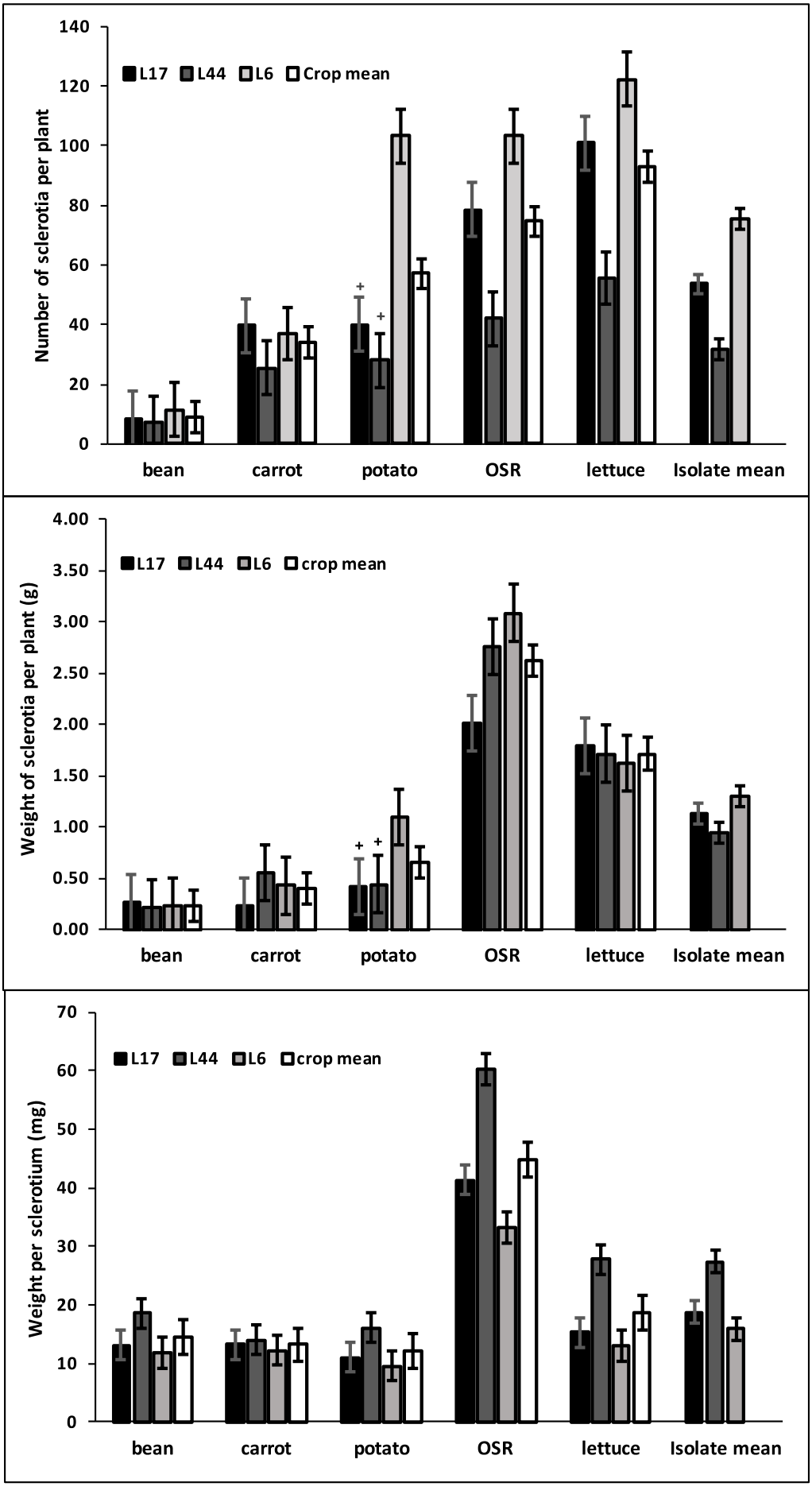
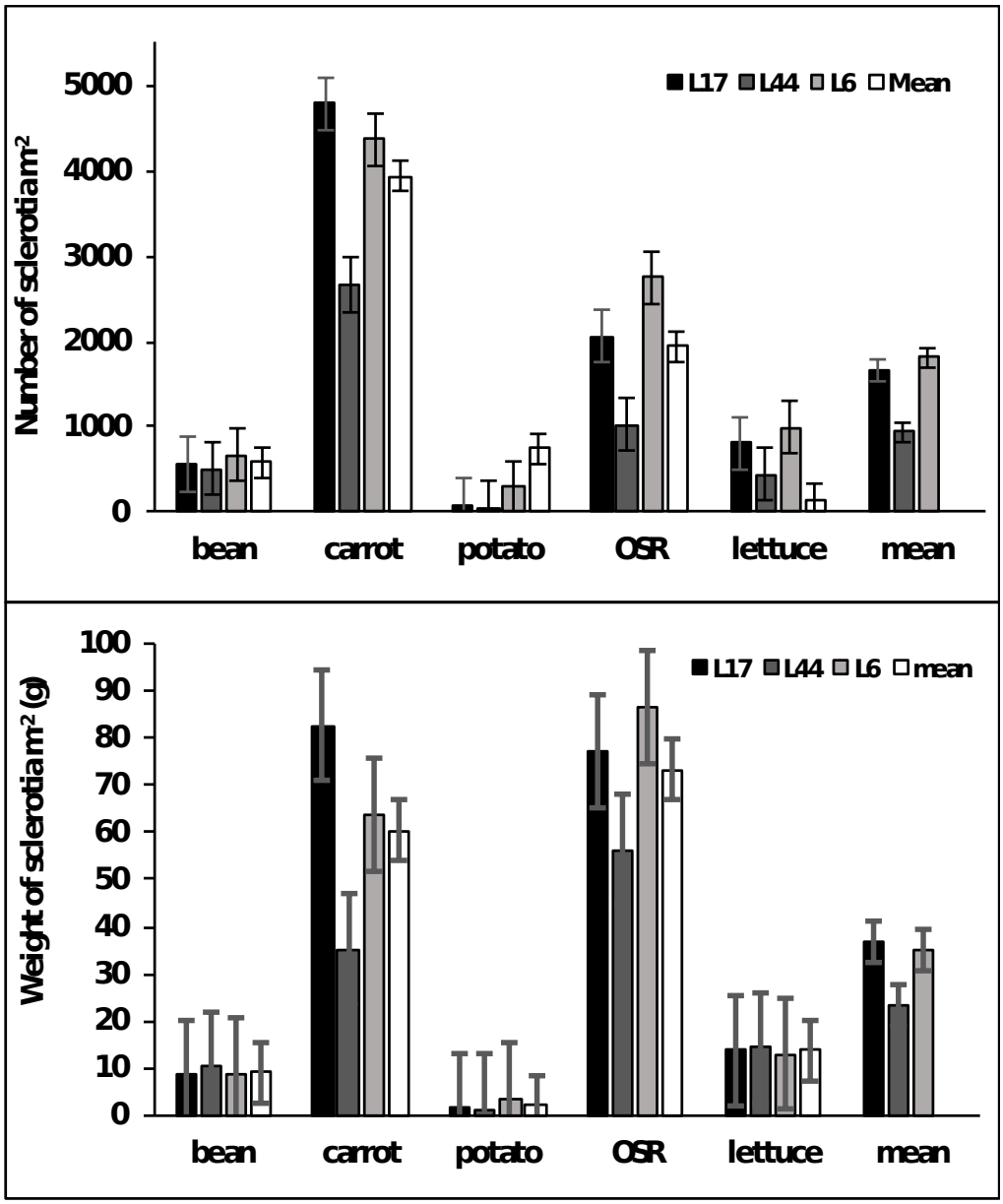

Number of sclerotia per plant
140
120
100
80
60
40
20
0
L17 L44 L6 Crop mean
bean
carrot
potato
OSR
lettuce
Isolate mean
Weight of sclerotia per plant (g)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
L17 L44 L6 crop mean
bean
carrot
potato
OSR
lettuce
Isolate mean
Weight per sclerotium (mg)
70
60
50
40
30
20
10
0
L17 L44 L6 crop mean
bean
carrot
potato
OSR
lettuce
Isolate mean

Fig. 3


Number of sclerotia m-2
5000
4000
3000
2000
1000
0
L17 L44 L6 Mean
bean
carrot
potato
OSR
lettuce
mean
Weight of sclerotia m-2 (g)
100
90
80
70
60
50
40
30
20
10
0
L17 L44 L6 mean
bean
carrot
potato
OSR
lettuce
mean