O'Brien, P.A. and Milroy, S.P. (2017) Towards biological control of Spongospora subterranea f. sp. subterranea, the causal agent of powdery scab in potato. Australasian Plant Pathology, 46 (1). pp. 1-10.

1 **Towards biological control of *Spongospora subterranea* f. sp. *subterranea*,**

2 **the causal agent of powdery scab in potato**

3

4 PA O'Brien* & SP Milroy. School of Veterinary & Life Sciences, Murdoch

5 University, Murdoch WA 6150 Australia

6 *Corresponding author email: P.Obrien@murdoch.edu.au

7

8 *Abstract:*

9 Powdery scab of potato, caused by the obligate biotrophic protozoan pathogen

10 *Spongospora subterranea* f.sp. *subterranea* (*Sss*), is a major problem in potato

11 growing areas throughout the world. It results in lesions (scabs) on the surface

12 of the tubers which renders them unmarketable. In recent years there has been

13 an increasing number of reports of the disease, many from new areas.

14 Management of the disease has proved difficult and relies on the integrated

15 application of a range of methods. Biocontrol is not currently used for the

16 management of powdery scab although the results of preliminary studies have

17 been encouraging. This review evaluates the potential for developing a

18 biocontrol strategy for powdery scab.

19

20

21 **Introduction**

22

23   Powdery scab of potato, caused by the biotrophic protozoan pathogen

24 *Spongospora subterranea* f.sp. *subterranea* (*Sss*), is a major problem in many

25 potato growing areas throughout the world. It results in lesions (scabs) on the

26 surface of tubers that are filled with a brown powder consisting of sporosori

27 (also referred to as sporeballs), hence the common name (Harrison et al. 1997).

28 Affected tubers have low acceptance at market and are down-graded or rejected

29 by traders, leading to reduced returns to growers and increased waste within the

30 industry. For producers of seed potatoes, the lesions may lead to rejection of

31 entire consignments as the disease is spread through infected tubers (Falloon et

32 al. 1996; Kirkham 1986). Economic losses in the fresh potato market are very

33   difficult to quantify. Annual losses to the Australian processing industry have

34   been estimated at A\$13.4 million (Wilson 2016).

35

36   In addition to producing lesions on tubers, the pathogen also infects roots,

37   a process that leads to reduced water and nutrient uptake and thus impaired

38   shoot and tuber growth (Falloon et al. 2016). Historically this aspect has

39   received less attention because tuber lesions are more evident, but root infection

40   is now considered to have the greater deleterious effect on crop production. In

41   field trials, impairment of water and nutrient utilisation can lead to a 25%

42   reduction in shoot dry weight, a 26% reduction in the number of tubers per plant

43   and a 42% reduction in tuber weight per plant (Falloon et al. 2016).

44   Susceptibility of cultivars to root infection is only loosely related to previously

45   determined susceptibility to powdery scab (i.e. development of tuber lesions)

46   and hence they are considered to be separate disease processes (Falloon et al.

47   2016; Nitzan et al. 2008). Root infection, with impaired water and nutrient

48   utilisation, can occur from early in the growth of plants, but gall formation and

49   tuber lesions only become evident at later stages of plant development (Falloon

50   et al. 2016). Gall formation, when severe, can also lead to impaired water and

51   nutrient utilisation (Johnson and Cummings 2015).

52

53   In addition to its direct impact on the host crop, *Sss* is also the vector of

54   the potato mop-top furovirus (Arif et al. 1995). Mop-top virus has been reported

55   to cause yield losses of between 30 and 60% (Carnegie et al. 2010) and tubers

56   expressing the characteristic 'spraing' symptoms are unacceptable at market.

57   The virus has spread around the world in the last 40 years and it can remain

58   infective in fields without cultivation of potatoes for many years (Kirk 2008;

59   Kalischuk et al. 2016) . It is speculated that alternative hosts may prolong

60   survival of the infectious virus although the host range of the virus is more

61   restricted than that of its vector (Kirk 2008).

62

63   The pathogen *Sss* has proved challenging to control, with no single

64   method reliably giving full control. Rather, management relies on the integrated

65   application of a range of tools (Falloon 2008). In this paper we will briefly review

66 the biology of the pathogen and current control methods as a basis for exploring

67 the potential to develop effective biological control options.

68

69

70 **Occurrence**

71

72   Powdery scab was first reported as a disease in Germany in 1841

73 (Harrison et al. 1997). Subsequently there were reports on occurrences of the

74 disease from many locations between 1846 and 1992 (Harrison et al. 1997). In

75 recent years there has been a resurgence in reports of the disease, many from

76 new areas such as Australia, New Zealand, Columbia, Pakistan, Korea and others

77 (Balendres et al. 2016; Harrison et al. 1997; Merz 2008). The resurgence in the

78 disease has been attributed to increased cultivation of potato cultivars with long

79 growing seasons, use of susceptible cultivars, increased irrigation of crops,

80 inadequate crop rotation, and de-registration of mercury based fungicides

81 (Braithwaite et al. 1994; Harrison et al. 1997; Merz 2008). As the disease is

82 transmitted through infected tubers, the increased transport of seed tubers

83 around the world may have exacerbated the spread of the disease (Gau et al.

84 2015). Increased awareness and increased efficiency in detection of the disease

85 may also have contributed to the increase in reporting.

86

87

88 **Taxonomy**

89

90   *Spongospora subterranea* (Wallr.) Lagerh f. sp. *subterranea* Tomlinson, the

91 causal agent of potato powdery scab, is a soil-borne obligate pathogen and a

92 plasmodiophorid characterized as having cruciform nuclear division,

93 multinucleate plasmodia, biflagellate zoospores and resting spores (Hutchison

94 and Kawchuk 1998). The taxonomic position of the plasmodiophorids has been

95 uncertain for some time. Traditionally they were placed in the fungi although

96 other researchers have argued for a protozoal origin (reviewed in (Qu and Christ

97 2004)). Analysis of SSU-rDNA sequences in five independent studies led to the

98 conflicting views that they are unrelated to any other eukaryotes (three studies)

99 or are related to the rhizopoda (two studies). The results of more recent

100   analyses now robustly place them within the eukaryote supergroup Rhizaria, as

101   a sister group to the omnivorous vampyrellid amoebae (Neuhauser et al. 2014).

102   Plasmodiophorids are the better known members of the group because they

103   include a number of plant parasites causing economically significant diseases of

104   crops including brassicas, potatoes, and grain crops (e.g. maize, rice, wheat,

105   sorghum). The most studied species is the clubroot-causing *Plasmodiophora*

106   *brassicae,* a parasite of crucifers which accounts for up to 10% loss of the

107   worldwide production of *Brassica* crops. Other well-studied species include

108   *Spongospora subterranea,* which causes powdery scab of potato and can serve as

109   a vector for Potato Mop Top Virus. Non- pathogenic species such as *Polymyxa*

110   *graminis* transmits economically important viruses to a number of grain plants

111   while *Polymyxa betae* is the vector for beet necrotic yellow vein virus, the cause

112   of sugar beet "rhizomania".

113

114

115   **Life Cycle**

116

117   The life cycle (Fig 1) has been described in the excellent reviews of

118   Harrison et al. (1997), (Merz 2008), and Balendres et al. (2016). In the asexual

119   or zoosporangial phase (Fig 1 inner circle) the host plant is infected by haploid

120   biflagellate zoospores that encyst on the root or tuber surfaces and penetrate the

121   plant tissue. Within the plant the zoospores develop into multicellular

122   plasmodia. After repeated cell divisions the plasmodia differentiate into

123   zoosporangia from which haploid biflagellate secondary zoospores are released.

124   These exit the sporangia through pores that extends through the sporangium

125   walls and the root surfaces resulting in release directly into the soil. The

126   secondary zoospores can initiate infections on other parts of the same plant such

127   as tubers, or on adjacent plants. This provides the basis for multiple re-infection

128   in a single growing season and thus the potential for very rapid inoculum build-

129   up.

130

131   In the sexual phase (Fig 1 outer circle) thick walled resting spores each

132   germinate to release a primary biflagellate haploid zoospore. Two haploid

zoospores may undergo cell fusion (plasmogamy) to form a binucleate zoospore that infects the plant (although this last step is still debated). This develops into a multinucleate plasmodium with binucleate cells. Eventually the nuclei fuse (karyogamy) and undergo meiosis to differentiate into thick walled spores within a structure known as a sporosorus. The resting spores are very resistant structures and can persist in soil for 4-5 years. The sexual stage has not been detected in *Sss* but has been described in the closely related species *Plasmodiophora brassica* (Tommerup and Ingram 1971).

The potential for rapid increases in inoculum level and the persistence of resistant spores in the soil are key considerations in the development of management strategies for Sss.

Pathogen Diversity

In considering the development of control strategies for Sssit is essential to understand the degree of genetic diversity in, and the genetic structure of the pathogen population. Various studies have shown the existence of genotypic variation, including between geographic locations. Analysis of ITS sequences showed that North American isolates, together with Australasian and some European collections formed a clonal population (group II) whilst South American and some European collections formed a separate group (group I) (Bulman et al. 2001; Gau et al. 2015; Qu and Christ 2004). The South American collections showed greater genetic diversity compared to collections from the rest of the world, suggesting that this is the centre of origin of the species (Gau et al. 2013; Gau et al. 2015) .

Qu and Christ (2004) found that their European (from Ireland and Scotland) collections were associated with particular potato cultivars. The European group I collections were all from cv. Saturna from different locations in Ireland and Scotland over several years whilst the European group II collections were from cv Kerrs Pink from locations in Ireland over several years. In some instances collections were obtained from both cultivars from the same area by

166   the same growers. Similar observations were reported by Gau et al. (2013). In

167   some Andean regions of South America short day relatives of the widely grown

168   long day potato species *Solanum tuberosum* ssp *tuberosum* are cultivated. These

169   are *S. tuberosum* ssp. *andigena*, and *S. tuberosum phureja*. South American

170   samples of *Sss* from *S.t. phureja* root galls formed one group, whilst samples from

171   tuber lesions on *S.t. tuberosum* and *S.t. andigena* formed another group. Samples

172   from the rest of the world formed a third group with a highly clonal structure.

173

174   Analysis of North American isolates with a suite of RFLP markers showed

175   that they clustered into two groups, one included isolates originating from

176   western North America., , and the second included isolates originating from

177   eastern North America (Qu and Christ 2006b).

178

179   The results of the different studies show that there is genotypic variation

180   across geographical regions. They also highlight the need for a large scale study

181   on a world wide population using multiple genetic loci such as microsatellite

182   markers (Dobrowolski et al. 2003) or RFLP markers (Qu and Christ 2006b). The

183   greater genetic diversity found in the South American population (Gau et al.

184   2015) suggests that this may be the centre of origin of the pathogen. Movement

185   of potato propagules from South America needs to be appropriately regulated to

186   minimize the risk of introducing new pathogen diversity into the world's

187   production regions.

188

189

190   **Current Control of Powdery Scab**

191

192   *Host resistance*

193

194   Although a number of potato cultivars with different levels of resistance

195   to *Sss* have been identified (Falloon et al. 2003), no cultivar is known to be

196   completely resistant to powdery scab (Falloon 2008; Hernandez Maldonado et al.

197   2013). Resistance varies from very resistant to very susceptible (quantitative)

198   suggesting a polygenic basis (Falloon 2008).   In general, more resistant cultivars

199   have fewer galls and fewer zoospores in their roots although the correlation is

200 not tight and exceptions exist (Falloon et al. 2003). Hernandez Maldonado et al.

201 (2013) compared *Sss* accumulation in roots and the numbers of galls that

202 developed on a resistant and a susceptible potato cultivar and found that

203 although there was little difference between the cultivars at early stages of

204 infection there was increasing divergence as the disease progressed. This

205 suggests that although the primary infection may be the same in both cultivars,

206 secondary infection may be restricted in the resistant cultivar.

207

208 *Chemical control*
209

210   There are a number of agro-chemicals that can be applied to seed tubers

211 or to the soil, at or before planting, that have been shown to reduce powdery

212 scab levels (reviewed in Falloon (2008)). Braithwaite et al. (1994) evaluated 25

213 fungicide treatments applied to severely infected tubers just before planting.

214 Plant emergence was assessed, and at maturity tubers were harvested and

215 scored for powdery scab infection. A number of the treatments reduced the

216 proportion of diseased tubers from 95% at planting to an average of 29% in the

217 subsequent crop compared with the untreated controls which declined to 70%.

218 Some of the other treatments resulted in phytotoxic effects. Treatment of tubers

219 with zinc sulfate and zinc oxide six weeks before planting also reduced infection

220 in the subsequent crop compared with untreated controls.

221

222   A subsequent study evaluated chemicals applied either as tuber dressings

223 or as in-furrow applications. The fungicides fluazinam, mancozeb, dichlorophen-

224 Na, were effective as tuber dressings of infected tubers, reducing the incidence of

225 powdery scab and increasing the yield of tubers (kg/plot) by up to 36% (Falloon

226 et al. 1996). The same study evaluated in-furrow treatments by planting

227 uninfected tubers of cvs Rua and Agria into heavily infested soil. Treatments

228 with fluazinam reduced powdery scab incidence, and increased yield of

229 marketable 'Rua' tubers by up to 55%, and 'Agria' tubers by 140%. High rates of

230 mancozeb also reduced incidence of the disease and increased marketable yield

231 of 'Rua' by 34%, and of 'Agria' by 68%. In-furrow treatment with zinc oxide and

232 foliar treatments with phosphorous acid did not control the disease.

233

234   *Crop rotation*

235

236   Long rotations with non-host species are recommended as the resting
237   spores of Sss can persist for many years in the soil (Falloon 2008). However, the
238   host range of *Sss* is much wider than was previously thought and includes many
239   non-solanaceous species (Qu and Christ 2006a). Of 26 species within 10 families
240   from monocotyledons and dicotyledons tested, 16 species were found to be
241   susceptible to Sss. Twelve species were newly recorded hosts for Sss*.* However
242   Qu and Christ (2006a) also observed that although some species were infected,
243   they did not producesporosori . It was suggested that these could possibly be
244   used to reduce the inoculum levels in soil. Sparrow et al. (2015) monitored
245   pathogen DNA levels in soils of South Eastern Australia over an eight years
246   period and suggested a minimum of five years between potato crops.

247

248   Larkin and Griffin (2007) evaluated the potential of rotation with brassica
249   crops to decrease the level of pathogen inoculum in soil and so reduce disease
250   severity in subsequent potato crops. Brassicas produce sulphur compounds,
251   glucosinolates, which break down to isothiocyanates that are toxic to a wide
252   range of phytopathogens. In a field trial, Indian mustard significantly reduced
253   *Sss* inoculum levels in the soil as measured by a bioassay. Crops of rapeseed or
254   yellow mustard were less effective. All three brassica species decreased the
255   severity of powdery scab by 25-39% relative to a standard oats rotation. The
256   brassicas also reduced the incidence of tubers with scabs by 19-40% with Indian
257   mustard again being the most effective. Indian mustard produces high levels of
258   glucosinolates, some of which convert to the most biologically active forms of
259   isothiocyanates produced by brassicas (Charron and Sams 1999). In the same
260   study, rotation with 'Lemtal' ryegrass led to similar reductions in disease
261   severity and incidence of tubers with lesions as were observed after rotation
262   with the brassicas. This suggests that factors other than the production of
263   isothiocyanates may have been responsible for the reduction in incidence and
264   severity.

265

266   As an alternative to direct pathogen inhibition, rotation crops may also

267 affect pathogens indirectly by influencing changes in the soil microbial

268 community. This may increase disease suppression by the soil. In a study to

269 examine the effects of rotation systems on soil microbial communities, Larkin

270 and Honeycutt (2006) demonstrated distinctive effects of specific rotation crops

271 and cropping sequences on these communities. They also found higher

272 populations of microorganisms that are generally beneficial to plants, such as

273 *Pseudomonas* spp. and *Trichoderma* spp., after planting barley, canola, and sweet

274 corn crops.

275

276 *Agronomic factors*

277

278   Agronomic factors, particularly soil nitrogen status and soil water content

279 were found to affect the severity of disease (Shah et al. 2014). The incidence

280 and/or severity of powdery scab were increased by nitrogen (nitrate and

281 ammonium) applications. Nitrogen application resulted in a greater amount of

282 *Sss* DNA in the soil and this effect was observed for two years after the trial. A

283 positive correlation between soil *Sss* DNA and disease severity was observed by

284 Brierley et al. (2013) and by Nakayama and Sayama (2013). However, Shah et al.

285 (2012) reported that in their study there was no consistently strong relationship

286 between the amount of inoculum at time of planting as measured by the number

287 of sporosori per g soil and disease incidence or severity at harvest. Irrigation

288 treatment also affected disease severity. An irrigation regime optimal for potato

289 growth resulted in greater severity, but not greater incidence, of powdery scab

290 than a constrained irrigation input (Shah et al. 2014). This is consistent with

291 higher soil moisture content facilitating the movement of zoospores through the

292 soil and increasing the chances of plant infection (Merz 2008; Balendres et al.

293 2016).

294

295

296 **Potential for Biocontrol**

297

298   Biocontrol, based on the use of an organism to restrict the ability of a

299 pathogen to cause disease is an aspect of plant disease control that has received

300 much attention in recent years. This is fuelled by the emergence of fungicide

301 resistance in pathogen populations, deregistration of fungicides, and growing

302 concerns about the use of chemicals in food production. In contrast biocontrol is

303 seen as a safe, non-toxic, renewable alternative. In some cases of biocontrol the

304 level of control rivals that achieved with chemicals although a lack of consistency

305 of control is often a concern.

306

307 *Identifying biocontrol agents*
308

309   There are few reports of studies aimed to identify potential biological

310 control agents (BCAs) for *Sss* in potato. The most extensive study is that of

311 Nakayama and Sayama (2013). They reported 54 – 70% suppression of disease

312 development over three years by application of an isolate of *Aspergillus*

313 *versicolour* to tubers. These values compared to 77 to 93% suppression by the

314 synthetic fungicide fluazinam applied in furrow. While the BCA was less effective

315 than the synthetic fungicide, the suppression was statistically significant.

316

317   Another approach was taken by Nielsen and Larsen (2004) who used

318 tomato as a model to examine the efficacy of commercially available biocontrol

319 products to reduce the infection of root hairs by primary zoospores of *Sss*. Each

320 agent was assessed in two experiments. The commercially produced biocontrol

321 products TRI 002 and Binab TF (both containing *Trichoderma harzianum*) gave a

322 statistically significant reduction in root colonization in both experiments. By

323 contrast the biocontrol product TRI 003 (also containing *Trichoderma*

324 *harzianum)* gave no significant reduction in either experiment. The product

325 FZB24, containing *Bacillus subtilis*, gave a reduced infection in only one of the

326 two experiments. In each experiment, all plant growth parameters examined

327 were markedly lower in the infected controls than the uninfected controls. While

328 some of the control agents increased the plant growth parameters above that in

329 the infected control, these increases were not consistent.

330

331   In the experiments of Nielsen and Larsen (2004) a very high inoculum

332 concentration of the pathogen was used; much greater than could be expected in

333 field soil or in soil adhering to the surface of seed tubers (e.g. 0-148 sporosori/g
334 of soil; (Brierley et al. 2013)). It is possible that more effective and more
335 consistent control could be obtained with more typical disease pressures;
336 biological control of *Plasmodiophora brassicae* has been found to be more
337 effective at lower pathogen pressure (Narisawa et al. 2005; Peng et al. 2011). It
338 was not known whether the BCAs tested by Nielsen and Larsen (2004) were
339 inhibitory to *Sss.* In contrast, in the study by Nakayama and Sayama (2013) soil
340 fungi were initially screened for inhibition of *Sss* infectivity using a bioassay.
341
342   Although research on biocontrol of *Sss* is limited, biocontrol of
343 *Plasmodiophora brassicae*, has been studied more extensively. A number of
344 studies have identified endophytes with the ability to suppress this pathogen.
345 These include *Bacillus subtilis*, *Gliocladium catenulatum*, *Heteroconium*
346 *chaetospira*, *Microbispora rosea* ssp *rosea*, *Streptomyces griseoruber*, *S.*
347 *griseoviridis*, *S. lydicus, S. olivochromogenes* and *Trichoderma atroviride, T.*
348 *harzianum and T. viride*. (Lahlali et al. 2014; Lee et al. 2008; Narisawa et al. 1998;
349 Peng et al. 2014; Peng et al. 2011; Wang et al. 2012; Wang et al. 2011). In some
350 studies a number of these potential biocontrol agents have shown levels of
351 suppression of clubroot greater than 85% and approaching the level of
352 effectiveness observed from synthetic fungicides.
353
354   The evidence from the limited studies that have been carried out suggests
355 that developing biocontrol strategies for plasmodiophorid diseases is possible.
356 However a problem common to all biocontrol strategies is the lack of consistency
357 of disease reduction. Several researchers have reported that using mixtures of
358 BCAs has increased the consistency of biocontrol across sites with different
359 conditions. Slininger et al. (2001) in their investigation into postharvest dry rot
360 of potato found that formulations of mixed BCAs performed more consistently
361 across 32 storage environments varying in cultivar, washing procedure,
362 temperature, harvest year, and storage time. Enhanced biocontrol using
363 mixtures of BCAs has been reported for control of late blight in potato (Slininger
364 et al. 2007), diseases of poplar (Gyenis et al. 2003), chilli (Muthukumar et al.
365 2011), and cucumber (Raupach and Kloepper 1998; Roberts et al. 2005). It is

also possible that different mixtures may need to be used in different climatic areas. Thus there is a need to identify a number of potential biocontrol agents. Mixtures do not always give increased control. In some cases there may be antagonism between the BCAs that results in reduced control compared to single strains. In evaluating agents for control of fire blight in pear, Stockwell et al. (2011) found that mixtures of *Pseudomonas fluorescens* A506 , *Pantoea vagus* C9-1 and *Pantoea agglomerans* Eh252 were less effective than the individual strains. The *Pantoea* strains exert their effects through the production of peptide antibiotics. In the mixture these were degraded by an extracellular protease produced by *P. fluorescens* A506. Roberts et al. (2005) also reported antagonism between BCA strains. They observed that populations of *Trichoderma virens* GL3 or GL321 were both substantially reduced after co-incubation with *Bacillus cepacia* BC-1 or *Serratia marcescens* isolates N1-14 or N2-4 in cucumber rhizospheres. These reports highlight the importance of considering possible antagonism between strains when developing biocontrol formulations. Co-cultivation *in vitro* can sometimes reveal inhibitory effects (Roberts et al. 2005) but not always. In the study by Stockwell et al. (2011) the antagonistic effects would not have been detected by co-cultivation as the BCAs themselves were not affected, only their potential action on the pathogen was disrupted.

BCAs are typically identified by screening rhizospheric or endophytic bacteria and fungi for inhibition of pathogen growth *in vitro,* followed by greenhouse trials of growth inhibiting isolates and finally field trials. The use of *in vitro* screening as an initial step has a number of significant limitations; most notably, it only identifies organism which act on the pathogen through a specific subset of mechanisms. Control of disease by a BCA occurs through a variety of mechanisms and in many cases direct contact with the pathogen is not necessary. Mechanisms of control include: detoxification of toxins produced by the pathogen (Newman et al. 2008), degradation of the pathogen cell wall leading to lysis (mycoparasitism) (Jan et al. 2011), production of antibiotics (Raaijmakers et al. 2002), antimicrobial surfactants (Raaijmakers et al. 2010), siderophores and volatiles (Santoyo et al. 2012) by the BCA, induction of plant defenses (Ting et al. 2012), stimulation of plant growth (van der Lelie et al.

399   2009), physical occlusion of the pathogen by occupying sites on root surfaces

400   that the pathogen would use for entry (Blumenstein et al. 2015), and biofilm

401   formation (Newman et al. 2008). A given BCA may use more than one

402   mechanism of inhibition, and different BCAs may exert their effects at different

403   times during the crop growing season. *In vitro* screening only identifies those

404   BCAs acting through the production of antibiotics or cell lysis. Further, in the

405   specific case of *Sss*, *in vitro* screening is not possible as the species is an obligate

406   biotroph. Thus initial screening would need to employ *in planta* methods similar

407   to those used by Nakayama and Sayama (2013).

408

409   *In vitro* inhibition or inhibition of disease development in greenhouse

410   trials does not always translate to effective disease management in the field

411   where weather, soil and biological variability are likely to be much greater.

412   Ultimately, the only realistic evaluation is by field trials. Given the range of

413   edaphic and environmental factors that can influence the effectiveness of BCAs

414   (as noted in the following section), the number and location of evaluation sites

415   needs to be selected to appropriately reflect the anticipated range of usage. As

416   the production of potato expands geographically into Mediterranean, sub-

417   tropical and even high altitude tropical areas, this aspect becomes more

418   significant. Further, given that soil microbial communities can vary dramatically

419   with soil type and land management, variation in these factors needs to be

420   captured also.

421

422   *Sss*, impacts potato very early in crop development (Hughes 1980; Taylor

423   et al. 1986). Screening methodology must reflect the need for protection to be

424   established by the time of plant emergence. With a focus solely on reducing tuber

425   symptoms, protection would need to persist to beyond tuber initiation; although

426   longer protection would be preferable as root damage continues to occur beyond

427   that stage (Hughes 1980), impacting host nutrient and water uptake.

428

429   *Stability across production environments*

430

431   Studies on the influence of environment on the efficacy of biological

432   control of soil-borne diseases demonstrate the influence of temperature (Jang et

433   al. 2011; Landa et al. 2001; Landa et al. 2004; Schmidt et al. 2004), soil water

434   status (Schmidt et al. 2004) and soil physical and chemical characteristics

435   (Ownley et al. 2003) on the levels of control achieved. Given the increasingly

436   diverse conditions and geographic locations under which potato is now

437   produced (Birch et al. 2012; Devaux et al. 2014), this will be an important

438   consideration.

439

440   No research has been reported that evaluates the influence of

441   environmental variables (either soil or weather) on the effectiveness of BCAs in

442   supressing *Sss* and little has been reported in relation to *P. brassicae*. Studying

443   the control of *P. brassicae* by *Heterconium chaetospira*, Narisawa et al. (2005)

444   found effective suppression under low to moderate moisture conditions but not

445   at high water status. On the other hand, Peng et al. (2011) found that periods of

446   dry soil impeded the effectiveness of a range of BCAs to varying degrees. *Bacillus*

447   *subtilis* and the synthetic fungicide, fluazinam were particularly sensitive.

448   Significantly, while the results of Zhou et al. (2014) showed variation in the

449   effectiveness of bacterial isolates in suppressing *P. brassicae* in Chinese cabbage,

450   the effectiveness of the synthetic fungicide also varied.

451

452   *Effective formulation of BCA*
453

454   The form in which a BCA is applied to a crop may affect its persistence and

455   thus its effectiveness. Resistant propagules such as bacterial endospores, fungal

456   conidia, chlamydospores or oospores are more persistent than vegetative

457   bacterial cells or fungal hyphal fragments (Schisler et al. 2004). For spore-

458   forming bacteria, yeasts, and fungi, the production system can be optimised for

459   the production of spores. Fermentation environments and culture age influence

460   the efficacy, stability and desiccation tolerance of many BCAs including fungi,

461   yeasts, and bacteria (Schisler et al. 2004). The fungal biocontrol agent

462   *Trichoderma harzianum* developed mycelium after four days at 28 ^{0}C and

463   chlamydospores after 10 days in liquid medium (potato dextrose broth) at 28℃

464   while on solid media (e.g., PDA, grains, wheat bran,) it produced conidia (Mishra

465   et al. 2012).

466

467   Persistence can be enhanced by mixing the BCA with additives that supply

468   nutrients, protect it from desiccation, from UV, and antagonistic organisms, and

469   increase its ability to stick to the surface of plants. The different types of

470   additives were reviewed by Schisler et al. (2004). The addition of calcium

471   carbonate to rice grain cultures of *Trichoderma martiale* stimulated conidia

472   production and enhanced the persistence of the BCA in the field and during

473   storage (Hanada et al. 2009). Formulation of strains of fluorescent *Pseudomonas*

474   with talc has been reported to enhance efficacy in control of Fusarium wilt in

475   banana (Saravanan et al. 2004) and tomato (Sarma et al. 2011). Inclusion of

476   chitin in the growth medium for the production of chitinolytic strains of *Serratia*

477   has been found to be effective and it is postulated that the chitin may also

478   stimulate the growth of other chitinolytic species in rhizospheres which would

479   help to prevent fungal infection (Kim et al. 2008).

480

481   Encapsulation of the BCA within a biodegradable matrix of protein (whey,

482   poly-Lysine) polysaccharide (e.g., cellulose, alginates, chitosan, pectin), or other

483   polymers such as lignin, protects the BCA from biotic and abiotic stress factors

484   and promotes shelf life and persistence in the environment. Overall

485   encapsulation results in greater disease control. The topic of encapsulation,

486   including the types of materials and how to make capsules, is reviewed by

487   Vemmer and Patel (2013).

488

489   *Methods of application of BCA*
490

491   A major factor in the efficacy of biocontrol agents is the method of

492   application. BCAs can be applied as seed (tuber) dressings, as soil-drenches or as

493   a foliar spray. For rhizospheric organisms that will encounter the pathogen

494   before it enters the host plant, and prevents entry either by occlusion or killing

495   the pathogen it is essential that the BCA is able to colonize root s as they develop.

496

497   For soil-borne diseases, soil drenches are most effective as they allow

498   extensive colonization of roots (Gossen et al. 2013). In-furrow applications or

499   seed dressings are alternatives although they may not be as effective in

500   providing high levels of inoculation. McLean et al. (2005) compared methods of

501   introducing *Trichoderma atroviridae* as a BCA for *Sclerotium cepivorum* (white

502   rot) in onions. In a glasshouse trial, they found that a pellet formulation

503   maintained greater levels of the BCA in the soil compared to solid-substrate or

504   seed-coating formulations. Pellets also gave more extensive colonization of root

505   systems of the onion seedlings. In a subsequent field trial a pellet formulation

506   resulted in a greater number of CFU/g of soil than a solid substrate form. The

507   pellet formulation also gave a more persistent inoculum.

508

509   Timing of the application may also have an important impact. In a trial of

510   suppression of the related clubroot pathogen *P. brassica* by the fungal BCA *H.*

511   *chaetospira*, it was found that soil application of a granular formulation of the

512   fungus reduced clubroot severity by > 80% relative to the control. However,

513   applying *H. chaetospira* at seeding was much less effective than earlier soil

514   application. More extensive root colonization by *H. chaetospira* resulted in

515   greater suppression of *P. brassicae* infection and subsequent clubroot

516   development (Lahlali et al. 2014) Thus, the greater suppression by the earlier

517   application may have resulted from more extensive root colonization.

518

519   In the case of *Sss*, protection is required from about the time of plant

520   emergence (Hughes 1980; Taylor et al. 1986). Thus, for any BCA to be effective,

521   early colonization of the below ground plant parts will be critical.

522

523   *Incorporation into disease management systems*
524

525   Falloon (2008) reviewed the development of integrated management

526   systems for *Sss*. There is currently no single management practice available that

527   provides full and reliable control of the disease. Effective management relies on

528   the coordinated use of a number of approaches. The main components listed by

529   Falloon (2008) were crop rotation, field selection, resistant cultivars, pathogen-

530   free planting material, appropriate pesticide use and sound crop management.

531   The use of biological control is compatible with the majority of these

532   components; however compatibility with agro-chemicals (fungicides and or

533   pesticides) is an obvious challenge.

534

535   Potato producers use a wide range of agro-chemicals as dressings for seed

536   tubers, applied either before or after storage, or as in-furrow applications at

537   plantings in bands up to 20 cm wide. Fungicides which are used against a range

538   of possible pathogens, are a particular issue. These vary from broad spectrum

539   treatments to products targeting specific disease organisms. Their mobility

540   within potato plants also ranges from contact to fully systemic. This will be a

541   major consideration for the incorporation of BCAs into the production system.

542   Clearly, the significance will depend on the particular chemical in use and its

543   mode of application on the one hand and the identity of the BCA and the mode of

544   inoculation on the other. For example, in-furrow application of fungicides may

545   interfere with the successful establishment of BCAs applied in-furrow or to the

546   seed piece depending on the identity of the BCA and the fungicide applied. On the

547   other hand broad-spectrum soil fumigants such as metam-sodium (sodium salt

548   of methyl dithiocarbamate) are still in use in some regions as a means of

549   reducing soil-borne diseases prior to planting potatoes. Although these

550   treatments are broad spectrum (Xie et al. 2015), there is likely to be limited

551   interference with BCAs applied to the seed potato or at planting as the fumigants

552   are released from the soil some weeks prior to planting. However, questions

553   have been raised regarding the impact of such fumigants on the size and

554   structure of soil microbial communities (De Cal et al. 2005; Macalady et al. 1998).

555   Significant alterations in community structure may have unforeseen

556   consequences for the success of the BCA, the pathogen, or soil functions.

557

558   Approaches to combining biocontrol agents and agrochemicals for use

559   against the target pathogen are important for IPM. Levels of disease suppression

560   achieved by combining the control methods are typically equal or superior to the

561   use of BCA alone (Deberdt et al. 2008; Hidalgo et al. 2003) (see discussion in

562   Hanada et al. (2009)).

563

564   Given that potato is vegetatively propagated, introducing an endophyte

565   into potato tubers *via* the parent seed crop may provide a level of protection

566   from non-systemic agrochemicals. This may provide a novel method of

567   combining biocontrol with seed-tuber dressings or in-furrow pesticide

568   applications in an integrated system. If feasible, this approach would also

569   provide a degree of protection for the BCA from environmental and soil

570   variability, as well as providing the earliest possible introduction of the BCA

571   which is important given the early impact of *Sss* on potato plants and tuber

572   quality. A structured experimental investigation is required.

573

574

575   *Is Successful Biological Control of Sss Possible?*

576

577   The limited work available suggests that efforts to identify organisms

578   capable of successfully suppressing disease development by *Sss* are likely to be

579   successful. To achieve this, a high throughput *in planta* assay will be required for

580   screening endophytic and rhizospheric organisms. The method described by

581   Nakayama and Sayama (2013) could be used for this purpose as could the

582   procedure of Nielsen and Larsen (2004). In this regard Andrea Ramirez et al.

583   (2013) have described a stem cutting assay to screen cultivars for resistance to

584   *Sss* that could be adapted to screen for disease control by microbial isolates.

585   Another possibility described by Merz et al. (2004)is a procedure to screen

586   potato cultivars for resistance using tissue culture plantlets.

587

588   However, identifying biological antagonists would appear to be the more

589   straightforward aspect of the research. The greater challenge lies in developing

590   a formulation and method of application that will provide adequate protection

591   consistently across production systems and geographic locations within the

592   intended range of use. Mixtures of BCAs may prove valuable in this respect.

593   Importantly, the formulation and mode of use will need to allow integration with

594   other disease control methods, especially the use of fungicides and pesticides.

595

596   Given the commercial significance of the disease processes caused by *Sss*

and the persistent challenge of effective control, a focused effort to explore the

potential to develop practical biological control systems for this pathogen would

appear warranted.

Acknowledgements

References

Andrea Ramirez L, Maria Zuluaga C, Paola Gonzalez E, Alejandro Marin M, Gonzalo Morales J, Eduardo Nustez C, Miguel Cotes J (2013) Using lateral stem cuttings to evaluate the resistance of *Solanum phureja* genotypes to *Spongospora subterranea* f. sp *subterranea*. Tropical Plant Pathology 38:303-312

Arif M, Torrance L, Reavy B (1995) Acquisition and transmission of potato mop-to furovirus by a culture of *Spongospora subterranea* f.sp. *subterranea* derived from a single cystosorus. Annals of Applied Biology 126:493-503. doi:10.1111/j.1744-7348.1995.tb05384.x

Balendres MA, Tegg RS, Wilson CR (2016) Key events in pathogenesis of *spongospora* diseases in potato: a review. Australasian Plant Pathology:1-12. doi:10.1007/s13313-016-0398-3

Birch PRJ, Bryan G, Fenton B, Gilroy EM, Hein I, Jones JT, Prashar A, Taylor MA, Torrance L, Toth IK (2012) Crops that feed the world 8: Potato: are the trends of increased global production sustainable? Food Security 4:477-508

Blumenstein K, Albrectsen BR, Martin JA, Hultberg M, Sieber TN, Helander M, Witzell J (2015) Nutritional niche overlap potentiates the use of endophytes in biocontrol of a tree disease. Biocontrol 60:655-667

Braithwaite M, Falloon RE, Genet RA, Wallace AR, Fletcher JD, Braam WF (1994) Control of powdery scab of potatoes with chemical seed tuber treatments. New Zealand Journal of Crop and Horticultural Science 22:121-128. doi:10.1080/01140671.1994.9513815

Brierley JL, Sullivan L, Wale SJ, Hilton AJ, Kiezebrink DT, Lees AK (2013) Relationship between *Spongospora subterranea* f.sp. *subterranea* soil inoculum level, host resistance and powdery scab on potato tubers in the field. Plant Pathology 62:413-420. doi:10.1111/j.1365-3059.2012.02649.x

641 Bulman SR, Kühn SF, Marshall JW, Schnepf E (2001) A Phylogenetic Analysis of
642   the SSU rRNA from Members of the Plasmodiophorida and Phagomyxida.
643   Protist 152:43-51. doi:http://dx.doi.org/10.1078/1434-4610-00042
644 Carnegie SF, Cameron AM, McCreath M (2010) Foliar symptoms caused by potato
645   mop-top virus on potato plants during vegetative propagation in Scotland
646   and their association with tuber yield, spraing and tuber infection. Potato
647   Research 53:83-93. doi:10.1007/s11540-010-9153-2
648 Charron CS, Sams CE (1999) Inhibition of *Pythium ultimum* and *Rhizoctonia*
649   *solani* by shredded leaves of Brassica species. J Am Soc Hort Sci 124:462-
650   467
651 De Cal A, Martinez-Treceño A, Salto T, López-Aranda JM, Melgarejo P (2005)
652   Effect of chemical fumigation on soil fungal communities in Spanish
653   strawberry nurseries. Applied Soil Ecology 28:47-56.
654   doi:http://dx.doi.org/10.1016/j.apsoil.2004.06.005
655 Deberdt P, Mfegue CV, Tondje PR, Bon MC, Ducamp M, Hurard C, Begoude BAD,
656   Ndoumbe-Nkeng M, Hebbar PK, Cilas C (2008) Impact of environmental
657   factors, chemical fungicide and biological control on cacao pod production
658   dynamics and black pod disease (*Phytophthora megakarya*) in Cameroon.
659   Biological Control 44:149-159. doi:10.1016/j.biocontrol.2007.10.026
660 Devaux A, Kromann P, Ortiz O (2014) Potatoes for sustainable global food
661   security. Potato Research 57:185-199. doi:10.1007/s11540-014-9265-1
662 Dobrowolski MP, Tommerup IC, Shearer BL, O'Brien PA (2003) Three clonal
663   lineages of *Phytophthora cinnamomi* in Australia revealed by
664   microsatellites. Phytopathology 93:695-704
665 Falloon RE (2008) Control of powdery scab of potato: Towards integrated
666   disease management American Journal of Potato Research 85:253-260.
667   doi:10.1007/s12230-008-9047-x
668 Falloon RE, Genet RA, Wallace AR, Butler RC (2003) Susceptibility of potato
669   (*Solanum tuberosum*) cultivars to powdery scab (caused by *Spongospora*
670   *subterranea* f. sp. *subterranea*), and relationships between tuber and root
671   infection. Australasian Plant Pathology 32:377-385.
672   doi:10.1071/AP03040
673 Falloon RE, Merz U, Butler RC, Curtin D, Lister RA, Thomas SM (2016) Root
674   infection of potato by *Spongospora subterranea*: Knowledge review and
675   evidence for decreased plant productivity. Plant Pathology 65:422-434.
676   doi:10.1111/ppa.12419
677 Falloon RE, Wallace AR, Braithwaite M, Genet RA, Nott HM, Fletcher JD, Braam
678   WF (1996) Assessment of seed tuber, in-furrow, and foliar chemical
679   treatments for control of powdery scab (*Spongospora subterranea* f.sp.
680   *subterranea*) of potato. New Zealand Journal of Crop and Horticultural
681   Science 24:341-353
682 Gau RD, Merz U, Falloon RE (2015) Infection risk potential of South American
683   *Spongospora subterranea* f.sp *subterranea* root gall and tuber lesion
684   inoculum on potato (*Solanum tuberosum* ssp *tuberosum*). American
685   Journal of Potato Research 92:109-116. doi:10.1007/s12230-014-9419-3
686 Gau RD, Merz U, Falloon RE, Brunner PC (2013) Global genetics and invasion
687   history of the potato powdery scab pathogen, *Spongospora subterranea*
688   f.sp *subterranea*. Plos One 8. doi:10.1371/journal.pone.0067944

689   Gossen BD, McDonald MR, Hwang SF, Strelkov SE, Peng G (2013) A comparison
690   of clubroot development and management on canola and Brassica
691   vegetables. Canadian Journal of Plant Pathology 35:175-191
692   Gyenis L, Anderson NA, Ostry ME (2003) Biological control of *Septoria* leaf spot
693   disease of hybrid Poplar in the field. Plant Disease 87:809-813
694   Hanada RE, Pomella AWV, Soberanis W, Loguercio LL, Pereira JO (2009)
695   Biocontrol potential of *Trichoderma martiale* against the black-pod
696   disease (*Phytophthora palmivora*) of cacao. Biological Control 50:143-149.
697   doi::10.1016/j.biocontrol.2009.04.005
698   Harrison JG, Searle RJ, Williams NA (1997) Powdery scab disease of potato - A
699   review. Plant Pathology 46:1-25. doi:10.1046/j.1365-3059.1997.d01-
700   214.x
701   Hernandez Maldonado ML, Falloon RE, Butler RC, Conner AJ, Bulman SR (2013)
702   *Spongospora subterranea* root infection assessed in two potato cultivars
703   differing in susceptibility to tuber powdery scab. Plant Pathology
704   62:1089-1096. doi:10.1111/ppa.12015
705   Hidalgo E, Bateman R, Krauss U, ten Hoopen M, Martinez A (2003) A field
706   investigation into delivery systems for agents to control *Moniliophthora*
707   *roreri*. European Journal of Plant Pathology 109:953-961.
708   doi:10.1023/B:EJPP.0000003746.16934.e2
709   Hughes I (1980) Powdery scab *Spongospora subterranea* of potatoes in
710   Queensland: occurrence, cultivar susceptibility, time of infection, effect of
711   soil pH, chemical control and temperature relations. Australian Journal of
712   Experimental Agriculture and Animal Husbandry 20:625-632.
713   doi:http://dx.doi.org/10.1071/EA9800625
714   Hutchison LJ, Kawchuk LM (1998) *Spongospora subterranea* f.sp. *subterranea*.
715   Canadian Journal of Plant Pathology-Revue Canadienne De
716   Phytopathologie 20:118-119
717   Jan AT, Azam M, Ali A, Haq QMR (2011) Novel approaches of beneficial
718   *Pseudomonas* in mitigation of plant diseases - an appraisal. Journal of
719   Plant Interactions 6:195-205. doi:10.1080/17429145.2010.541944
720   Jang MH, Ahn SY, Kim SH, Noh JH, Yun HK (2011) Evaluation of grapevine
721   varietal resistance to anthracnose through treating culture filtrates from
722   *Elsinoe ampelina*. Horticulture Environment and Biotechnology 52:152-
723   157
724   Johnson DA, Cummings TF (2015) Effect of Powdery Scab Root Galls on Yield of
725   Potato. Plant Disease 99:1396-1403. doi:10.1094/pdis-11-14-1170-re
726   Kalischuk M, Lynn J, Kawchuk L (2016) First report of potato mop-top virus
727   infecting potatoes in Alberta. Plant Disease 100:2544-2544.
728   doi:10.1094/PDIS-06-16-0902-PDN
729   Kim YC, Jung H, Kim KY, Park SK (2008) An effective biocontrol bioformulation
730   against *Phytophthora* blight of pepper using growth mixtures of combined
731   chitinolytic bacteria under different field conditions. European Journal of
732   Plant Pathology 120:373-382
733   Kirk HG (2008) Mop-top virus, relationship to its vector. American Journal of
734   Potato Research 85:261-265. doi:10.1007/s12230-008-9021-7
735   Kirkham R (1986) Screening for resistance to powdery scab disease of potatoes.
736   Australian Journal of Experimental Agriculture 26:245-247.
737   doi:http://dx.doi.org/10.1071/EA9860245

738 Lahlali R, McGregor L, Song T, Gossen BD, Narisawa K, Peng G (2014)
739   *Heteroconium chaetospira* induces resistance to clubroot via upregulation
740   of host genes involved in jasmonic acid, ethylene, and auxin Biosynthesis.
741   PLoS ONE 9:e94144. doi:10.1371/journal.pone.0094144
742 Landa BB, Navas-Cortes JA, Hervas A, Jimenez-Diaz RM (2001) Influence of
743   temperature and inoculum density of *Fusarium oxysporum* f. sp *ciceris* on
744   suppression of fusarium wilt of chickpea by rhizosphere bacteria.
745   Phytopathology 91:807-816. doi:10.1094/phyto.2001.91.8.807
746 Landa BB, Navas-Cortés JA, Jiménez-Díaz RM (2004) Influence of temperature on
747   plant–rhizobacteria interactions related to biocontrol potential for
748   suppression of fusarium wilt of chickpea. Plant Pathology 53:341-352.
749   doi:10.1111/j.0032-0862.2004.01008.x
750 Larkin RP, Griffin TS (2007) Control of soilborne potato diseases using Brassica
751   green manures. Crop Protection 26:1067-1077.
752   doi:10.1016/j.cropro.2006.10.004
753 Larkin RP, Honeycutt CW (2006) Effects of different 3-year cropping systems on
754   soil microbial communities and Rhizoctonia diseases of potato.
755   Phytopathology 96:68-79. doi:10.1094/PHYTO-96-0068
756 Lee SO, Choi GJ, Choi YH, Jang KS, Park D-J, Kim C-J, Kim J-C (2008) Isolation and
757   characterization of endophytic actinomycetes from chinese cabbage roots
758   as antagonists to *Plasmodiophora brassicae*. Journal of Microbiology and
759   Biotechnology 18:1741-1746. doi:10.4014/jmb.0800.108
760 Macalady JL, Fuller ME, Scow KM (1998) Effects of metam sodium fumigation on
761   soil microbial activity and community structure. Journal of Environmental
762   Quality 27:54-63
763 McLean KL, Swaminathan J, Frampton CM, Hunt JS, Ridgway HJ, Stewart A (2005)
764   Effect of formulation on the rhizosphere competence and biocontrol
765   ability of *Trichoderma atroviride* C52. Plant Pathology 54:212-218.
766   doi:10.1111/j.1365-3059.2005.01158.x
767 Merz U (2008) Powdery scab of potato - Occurrence, life cycle and epidemiology.
768   American Journal of Potato Research 85:241-246. doi:10.1007/s12230-
769   008-9019-1
770 Merz U, Martinez V, Schwarzel R (2004) The potential for the rapid screening of
771   potato cultivars (*Solanum tuberosum*) for resistance to powdery scab
772   (*Spongospora subterranea*) using a laboratory bioassay. European Journal
773   of Plant Pathology 110:71-77. doi:10.1023/B:EJPP.0000010123.21255.d1
774 Mishra DS, Prajapati CR, Gupta AK, Sharma SD (2012) Relative bio-efficacy and
775   shelf-life of mycelial fragments, conidia and chlamydospores of
776   *Trichoderma harzianum*. Vegetos 25:225-232
777 Muthukumar A, Eswaran A, Sangeetha G (2011) Induction of systemic resistance
778   by mixtures of fungal and endophytic bacterial isolates against *Pythium*
779   *aphanidermatum*. Acta Physiologiae Plantarum:1-12
780 Nakayama T, Sayama M (2013) Suppression of potato powdery scab caused by
781   *Spongospora subterranea* using an antagonistic fungus *Aspergillus*
782   *versicolor* isolated from potato roots Conference poster. Proceedings of
783   the Ninth Symposium of the International Working Group on Plant
784   Viruses with Fungal Vectors, Obihiro, Hokkaido, Japan, 19-22 August
785   2013:53-54

786   Narisawa K, Shimura M, Usuki F, Fukuhara S, Hashiba T (2005) Effects of
787   pathogen density, soil moisture, and soil pH on biological control of
788   clubroot in Chinese cabbage by *Heteroconium chaetospira*. Plant Disease
789   89:285-290. doi:10.1094/pd-89-0285
790   Narisawa K, Tokumasu S, Hashiba T (1998) Suppression of clubroot formation in
791   Chinese cabbage by the root endophytic fungus, *Heteroconium*
792   *chaetospira*. Plant Pathology 47:206-210. doi:10.1046/j.1365-
793   3059.1998.00225.x
794   Neuhauser S, Kirchmair M, Bulman S, Bass D (2014) Cross-kingdom host shifts of
795   phytomyxid parasites. BMC Evolutionary Biology 14:1-13.
796   doi:10.1186/1471-2148-14-33
797   Newman KL, Chatterjee S, Ho KA, Lindow SE (2008) Virulence of plant
798   pathogenic bacteria attenuated by degradation of fatty acid cell-to-cell
799   signaling factors. Molecular Plant-Microbe Interactions 21:326-334.
800   doi:10.1094/mpmi-21-3-0326
801   Nielsen SL, Larsen J (2004) Two *Trichoderma harzianum*-based bio-control
802   agents reduce tomato root infection with *Spongospora subterranea*
803   (Wallr.) Lagerh., f. sp *subterranea*, the vector of Potato mop-top virus.
804   Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant
805   Diseases and Protection 111:145-150
806   Nitzan N, Cummings TF, Johnson DA, Miller JS, Batchelor DL, Olsen C, Quick RA,
807   Brown CR (2008) Resistance to Root Galling Caused by the Powdery Scab
808   Pathogen *Spongospora subterranea* in Potato. Plant Disease 92:1643-
809   1649. doi:10.1094/PDIS-92-12-1643
810   Ownley BH, Duffy BK, Weller DM (2003) Identification and manipulation of soil
811   properties to improve the biological control performance of phenazine-
812   producing *Pseudomonas fluorescens*. Applied and Environmental
813   Microbiology 69:3333-3343
814   Peng G, Lahlali R, Hwang S-F, Pageau D, Hynes RK, McDonald MR, Gossen BD,
815   Strelkov SE (2014) Crop rotation, cultivar resistance, and
816   fungicides/biofungicides for managing clubroot (*Plasmodiophora*
817   *brassicae*) on canola. Canadian Journal of Plant Pathology 36:99-112.
818   doi:10.1080/07060661.2013.860398
819   Peng G, McGregor L, Lahlali R, Gossen BD, Hwang SF, Adhikari KK, Strelkov SE,
820   McDonald MR (2011) Potential biological control of clubroot on canola
821   and crucifer vegetable crops. Plant Pathology 60:566-574.
822   doi:10.1111/j.1365-3059.2010.02400.x
823   Qu X, Christ BJ (2006a) The host range of *Spongospora subterranea* f. sp
824   *subterranea* in the United States. American Journal of Potato Research
825   83:343-347
826   Qu XS, Christ BJ (2004) Genetic variation and phylogeny of *Spongospora*
827   *subterranea* f.sp *subterranea* based on ribosomal DNA sequence analysis.
828   American Journal of Potato Research 81:385-394
829   Qu XS, Christ BJ (2006b) Single cystosorus isolate production and restriction
830   fragment length polymorphism characterization of the obligate biotroph
831   *Spongospora subterranea* f. sp *subterranea*. Phytopathology 96:1157-
832   1163. doi:10.1094/phyto-96-1157

833 Raaijmakers J, Vlami M, de Souza J (2002) Antibiotic production by bacterial
834   biocontrol agents. Antonie van Leeuwenhoek 81:537-547.
835   doi:10.1023/A:1020501420831
836 Raaijmakers JM, De Bruijn I, Nybroe O, Ongena M (2010) Natural functions of
837   lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and
838   antibiotics. FEMS Microbiology Reviews 34:1037-1062.
839   doi:10.1111/j.1574-6976.2010.00221.x
840 Raupach GS, Kloepper JW (1998) Mixtures of plant growth-promoting
841   rhizobacteria enhance biological control of multiple cucumber pathogens.
842   Phytopathology 88:1158-1164. doi:10.1094/PHYTO.1998.88.11.1158
843 Roberts DP, Lohrke SM, Meyer SLF, Buyer JS, Bowers JH, Baker CJ, Li W, de Souza
844   JT, Lewis JA, Chung S (2005) Biocontrol agents applied individually and in
845   combination for suppression of soilborne diseases of cucumber. Crop
846   Protection 24:141-155
847 Santoyo G, Orozco-Mosqueda MD, Govindappa M (2012) Mechanisms of
848   biocontrol and plant growth-promoting activity in soil bacterial species of
849   *Bacillus* and *Pseudomonas*: a review. Biocontrol Science and Technology
850   22:855-872
851 Saravanan T, Muthusamy M, Marimuthu T (2004) Effect of *Pseudomonas*
852   *fluorescens* on fusarium wilt pathogen in banana rhizosphere. Journal of
853   Biological Sciences 4:192-198
854 Sarma M, Kumar V, Saharan K, Srivastava R, Sharma AK, Prakash A, Sahai V,
855   Bisaria VS (2011) Application of inorganic carrier-based formulations of
856   fluorescent pseudomonads and *Piriformospora indica* on tomato plants
857   and evaluation of their efficacy. Journal of Applied Microbiology 111:456-
858   466
859 Schisler DA, Slininger PJ, Behle RW, Jackson MA (2004) Formulation of *Bacillus*
860   spp. for biological control of plant diseases. Phytopathology 94:1267-
861   1271. doi:10.1094/PHYTO.2004.94.11.1267
862 Schmidt CS, Agostini F, Leifert C, Killham K, Mullins CE (2004) Influence of soil
863   temperature and matric potential on sugar beet seedling colonization and
864   suppression of pythium damping-off by the antagonistic bacteria
865   *Pseudomonas fluorescens* and *Bacillus subtilis*. Phytopathology 94:351-
866   363. doi:10.1094/PHYTO.2004.94.4.351
867 Shah FA, Falloon RE, Butler RC, Lister RA (2012) Low amounts of *Spongospora*
868   *subterranea* sporosorus inoculum cause severe powdery scab, root galling
869   and reduced water use in potato (*Solanum tuberosum*). Australasian Plant
870   Pathology 41:219-228. doi:10.1007/s13313-011-0110-6
871 Shah FA, Falloon RE, Butler RC, Lister RA, Thomas SM, Curtin D (2014)
872   Agronomic factors affect powdery scab of potato and amounts of
873   *Spongospora subterranea* DNA in soil. Australasian Plant Pathology
874   43:679-689. doi:10.1007/s13313-014-0317-4
875 Slininger PJ, Schisler DA, Eirjcsson LD, Brandt TL, Frazier MJ, Woodell LK, Olsen
876   NL, Kleinkopf GE (2007) Biological control of post-harvest late blight of
877   potatoes. Biocontrol Science and Technology 17:647-663.
878   doi:10.1080/09583150701408881
879 Slininger PJ, Schisler DA, Kleinkopf GE (2001) Combinations of dry rot
880   antagonistic bacteria enhance biological control consistency in stored
881   potatoes. Phytopathology 91:S83

882   Sparrow LA, Rettke M, Corkrey SR (2015) Eight years of annual monitoring of
883   DNA of soil-borne potato pathogens in farm soils in south eastern
884   Australia. Australasian Plant Pathology 44:191-203. doi:10.1007/s13313-
885   014-0340-5
886   Stockwell VO, Johnson KB, Sugar D, Loper JE (2011) Mechanistically compatible
887   mixtures of bacterial antagonists improve biological control of fire blight
888   of pear. Phytopathology 101:113-123
889   Taylor PA, Flett SP, de Boer RF, Marshall DR (1986) Effect of irrigation regimes
890   on powdery scab disease and yield of potatoes. Australian Journal of
891   Experimental Agriculture 26:745-750
892   Ting ASY, Mah SW, Tee CS (2012) Evaluating the feasibility of induced host
893   resistance by endophytic isolate *Penicillium citrinum* BTF08 as a control
894   mechanism for *Fusarium* wilt in banana plantlets. Biological Control
895   61:155-159. doi:10.1016/j.biocontrol.2012.01.010
896   Tommerup IC, Ingram DS (1971) The life cycle of *Plasmodiophora brassicae*
897   woron. In brassica tissue cultures and in intact roots. New Phytol 70:327-
898   332
899   van der Lelie D, Taghavi S, Monchy S, Schwender J, Miller L, Ferrieri R, Rogers A,
900   Wu X, Zhu W, Weyens N, Vangronsveld J, Newman L (2009) Poplar and its
901   bacterial endophytes: coexistence and harmony. Critical Reviews in Plant
902   Sciences 28:346-358
903   Vemmer M, Patel AV (2013) Review of encapsulation methods suitable for
904   microbial biological control agents. Biological Control 67:380-389.
905   doi:10.1016/j.biocontrol.2013.09.003
906   Wang J, Huang Y, Lin S, Liu F, Song Q, Peng Y, Zhao L (2012) A strain of
907   *Streptomyces griseoruber* isolated from rhizospheric soil of Chinese
908   cabbage as antagonist to *Plasmodiophora brassicae*. Annals of
909   Microbiology 62:247-253. doi:10.1007/s13213-011-0253-2
910   Wang S, Wu H, Zhan J, Xia Y, Gao S, Wang W, Xue P, Gao X (2011) The role of
911   synergistic action and molecular mechanism in the effect of genetically
912   engineered strain *Bacillus subtilis* OKBHF in enhancing tomato growth
913   and cucumber mosaic virus resistance. Biocontrol 56:113-121
914   Wilson CR (2016) Plant pathogens – the great thieves of vegetable value. Acta
915   Horticulturae (in press)
916   Xie H, Yan D, Mao L, Wang Q, Li Y, Ouyang C, Guo M, Cao A (2015) Evaluation of
917   methyl bromide alternatives efficacy against soil-borne pathogens,
918   nematodes and soil microbial community. PLoS ONE 10:e0117980
919   Zhou LH, Zhang LH, He YQ, Liu F, Li M, Wang ZS, Ji GH (2014) Isolation and
920   characterization of bacterial isolates for biological control of clubroot on
921   Chinese cabbage. European Journal of Plant Pathology 140:159-168
922
923
924

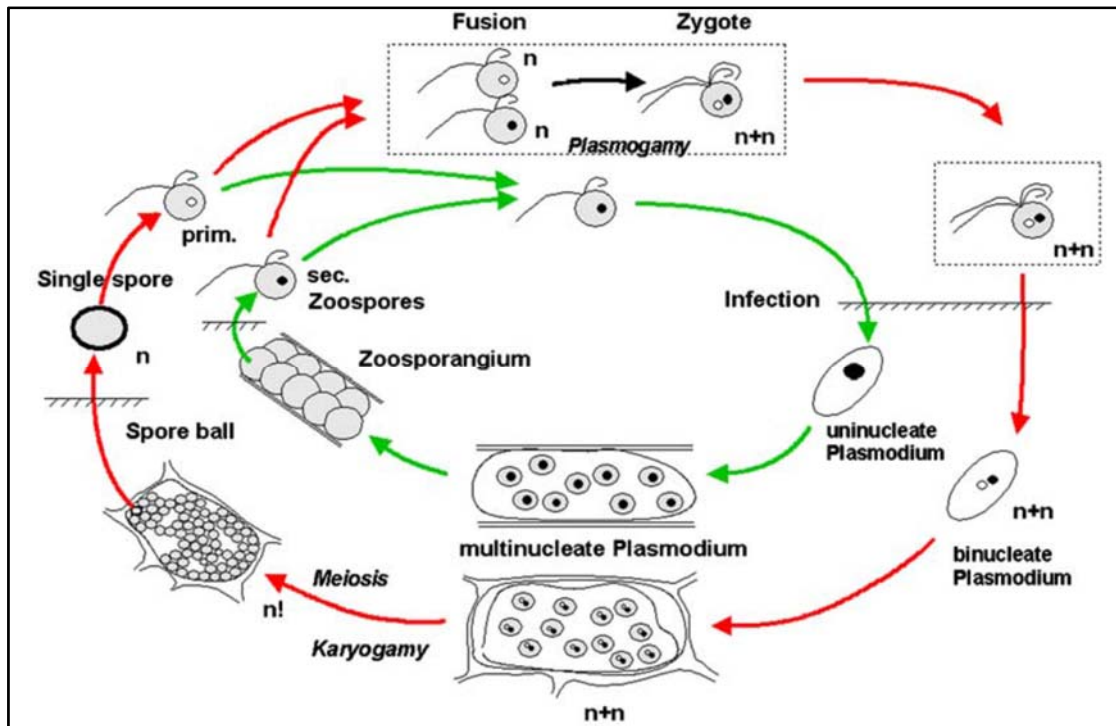925   **Captions for Figures**
926
927

928

929   Figure 1. Tentative life cycle of *Spongospora subterranea* f.sp. *subterranea*. The

930   outer path represents sexual reproduction and the inner path represents asexual

931   reproduction. (Reproduced from Merz (2008) with permission of Springer).

932
933

934   Fig 1:
935