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1 **Resistance to *Sclerotinia sclerotiorum* in wild *Brassica* species and the**
2 **importance of *Sclerotinia subarctica* as a *Brassica* pathogen**

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18
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20 **Abstract**

21

22 *Brassica* crops are of global importance with oilseed rape (*Brassica napus*) accounting for 13%
23 of edible oil production. All *Brassica* are susceptible to Sclerotinia stem rot, caused by
24 *Sclerotinia sclerotiorum*, a generalist fungal pathogen causing disease in over 400 plant
25 species. Generally, sources of plant resistance result in partial control of the pathogen although
26 some studies have identified wild *Brassica* species that are highly resistant. The related
27 pathogen *S. subarctica* has also been reported on *Brassica* but its aggressiveness in relation to
28 *S. sclerotiorum* is unknown. In this study, detached leaf and petiole assays were used to identify
29 new sources of resistance to *S. sclerotiorum* within a wild *Brassica* C genome diversity set.
30 High level resistance was observed in *B. incana* and *B. cretica* in petiole assays, while wild *B.*
31 *oleracea* and *B. incana* lines were the most resistant in leaf assays. A *B. bourgeai* line showed
32 both partial petiole and leaf resistance. Although there was no correlation between the two
33 assays, resistance in the detached petiole assay was correlated with stem resistance in mature
34 plants. When tested on commercial cultivars of *B. napus*, *B. oleracea* and *B. rapa*, selected
35 isolates of *S. subarctica* exhibited comparable aggressiveness to *S. sclerotiorum* indicating it
36 can be a significant pathogen of *Brassica*. This is the first study to identify *B. cretica* as a
37 source of resistance to *S. sclerotiorum* and to report resistance in other wild *Brassica* species
38 to a UK isolate, hence providing resources for breeding of resistant cultivars suitable for
39 Europe.

40 **Introduction**

41

42 Oilseed *Brassica* crops such as oilseed rape and mustard are important commodities in Europe,
43 India, Australia, China and Canada, contributing 13% of the total world's production of edible
44 oil (Carr, 1990) while other *Brassica* species such as cabbage, cauliflower, broccoli, and turnip,
45 are major food crops which make a significant contribution to nutrition and health (Zhang *et al.*,
46 1992). All Brassicas are susceptible to Sclerotinia stem rot (SSR), caused by *Sclerotinia*
47 *sclerotiorum*. As a generalist necrotrophic pathogen which causes disease on over 400 plant
48 species (Boland & Hall, 1994), the fungus is also a serious threat to many other economically
49 important crops worldwide including soybean, sunflower, peas, beans, carrot, lettuce and
50 potatoes (Mei *et al.*, 2013, Uloth *et al.*, 2013, Derbyshire & Denton-Giles, 2016).

51 Oilseed rape (also known as canola; *Brassica napus*) is one of the most widely grown
52 *Brassica* species where SSR routinely results in serious losses, with incidence in the range of
53 10-20% in Canada, Australia, USA and Europe (Derbyshire & Denton-Giles, 2016). Soilborne
54 sclerotia of *S. sclerotiorum* germinate to produce apothecia and subsequent release of
55 ascospores results in infected petals which initiate lesions on the stems, leading to lodging and
56 significantly reduced yields (Derbyshire & Denton-Giles, 2016). In India, substantial losses
57 due to SSR have been recorded for other *Brassica* species, particularly for mustard (*B. juncea*)
58 which is widely grown, and where yield losses of 37-92% have been recorded in the Rajasthan
59 region (Shivpuri *et al.*, 2000).

60 Currently, there are no *Brassica* crop varieties with high levels of resistance to SSR
61 commercially available. Identifying sources of resistance in *Brassica* is challenging as there
62 can be considerable variability in plant screening assays depending on conditions, plant growth
63 stage and *S. sclerotiorum* isolate (Garg *et al.*, 2010b, Uloth *et al.*, 2013, Ding *et al.*, 2015,
64 Taylor *et al.*, 2015). Despite these problems, some sources of partial resistance have been

65 identified and mapped in *B. napus* (Zhao *et al.*, 2006, Li *et al.*, 2009, Yin *et al.*, 2010, Taylor
66 *et al.*, 2015, Gyawali *et al.*, 2016, Wu *et al.*, 2016). However, higher level resistance to SSR
67 has been identified in more diverse cruciferous plants including wild species (Navabi *et al.*,
68 2010, Mei *et al.*, 2011, Uloth *et al.*, 2013). A study in India reported that stem lesions caused
69 by *S. sclerotiorum* were eight times smaller in *B. napus* and *B. juncea* introgression lines
70 derived from wild germplasm (*Erucastrum cardamanoides*, *B. fruticulosa*, *Diplotaxis*
71 *tenuisiliqua* and *E. abyssinicum*) compared to standard susceptible *B. napus* and *B. juncea*
72 lines (Garg *et al.*, 2010a). The only recent resistance study carried out in Europe used a *B.*
73 *napus* diversity set and identified lines partially resistant to SSR (Taylor *et al.*, 2015). Prior to
74 this, a study in Ireland reported an increased level of *S. sclerotiorum* resistance in a
75 mutagenized *B. napus* population (Mullins *et al.*, 1999).

76 Several different approaches have been used to screen for resistance to SSR, which in
77 some cases have produced conflicting results. The main methods reported include stem
78 inoculations with a toothpick (Zhao & Meng, 2003, Yin *et al.*, 2010), petiole inoculations with
79 an agar plug (Zhao *et al.*, 2006) or infected wheat grain (Taylor *et al.*, 2015), detached leaf
80 inoculations using agar plugs (Zhao & Meng, 2003) or mycelial fragments on attached
81 cotyledons (Garg *et al.*, 2008) and detached stem inoculations using agar plugs (Mei *et al.*,
82 2012). Arguably the most robust test for field resistance in OSR which has been widely
83 employed is the inoculation of mature plant stems using an agar plug (Buchwaldt *et al.* 2005).
84 Using this method, it was shown that lesion size is strongly correlated with plant death, and
85 hence directly linked to yield (Li *et al.*, 2006). The problem with this assay (and others) is that
86 it takes a very long time to carry out, especially if replicate experiments are required; hence it
87 might take several years to complete a robust resistance screen. Therefore, the development of
88 more rapid assays is desirable as long as they relate to mature plant / field resistance.

89 Although *S. sclerotiorum* is the major pathogen causing SSR on *Brassica*, the related
90 species *S. subarctica* (originally termed *Sclerotinia* sp. 1), first identified on wild plants and
91 potato in Norway (Holst-Jensen *et al.*, 1998), can cause identical symptoms to *S. sclerotiorum*
92 but appears confined to northern latitudes (Clarkson *et al.*, 2017). *S. subarctica* has been
93 identified on lettuce, cabbage, bean and potato in Alaska (Winton *et al.*, 2006), and was first
94 reported in England on *Ranunculus acris* (meadow buttercup) where the same isolate was
95 shown to be pathogenic on *B. napus* (Clarkson *et al.*, 2010). More recently, the pathogen has
96 been found on further crop plants including carrot, celery root, Jerusalem artichoke, pea, swede,
97 and turnip rape (*Brassica rapa* subsp. *oleifera*) in Scotland and Norway (Brodal *et al.*, 2016,
98 Clarkson *et al.*, 2017). Hence, *S. subarctica* appears to have a similarly broad host range to *S.*
99 *sclerotiorum* and is a significant pathogen in some northern countries. However, an initial study
100 comparing the aggressiveness of a single *S. subarctica* isolate with *S. sclerotiorum* suggested
101 that *S. subarctica* was a weaker pathogen on three *Brassica* spp. (Taylor *et al.*, 2015).

102 The aim of this study was to screen wild *Brassica* species for resistance to *S.*
103 *sclerotiorum* to determine if new and higher level sources of resistance could be identified
104 compared to those identified previously in *B. napus* (Taylor *et al.*, 2015). To achieve this, and
105 overcome the problem of highly variable wild *Brassica* morphotypes, improved detached
106 petiole and detached leaf assays were developed. In addition, to determine the importance of
107 *S. subarctica* as a pathogen, the aggressiveness of 12 isolates was compared with three
108 previously characterised *S. sclerotiorum* isolates on different *Brassica* species.

109 **Materials and Methods**

110

111 ***Sclerotinia* isolates and ascospore production**

112 The four *S. sclerotiorum* isolates used in this study were obtained from infected lettuce (L6,
113 L44), pea (P7) and buttercup (*Ranunculus acris*, DG4) from different locations in England.
114 These isolates also represented different genotypes as identified previously using microsatellite
115 markers (Clarkson *et al.*, 2017; Table 1). The pathogenicity of these isolates was tested
116 previously against three *Brassica* spp. with L6 and P7 identified as aggressive, L44 as
117 intermediate and DG4 as weak in terms of their virulence (Taylor *et al.*, 2015). The 12 *S.*
118 *subarctica* isolates were obtained from England, Scotland, Norway and Sweden from buttercup
119 (six isolates), pea (two isolates), lettuce (two isolates), potato (one isolate) and swede (one
120 isolate) and also represented different microsatellite genotypes (Clarkson *et al.*, 2017; Table
121 1). Cultures of each isolate were initiated from stock sclerotia maintained at 5°C; a single
122 sclerotium was bisected and placed on potato dextrose agar (PDA) or glucose rich medium (10
123 g peptone, 20 g glucose, 18 g agar, 0.5 g KH₂PO₄, 1 L H₂O, adjusted to pH 4.0) and incubated
124 at 20°C for 3-4 days to produce actively growing colonies. These were then further subcultured
125 onto PDA plates and grown for 2 days at 20°C to provide actively growing mycelium for
126 petiole inoculations.

127 *S. sclerotiorum* ascospores for leaf inoculations were produced as described by
128 Clarkson *et al.*, (2014). Briefly, this involved burying cold-conditioned sclerotia (isolate L6)
129 in moist, pasteurised compost and incubating at 15°C to stimulate germination and production
130 of apothecia. Ascospores were then collected onto a filter paper using a suction pump and
131 stored at 4°C until use.

132 ***Brassica* lines**

133 All *Brassica* lines tested for resistance to *S. sclerotiorum* were derived from the Warwick
134 Genetic Resources Unit and a wild *Brassica* ‘C genome’ diversity set (Table 2). The full
135 diversity set comprises 89 founder accessions representing 14 different species and also
136 includes fixed doubled haploid lines for 35 accessions which were crossed with a compatible
137 rapid cycling line to overcome self-incompatibility (Pink *et al.*, 2008).

138

139 **Detached leaf and petiole assays**

140 Previously, *B. napus* lines were screened for resistance to *S. sclerotiorum* using both immature
141 and mature plants assays (Taylor *et al.*, 2015). In the former test, plants at the 7-9 leaf stage
142 were inoculated by placing a wheat grain colonised by *S. sclerotiorum* in a leaf axil and
143 assessing severity of infection based on leaf wilting and lesion development while the latter
144 involved inoculating the stem of mature flowering plants with an agar plug of mycelium and
145 measuring lesion size over time (Taylor *et al.*, 2015). However, neither of these methods were
146 suitable for the wild *Brassica* lines in this study because of their diverse morphology and
147 differences in both their growth rate and ability to produce elongated stems. Therefore, two
148 other assays were employed, comprising inoculation of detached petioles with agar plugs of
149 mycelium and inoculation of detached leaves with *S. sclerotiorum* ascospores.

150 The detached petiole assay was based on the method of Mei *et al.*, (2012). *Brassica*
151 plants were grown in a glasshouse (20°C, 16 h photoperiod) until they had eight true leaves
152 (approximately 14 weeks). Petioles from side stem branches (the three oldest non-senescent
153 branches) were then excised at approx. 1 cm from the main stem and a 10 cm section of petiole
154 prepared. Both ends were sealed with parafilm and the petioles placed on moist
155 chromatography paper (Whatman 3MM, Fisher Scientific, UK) in a clear plastic box (three
156 stems per box). An agar plug (4 mm) taken from the leading edge of an actively growing

157 colony of *S. sclerotiorum* isolate L6 on glucose rich medium was then placed mycelium side
158 down in the centre of each of the detached petioles. Following incubation at 15°C for 3 days in
159 a controlled environment room (12 h photoperiod), the length of the resultant lesions was
160 measured. Mock (control) inoculations were set up using 4 mm plugs of clean glucose rich
161 agar.

162 The detached leaf assay was based on the methods published by Garg *et al.*, (2008) and
163 Mei *et al.*, (2011) with the exception that ascospores were used rather than mycelium. *Brassica*
164 plants were grown in a glasshouse (20°C, 16 h photoperiod) until the first two true leaf stage
165 (approx. 4 weeks) after which leaves 1 and 2 were detached from the test plant, blotted dry and
166 placed on tap water agar (8 g L⁻¹) in a propagator (35 x 23 cm, Sankey, UK) containing 600 ml
167 of agar, 24 leaves per propagator. Inoculum was prepared by placing a section (approx. 4.5
168 cm²) of filter paper containing ascospores of *S. sclerotiorum* isolate L6 in a 50 ml tube
169 containing 8 ml of sterile 50% potato dextrose broth (PDB; Formedium, UK). The tube was
170 then shaken vigorously for approx. 1 min to break up the filter paper and the slurry filtered
171 through Miracloth (Merck Millipore, UK) to remove paper fragments. The resultant spore
172 suspension was adjusted to a concentration of 1 x 10⁵ ml⁻¹ using a haemocytometer. Two 15
173 µl drops of this ascospore suspension were pipetted onto the adaxial side of each *Brassica* leaf
174 (one on each side of the mid vein), and incubated at 20°C for 3 days in a controlled environment
175 room (12 hour photoperiod). Leaves were then photographed and the area of each lesion
176 measured using ImageJ software (Schneider *et al.*, 2012). Mock (control) inoculations were set
177 up using 50% PDB only.

178 **Screening wild *Brassica* lines for resistance to *S. sclerotiorum***

179

180 ***Resistance screen 1***

181 49 lines from the wild *Brassica* ‘C genome’ diversity set and seven *B. carinata* lines from the
182 Warwick Genetic Resources Unit were screened for *S. sclerotiorum* resistance using both leaf
183 and petiole assays as described above along with three previously tested *B. napus* lines (line
184 57, susceptible; line 58, highly susceptible; line 59, partially resistant; Table 2; Taylor *et al.*,
185 2015). For the petiole assay, four replicate experiments were carried out using two plants per
186 line and three inoculated petioles per plant for each experiment giving a total of 24
187 measurements per line for the statistical analysis. Each box contained all three petioles from a
188 single plant, boxes were positioned in the growth room using an alpha lattice design and data
189 were analysed by ANOVA using Genstat 18th Edition (Payne *et al.*, 2009) with replicate
190 experiment, position and replicate plant included as factors. For the leaf assay, there were four
191 replicate experiments, each consisting of eight inoculated leaves (from four plants) for each
192 line giving a total of 64 measurements per line for the statistical analysis. Each propagator
193 contained all eight leaves from three different lines, and positions within the growth room were
194 randomised using an alpha lattice design with data analysed by ANOVA using Genstat as
195 described for the detached petiole assay. Differences in lesion size (petiole assay) or lesion area
196 (leaf assay) between lines were considered significant if they were larger than the overall
197 calculated LSD value ($P < 0.05$).

198

199 ***Resistance screen 2***

200 The 20 *B. napus* lines (61-77; Table 2) which had shown a range of resistance / susceptibility
201 responses in previous mature plant tests (Taylor *et al.*, 2015) were screened for *S. sclerotiorum*
202 resistance using both leaf and petiole assays as well as five selected lines from resistance screen

203 1 (Line 3, partial resistance in both petiole and leaf assays; Lines 9 and 14, high level resistance
204 in petiole assay; Lines 10 and 19, susceptible in both assays), seven fixed doubled haploid lines
205 derived from Lines 3, 9, 14 through crossing with a rapid cycling *Brassica* line as well as this
206 parent line itself (Table 2). Replication, experimental design and data analysis were as
207 described for resistance screen 1.

208 Spearman's rank correlations were calculated using Genstat in order to examine the
209 relationship between leaf and petiole lesion size for both *S. sclerotiorum* resistance screens.
210 Similarly, the lesion sizes previously recorded for the 20 *B. napus* lines in the previous mature
211 plant test (Taylor *et al.*, 2015) were compared with the data from the detached leaf and petiole
212 assays of the same lines in resistance screen 2. Correlations between resistance screens 1 and
213 2 were also analysed in the same way.

214

215 **Comparison of aggressiveness of *S. subarctica* and *S. sclerotiorum* isolates**

216 The aggressiveness of 12 *S. subarctica* isolates was compared with three *S. sclerotiorum*
217 isolates (Table 1) that were previously identified as being of high (P7), medium (L44) or low
218 (DG4) aggressiveness on semi-mature plants of *B. napus* (oilseed rape cv. Temple), *B. oleracea*
219 (broccoli cv. Beaumont), and *B. rapa* (turnip cv. Manchester; Taylor *et al.*, 2015). This was
220 done using the detached petiole assay and the same *B. napus*, *B. oleracea* and *B. rapa* host
221 cultivars as used previously. Three replicate experiments were carried out using two plants per
222 treatment and three petioles per plant for each experiment. Each box contained all three petioles
223 from a single plant, boxes were positioned in the growth room using a randomised block design
224 and data analysed using ANOVA in Genstat with replicate, block and position as factors.
225 ANOVAs were carried out to assess the effect of isolate, *Brassica* type and any interaction.
226 Differences in lesion size between isolates were considered significant if they were larger than

227 the overall calculated LSD value ($P < 0.05$). To generate means of *Brassica* type, isolate was
228 removed as a factor from the ANOVA analysis.

229 **Results**

230

231 **Screening wild *Brassica* lines for resistance to *S. sclerotiorum***

232

233 ***Resistance screen 1***

234 In the detached petiole assay, significant differences in lesion size were identified between the
235 60 *Brassica* lines ($P < 0.001$, Fig. 1a; Fig. 2abc). The most resistant lines were 14 (*B. incana*)
236 and 9 (*B. cretica*) which had mean lesion sizes of 3.1 mm and 11.4 mm, respectively. The most
237 susceptible lines were 55 and 54 (both *B. carinata*) with mean lesion sizes of 94.4 and 88.1
238 mm, respectively. The majority of the wild *Brassica* lines (47 out of 56) were significantly
239 more resistant to *S. sclerotiorum* than the elite winter OSR cultivar Temple (line 57, mean
240 lesion size 71.9 mm, Fig. 1a). Of the top ten most resistant lines, five were *B. incana* and two
241 were *B. cretica*.

242 In the detached leaf assay, significant differences in lesion areas were identified
243 between the 60 *Brassica* lines ($P < 0.001$, Fig. 1b, Fig. 2 def). The most resistant lines in this
244 assay were 39 (wild *B. oleracea*) and 17 (*B. incana*) with mean lesion areas of 32.2 and 50.4
245 mm², respectively (Fig. 1b). The most susceptible lines were 18 (*B. incana*) and 7 (*B. cretica*)
246 with mean lesion areas of 221.2 and 212.6 mm², respectively. Only line 39 (wild *B. oleracea*)
247 was significantly more resistant to *S. sclerotiorum* than the elite winter OSR cultivar Temple
248 (line 57, mean lesion area 76.4mm², Fig. 1b). Of the top ten most resistant lines, four were
249 wild *B. oleracea*.

250 There was no significant correlation between leaf and petiole resistance to *S.*
251 *sclerotiorum* in the two assays ($r = -0.021$, $P = 0.87$, Fig. S1a). However, some lines performed
252 well in both tests, in particular line 3 (*B. bourgeai*, lesion sizes 19.4mm and 62.2mm² in petiole
253 and leaf assays, respectively, Fig. 1ab).

254 **Resistance screen 2**

255 In the detached petiole assay, significant differences in lesion size were identified between the
256 33 *Brassica* lines ($P < 0.001$, Fig. 3a). The most resistant lines were 14 (*B. incana*) and 81 (*B.*
257 *cretica*, DH line) with mean lesion sizes of 21.3 and 29.4 mm, respectively. The data was
258 consistent and significantly correlated with the results of resistance screen 1 for the eight lines
259 that were evaluated in both tests ($r = 0.75$, $P = 0.012$, Fig. S1b), with lines 14, 3 and 9 identified
260 as being more resistant to *S. sclerotiorum*. Line 14 showed a very high level of resistance in
261 both resistance screens 1 and 2 with mean petiole lesion sizes of 3.1 mm and 21.3 mm,
262 respectively. Overall lesion sizes were greater in screen 2 than in screen 1.

263 In the detached leaf assay, significant differences in lesion area were observed between
264 the 33 *Brassica* lines ($P < 0.001$, Fig. 3b) with the most resistant lines identified as 63 and 59
265 (both *B. napus*) with mean lesion areas of 28.4 and 33.4 mm², respectively (Fig. 3b). Again,
266 the data was broadly consistent with resistance screen 1 but the correlation fell just below the
267 level of significance ($r = 0.48$, $P = 0.054$, Fig. S1c). Line 14, which showed a high level of
268 petiole resistance in both resistance screens, was only partially resistant in the leaf assay (mean
269 lesion area 64.4 mm²) and quite susceptible in resistance screen 1 (mean lesion area 147.6
270 mm²). Again, as for resistance screen 1, no significant correlation was found between leaf and
271 petiole resistance ($r = -0.24$, $P = 0.051$, Fig S1d).

272 As the 20 *B. napus* lines used in resistance screen 2 had previously been evaluated for
273 *S. sclerotiorum* resistance in a mature plant test (Taylor *et al.*, 2015), direct comparisons could
274 be made with the detached leaf and stem assays reported here. A significant correlation was
275 evident between lesion size in the previous mature plant data and the detached petiole assay (r
276 $= 0.50$, $P = 0.009$, Fig. S1e), but not with those from the detached leaf assay ($r = 0.14$, $P =$
277 0.15 , Fig. S1f).

278

279 **Comparison of aggressiveness of *S. subarctica* and *S. sclerotiorum* isolates**

280

281 Using the detached petiole assay, 11 of the 12 *S. subarctica* isolates were pathogenic on
282 broccoli, turnip and OSR (Fig. 4a) with isolate ENG34 (from buttercup) failing to initiate
283 lesions. This isolate was also noted to be slow-growing in culture. There were significant
284 differences in lesion size between the different crop types ($P < 0.001$) and pathogen isolates (P
285 < 0.001) and a crop type x isolate interaction was also observed ($P < 0.001$). Overall, the
286 majority of the *S. subarctica* isolates were significantly less aggressive than the *S. sclerotiorum*
287 isolates (Fig. 4a). On broccoli, *S. sclerotiorum* P7 was the most aggressive isolate resulting in
288 a mean lesion size of 64.1 mm but this was not significantly different from *S. subarctica* isolates
289 SC25 (mean lesion size 61.9 mm) and SC61 (mean lesion size 51.6 mm, Fig. 4a). On OSR, *S.*
290 *subarctica* SC61 was the most aggressive isolate, resulting in a significantly greater mean
291 lesion size (55.1 mm) than any other isolate (Fig. 4a). On turnip, *S. sclerotiorum* isolate P7
292 was again the most aggressive isolate resulting in a significantly greater mean lesion size (93.9
293 mm) than any other isolate (Fig. 4a). The most aggressive *S. subarctica* isolate was SC61
294 (mean lesion size 74.3mm), significantly greater than any other *S. subarctica* isolate but not
295 significantly different from *S. sclerotiorum* isolates L44 and DG4 (Fig. 4a). Across all crop
296 types, *S. subarctica* ENG10 from buttercup was consistently the least aggressive isolate.
297 Comparing susceptibility to *S. subarctica* across crop types, significant differences were
298 observed ($P < 0.001$) with turnip being the most susceptible (Fig. 4b). The order of
299 susceptibility between OSR and broccoli however varied between isolates.

300 **Discussion**

301

302 There have been few studies examining wild *Brassica* species as sources of resistance to *S.*
303 *sclerotiorum* and none have investigated resistance to a UK or European isolate. In this study,
304 two rapid and reproducible assays identified a high level of resistance to a UK isolate within a
305 variety of wild *Brassica* lines, particularly line 14 (*B. incana*) and line 81 (*B. cretica*; DH of
306 line 9) which both showed a high level of petiole resistance. Line 14, also exhibited partial leaf
307 resistance in resistance screen 2. As line 81 is a DH line, the resistance should be genetically
308 fixed, which should allow a more straight-forward route for introgression into *B. napus*. In
309 resistance screen 2, lines 14 and 81 exhibited a significantly higher level of resistance in petiole
310 tests compared with *B. napus* line 62, a line which was previously identified as the most
311 resistant within 96 lines from a *B. napus* diversity set (line 69; Taylor *et al.*, 2015). To our
312 knowledge, this is the first report of *S. sclerotiorum* resistance in *B. cretica*, which hence
313 provides another potential source of useful breeding material. *B. cretica* has not been widely
314 studied and is lacking in genomic information although it has been reported that this species
315 did not demonstrate any resistance to *Verticillium* wilt (Happstadius *et al.*, 2003). Furthermore,
316 47 lines (in resistance screen 1) were significantly more resistant than the elite winter OSR
317 cultivar Temple, hence providing a range of potential sources of resistance.

318 In this study, lesion sizes in the petiole tests ranged from 0.3 cm in the most resistant
319 line to 9.4 cm in the most susceptible. By comparison, studies using a similar method resulted
320 in lesion sizes of 2.2 to 6.6 cm for cultivated and wild species of *B. rapa*, *B. oleracea*, *B. napus*,
321 *B. juncea* and *B. carinata* (Mei *et al.*, 2012), 2.5-10 cm in *B. oleracea* (Mei *et al.*, 2013), 3.1-
322 13.0 cm in *B. napus* (Wei *et al.*, 2014) and 3.5-8.2 cm in *B. napus* lines with resistance
323 introgressed from *B. oleracea* (Ding *et al.*, 2013). These results suggest that firstly, the most
324 resistant lines identified here are comparable to, if not more resistant than, those reported by

325 other researchers. Secondly the results confirm previous reports that higher levels of resistance
326 can more often be found in wild *Brassica* compared to cultivated species (Mei *et al.*, 2011,
327 Uloth *et al.*, 2013, Ding *et al.*, 2015, You *et al.*, 2016). Five of the ten most resistant lines in
328 the petiole test were *B. incana* which was also identified as a source of SSR resistance by Mei
329 *et al.*, (2011) following toothpick inoculation of mature plant stems from a wide range of
330 *Brassica* species. In another study, where stems of mature plants were inoculated with agar
331 plugs of *S. sclerotiorum*, *B. incana* was again shown to have good resistance to SSR although
332 higher levels were found in *Raphanus raphanistrum*, *B. carinata* and *R. sativus* (Uloth *et al.*,
333 2013). Using the same method, good resistance has also been identified in lines of *B. nigra*
334 and *B. carinata* (Navabi *et al.*, 2010). Overall therefore, there is strong evidence that *B. incana*
335 lines can provide useful sources of resistance against *S. sclerotiorum* while added value may
336 also be gained through its resistance to cabbage whitefly (Pelgrom *et al.*, 2015). In contrast to
337 some of the above studies however, none of the *B. carinata* lines used exhibited resistance to
338 SSR in the tests reported here. This may be due to differences between the *S. sclerotiorum*
339 isolates from the UK and Canada or differences between *B. carinata* accessions. It has been
340 observed previously that different accessions of *B. carinata* and other wild species can be either
341 highly resistant or highly susceptible to SSR (Uloth *et al.*, 2013).

342 Whilst resistance to SSR has been found in wild *Brassica* species previously, this is the
343 first report of resistance to a UK isolate of *S. sclerotiorum*, hence confirming that such wild
344 sources of resistance could be suitable for development for *Brassica* crops in the UK and
345 potentially the rest of Europe. It has been suggested previously that it is critically important to
346 identify resistance to ‘local’ isolates of *S. sclerotiorum* (Taylor *et al.*, 2015). This may be
347 because the pathogen is highly diverse and although a few genotypes are widespread within
348 countries and very occasionally between countries, the majority are confined to specific fields
349 or growing areas (Clarkson *et al.*, 2017). The importance of using local isolates in resistance

350 screening programmes was demonstrated in a previous study where Mystic, a *B. napus* cultivar
351 shown to be consistently resistant to *S. sclerotiorum* isolates from Australia (Garg *et al.*, 2008,
352 Garg *et al.*, 2010a, Uloth *et al.*, 2013) was highly susceptible to isolates from the UK (Taylor
353 *et al.*, 2015). Moreover, *S. sclerotiorum* isolates from different regions may also vary in their
354 response to environmental conditions under which the resistance test is performed. This effect
355 was demonstrated by one study where lines which had previously been shown to be resistant
356 were found to be highly susceptible to the same *S. sclerotiorum* isolate (You *et al.*, 2016, Uloth
357 *et al.*, 2013).

358 In addition to the detached petiole tests, the same set of *Brassica* lines was assessed for
359 *S. sclerotiorum* resistance using a detached leaf assay. In contrast to all previous detached leaf
360 studies which have used either an agar plug (Mei *et al.*, 2011) or macerated mycelial fragments
361 on attached cotyledons as inoculum (Garg *et al.*, 2008), ascospores were used which are
362 normally the primary source of *S. sclerotiorum* infections in the field. Although ascospores
363 take a significant amount of time to produce, they can be stored for several years on dry filter
364 paper at 4°C. Using this test, only a single wild *Brassica* line (39, wild *B. oleracea*) was
365 significantly more resistant than the commercial *B. napus* variety Temple (line 57). *B. napus*
366 line 59 which showed consistent resistance over the two leaf assays also showed partial
367 resistance in previous work using stem inoculation of mature plants (line 83; Taylor *et al.*,
368 2015). As observed in the petiole tests, some *S. sclerotiorum* resistance was also evident in *B.*
369 *incana* lines using the leaf test although a different line (line 17) was the most resistant. This
370 again indicates the value of this species as a source of resistance, further supporting the results
371 of previous work (Mei *et al.*, 2011, Mei *et al.*, 2013, Ding *et al.*, 2015). This is also the first
372 study to identify partial leaf resistance to *S. sclerotiorum* in wild *B. oleracea*, *B. macrocarpa*,
373 *B. vilosa* and *B. bourgeai* and to our knowledge, this is also the first study to investigate leaf
374 or stem resistance in *B. hilarionis*, *B. macrocarpa* and *B. atlantica*. Whilst no high level

375 resistance was observed in these species, some moderate stem resistance was observed in *B.*
376 *atlantica* and *B. macrocarpa*, potentially presenting alternative sources of resistance for future
377 breeding programmes. This resistance would need to be introgressed into *B. napus*, something
378 which has been done successfully for resistance from wild crucifers (Garg *et al.*, 2010a).

379 No correlation was found between leaf and stem resistance to *S. sclerotiorum* in this
380 study, suggesting that resistance in these two different tissue types may be controlled by
381 different genes or pathways. This confirms the results of previous work where results using a
382 toothpick inoculation method on mature *B. napus* stems were different from those using an
383 agar plug method on detached leaves (Zhao & Meng, 2003). Similarly, Uloth *et al.*, (2013) and
384 You *et al.*, (2016) observed different responses between stems of field grown mature *Brassica*
385 plants inoculated with agar plugs of *S. sclerotiorum* and leaves of the same plants which had
386 been naturally infected by ascospores, and also concluded that genetic control of leaf and stem
387 resistance is probably different. In contrast, two studies have demonstrated weak correlations
388 between the size of lesions produced on detached leaves inoculated with agar plugs, compared
389 with those resulting from inoculation of mature stems using a toothpick method (Mei *et al.*,
390 2011) or detached stems inoculated with an agar plug (Mei *et al.*, 2013).

391 Although the detached stem/petiole inoculation method has been widely used in
392 screening for *S. sclerotiorum* resistance, few studies have compared this approach with stem
393 inoculation of intact mature plants. Using a set of 20 *B. napus* lines, a significant correlation
394 was observed between results from the detached petiole test here and those from the stem
395 inoculation of mature plants in a previous study ($r = 0.50$; Taylor *et al.*, 2015) while the only
396 previous comparison reported correlations of 0.21 or 0.29 (Wei *et al.*, 2014). The detached
397 petiole method is therefore a valid, and rapid resistance screening approach which could be
398 employed as a primary screen for large numbers of breeding lines in order to pre-select a
399 smaller number of lines for testing using the mature stem inoculation method. The detached

400 petiole assay is also particularly applicable to screening for resistance relevant to OSR where
401 infections are generally initiated in the stem while the detached leaf assay may be more
402 applicable to leafy *Brassica* crops.

403 This is also the first study to evaluate the relative aggressiveness of a range of *S.*
404 *subarctica* isolates and used the same *Brassica* species and cultivars employed previously to
405 compare aggressiveness of *S. sclerotiorum* isolates (Taylor *et al.*, 2015). This involved
406 inoculation of stems of immature plants rather than detached petioles, but results were
407 consistent in that P7 was generally the most aggressive of the three *S. sclerotiorum* isolates
408 included in both studies and *B. rapa* (turnip) was the most susceptible of the three *Brassica*
409 species. It was shown for the first time that *S. subarctica* isolates exhibit a range of
410 aggressiveness across *Brassica* species as observed previously in studies with *S. sclerotiorum*
411 on different host plants (Ekins *et al.*, 2007, Otto-Hanson *et al.*, 2011, Taylor *et al.*, 2015). The
412 reasons for this are unclear although for *S. sclerotiorum* it has been suggested that differences
413 between isolates in oxalic acid production may be responsible (Durman *et al.*, 2005). Although
414 it is not known whether oxalic acid is produced by *S. subarctica*, as indicated above,
415 populations are similarly genetically diverse as reported for *S. sclerotiorum* (Clarkson *et al.*,
416 2017) which could account for this biological variation. Overall however, the majority of the
417 *S. subarctica* isolates were moderately or weakly aggressive compared to *S. sclerotiorum*,
418 especially those isolated from wild buttercup and this was also a trend observed previously for
419 *S. sclerotiorum* isolates (Taylor *et al.*, 2015). Again, in studies with *S. sclerotiorum* it has been
420 suggested that isolates from different hosts may vary in their ability to produce oxalic acid; for
421 instance, isolates collected from lettuce produced less oxalic acid resulting in smaller lesions
422 compared with isolates collected from sunflower or soybean (Durman *et al.*, 2005). However,
423 further studies with a greater number of isolates from different hosts would be required to test
424 this hypothesis for *S. subarctica*. In contrast, two *S. subarctica* isolates (SC25, SC61) from

425 swede and potato were consistently highly aggressive across the *Brassica* hosts and were
426 comparable with *S. sclerotiorum* isolates. This suggests that some *S. subarctica* isolates at least
427 constitute a similar threat to crop plants and hence this pathogen should be included in plant
428 resistance screening for crops grown in northern latitudes where *S. subarctica* is most
429 prevalent.

430 In conclusion, wild *Brassica* lines showing high level resistance to *S. sclerotiorum* have
431 been identified using rapid detached leaf and petiole assays, which constitute a useful resource
432 for future breeding programmes of relevance to the UK and potentially Europe. It was also
433 shown that although isolates of *S. subarctica* vary in their aggressiveness, some can cause
434 significant disease on *Brassica* that is comparable to the most aggressive *S. sclerotiorum*
435 isolates.

436

437

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564 **Table 1:** List of *Sclerotinia* isolates used in this study.

| Isolate code | Origin ¹ | Host | Year isolated | Haplotype ² |
|---------------------------------|------------------------------|-----------|---------------|------------------------|
| <i>Sclerotinia sclerotiorum</i> | | | | |
| DG4 (ENG 91) | Warwickshire, England (DG2) | Buttercup | 2009 | 176 |
| L6 (ENG 189) | West Sussex, England (LE1) | Lettuce | 2005 | 3 |
| L44 (ENG 185) | West Sussex, England (LE1) | Lettuce | 2005 | 78 |
| P7 (ENG 254) | Herefordshire, England (PE1) | Pea | 2009 | 1 |
| <i>Sclerotinia subarctica</i> | | | | |
| HE1 (ENG 20) | Herefordshire, England | Buttercup | 2009 | 1 |
| ENG10 | Herefordshire, England | Buttercup | 2011 | 4 |
| ENG8 | Herefordshire, England | Buttercup | 2011 | 8 |
| ENG34 | Herefordshire, England | Buttercup | 2009 | 6 |
| SC25 | Isla Bend, Scotland | Potato | 2012 | 2 |
| SC52 | Fife, Scotland | Buttercup | 2012 | 5 |
| SC58 | Fife, Scotland | Buttercup | 2012 | 9 |
| SC63 | Perthshire, Scotland | Pea | 2012 | 3 |
| SC70 | Perthshire, Scotland | Pea | 2012 | 11 |
| SC61 | East Lothian Scotland | Swede | 2012 | 68 |
| LST3 | Tranägen, Sweden | Lettuce | 2012 | ND |
| NOR41 | Rogaland, Norway | Lettuce | 2012 | 42 |

565

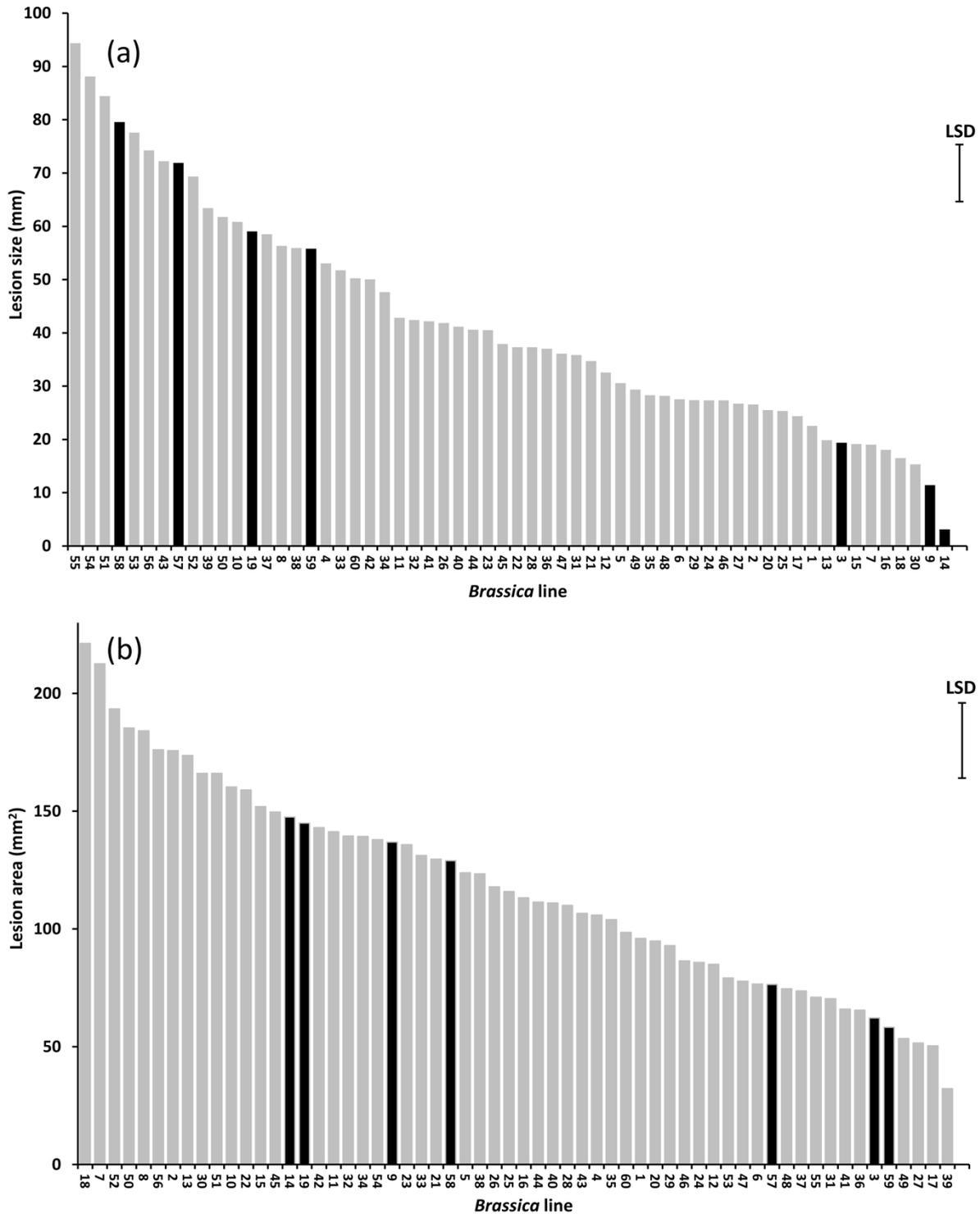
566 ¹ Locations followed by codes in brackets refer to populations characterised by Clarkson *et al.*,
 567 (2013).

568 ² Microsatellite haplotype as designated by Clarkson *et al.* (2013) for *S. sclerotiorum* and
 569 Clarkson *et al.* (2017) for *S. subarctica*.

570 **Table 2:** List of *Brassica* lines used in this study.

| Line No. | Brassica species ¹ | Resistance screen no. | Line No. | Brassica species ¹ | Resistance screen no. |
|----------|-------------------------------|-----------------------|----------|---|-----------------------|
| 1 | <i>B. atlantica</i> | 1 | 45 | <i>B. rupestris</i> | 1 |
| 2 | <i>B. atlantica</i> | 1 | 46 | <i>B. vilosa</i> | 1 |
| 3 | <i>B. bourgeai</i> | 1&2 | 47 | <i>B. vilosa</i> | 1 |
| 4 | <i>B. cretica</i> | 1 | 48 | <i>B. vilosa</i> | 1 |
| 5 | <i>B. cretica</i> | 1 | 49 | <i>B. vilosa</i> | 1 |
| 6 | <i>B. cretica</i> | 1 | 50 | <i>B. carinata</i> | 1 |
| 7 | <i>B. cretica</i> | 1 | 51 | <i>B. carinata</i> | 1 |
| 8 | <i>B. cretica</i> | 1 | 52 | <i>B. carinata</i> | 1 |
| 9 | <i>B. cretica</i> | 1&2 | 53 | <i>B. carinata</i> | 1 |
| 10 | <i>B. hilarionis</i> | 1&2 | 54 | <i>B. carinata</i> | 1 |
| 11 | <i>B. hilarionis</i> | 1 | 55 | <i>B. carinata</i> | 1 |
| 12 | <i>B. incana</i> | 1 | 56 | <i>B. carinata</i> | 1 |
| 13 | <i>B. incana</i> | 1 | 57 | <i>B. napus</i> (27, cv. Temple) | 1&2 |
| 14 | <i>B. incana</i> | 1&2 | 58 | <i>B. napus</i> (41) | 1&2 |
| 15 | <i>B. incana</i> | 1 | 59 | <i>B. napus</i> (83) | 1&2 |
| 16 | <i>B. incana</i> | 1 | 60 | <i>B. napus</i> | 1 |
| 17 | <i>B. incana</i> | 1 | 61 | <i>B. napus</i> (18) | 2 |
| 18 | <i>B. incana</i> | 1 | 62 | <i>B. napus</i> (69) | 2 |
| 19 | <i>B. insularis</i> | 1&2 | 63 | <i>B. napus</i> (87) | 2 |
| 20 | <i>B. macrocarpa</i> | 1 | 64 | <i>B. napus</i> (36) | 2 |
| 21 | <i>B. macrocarpa</i> | 1 | 65 | <i>B. napus</i> (8) | 2 |
| 22 | <i>B. macrocarpa</i> | 1 | 66 | <i>B. napus</i> (91) | 2 |
| 23 | <i>B. macrocarpa</i> | 1 | 67 | <i>B. napus</i> (20) | 2 |
| 24 | <i>B. macrocarpa</i> | 1 | 68 | <i>B. napus</i> (33) | 2 |
| 25 | <i>B. macrocarpa</i> | 1 | 69 | <i>B. napus</i> (60) | 2 |
| 26 | <i>B. macrocarpa</i> | 1 | 70 | <i>B. napus</i> (3) | 2 |
| 27 | <i>B. macrocarpa</i> | 1 | 71 | <i>B. napus</i> (37) | 2 |
| 28 | <i>B. macrocarpa</i> | 1 | 72 | <i>B. napus</i> (56) | 2 |
| 29 | <i>B. macrocarpa</i> | 1 | 73 | <i>B. napus</i> (74) | 2 |
| 30 | wild <i>B. oleracea</i> | 1 | 74 | <i>B. napus</i> (11) | 2 |
| 31 | wild <i>B. oleracea</i> | 1 | 75 | <i>B. napus</i> (89) | 2 |
| 32 | wild <i>B. oleracea</i> | 1 | 76 | <i>B. napus</i> (17) | 2 |
| 33 | wild <i>B. oleracea</i> | 1 | 77 | <i>B. napus</i> (19) | 2 |
| 34 | wild <i>B. oleracea</i> | 1 | 78 | <i>B. bourgeai</i> (DH of line 3) | 2 |
| 35 | wild <i>B. oleracea</i> | 1 | 79 | <i>B. bourgeai</i> (DH of line 3) | 2 |
| 36 | wild <i>B. oleracea</i> | 1 | 80 | <i>B. cretica</i> (DH of line 9) | 2 |
| 37 | wild <i>B. oleracea</i> | 1 | 81 | <i>B. cretica</i> (DH of line 9) | 2 |
| 38 | wild <i>B. oleracea</i> | 1 | 82 | <i>B. cretica</i> (DH of line 9) | 2 |
| 39 | wild <i>B. oleracea</i> | 1 | 83 | <i>B. cretica</i> (DH of line 9) | 2 |
| 40 | wild <i>B. oleracea</i> | 1 | 84 | <i>B. incana</i> (DH of line 14) | 2 |
| 41 | wild <i>B. oleracea</i> | 1 | 85 | <i>B. oleracea</i> (rapid cycling line) | 2 |
| 42 | <i>B. montana</i> | 1 | 45 | <i>B. rupestris</i> | 1 |
| 43 | <i>B. montana</i> | 1 | 46 | <i>B. vilosa</i> | 1 |
| 44 | <i>B. rupestris</i> | 1 | 47 | <i>B. vilosa</i> | 1 |

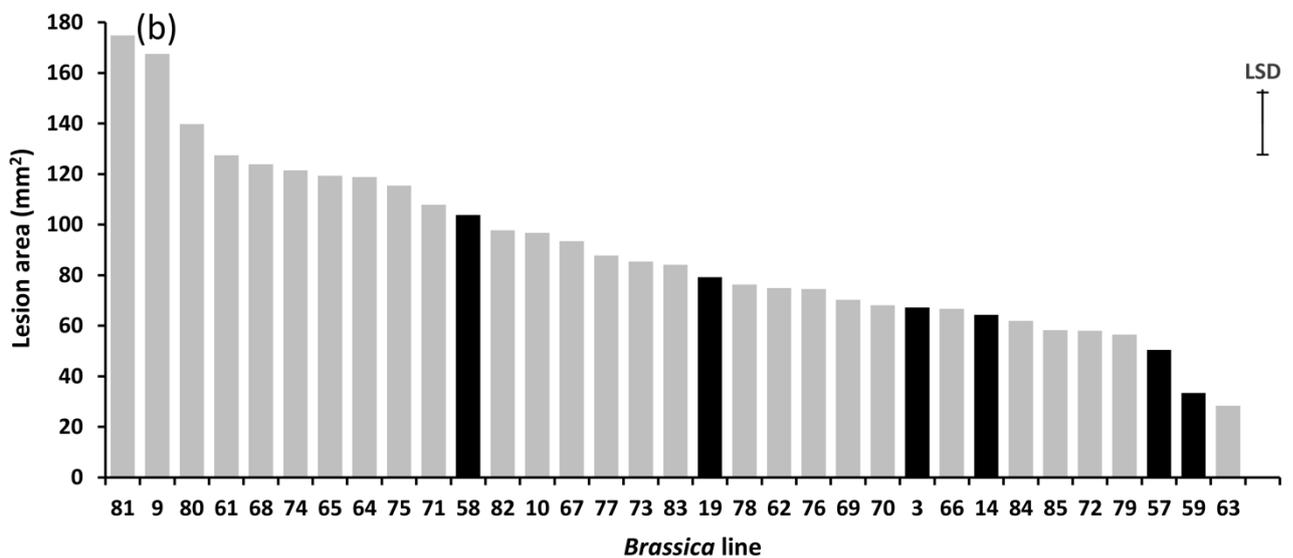
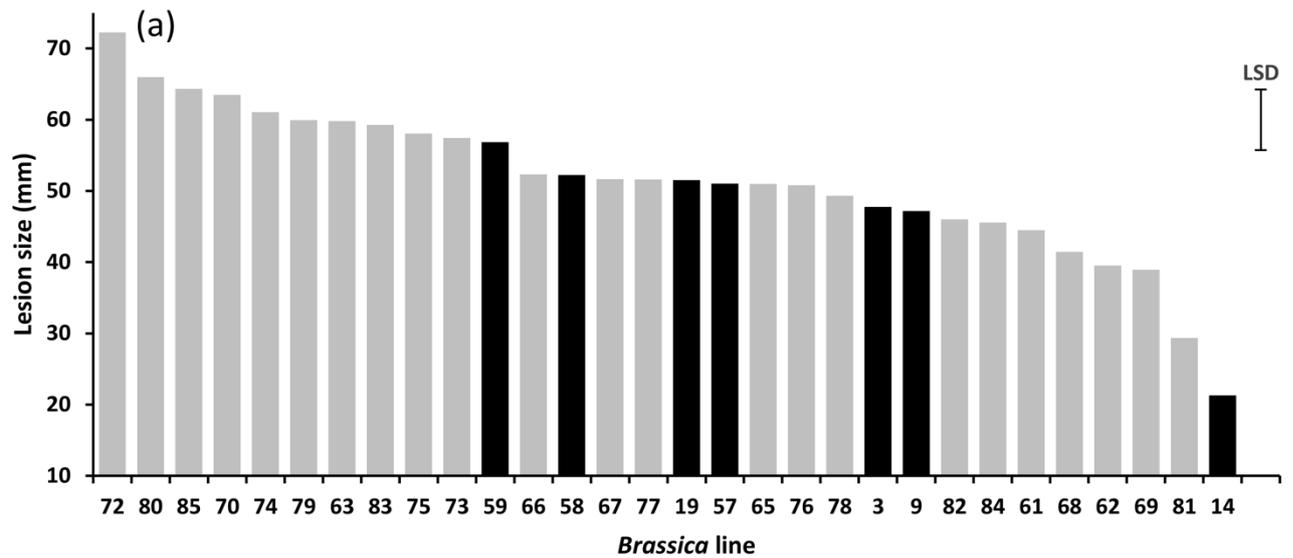
571 ¹ Numbers in brackets refer to previous line number designation in Taylor *et al.*, (2015)



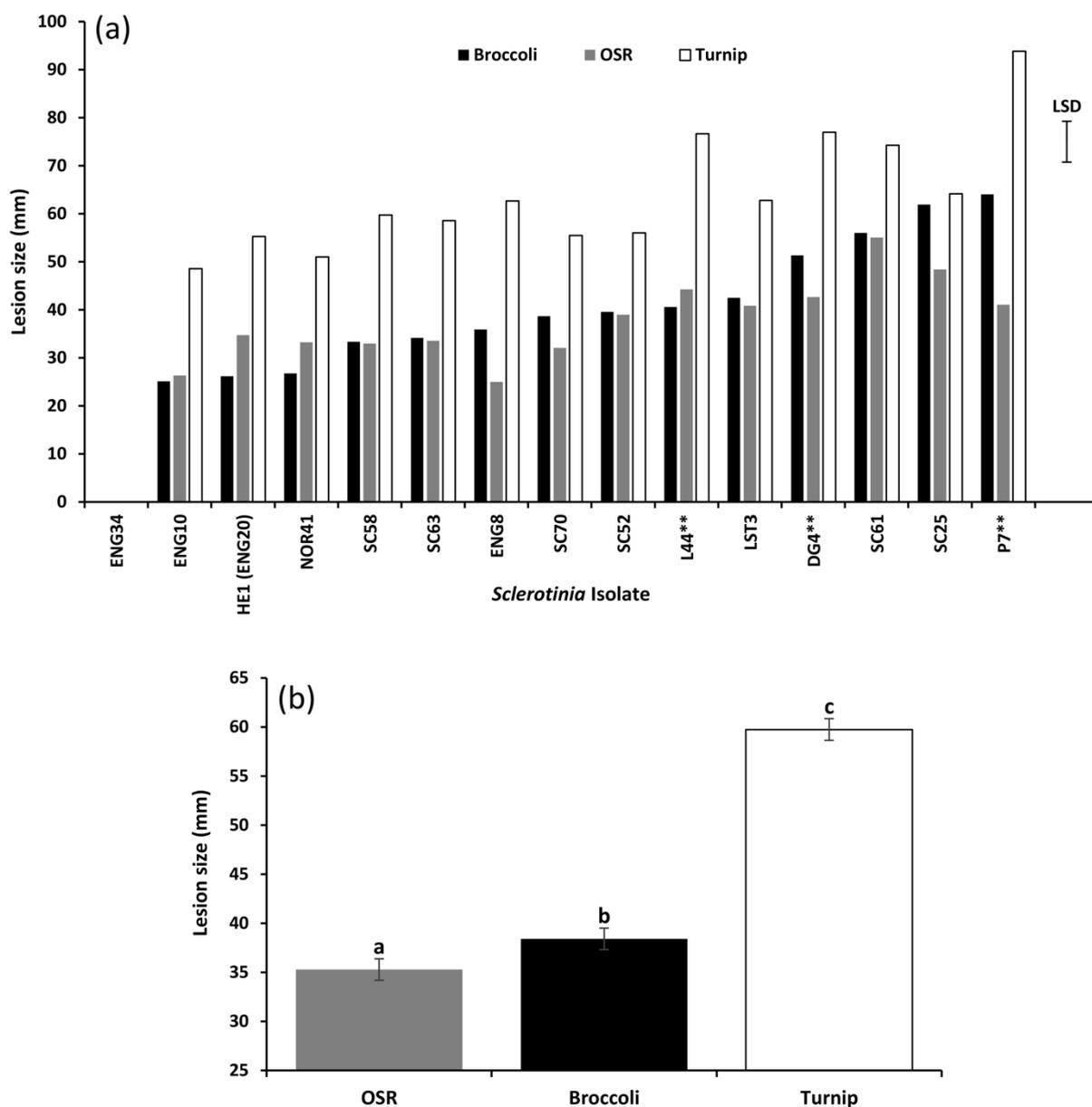
572 **Figure 1:** Mean lesion size/area for 60 *Brassica* lines following inoculation with *S.*
573 *sclerotiorum* for (a) detached petiole and (b) detached leaf assays in resistance screen 1.
574 Error bars represent the least significant difference (LSD, 5% level) following ANOVA
575 analyses. Black bars indicate lines used in both resistance screens 1 and 2.



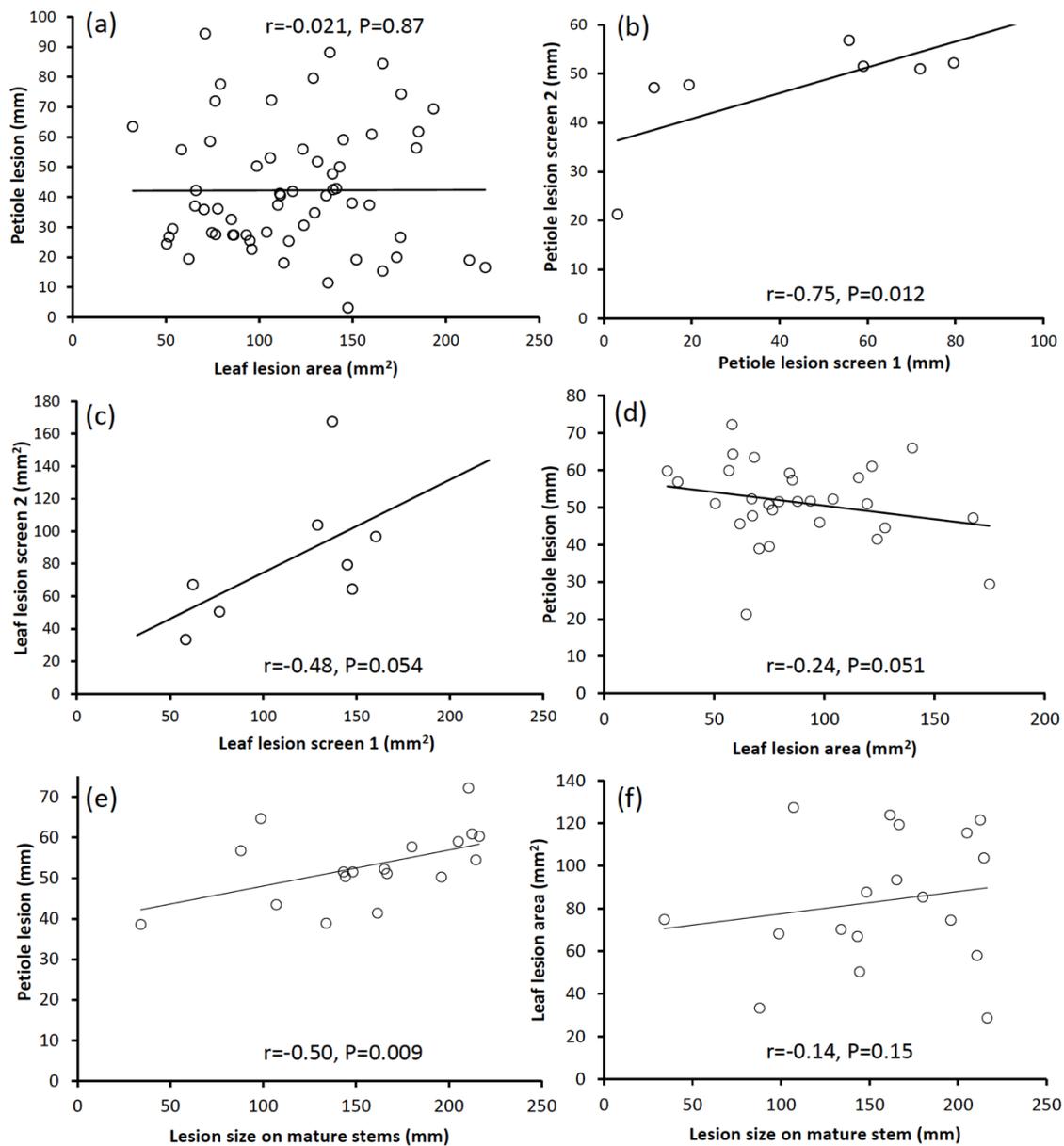
576 **Figure 2:** Photographs of petioles (a-c) and leaves (d-f) of *Brassica* lines inoculated with *S.*
577 *sclerotiorum* illustrating the range of phenotypes. (a) line 14, highly resistant; (b) line 29,
578 intermediate resistance; (c) line 43, highly susceptible; (d) line 39, highly resistant; (e) line
579 23, intermediate resistance; (f) line 52, highly susceptible.



580 **Figure 3:** Mean lesion size/area for 20 *B. napus* lines, 12 selected wild *Brassica* lines and a
 581 rapid cycling *B. oleracea* line following inoculation with *S. sclerotiorum* for (a) detached
 582 petiole and (b) detached leaf assays in resistance screen 2. Error bars represent the least
 583 significant difference (LSD, 5% level) following ANOVA analyses. Black bars indicate lines
 584 used in both resistance screens 1 and 2.



585 **Figure 4:** Mean lesion size for *B. napus* (oilseed rape cv. Temple), *B. oleracea* (broccoli cv.
586 Beaumont), and *B. rapa* (turnip cv. Manchester) inoculated with three *S. sclerotiorum* and 12
587 *S. subarctica* isolates (a) for all isolates; (b) for 12 *S. subarctica* isolates across each crop
588 type in detached petiole tests. Error bars in (a) represent the least significant difference
589 (LSD, 5% level) following ANOVA analyses. Letters in (b) indicate a significant difference
590 based on LSD values following ANOVA analysis. ** indicates *S. sclerotiorum* isolates.



591 **Figure S1:** Correlation plots for traits measured in this study. (a) *S. sclerotiorum* lesion size
 592 on petioles vs lesion area on leaves for resistance screen 1; (b) comparison of *S. sclerotiorum*
 593 lesion size on petioles in resistance screens 1 and 2; (c) comparison of *S. sclerotiorum* lesion
 594 area on leaves between resistance screens 1 and 2; (d) *S. sclerotiorum* lesion size on petioles
 595 vs lesion area on leaves for resistance screen 2; (e) comparison of *S. sclerotiorum* lesion size
 596 on petioles and mature plant stems (Taylor et al., 2015) for 20 *B. napus* lines; (f) comparison
 597 of *S. sclerotiorum* lesion area on leaves and lesion size on mature plant stems (Taylor et al.,
 598 2015) for 20 *B. napus* lines.