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The control of Sclerotinia stem rot on oilseed rape (*Brassica napus*): Current practices and future opportunities.

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15 Abstract

16 *Sclerotinia* stem rot (SSR) caused by the phytopathogenic fungus *Sclerotinia sclerotiorum* is
17 a major disease of oilseed rape (*Brassica napus*). During infection, large, white/grey lesions
18 form on the stems of the host plant, perturbing seed development and decreasing yield. Due
19 to its ability to produce long term storage structures called sclerotia, *S. sclerotiorum* inoculum
20 can persist for long periods in the soil. Current SSR control relies heavily on cultural
21 practices and fungicide treatments. Cultural control practices aim to reduce the number of
22 sclerotia in the soil or create conditions that are unfavourable for disease development. These
23 methods of control are under increased pressure in some regions, as rotations tighten and
24 inoculum levels increase. Despite their ability to efficiently kill *S. sclerotiorum*, preventative
25 fungicides remain an expensive gamble for SSR control, as their effectiveness is highly
26 dependent on the ability to predict the establishment of microscopic infections in the crop.
27 Failure to correctly time fungicide applications can result in a substantial cost to the grower.
28 This review describes the scientific literature pertaining to current SSR control practices.
29 Furthermore, it details recent advances in alternative SSR control methods including the
30 generation of resistant varieties through genetic modification and traditional breeding, and
31 biocontrol. The review concludes with a future directive for SSR control on oilseed rape.

32 Introduction

33 Oilseed rape (*Brassica napus* L.) is an allotetraploid crop species derived from natural
34 hybridization of the diploid progenitor species *B. oleracea* and *B. rapa* (Chalhoub *et al.*,
35 2014). The oil-rich seeds of oilseed rape are routinely processed into edible oil, biodiesel and
36 high quality animal feed. Global oilseed rape production has increased > 8 fold since 1975
37 and the crop has gone from being the sixth to the second most important oilseed crop in that
38 period (FAOSTAT, 2015). Canada, China, India, Germany, France and Australia are the
39 leading oilseed rape producing countries, accounting for 75% of the total worldwide
40 production in 2013 (FAOSTAT, 2015).

41 Historically, oilseed rape breeding efforts have primarily focused on reducing erucic acid and
42 glucosinolate concentrations in seeds. Erucic acid has been shown to be cytotoxic to
43 mammals (Charlton *et al.*, 1975; Srivastava *et al.*, 1975), whereas glucosinolates negatively
44 affect *B. napus* meal palatability in stock (Mithen, 1992). Extensive breeding efforts in
45 Canada in the 1970s led to the development of cultivars with < 2% erucic acid and < 30
46 μmol/g glucosinolates (Cowling, 2007). These cultivars were termed ‘double low’ and were
47 ascribed the trade name ‘Canola’. Canola is the primary form of oilseed rape grown for
48 human consumption and animal feed. For the purposes of this review the term ‘canola’ will
49 be used henceforth to describe the most widely grown form of ‘double low’ oilseed rape (*B.*
50 *napus*). However, it should be noted that ‘double low’ *B. rapa* and *B. juncea*-based cultivars
51 have also been attributed the trade name canola, although they are not as widely grown
52 (Rempel *et al.*, 2014).

53 The fungal phytopathogen *Sclerotinia sclerotiorum* is the causal agent of Sclerotinia stem rot
54 (SSR) in canola. *S. sclerotiorum* is present in all major canola growing regions and is
55 generally considered one of the most economically damaging canola pathogens (Kharbanda

56 and Tewari, 1996). The stems of canola plants infected with *S. sclerotiorum* are liable to
57 lodge during flowering and seed filling, which can cause a significant loss of crop yield.

58 SSR symptoms include the formation of bleached, greyish lesions on the main stem, branches
59 or pods of the canola plant; the presence of hard, melanised, black sclerotia within the cortex
60 of infected stems; and early flowering and wilting of plant tissue in the terminal parts of the
61 infected stems (Khangura and Beard, 2015). Though sporadic in occurrence, given favourable
62 environmental conditions *S. sclerotiorum* infestation can be very high in canola crops. In
63 Canada, provincial average incidences of SSR are often rated at 10-20 % (Anonymous,
64 2009), though field-specific incidence can be much higher. For example, in the results of a
65 2010 survey of diseases in Saskatchewan, 94 % incidence within an individual canola crop
66 was recorded (Dokken-Bouchard *et al.*, 2010). Comparable levels of SSR incidence have
67 been reported in the canola growing regions of Australia and the USA (Hind *et al.*, 2003; del
68 Rio *et al.*, 2007). In all of these regions, disease caused by *S. sclerotiorum* is thought to be
69 exacerbated by high levels of precipitation, which aids in the establishment of *S. sclerotiorum*
70 *in planta* (Koch *et al.*, 2007). Based on calculations derived from del Rio (del Rio *et al.*,
71 2007) an average SSR incidence of 10 to 20%, such as that reported for Canadian provinces,
72 equates to an approximate average yield loss of 5 to 10%.

73 Current SSR control methods rely primarily on cultural control practices and fungicide
74 application (Bardin and Huang, 2001; Murray and Brennan, 2012). The majority of cultural
75 control practices aim to reduce the level of *S. sclerotiorum* inoculum in the soil, or create a
76 local environment that is non-conducive to SSR development. Fungicides are routinely used
77 to impede the establishment of *S. sclerotiorum* germlings on the floral tissue of canola in an
78 attempt to break the disease cycle (Turkington and Morrall, 1993). However, the efficacy of
79 these fungicide applications is dependent on being able to predict when fungal ascospores are

80 first present on floral tissue. Failure to apply fungicides at the most effective time can result
81 in an economic cost to the grower.

82 As global canola production becomes more intensive, it is likely that improved control
83 methods will be required to maintain economically viable levels of SSR. The following
84 review will summarize current and prospective control methods and how they interfere with
85 the infection cycle of *S. sclerotiorum* on canola. Secondly, the limitations and strengths
86 associated with cultural and fungicide-based control methods in the context of modern canola
87 production will be discussed. Thirdly, the potential for new viral and non-viral based
88 biocontrol agents to control SSR will be evaluated. Finally, progress on the development of
89 genetically resistant canola plants via both natural and transgenic processes will be presented.

90

91 The *Sclerotinia sclerotiorum* infection cycle on canola

92 *S. sclerotiorum* belongs to the family Sclerotiniaceae, which is in the order Helotiales within
93 the fungal phylum Ascomycota. The development of melanised hyphal aggregates (termed
94 sclerotia) that act as resting structures, is a characteristic shared by all members of the
95 Sclerotiniaceae (Bolton *et al.*, 2006). Sclerotia form at the end of the infection cycle and can
96 appear in infested canola stubble. These structures may survive for several years (Khangura
97 and Beard, 2015), reducing the efficacy of break-cropping as a potential means of control.
98 When favourable environmental conditions are present, sclerotia germinate either
99 myceliogenically or carpogenically (Bardin and Huang, 2001). Temperature has a major
100 effect on sclerotial germination, which does not occur at 10 °C and is partially inhibited at 15
101 °C (Jones and Gray, 1973). The two kinds of germination produce distinct diseases. Sclerotia
102 that germinate myceliogenically produce mycelium that infects ground-level tissues of
103 susceptible plants, whereas those that germinate carpogenically produce fruiting bodies

104 (apothecia) that are laden with fungal spores (ascospores), which are dispersed via air
105 currents to infect aboveground tissues (Bardin and Huang, 2001). Ascospores require
106 senescent tissues in order to infect plants (Jamaux *et al*, 1995). In canola, this is often petals
107 that have fallen onto leaves or into leaf axils (Turkington and Morrall, 1993). Of the two
108 modes of germination, carpogenic is most common and is what produces the inoculum
109 contributing to SSR outbreaks in canola (Bom and Boland, 2000; Clarkson *et al*., 2007).

110 It is thought that colonisation of senescing petals allows *S. sclerotiorum* to build up sufficient
111 nutrient levels to initiate infection (Garg *et al*, 2010). Following petal colonisation, the fungus
112 produces aggregates of appressoria (fungal penetration structures deriving from hyphal tips)
113 termed infection cushions, which are used to penetrate green tissues of the plant (Jamaux *et*
114 *al*, 1995). Subsequent infectious growth coincides with the production of oxalic acid, which
115 is thought to be a major virulence factor. This compound has been shown to produce various
116 effects on molecular components of both host and pathogen (reviewed in Hegedus and
117 Rimmer, 2005). In the host, oxalic acid leads to the generation of low pH in the middle
118 lamella, which causes sequestration of calcium ions, and interference with stomatal closure
119 via accumulation of potassium and starch hydrolysis. In the pathogen, oxalic acid is thought
120 to directly enhance the activity of pectolytic enzymes involved in degradation of host tissue.

121 As infection proceeds, brown necrotic lesions appear around penetration sites and spread
122 wherever the fungus grows *in planta*. When a canola plant is heavily infected, lodging may
123 occur as necrotic lesions girdle the stem and cause it to lose its rigidity; this is the major
124 cause of SSR-induced yield loss in canola (Khangura and Beard, 2015). There are currently a
125 number of disease intervention measures that target the various stages of the *S. sclerotiorum*
126 infection cycle (Figure 1). Some of these are theoretical, having only been demonstrated
127 experimentally, and others are currently used by growers. The different types of SSR control

128 measures include cultural control, fungicides, biological control, genetic resistance breeding
129 and genetic modification (which is currently theoretical).

130

131 Cultural control practices for SSR

132 Cultural control practices promote local conditions that are unfavourable for pathogen
133 survival and disease development (Howard, 1996). For cultural control practices to be
134 effective, an understanding of the basic biology of the target pathogen is essential. The
135 control of SSR in canola relies heavily on cultural control practices (Kharbanda and Tewari,
136 1996; Murray and Brennan, 2012). These practices generally aim to reduce the number of
137 sclerotia in the soil, or create a local environment that is non-conducive to SSR development.

138 *S. sclerotiorum* spends around 90% of its lifecycle as sclerotia in the soil (Adams and Ayers,
139 1979). Under suitable environmental conditions, sclerotia germinate and develop inoculum-
140 laden apothecia (Bolton *et al*., 2006). A popular way for canola growers to manage the
141 number of sclerotia within their fields is via crop rotation (Kharbanda and Tewari, 1996). By
142 rotating *Brassica napus* with crops that aren't susceptible to *S. sclerotiorum* (nonhost crops),
143 the annual life cycle of *S. sclerotiorum* can be disrupted, reducing the annual number of
144 sclerotia that enter the soil 'sclerotia bank'. The current advice for Australian canola growers
145 is a 1 in 4 year rotation with a nonhost crop species such as wheat or barley (Anonymous,
146 2013). However, this advice should only serve as a guide as it has been suggested that
147 individual sclerotia of *S. sclerotiorum* can remain viable within a field environment for at
148 least 7 years (Adams and Ayers, 1979; Kora *et al*., 2008). A study in Canada showed that
149 sclerotia isolated from an oilseed rape field that had been over-sown with barley for three
150 successive years remained viable and were able to germinate and develop apothecia
151 (Williams and Stelfox, 1980). The build-up of sclerotia in the soil is further exacerbated when

152 a host crop is grown for consecutive years without rotation (Adams and Ayers, 1979).
153 Rotation length is a key cultural control component that needs to be carefully considered
154 when attempting to limit SSR incidence.

155 Crop rotation is only effective if the lifecycle of the target pathogen is interrupted. One of the
156 challenges with *S. sclerotiorum* is that it can complete its lifecycle on more than 400 different
157 host species, including common crop weeds such as Wild radish (*Raphanus raphanistrum*),
158 Shepherd's purse (*Capsella bursa-pastoris*), Canada thistle (*Cirsium arvense*), Field sow-
159 thistle (*Sonchus arvensis*) and Dandelion (*Taraxacum officinale*) (Boland and Hall, 1994).
160 For crop rotation to be effective against *S. sclerotiorum* it must be coupled with an efficient
161 weed control programme which minimizes the chances of a 'green bridge' establishing and
162 allowing *S. sclerotiorum* to persist in fields when canola is not in rotation.

163 The effect of tilling on the survival of sclerotia in the soil has been extensively studied.
164 Tilling practices reduce the ability of sclerotia to produce apothecia by burying them deep in
165 the soil (Kharbanda and Tewari, 1996). Abawi and Grogan (1979), reported that sclerotia of
166 *S. sclerotiorum* are functional only in the top 2-3 cm of soil as apothecial stipes are unable to
167 grow longer than 3 cm. Williams and Stelfox (1980), confirmed that ploughing sclerotia to a
168 depth of 7.6 - 15 cm reduced germination and apothecium production the following year.
169 However, the survival rate of *S. sclerotiorum* sclerotia is enhanced when sclerotia are buried
170 deep in the soil (Merriman *et al*., 1979). Successive years of tilling would only bring
171 previously buried sclerotia back to the soil surface. Therefore, this technique is more
172 effective if the sclerotia are buried and remain buried until sclerotial viability is reduced.

173 Another technique that serves to reduce the viability of sclerotia in the soil involves
174 manipulating the soil-water content. High soil moisture has been shown to negatively affect
175 the survival rates of sclerotia (Teo *et al*., 1989; Wu *et al*., 2008). In extreme cases where

176 flooding has occurred for more than 24 consecutive days, sclerotial viability has been
177 severely affected (Moore, 1949). Alternatively, limiting water availability within the top 5 cm
178 of soil can also have an effect on sclerotial viability (Kora *et al*., 2008). Manipulating soil-
179 water content for SSR control would be restricted to cropping systems that utilize controlled
180 irrigation schemes. Furthermore, these control practices would need to be implemented
181 between cropping seasons as the soil-water environments described are likely to be
182 detrimental to the viability of the crop plants themselves.
183 Additionally, chemical amendment of soil has also been shown to be effective at reducing
184 carpogenic germination of sclerotia. Namely, the commercially available fertiliser Perlka ®
185 (calcium cyanamide) was shown to significantly reduce the number of apothecia in four field
186 trials by up to 87% (Huang *et al*, 2006).
187 Several cultural control practices focus on modulating the local field environment so that
188 conditions are non-conducive to SSR development. Relative humidity is proposed to be a key
189 factor affecting the epidemiology of *S. sclerotiorum* in *B. napus* crops. Analysis of data
190 collected from local weather stations, showed that proximal relative humidity readings
191 greater than 80% correlate well with SSR development in the crop (Koch *et al*., 2007). High
192 relative humidity is especially problematic during flowering, as senescing flower petals are
193 the primary target of *S. sclerotiorum* ascospores. Relative humidity within the crop can be
194 reduced by allowing air flow between individual plants. This is achieved by using suitable
195 row widths and seeding rates during sowing.

196 Registered fungicides, fungicide resistance and the evaluation of other chemistries

197 In addition to cultural control, fungicides are widely used to control SSR (Khangura and
198 Beard, 2015). Globally, several different fungicide classes have proved effective for the
199 control of *S. sclerotiorum*, including anilinopyrimidines (Benigni and Bompeix, 2010),
200 benzimidazoles (Attanayake *et al*., 2011), dicarboxamides (Matheron and Matejka, 1989),

201 demethylation inhibitors (DMIs) (Li *et al*., 2015b), quinone outside inhibitors (QoIs,
202 otherwise known as strobilurins) (Mueller *et al*., 2002; Xu *et al*., 2014) and succinate
203 dehydrogenase inhibitors (SDHIs) (Stammler *et al*., 2007; Ma *et al*., 2009b). Others have
204 been tested *in vitro* for activity against *S. sclerotiorum* but have not yet been utilised in the
205 field, for instance the phenylpyrroles (Kuang *et al*., 2011).

206 These fungicide classes exhibit diverse modes of action: the anilinopyrimidines have been
207 reported to inhibit methionine biosynthesis (Masner *et al*., 1994), and the dicarboxamides are
208 thought to inhibit osmotic signal transduction (Yamaguchi and Fujimura, 2005); though the
209 precise molecular mechanisms of these activities are not known. The benzimidazoles bind
210 tubulin, which disrupts microtubule formation (Lacey, 1990). The DMIs inhibit the fungal
211 sterol 14-α demethylase enzyme CYP51, which is essential for biosynthesis of membrane
212 sterols (Hitchcock *et al*., 1990). The QoIs and SDHIs inhibit mitochondrial respiration by
213 binding to the quinone outside site of the mitochondrial cytochrome b subunit and the
214 mitochondrial enzyme succinate dehydrogenase respectively (Bartlett *et al*., 2004; Leroux *et*
215 *al*., 2010). Finally, similarly to the dicarboxamides, the phenylpyrroles inhibit osmotic signal
216 transduction by inhibiting a specific protein kinase (Pillonel and Meyer, 1997).

217 Thus there is a broad range of fungicides that can be used for the prevention of SSR in
218 canola, though not all are registered for use on canola in all growing regions. For instance, in
219 Australia only members of two of the six fungicide classes mentioned are currently registered
220 for use on canola (Table 1).

221 Since the majority of the fungicide classes listed above are known to exhibit only single-site
222 activity, the propensity for development of fungal resistance to them is relatively high.
223 Indeed, for each of the fungicide classes mentioned there are resistant strains of pathogenic
224 fungi known (Ishii *et al*., 2001; Myresiotis *et al*., 2007; Avenot *et al*., 2008; Banno *et al*.,

225 2008; Fernandez-Ortuno *et al*., 2013; Chen *et al*., 2014b; Frenkel *et al*., 2015), which in many
226 instances appeared within just a few years of fungicide introduction (Fraaije *et al*., 2002;
227 Stevenson *et al*., 2008). However, resistance in *S. sclerotiorum* has only been reported
228 globally for the benzimidazoles and dicarboxamides (Gossen *et al*., 2001; Ma *et al*., 2009a)
229 and in both instances strains resistant to these fungicides appeared more than a decade after
230 the introduction of these fungicides.

231 The reason that *S. sclerotiorum* appears to have a low propensity for fungicide resistance
232 development may be due to its monocyclic infection cycle. By definition, this kind of
233 infection proceeds from a single source of inoculum per growing season (Maddison *et al*,
234 1996). This effectively diminishes population size and genetic potential, as secondary spread
235 of the disease occurs via expansion of multinucleate mycelium through mitotic division.
236 Indeed, it has been demonstrated using a predictive model that monocyclic vs polycyclic
237 infection-strategy is an important determinant of the risk of fungicide resistance development
238 in plant pathogens (Grimmer *et al*., 2015). Furthermore, *S. sclerotiorum* is a homothallic
239 species thought to reproduce predominantly through self-fertilisation. This is thought to be
240 what has led to observations of its clonal population structure marked by a relatively low
241 level of genetic diversity on a local scale (Kohli and Kohn, 1998).

242 Evidence would suggest then, that fungicides are a useful long term control method for *S.*
243 *sclerotiorum* as it is unlikely to develop resistance quickly, especially if fungicides with
244 varying modes of action are used. However, there is still a need for the testing and registering
245 of a more diverse set of fungicides in regions where chemistries with limited modes of action
246 are used. This is because however small the risk of resistance development may be, it is still
247 present and could pose a potential threat to future crop yields if fungicide inputs are not well-
248 managed (Gossen *et al*., 2001; Ma *et al*., 2009b). Furthermore, selective pressure caused by
249 over-use of a small number of registered fungicides on canola may create resistance in other

250 canola-infecting species such as *Alternaria brassicicola*, which has already developed
251 resistance to several fungicide classes (Iacomi-Vasilescu *et al*., 2004).

252 Additionally, in some instances unregistered fungicides have shown a greater potential than
253 those currently registered for the control of SSR. One example is the phenylpyrrole
254 compound fludioxonil, which was shown to exhibit greater activity against Chinese *S.*
255 *sclerotiorum* isolates than iprodione, which is a commonly applied fungicide in China
256 (Kuang *et al*., 2011). In the future, testing of alternative fungicides for the prevention of SSR
257 on canola may be important for disease control in regions where there is limited availability
258 of registered fungicides. This kind of research is unlikely to be conducted by pesticide-
259 manufacturing companies as unnecessary registration of new fungicides for use in smaller
260 fungicide-markets (for instance Australia) when active ingredients have not been
261 compromised could lead to market failure and economic losses incurred by the registration
262 process. Thus, it should be an important part of government-led research on SSR control in
263 the future.

264

265 Predicting time of fungicide application for optimal control of SSR.

266 Due to environmental constraints on fungal growth and plant infection, outbreaks of SSR are
267 difficult to predict and deciding necessity and timing of fungicide-sprays for the disease
268 presents a challenge to growers.

269 If fungicides are sprayed prophylactically regardless of potential disease outcome, years in
270 which the level of SSR incidence would otherwise be low or absent will incur economic
271 losses due to unnecessary fungicide input. As a result of this economic consideration, there
272 has been a great deal of research focussed on the development of mathematical models and
273 sampling methods that can be used to predict SSR outbreaks.

274 Numerous quantitative variables that affect SSR severity in various crops have been
275 described, including the number and spatial distribution of apothecia, amount of airborne
276 inoculum, frequency of petal infestation, soil moisture content and relative humidity (Hunter
277 *et al*., 1984; Gugel and Morrall, 1986; Boland and Hall, 1988; McCartney, 1999). These
278 variables are inextricably linked to one another and other qualitative variables such as
279 weather forecast and cropping history. Several attempts have been made at using these
280 variables individually or in combinations to forecast SSR outbreaks in canola.

281 During the early 1980s a checklist was devised to aid in optimisation of fungicide spray
282 decisions on canola, based on factors thought to affect SSR incidence and grain price
283 (Thomas, 1984). Around the same time, in Denmark a forecasting scheme was developed
284 based on controlled germination of sclerotia. This involved collection of sclerotia from
285 infested oilseed rape fields followed by priming for germination and sowing at designated
286 depots. The percentage of germinated sclerotia in a given depot was then used to forecast
287 SSR outbreaks in oilseed rape crops within a 15 km radius (Buchwaldt, 1986). However,
288 there are several disadvantages to this approach including its lack of predictive power for
289 individual fields with different historical cropping regimes and thus numbers of sclerotia, its
290 inability to account for ascospore formation and spread and its subjectivity to local
291 environmental conditions. In order to enhance the predictive power of such a scheme,
292 attempts have also been made to measure ascospore release from sclerotial depots (Koch *et*
293 *al*., 2007).

294 Another approach that was investigated in the late 1980s and early 1990s is the use of the
295 amount of canola petal infestation to forecast SSR outbreaks. Turkington and Morrall (1993),
296 incubated canola petals on agar plates in order to determine whether *S. sclerotiorum* would
297 grow from them, allowing for a proxy assessment of disease presence/absence. Using this
298 method they were able to show that the percentage of petals infested with *S. sclerotiorum*

299 could be used to accurately predict SSR severity in 74 % of cases, based on results from
300 several consecutive field trials conducted in Canada. Presumably as a result of these findings,
301 petal testing kits were developed and made commercially available to Canadian canola
302 growers in the early 1990s. These consisted of plates containing agar medium suitable for *S.*
303 *sclerotiorum* growth and directions for use (Morrall, 1991). Similar petal testing kits were
304 commercialised in Australia though they were sold with the caveat that they were only useful
305 to determine presence/absence of *S. sclerotiorum* in the canola crop and not for the prediction
306 of SSR severity (Hind-Lanoiselet, 2004).

307 Though petal testing has been shown to be useful for the prediction of SSR, it loses its
308 predictive power when environmental conditions are not conducive to *S. sclerotiorum*
309 infection. Attempts at countering this were made by Turkington and Morrall (1993), who
310 suggested incorporation of environmental variables such as light penetration, leaf area index
311 and crop height into petal infestation-based forecasting systems. This study also showed that
312 the amount of petal infestation varied between early, full and late bloom, with a general trend
313 towards increased infestation as flowers developed. However, issues were raised by the
314 authors concerning the facilitation of measurement of environmental variables and the
315 practicality of a sustained petal sampling effort covering different bloom stages for growers.

316 Another attempt at consolidating environmental variables with petal infestation for SSR
317 forecasting was made by Bom and Boland (2000). In this study, a simpler predictive model
318 based on both level of petal infestation and relative soil moisture content was developed.
319 However, another problem with the petal testing method is that estimation of petal infestation
320 may itself be influenced by environmental variables. For instance, it has been shown that
321 collection of wet petals or collection of petals immediately prior to heavy rainfall can lead to
322 underestimation of petal infestation (Turkington *et al.*, 1991), as can collection of petals in
323 the morning as opposed to during the afternoon (Turkington and Morrall, 1993).

324 Perhaps because of their ease of implementation, models that rely on more easily determined
325 factors such as cropping history, disease incidence in previous years and weather forecast are
326 currently the most widely used by growers to aid in making decisions surrounding fungicide
327 application. After evaluating checklist questions that were in use in Sweden at the time,
328 Twengstrom *et al*. (1998) developed a risk points table-based prediction model using the
329 factors 'number of years since last oilseed rape crop', 'disease incidence in last *S.*
330 *sclerotiorum* host crop', 'crop density', 'rain in the last two weeks before flowering',
331 'weather forecast' and 'regional risk for apothecium development'. At a given threshold of
332 points, this model gave accurate spray recommendations for 75 % of fields that required
333 fungicide input and 84 % for fields that did not.

334 Though various SSR risk assessment schemes based on information from the cited studies
335 were adopted by oilseed growers, a centralised assessment scheme for oilseed growers in a
336 given region was not developed until 2007 by Koch *et al.* in Germany (Koch *et al*., 2007).
337 This model, named SkleroPro, worked by creating a regional assessment of disease risk based
338 on environmental variables acquired from weather stations such as relative humidity and
339 rainfall, which could then be built upon to determine field-site specific risk based on
340 parameters such as cropping history, expected yield, produce price and spray cost. Spray
341 recommendations could be automatically determined by feeding data for these parameters
342 through an internet-based platform. This model was able to provide accurate
343 recommendations in 70-81 % of cases based on historical data and field trials. However, it
344 was conceded by the authors that the model did not account for extrinsic inoculum levels and
345 was therefore most suitable for regions in which *S. sclerotiorum* inoculum was continually
346 present at a high level.

347 An alternative to assessing petal infestation to predict SSR severity in canola is assessment of
348 inoculum levels detected in air samples adjacent to field sites, which has previously been

349 achieved via the use of Burkard spore traps (McCartney, 1999). Traditional quantification
350 methods, i.e. microscopic analysis of trapped spores, were superseded by a polymerase chain
351 reaction-based detection method in 2002 (Freeman *et al*., 2002). This method can be used to
352 determine whether *S. sclerotiorum* DNA is present within the spore trap, which is used as a
353 proxy assessment of its presence/absence within the field. This method itself was improved
354 upon in 2009 with the development of a quantitative real-time PCR (qPCR)-based assay
355 (Rogers *et al*., 2009). This new method proved sensitive enough to detect as little as 0.5 pg of
356 *S. sclerotiorum* DNA, which is equivalent to approximately 1.5 ascospores. This lends itself
357 well to deployment of widely dispersed spore traps for detection of *S. sclerotiorum* in larger
358 regions rather than within single fields, which may be useful for a more coordinated effort to
359 reduce the impact of SSR in canola.

360 An improvement upon PCR-based detection systems is the SYield sensor system
361 commercially available from Syngenta. This system is able to automatically detect and
362 quantify the amount of airborne *S. sclerotiorum* inoculum through monitoring of oxalic acid
363 levels in a growth medium. Information from the spore traps can be transmitted wirelessly to
364 give growers alerts at times when disease inoculum levels are high, which could aid in timing
365 of fungicide sprays (Roberts, 2013; Scuffell, 2013).

366 The models mentioned thus far were developed in temperate growing regions. Thus, there is
367 the possibility that they are not applicable to the arid or semi-tropical growing regions of
368 countries such as Australia and China. For example, in Western Australia, it was found that
369 relative humidity and rainfall were the variables with the biggest impact on SSR severity.
370 Average temperature did not affect disease severity, as temperatures are almost always
371 optimum for fungal growth in this region. From this research, a regional SSR forecasting
372 model is currently being developed for Australian conditions (Lee, 2014).

373

374 Biological control

375 Over the past 20 years there has been a concerted effort to identify biological control agents
376 (BCAs) of *S. sclerotiorum*. Despite a frenetic rate of discovery of potential BCAs, only a
377 small number have been commercialized (Zeng *et al*., 2012a). Most of the reported BCAs lie
378 within the fungal and bacterial kingdoms. However, it has recently been shown that viral
379 particles can also be used to perturb the growth of *S. sclerotiorum* (Yu *et al*., 2013). Within
380 this section, past efforts, successes and future potential for the biocontrol of *S. sclerotiorum*
381 on *B. napus* are summarized.

382 Many of the organisms identified as being parasitic to *S. sclerotiorum* are also fungi.
383 *Trichoderma harzianum* parasitises both the sclerotial and hyphal growth stages of *S.*
384 *sclerotiorum* (Abdullah *et al*., 2008; Troian *et al*., 2014). Analysis of gene expression during
385 these parasitizing events suggests that fungal cell wall-degrading enzymes are actively
386 produced by *T. harzianum*. Functional studies have demonstrated the importance of the
387 endogenous *T. harzianum* cell wall-degrading chitinase *Chit42*. *T. harzianum* isolates that
388 were transformed with a constitutively expressed transgene of *Chit42* had higher levels of
389 chitinase activity than WT isolates, making them more able to inhibit the growth of *S.*
390 *sclerotiorum* (Kowsari *et al*., 2014). The fungus *Ulocladium atrum* was shown to have an
391 antagonistic effect on germinating ascospores of *S. sclerotiorum* (Li *et al*., 2003). Co-
392 inoculation of canola petals with *U. atrum* and *S. sclerotiorum* reduced the amount of SSR
393 development as compared to inoculation with *S. sclerotiorum* alone.

394 The most commonly studied mycoparasite for the biocontrol of *S. sclerotiorum* is
395 *Coniothyrium minitans*. Like *T. harzianum*, *C. minitans* parasitizes the sclerotia and mycelia
396 of *S. sclerotiorum* (Whipps and Gerlagh, 1992; McQuilken *et al*., 1995; McLaren *et al*.,

397 1996). The key to the success of *C. minitans* as a BCA is its ability to persist and spread
398 within the soil. *C. minitans* is relatively resilient to annual fluctuations in temperature and
399 soil-moisture content. In non-irrigated soil with ambient air temperatures ranging from -0.1 to
400 39°C, *C. minitans* was able to persist for a period of 750 days (Yang *et al*., 2010). The
401 conidia of *C. minitans* can be easily spread through the soil by free water (Yang *et al*., 2009).
402 It has been shown that active spreading of *C. minitans* during the seedling stage of canola can
403 reduce the amount of carpogenic germination of *S. sclerotiorum* later in the growing season
404 (Yang *et al*., 2009). Parasitisation of *S. sclerotiorum* by *C. minitans* likely involves the
405 breakdown of oxalic acid, a known pathogenicity factor of *S. sclerotiorum* (Cessna *et al*.,
406 2000). A study by Ren *et al*. (2007) showed that *C. minitans* actively breaks down oxalic acid
407 resulting in localized changes in pH. It is hypothesized that the induced change in pH of the
408 infected tissue stimulates the production of cell-wall degrading enzymes. The role of oxalic
409 acid in the mycoparasatism of *C. minitans* on *S.* sclerotiorum was further confirmed by
410 studying oxalate decarboxylase deficient mutants of *C. minitans*. These mutant strains had
411 lost the ability to break down oxalic acid. When co-incubated with *S. sclerotiorum* the
412 *Cmoxdc1* mutant had a reduced ability to infect the mycelia of *S. sclerotiorum* as compared to
413 wild-type *C. minitans* (Zeng *et al*., 2014). In addition to its ability to break down oxalic acid,
414 *C. minitans* is also proposed to secrete antifungal compounds. Yang *et al.* (Yang *et al*., 2007)
415 showed that substances present in the culture filtrate of *C. minitans* were active against
416 mycelial growth and ascospore germination of *S. sclerotiorum*.

417 In addition to parasitic fungi, many bacterial mycoparasites have been identified as potential
418 SSR BCAs, including *Streptomyces platensis* (Wan *et al*., 2008), *S. lydicus* (Zeng *et al*.,
419 2012a), *Bacillus subtilis* (Hu *et al*., 2005; Hou *et al*., 2006; Hu *et al*., 2011; Chen *et al*.,
420 2014a; Gao *et al*., 2014; Hu *et al*., 2014a), *B. amyloliquefaciens* (Abdullah *et al*., 2008; Wu *et*
421 *al*., 2014), *B. megaterium* (Hu *et al*., 2013), *Pseudomonas fluorescens* (Aeron *et al*., 2011; Li

422 *et al*., 2011), *P. chlororaphis* (Poritsanos *et al*., 2006; Fernando *et al*., 2007; Selin *et al*.,
423 2010) and *Serratia plymuthica* (Thaning *et al*., 2001). In contrast to the fungal BCAs
424 described above, the majority of characterized bacterial mycoparasites target the ascospores
425 and growing hyphae of *S. sclerotiorum* (Savchuk and Fernando, 2004). Typically, broth
426 cultures and/or cell suspensions of the potential bacterial BCA are applied to the above-
427 ground parts of canola plants using spray inoculation techniques. Scanning electron
428 microscopy (SEM) images of oilseed rape leaves that had been pre-inoculated with both *S.*
429 *sclerotiorum* and *B. subtilis* EDR4 showed *S. sclerotiorum* hyphae with abnormal growth,
430 cytoplasm leakage and fewer infection cushions as compared to negative controls (Chen *et*
431 *al*., 2014a). *S. sclerotiorum* mycelia have also been shown to become malformed when
432 incubated with *P. fluorescens*. As a result, the growth of *S. sclerotiorum* was reduced by
433 84.4% (Li *et al*., 2011). Independent spray inoculation assays with *B. amyloliquefaciens*
434 NJZJSB3 (Wu *et al*., 2014) and *B. subtilis* Em7 (Gao *et al*., 2014) resulted in decreased
435 incidence of SSR disease by 83.3% and 50- 70% respectively. The levels of SSR control
436 achieved in these experiments is comparable to the control achieved by synthetic fungicides
437 (Gao *et al*., 2014).

438 As an alternative to spray techniques, bacterial BCAs of *S. sclerotiorum* can also be applied
439 as seed pellets and seed wraps/coats. These protective structures increase the viability of the
440 BCAs over time and can also promote root and rhizosphere colonization (Hu *et al*., 2005;
441 Aeron *et al*., 2011). Some of the bacterial BCAs that have been applied as seed coats have
442 had the added effect of increasing plant growth and yield. *B. megaterium* A6, *B. subtilis* Tu-
443 100 and *P. fluorescens* PS all exhibit plant growth promoting effects in conjunction with the
444 ability to reduce the incidence of SSR (Aeron *et al*., 2011; Hu *et al*., 2011; Hu *et al*., 2013).

445 Three of the aforementioned biological control agents have been commercialized for use
446 against *Sclerotinia sclerotiorum* (Table 2.). Formulations of the fungi *C. minitans*

447 CON/M/91-08 (Contans® WG) and *T. harzianum* T-22 (PlantShield® HC) have been
448 designed for sclerotial control whereas the bacterial formulation of *B. subtilis* QST-713
449 (Serenade® MAX) has been designed to target fungal growth in the phyllosphere
450 (Walgenbach, 2009). A study comparing the ability of each of the three commercial BCAs to
451 inhibit the germination of *S. sclerotiorum* sclerotia showed that *C. minitans* (Contans® WG)
452 was most effective, reducing the viability of sclerotia of *S. sclerotiorum* in the soil by 95.3%
453 and reducing overall disease severity by 68.5% (Zeng *et al*., 2012a). In an independent study,
454 applications of Contans® WG to oilseed rape field trials resulted in a marked reduction in
455 SSR disease incidence as compared to untreated controls (Hedke *et al*., 1999).

456 The ability of BCAs to maintain an effective concentration in the field is often questionable
457 and depends largely on environmental conditions (Hu *et al*., 2011). However, BCAs are often
458 viewed as environmentally friendly alternatives to common fungicides (Fernando *et al*.,
459 2004). Contans® WG has been quoted as being relatively cheap as compared to other
460 biocontrol agents (Hedke *et al*., 1999). Despite this, Contans® WG remains the most
461 promising non-cultural control option for killing field-borne sclerotia of *S. sclerotiorum*.

462 The use of mycoviruses for the control of *S. sclerotiorum* is an exciting new discovery in the
463 field of biological control research (Xie and Jiang, 2014). A breakthrough paper by Yu *et al.*
464 (Yu *et al*., 2013) demonstrated that viral particles from the *S. sclerotiorum* hypovirulence-
465 associated DNA virus-1 (SsHADV-1) were able to limit *S. sclerotiorum* infection when
466 applied to infected *Arabidopsis thaliana* plants. Furthermore, when a fragmented suspension
467 of virus-infected *S. sclerotiorum* hyphae were applied to aerial parts of *B. napus* the incidence
468 and severity of SSR was reduced. Despite limitations with the spread of these viral particles
469 via host-plant cells, evidence suggests that they can be transmitted between fungi irrespective
470 of vegetative compatibility. The suitability of these viruses as potential BCAs is further
471 promoted by the fact that they have a limited host range (Yu *et al*., 2013). For example,

472 SsHADV-1 can infect species within the *Sclerotinia* genus but is unable to infect *Botrytis*
473 *cinerea*, a closely related member of the Sclerotiniaceae. The advent of high throughput
474 sequencing has made virus discovery simpler and has aided in the discovery of additional
475 hypovirulence-associated viruses of *S. sclerotiorum* that could be developed as potential
476 BCAs (Jiang *et al.*, 2013a; Hu *et al.*, 2014b; Xiao *et al.*, 2014). Although the cost
477 effectiveness of viral BCAs is still to be determined, they offer an exciting new opportunity
478 for the future control of SSR in canola.

479 Thus there exist a number of potential and already commercialised options for the biocontrol
480 of SSR in canola. However, amongst those already commercialised, reports of efficacy are
481 inconsistent. Though Zeng *et al* (2012a) report that *C. minitans* reduced sclerotia in the soil
482 by 95.3 %, in a similar study by the same authors (Zeng *et al*, 2012b), it was reported that *C.*
483 *minitans* only reduced the number of sclerotia by 50 %. It has also been shown that different
484 strains of *S. sclerotiorum* that produce varying amounts of oxalate are differentially
485 susceptible to *C. minitans* (Yongbing *et al*, 2011). Additionally, in a study by Sumida *et al*
486 (2015), application of *T. harzianum* did not inhibit sclerotial germination relative to the
487 untreated control, despite several reports of its efficacy (de Aguiar, 2014; Zeng, 2012a).

488 Another consideration surrounding the potential use of BCAs for control of disease is their
489 cost benefit ratio relative to conventional methods. For products such as Contans® WG,
490 which may require repeated seasonal applications to achieve its full impact, it is likely that
491 uptake by growers is strongly affected by such a consideration (Faechner, 2015). Despite
492 these hurdles, the fact that several biocontrol agents exist and are registered for crop
493 protection in various countries is evidence that there is at least a limited amount of grower
494 uptake. It is likely that these kinds of products will serve in future, as they do today, as a
495 valuable tool that can act synergistically as a part of integrated pest management schemes for
496 SSR. It is unlikely however, that they will act as a complete solution for the disease.

497

498 ## 6. Genetic resistance

499 Utilizing natural host-resistance through breeding is the most preferred option for controlling
500 crop disease, including SSR on canola (Moose and Mumm, 2008). Studies concerned with
501 identifying SSR resistance in *B. napus* cultivars have primarily utilized *in vitro* bioassays to
502 identify cultivars that are more able to resist *S. sclerotiorum* infection (Garg *et al*., 2008;
503 Garg *et al*., 2010a; Garg *et al*., 2010b; Garg *et al*., 2010c; Uloth *et al*., 2013; Uloth *et al*.,
504 2014; Taylor *et al*., 2015). Several studies have gone a step further and have mapped genetic
505 loci that are associated with SSR resistance in *B. napus* (Table 3). Despite the discovery of
506 resistance-associated QTLs, the development of completely resistant *B. napus* cultivars
507 through breeding is yet to be accomplished. Reasons for this lack of transition from the lab
508 into the field will be examined. Furthermore, the potential of introgressed and apetalous
509 cultivars of *B. napus* to resist SSR will be discussed.

510 The most popular method for screening *B. napus* cultivars for resistance to *Sclerotinia*
511 *sclerotiorum* involves performing pathogen bioassays. A common approach involves
512 inoculating *B. napus* cultivars with *S. sclerotiorum* and incubating infected plants in a
513 standardized environment. Following a defined period of time, measurements are taken to
514 determine disease severity. Despite this standardized process, a large amount of technical
515 variation exists between different studies. This makes it difficult to compare the results of
516 similar studies. In some cases *B. napus* cultivars may be designated as partially resistant in
517 one study and susceptible in another. For example the *B. napus* cultivar Mystic has produced
518 contradictory phenotypes in alternative studies (Uloth *et al*., 2013; Taylor *et al*., 2015).

519 The lack of standardization between these studies is likely to be partly due to technical
520 differences associated with how the pathogen is inoculated onto *B. napus*. A common

521 technique is to introduce inoculum into the stem of *B. napus* by piercing the stem with an
522 infested toothpick (Zhao and Meng, 2003). The validity of this technique is questionable as
523 this method eliminates host resistance that is mediated by the plant's epidermal cells. These
524 cells are well known responders to microbial invasion and are the sites of phytoalexin
525 accumulation and cell wall apposition formation in canola (Garg *et al.*, 2010c).

526 Ideally, the form of inoculum used for pathogen bioassays would be similar to the inoculum
527 that induces SSR in the wild i.e. ascospores and infected petals (Figure 1). The use of
528 ascospores as a form of inoculum is technically challenging as it is difficult to routinely
529 produce ascospores in the laboratory. Furthermore, past studies have shown that artificial
530 inoculation of canola plants with ascospores can result in inconsistent infection across trials
531 (Bradley *et al.*, 2006). As an alternative to ascospore inoculation, infected substrates that
532 resemble infected petals are commonly used, including infected wheat grain (Taylor *et al.*,
533 2015) and infected potato dextrose agar (PDA) plugs (Garg *et al.*, 2013). A study on *S.*
534 *sclerotiorum* infection in Arabidopsis showed that the substrate that is used for inoculation
535 greatly influences the resulting disease severity ratings (Guo and Stotz, 2007). Therefore, it is
536 likely that the choice of inoculum also affects the level of disease severity observed for
537 assays with canola tissue.

538 *S. sclerotiorum* pathogenicity assays on *B. napus* are often limited to a single infectious
539 isolate. This is concerning as it has been suggested that different *S. sclerotiorum* pathotypes
540 exist (Ge *et al.*, 2012). If *S. sclerotiorum* does indeed have pathotypes, then resistance data
541 generated from a single isolate may not correlate with resistance in the field, where multiple
542 genotypes exist. Taylor *et al* (Taylor *et al.*, 2015) validated the choice of their assayed isolate
543 using a pre-screening population of *Brassica* species. Through haplotyping and assessment of
544 aggressiveness on this test population, a highly aggressive and weakly aggressive *S.*
545 *sclerotiorum* isolate were able to be selected for all subsequent bioassays. In order to make

546 the results of *in vitro* bioassays more relevant to a multi-pathotype field environment
547 bioassays should consider using multiple isolates that have distinct genotypes and pathotypes.

548 Substantial efforts have been made to map genetic loci that contribute to SSR resistance in
549 canola. To date, the majority of published studies have used a bi-parental mapping approach
550 which involves genotyping and phenotyping populations derived from a cross between a
551 partially resistant parent and a susceptible parent (Table 3). Apart from the Wei *et al* study
552 (Wei *et al.*, 2014), all of these mapping studies used phenotyping data that was collected from
553 *in vitro* pathogen bioassays.

554 For many of the mapping studies, resistance was assayed at multiple *B. napus* life stages. The
555 majority of major QTLs were detected in only a single life stage. However, Wu *et al* (Wu *et*
556 *al.*, 2013) identified a QTL that was present in eight week old plants as well as mature plants.
557 These studies suggest that a proportion of SSR resistance is likely to be development-
558 specific. In concordance with what is known about the disease cycle, the most useful QTLs
559 for resistance breeding are likely to be those that are identified in mature, flowering plants.

560 Recent completion of the *Brassica napus* genome (Chalhoub, 2014) has enabled direct
561 comparisons to be made between documented SSR-resistance mapping studies (Li *et al.*,
562 2015a). Li *et al* show that several independent mapping studies identified the same QTLs for
563 resistance to *S. sclerotiorum* in *B. napus*. The QTLs SRC6 and Sll16 are an example of
564 overlapping inter-project QTLs (Zhao *et al.*, 2006; Wu *et al.*, 2013). Based on differential
565 gene expression analysis of the parent genotypes, the candidate resistance gene BnaC.IGMT5
566 was identified within the SRC6 locus (Wu *et al.*, 2013). This gene is homologous to the
567 *Arabidopsis thaliana* glucosinolate methyltransferase gene and is a candidate SSR resistance
568 gene (At1g76790). Interestingly, this QTL does not overlap with major QTLs associated with
569 leaf and seed glucosinolate content in *B. napus* (Feng *et al.*, 2012; Harper *et al.*, 2012; Lu *et*

570 *al*., 2014). Specific glucosinolates have been shown to have different activities against *S.*
571 *sclerotiorum* (Fan *et al*., 2008). Therefore, it could be speculated that BnaC.IGMT may
572 influence the repertoire of glucosinolates that are produced by a canola plant rather than the
573 overall number of glucosinolates.

574 To date there are few publications that use association mapping to identify resistance to *S.*
575 *sclerotiorum*. As opposed to bi-parental mapping techniques, these studies are not limited by
576 the genetic diversity restricted within two individual canola plants and therefore represent a
577 more comprehensive way of identifying QTLs for SSR resistance in *B. napus*. Genome wide
578 association studies (GWAS) have been performed for SSR in soybean (Bastien *et al*., 2014)
579 and sunflower (Fusari *et al*., 2012), as well as pod shatter resistance in canola (Raman *et al*.,
580 2014). The recently developed *B. napus* GWAS ERANET-ASSYST population could be
581 utilized for future SSR resistance studies (Schiessl *et al*., 2014).

582 Proteomic analyses have also been used in an attempt to identify genetic components of *B.*
583 *napus* that correlate with disease resistance phenotypes. Wen *et al*. (Wen *et al*., 2013)
584 identified 20 proteins with a putative role in disease resistance, including a glycine-rich
585 protein, a trypsin inhibitor protein, two heat shock proteins and a thiol methyltransferase. A
586 similar study by Garg *et al*. (2013) identified several proteins with putative roles in primary
587 metabolic pathways, antioxidant defence, ethylene biosynthesis, pathogenesis, protein
588 synthesis and protein folding. These genes still require functional characterization.

589 The lack of discovery of genetic resistance in current canola populations has prompted
590 researchers to search for genetic resistance to *S. sclerotiorum* within close relatives of *B.*
591 *napus*, with the intention to introgress resistance across the species barrier. The Brassica
592 species *B. rapa* var. *chinensis*, *B. oleracea*, *B. rupestris*, *B. incana*, *B. insularis*, and *B. villosa*
593 have all been shown to harbor high levels of resistance to *S. sclerotiorum* (Mei *et al*., 2013;

594 Uloth *et al*., 2013; Uloth *et al*., 2014). Many of the resistance responses observed in *B.*
595 *oleracea* were shown to occur at the point of attempted penetration (Uloth *et al*., 2014). This
596 indicates that the epidermal cell wall is important for resisting infection in the C genome of
597 *B. napus* and suggests that assays that directly wound tissue are likely to be circumventing
598 this resistance response.

599 Attempts have been made to introgress genes from *B. oleracea* (Ding *et al*., 2013), as well as
600 genes from other wild crucifers that lie outside the *Brassica* genus. Promising results have
601 been reported for *B. napus* lines that have introgressed genetic material from *Erucastrum*
602 *cardaminoides*, *Diplotaxis tenuisiliqua*, *E. abyssinicum*, *Capsella bursa-pastoris* and *Sinapsis*
603 *arvensis* (Chen *et al*., 2007; Garg *et al*., 2010a; Wei *et al*., 2010). The challenge now lies with
604 genetically moulding these lines into suitable crop cultivars that retain the characteristics of
605 qualified canola cultivars.

606 Apetalous *B. napus* plants have a genetic mutation that affects their ability to develop petals
607 (Zhao and Wang, 2004). These mutant plants develop less SSR, presumably as a result of the
608 lack of primary infection substrate available for *S. sclerotiorum* establishment (Zhao and
609 Wang, 2004). In glasshouse experiments, the reduction in petal numbers appeared to correlate
610 with a reduction in disease incidence (Young and Werner, 2012). However the ability of
611 apetalous cultivars to perform better than full-petalled cultivars in the field is not decisive. On
612 average both apetalous and fully-petalled cultivars are equally infected by *S. sclerotiorum*
613 (Young and Werner, 2012).

614 The development of SSR-resistant canola lines is challenging as key traits like low erucic
615 acid content, and low glucosinolate content must not be compromised in newly developed
616 varieties. Although there is potential for introgressed genes to increase resistance levels, the

617 development of these varieties will be costly and the durability of the introgressed resistance
618 is undetermined.

619 Additionally, in many of the studies in which QTLs have been identified via the use of
620 biparental mapping populations, the differentiation between ‘susceptible’ and ‘resistant’ or
621 ‘partially resistant’ parents is questionable. For example, Wu *et al* (2013) report 10 QTLs for
622 adult plant resistance and three for seedling resistance in a doubled haploid population
623 derived from a cross between the *B. napus* cultivars ‘J7005’ and ‘Hua 5’. However, in this
624 study, though the susceptible parent Hua 5 was clearly highly susceptible to the disease –
625 whole plants were overcome by fungal infection – the partially resistant parent still exhibited
626 a relatively large amount of disease – lesions were between 10 and 15 cm seven days post
627 inoculation. Similarly, in the cited study by Yin *et al* (2010), QTLs were identified in a
628 doubled-haploid population derived from a susceptible parent with an average lesion size of
629 approximately 5-6 cm and a partially susceptible parent with an average lesion size of 3-4 cm
630 after 7 days. Thus, although there may be evidence of some resistance to *S. sclerotiorum* in *B.*
631 *napus* germplasm, extensive characterisation of pathogen biology and the molecular
632 processes involved in infection are needed to elucidate whether major improvements to
633 canola yield can be made via conventional breeding.

634

635 Potential transgene solutions

636 The previously mentioned issues with prediction of SSR outbreaks and lack of resistant
637 germplasm together present a strong case for the use of genetic engineering to combat SSR in
638 canola. Before this can occur, transgenes must be tested for their efficacy against SSR and
639 their impacts on the environment and consumers.

640 One strategy that could mitigate the impacts of transgenes on the environment and consumers
641 is the use of only those genes that affect more specific aspects of the molecular biology of
642 major pathogens. The wealth of research detailing the molecular basis of infection by
643 numerous species, including *S. sclerotiorum,* has facilitated the discovery of a number of
644 potential transgene solutions for countering fungal disease in *B. napus*. Some of these are
645 more specific to SSR resistance and others have shown efficacy against a range of pathogenic
646 fungi, including *S. sclerotiorum*. The following sections detail studies that have thus far
647 produced transgenic plants with enhanced SSR resistance with a specific focus on what
648 has/has not been tested in *B. napus*. Other potential avenues for engineering resistance to *S.*
649 *sclerotiorum* are also discussed.

650 *Oxalic acid degrading enzymes*

651 An important virulence determinant in *S. sclerotiorum* is oxalic acid (Bolton *et al*., 2006).
652 The link between this compound and *S. sclerotiorum* pathogenicity has been known for
653 decades (Noyes and Hancock, 1981; Marciano *et al*., 1983; Magro *et al*., 1984; Tu, 1985).
654 Several studies have demonstrated that heterologous expression of oxalate oxidase (OxO), a
655 gene encoding an enzyme capable of breaking down oxalic acid, can increase SSR resistance
656 in susceptible host species including *B. napus,* tomato (*Solanum lycopersicum*) and soybean
657 (*Glycine max*) (Donaldson *et al*., 2001; Simmonds *et al*., 2001; Hu *et al*., 2003; 2004; Dong
658 *et al*., 2008; Walz *et al*., 2008; Calla *et al*., 2014).

659 The plant OxOs are members of the germin family of proteins, a subset of the cupin
660 superfamily (Dunwell, 1998; Davidson *et al*., 2009). The former is distributed throughout the
661 plant kingdom, and the latter is distributed throughout all kingdoms. Germins with OxO
662 activity have so far only been discovered in what have been referred to by Hill (1937) as the
663 'true cereals', including wheat, rye, barley, oats, maize and rice; though other cupins with

664 OxO activity have also been found in bacteria and fungi (Escutia *et al*., 2005). These
665 enzymes catalyse conversion of oxalic acid into CO_2 and H_2O_2 (Dumas *et al*., 1993). In
666 addition to having a direct effect on pathogens like *S. sclerotiorum*, resulting from their
667 ability to break down oxalic acid, the H_2O_2 produced as a result of their catalysis is thought to
668 be involved in basal defence responses such as signalling cascades and cross-linking of cell
669 wall polymers during the process of lignification (Dumas *et al*., 1995).

670 Thompson *et al*. (1995) expressed OxO purified from barley roots in *B. napus*. This led to
671 resistance against wilting induced by application of purified oxalic acid. Resistance to SSR
672 was not demonstrated in a *B. napus* OxO transgenic line until 2008 (Dong *et al*., 2008). In the
673 latter study, which focussed on an OxO from wheat (*Triticum aestivum*) named *gf-2.1*, it was
674 shown that lesion size in transgenic *B. napus* was reduced by up to 44 % when artificially
675 challenged in detached leaf assays and disease incidence was reduced by 80 % in the field
676 (Dong *et al*., 2008). In both tomato and soybean, transgenic OxO-expressing lines also
677 exhibited increased resistance in the field (Cober *et al*., 2003; Walz *et al*., 2008).

678 Though heterologous expression of OxO may appear promising for enhancing resistance to *S.*
679 *sclerotiorum*, a caveat that surrounds its potential agricultural application is the observation
680 that germin proteins can act as allergens in humans (Jensen-Jarolim *et al*., 2002). A possible
681 alternative to OxO then, is oxalate decarboxylase (OxDC), which is another member of the
682 cupin superfamily (Khuri *et al*., 2001). This enzyme also catalyses breakdown of oxalic acid,
683 though its products are formic acid and CO_2 (Muthusamy *et al*., 2006). OxDC has so far been
684 purified from several microbial fungi, and has been expressed in a number of SSR-
685 susceptible species including lettuce, soybean, tobacco and tomato (Kesarwani *et al*., 2000;
686 Dias *et al*., 2006). In all the cited studies resistance to SSR was high, and in some cases no
687 lesions at all were produced in transgenic lines challenged with WT *S. sclerotiorum*.
688 However, expression of an OxDC remains to be attempted in *B. napus*. Furthermore,

689 although there is no mention of allergenic properties of OxDC in the scientific literature,
690 there is a possibility that this is a reflection of a lack of research attention rather than an
691 absence of allergenicity.

692 *Direct inhibitors of fungal growth*

693 Innate immunity is something that all living organisms possess, and an important part of it is
694 the production of antimicrobial peptides (AMPs) (Ganz, 2003). These cysteine-rich proteins,
695 which are usually less than 50 amino acids long, are thought to have appeared early during
696 evolutionary history – approximately 1.6 billion years ago – and have been identified in taxa
697 in all biological kingdoms. Most known AMPs have a broad spectrum of activity and are
698 active against both prokaryotic and eukaryotic microbes, though there are also those that are
699 specifically active against fungi. In most cases, activity is determined by the positively
700 charged nature of the AMP. This allows for it to interact with negatively charged
701 phospholipids or amino acid residues in microbial membranes. Subtle differences in
702 membrane and protein structure in different microbial species determine the effectiveness of
703 the AMP against them (Broekaert *et al*., 1997).

704 AMPs from heterologous organisms have been expressed in several SSR-susceptible species,
705 including *B. napus* and the closely related species *B. juncea,* and tested for their ability to
706 enhance resistance to *S. sclerotiorum* infection. In *B. napus*, expression of a 102 amino acid
707 AMP-encoding sequence (*PmAMP1*) from white pine (*Pinus monticola*) led to an
708 approximately 80 % reduction in lesion length when challenged with *S. sclerotiorum.*
709 Additionally, transgenic lines generated in this study were resistant to the major *B. napus*
710 pathogens *Alternaria brassicae* and *Leptosphaeria maculans* (Verma *et al*., 2012). In *B.*
711 *juncea,* expression of a gene encoding a chimeric peptide (designated MsrA1) containing
712 sequences from cecropin A (a proteinaceous toxin isolated from *Hyalophora cecropia*) and

713 melittin (a key proteinaceous toxin component of bee venom) led to percentage of disease
714 protection (as defined by (Mondal *et al*., 2007)) ranging from 56 to 76.5 %. Furthermore, like
715 *B. napus* lines expressing *PmAMP1*, the transgenic *B. juncea* plants also exhibited increased
716 resistance to the fungal pathogen *A. brassicae* (Rustagi *et al*., 2014).

717 In addition to these studies, AMPs of the plant-specific lipid transfer protein (LTP) class have
718 also been tested in a number of *S. sclerotiorum*-susceptible species. LTPs are so named for
719 their ability to transfer phospholipids between biological membranes and are thought to
720 function both in biosynthesis of essential cellular constituents within cell walls and
721 membranes and defence against pathogens. Though it is not known how they inhibit the
722 growth of invading microbes, the mechanism is thought to be independent of their ability to
723 bind lipids (Garcia-Olmedo *et al*., 1995).

724 In *B. napus*, two recent studies have described enhanced SSR resistance through LTP
725 transgene expression. In the first of these, ectopic expression of an LTP from Brazilian
726 upland rice (*Oryza sativa*) was shown to reduce lesion size by approximately 50 % when
727 leaves were challenged with *S. sclerotiorum*. Intriguingly, when seeds were exposed to oxalic
728 acid *in vitro*, transgenic lines exhibited a greater germination rate than WT. It was
729 hypothesised that reduced levels of MDA, the final product of lipid peroxidation, associated
730 with introduction of the LTP sequence and led to improved resistance to environmental
731 stress. This is in accordance with previous studies on MDA content of plant tissues and
732 effects on abiotic stress resistance (Fan *et al*., 2013). In the second study, ectopic expression
733 of an LTP from motherwort (*Leonurus japonicus*) led to an approximately 20 % lesion size
734 reduction when leaves were exposed to *S. sclerotiorum*. Additionally, it was shown that
735 common signatures of plant defences such as H_2O_2 and *PR-2* expression were elevated in the
736 transgenic lines (Jiang *et al*., 2013b). This hints at a signalling role for LTPs in defence

737 against fungal diseases (discussed in a later section) as well as their potential for direct
738 inhibition of fungal growth.

739 In addition to the AMPs, another important group of transgenes that have been shown to
740 directly interfere with the growth of pathogens is the chitinases. These genes encode enzymes
741 that break down the fungal cell wall polymer chitin. In many plants they play an important
742 part in defence against pathogens. However in many cases, induction of chitinase expression
743 is thought to either be too slow, too weak or effectively countered by pathogen defences, e.g.
744 the LysM proteins of *Zymoseptoria tritici* (Marshall *et al*, 2011) and Avr4 of *Cladosporium*
745 *fulvum* (van den Burg *et al*, 2006), for the enzymes to have any impact on disease
746 development. One approach that could enhance the use of chitinases in generation of disease
747 resistance is the constitutive ectopic expression of those with a high degree of activity against
748 pathogens of the recipient organism.

749 Such an approach was used in an early study, where it was shown that constitutive expression
750 of a chimeric chitinase from tomato (*Lycopersicum solanum*) and tobacco (*Nicotiana*
751 *benthamiana*) under the cauliflower mosaic virus 35S promoter in *B. napus* leads to increased
752 resistance to a number of pathogens, including *S. sclerotiorum*. Resistance conferred by these
753 genes varied between transgenic lines and between pathogens, though for the best two SSR-
754 resistant transgenic lines, stem lesions were reduced by 70 and 79 % (Grison *et al*., 1996).
755 Since then, two studies have documented constitutive expression of different chitinases in *B.*
756 *napus*, resulting in significant levels of SSR resistance. In the first of these studies, a chimeric
757 protein containing both chitinase and sporamin sequences was shown to reduce lesion size by
758 more than 50 % in transgenic plants when they were challenged with *S. sclerotiorum* (Liu *et*
759 *al*., 2011). The rationale for adding a sporamin-encoding sequence to the expression vector in
760 this study was to simultaneously enhance fungal infection and insect resistance, sporamin
761 having been characterised as a potent inhibitor of invertebrate pests in other species (Cai *et*

762 *al*., 2003; Chen *et al*., 2006). In the second of these studies, a chitinase gene (*Chit33*) from
763 the fungus *Trichoderma viride* (which is able to feed on other fungi by utilising chitin) was
764 expressed leading to reductions in lesion size in transgenic lines of between 30 % and 62.5 %
765 (Solgi *et al*., 2015).

766 *Modulators of host physiology*

767 Programmed cell death is a prominent feature of many plant-pathogen interactions and
768 depending on whether the invading species is necrotrophic or biotrophic, it may be most
769 commonly associated with susceptibility or resistance respectively (Deller *et al*, 2011).
770 During the interaction between *S. sclerotiorum* and its host species, it is thought that oxalic
771 acid is a major elicitor of apoptosis, leading to host susceptibility.

772 One way of preventing infection by necrotrophic pathogens such as *S. sclerotiorum* could be
773 the ectopic expression of genes that prevent apoptosis of host cells. This was first
774 demonstrated to be a potential means of plant disease control in tobacco (*Nicotiana*
775 *benthamiana*) (Dickman *et al*., 2001). In this study, four regulators of cell death from humans
776 (*bcl-2* and *bcl-xl*), *Caenorhabditis elegans* (*ced-9*) and Baculovirus (*op-iap*) were expressed
777 individually and tested for their abilities to confer resistance to *S. sclerotiorum,* and the
778 fungal pathogens *Botrytis cinerea* and *Cercospora nicotianae*. When challenged with *S.*
779 *sclerotiorum* or *B. cinerea*, disease was completely absent in most cases for all tobacco leaves
780 expressing the tested genes. However, when challenged with *C. nicotianae*, only *ced-9*-
781 expressing tobacco plants were completely resistant, whereas others expressing *bcl-2, bcl-xl*
782 and *op-iap* exhibited significantly reduced symptoms.

783 Though expression of the above-mentioned anti-apoptotic genes in tobacco greatly enhanced
784 resistance to SSR and several other diseases, like OxDC, these genes have yet to be expressed
785 and tested in *B. napus*. If they were to enhance resistance to both *S. sclerotiorum* and other

786 major necrotrophic *B. napus* pathogens such as *A. brassicicola, Erysiphae brassicicola* and *L.*
787 *maculans,* they could be important candidates for a transgenic solution to yield improvement
788 in this crop.

789

790 Conclusions

791 This review has described current cultural and fungicide control practices for managing SSR
792 in canola. Limitations associated with the ability of current control practices to reduce
793 inoculum in the soil and correctly time fungicide applications have been highlighted as areas
794 that require more focused research. Studies on the antagonistic behaviour of *C. minitans* on
795 sclerotia of *S. sclerotiorum* have led to the development of the commercially available
796 biocontrol agent Contans® WG. This product has been shown to cause marked decreases in
797 sclerotia viability and offers an alternative method for controlling sclerotia levels in the soil.
798 There is new excitement about the potential of viral-particles to act as *S. sclerotiorum*
799 antagonists (Yu *et al*., 2013; Hu *et al*., 2014b; Xie and Jiang, 2014). Whether these
800 discoveries will lead to the development of viral-based biocontrol agents for SSR control
801 remains to be seen. Quantitative trait loci have been identified in *B. napus* for resistance to *S.*
802 *sclerotiorum* many times (Table 3), although it has only become possible with the publication
803 of the *B napus* genome (Chalhoub *et al*., 2014) to directly compare the positions of these
804 independently identified QTLs (Li *et al*., 2015a). Indeed, two such QTLs were independently
805 identified in separate studies, elevating the likelihood that these QTLs are involved in
806 resistance to *S. sclerotiorum*. SSR resistance has also been identified in wild relatives of *B.*
807 *napus* (Chen *et al*., 2007; Garg *et al*., 2010a; Wei *et al*., 2010). The opportunity to introgress
808 resistance genes from wild species from the Brassicaceae into *B. napus* is a genuine option.

809 Unfortunately, it is likely to take considerable time and capitol to achieve canola-quality crop
810 varieties using this method.

811 Although fungicides are a viable option for the control of Sclerotinia, in some regions (most
812 notably Australia) chemistries with only a few modes of action are registered. Additionally,
813 fungicide spray timing for optimum efficacy against SSR presents a hurdle to growers.
814 Therefore, future research should be directed towards diversification of fungicide chemistries
815 in smaller fungicide markets and continued improvement of disease forecasting systems.

816 The development of *Brassica napus* GWAS populations (Bus *et al*., 2011) together with
817 modern genotype-by-sequencing methods have now made it possible to screen genetically
818 diverse populations of canola for QTLs associated with traits like pod shatter resistance
819 (Raman *et al*., 2014). These technologies could just as easily be used to screen for resistance
820 to *S. sclerotiorum.* In order to remove any bias associated with bioassay screening, isolate
821 genotype and artificial growth conditions, the ideal experiment would be conducted in the
822 context of a field trial. Studies using GWAS populations of canola would aim to confirm the
823 importance of known *S. sclerotiorum* resistance QTLs, identify new QTLs and ultimately
824 prove whether economically significant resistance exists within the world's canola
825 germplasm. If the null hypothesis - that there is no commercially viable resistance to *S.*
826 *sclerotiorum* in canola germplasm - is proven, there would be precedence to move away from
827 examining canola for genetic resistance to *S. sclerotiorum* to other more promising research
828 areas.

829 An alternative to searching for and promoting natural resistance to *S. sclerotiorum* is to
830 construct genetically resistant canola using transgenic technology. Transgenic hybrid canola
831 is already grown in the USA, Canada, Australia and Chile (ISAAA Brief No 46-2013
832 website), where it is marketed as a more cost-effective alternative to traditional open-

833 pollinated canola varieties. Perhaps one of the most exciting and applicable new transgenic
834 technologies for controlling crop diseases is host-induced gene silencing (HIGS) (Koch and
835 Kogel, 2014). HIGS works by expressing pre-cursor microRNA constructs *in planta* that are
836 then processed by the host's RNAi machinery into microRNAs that move into the pathogen
837 and suppress the expression of specific fungal genes. In general, this technology can provide
838 the plant with its own integrated nucleic acid-based fungicide. A recent paper has
839 demonstrated that transgenic tobacco leaves, transformed with a micro-RNA that targets a
840 chitin synthase gene of *S. sclerotiorum,* exhibited an 87 % disease reduction (Andrade *et al.*,
841 2015). This technology is gene-specific and could be adapted to target the same "required-
842 for-life" genes whose products modern fungicides are designed to target. Indeed, the
843 conserved *Cyp51* gene of *Fusarium* species has been successfully targeted using HIGS,
844 resulting in complete immunity to *F. graminearum* (Koch *et al.*, 2013).

845 In conjunction with an applied approach to *S. sclerotiorum* control, substantially more
846 fundamental research aimed at understanding the patho-biology of *S. sclerotiorum* is
847 required. Important questions like; "Why can *S. sclerotiorum* infect so many diverse hosts?",
848 "Why are monocotyledonous plants more resistant to *S. sclerotiorum* infection?" and "What
849 are the major pathogenicity factors of *S. sclerotiorum*?" need to be addressed so that
850 insightful new ideas about how to control *S. sclerotiorum* can be devised.

851

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855

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1436 Figure 1.

1437 The *Sclerotinia sclerotiorum* infection cycle and potential points for disease intervention.
1438 Ascospores are released from germinating apothecia and wind-dispersed to aboveground
1439 tissues. Ascospores germinate and primarily infect senescing petal tissue. Infected petals
1440 abscise and lodge in leaf axils allowing *S. sclerotiorum* to penetrate and infect stems,
1441 resulting in SSR. Following infection, sclerotia are formed, which may persist in the soil for
1442 several years. Different control measures may be implemented at different points in the
1443 infection cycle to control SSR, these measures include: cultural control (C), biocontrol (BC),
1444 fungicides (F) and genetic resistance (GR). Control measures that are underlined are currently
1445 commonly used for SSR control. Reference to specific details associated with each control
1446 measure can be found within corresponding sections of this review.

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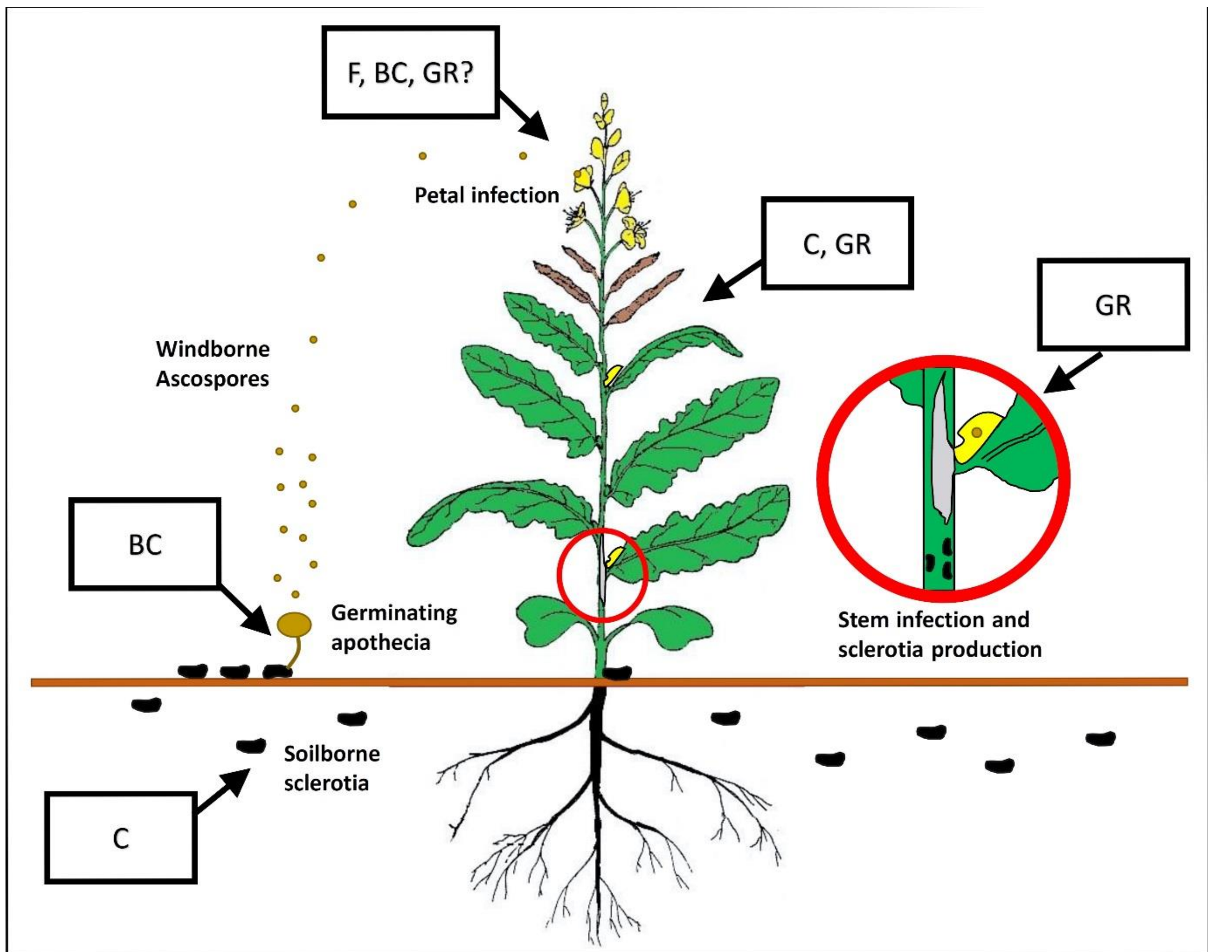

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1450 Table 2 Commercially available biocontrol agents for the control of SSR in canola.

Commercial product	Kingdom	Organism and strain	Strain	Host target tissue
Contans® WG	Fungi	*Coniothyrium minitans*	CON/M/91-08	Sclerotia
PlantShield® HC	Fungi	*Trichoderma harzianum*	T-22	Sclerotia
Serenade® MAX	Bacteria	*Bacillus subtilis*	QST-713	Ascospores, Mycelia

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1460 Table 3 A collation of QTL described for resistance to *Sclerotinia sclerotiorum* in *Brassica*

1461 *napus.*

Study	Mapping population(s)	Inoculated organ	Inoculation method	Disease quantification method	Major QTLs	Overlapping QTLs
Zhao & Meng, 2003	H5200 x Ning RS-1 (F2:3, *n* = 128)	Excised leaf	Agar plug	Lesion size	qLRS1, qLRS2, qLRS3.	None
		Stem	Toothpick	Lesion size	qSRM1, qSRM2, qSRM3	
Zhao et al., 2006	RV298 x P1804 (DH, *n* =152)	Petiole	Agar plug	Lesion size	Sll2, Sll5, Sll12, Sll14a, Sll14b, Sll16*, Sll19.	Sll12 and Dw12, Sll16 and Dw16
		Petiole	Agar plug	Days to wilt	Dw3, Dw12, Dw16, Dw19.	
	Major x Stellar (DH, *n* = 104)	Petiole	Agar plug	Lesion size	Sll3.	Sll3 and Dw3
		Petiole	Agar plug	Days to wilt	Dw3.	
Yin et al., 2010	DH821 x DHBao604 (DH, *n* = 72)	Stem and Petal	Toothpick	Lesion size	StN10a, StN11a, StN11b, StN7a, StN10b, StN10c, StN4, StN7b, StN12a, StN12b,	StN4 and SaN4
		Stem	Agar plug	Lesion size	SaN4, SaN3a, SaN3b, SaN7c, SaLG11a, SaN17a, SaN17b, SaN6, SaN1, SaLG11b.	
		Petal	Mycelium	Lesion size	PaN12.	
Wu et al., 2013	J7005 x Huashuang 5 (DH, *n* = 190)	Excised leaf	Agar plug	Lesions size	LRA3, LRA9*, LRC5.	LRA3 and SRA3, LRA9 and SRA9
		Stem	Agar plug	Lesions size	SRA1, SRA2, SRA3, SRA9*, SRC6*, SRC8a, SRA6, SRA8, SRC7, SRC8b.	
Wei et al., 2014	Express x SWU 7 (DH, *n* = 261)	Excised stem	Agar plug	Lesion size	qSR11-1*, qSR11-2, qSR10-1, qSR10-2, qSR10-3.	qFR11-4, qFR10-2, qSR11-2, qSR10-3, qFR11-3 and qFR10-1
		Stem (Field)	Natural inoculation	Infected /uninfected	qFR10-1, qFR10-2, qFR11-1, qFR11-2 qFR11-3, qFR11-4.	

1462 *Based on comparative analysis by Li et al. the independently discovered QTLs Sll16 (Zhao
1463 et al., 2006) and SRC6 (Wu et al., 2013) overlap, as do the independently discovered QTLs
1464 LRA9, SRA9 (Wu et al., 2013) and qSR11-1 (Wei et al., 2014).

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