Towards biological control of *Spongospora subterranea* f. sp. *subterranea*,
the causal agent of powdery scab in potato

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Abstract:

Powdery scab of potato, caused by the obligate biotrophic protozoan pathogen
*Spongospora subterranea* f.sp. *subterranea* (Sss), is a major problem in potato
growing areas throughout the world. It results in lesions (scabs) on the surface
of the tubers which renders them unmarketable. In recent years there has been
an increasing number of reports of the disease, many from new areas.

Management of the disease has proved difficult and relies on the integrated
application of a range of methods. Biocontrol is not currently used for the
management of powdery scab although the results of preliminary studies have
been encouraging. This review evaluates the potential for developing a
biocontrol strategy for powdery scab.

Introduction

Powdery scab of potato, caused by the biotrophic protozoan pathogen
*Spongospora subterranea* f.sp. *subterranea* (Sss), is a major problem in many
potato growing areas throughout the world. It results in lesions (scabs) on the
surface of tubers that are filled with a brown powder consisting of sporosori
(also referred to as sporeballs), hence the common name (Harrison et al. 1997).
Affected tubers have low acceptance at market and are down-graded or rejected
by traders, leading to reduced returns to growers and increased waste within the
industry. For producers of seed potatoes, the lesions may lead to rejection of
entire consignments as the disease is spread through infected tubers (Falloon et
al. 1996; Kirkham 1986). Economic losses in the fresh potato market are very
difficult to quantify. Annual losses to the Australian processing industry have
been estimated at A$13.4 million (Wilson 2016).

In addition to producing lesions on tubers, the pathogen also infects roots,
a process that leads to reduced water and nutrient uptake and thus impaired
shoot and tuber growth (Falloon et al. 2016). Historically this aspect has
received less attention because tuber lesions are more evident, but root infection
is now considered to have the greater deleterious effect on crop production. In
field trials, impairment of water and nutrient utilisation can lead to a 25%
reduction in shoot dry weight, a 26% reduction in the number of tubers per plant
and a 42% reduction in tuber weight per plant (Falloon et al. 2016).

Susceptibility of cultivars to root infection is only loosely related to previously
determined susceptibility to powdery scab (i.e. development of tuber lesions)
and hence they are considered to be separate disease processes (Falloon et al.
2016; Nitzan et al. 2008). Root infection, with impaired water and nutrient
utilisation, can occur from early in the growth of plants, but gall formation and
tuber lesions only become evident at later stages of plant development (Falloon
et al. 2016). Gall formation, when severe, can also lead to impaired water and
nutrient utilisation (Johnson and Cummings 2015).

In addition to its direct impact on the host crop, Sss is also the vector of
the potato mop-top furovirus (Arif et al. 1995). Mop-top virus has been reported
to cause yield losses of between 30 and 60% (Carnegie et al. 2010) and tubers
expressing the characteristic ‘spraing’ symptoms are unacceptable at market.
The virus has spread around the world in the last 40 years and it can remain
infective in fields without cultivation of potatoes for many years (Kirk 2008;
Kalischuk et al. 2016). It is speculated that alternative hosts may prolong
survival of the infectious virus although the host range of the virus is more
restricted than that of its vector (Kirk 2008).

The pathogen Sss has proved challenging to control, with no single
method reliably giving full control. Rather, management relies on the integrated
application of a range of tools (Falloon 2008). In this paper we will briefly review
the biology of the pathogen and current control methods as a basis for exploring
the potential to develop effective biological control options.

Occurrence

Powdery scab was first reported as a disease in Germany in 1841 (Harrison et al. 1997). Subsequently there were reports on occurrences of the disease from many locations between 1846 and 1992 (Harrison et al. 1997). In recent years there has been a resurgence in reports of the disease, many from new areas such as Australia, New Zealand, Columbia, Pakistan, Korea and others (Balendres et al. 2016; Harrison et al. 1997; Merz 2008). The resurgence in the disease has been attributed to increased cultivation of potato cultivars with long growing seasons, use of susceptible cultivars, increased irrigation of crops, inadequate crop rotation, and de-registration of mercury based fungicides (Braithwaite et al. 1994; Harrison et al. 1997; Merz 2008). As the disease is transmitted through infected tubers, the increased transport of seed tubers around the world may have exacerbated the spread of the disease (Gau et al. 2015). Increased awareness and increased efficiency in detection of the disease may also have contributed to the increase in reporting.

Taxonomy

*Spongospora subterranea* (Wallr.) Lagerh. f. sp. *subterranea* Tomlinson, the causal agent of potato powdery scab, is a soil-borne obligate pathogen and a plasmodiophorid characterized as having cruciform nuclear division, multinucleate plasmodia, biflagellate zoospores and resting spores (Hutchison and Kawchuk 1998). The taxonomic position of the plasmodiophorids has been uncertain for some time. Traditionally they were placed in the fungi although other researchers have argued for a protozoal origin (reviewed in (Qu and Christ 2004)). Analysis of SSU-rDNA sequences in five independent studies led to the conflicting views that they are unrelated to any other eukaryotes (three studies) or are related to the rhizopoda (two studies). The results of more recent
analyses now robustly place them within the eukaryote supergroup Rhizaria, as a sister group to the omnivorous vampyrellid amoebae (Neuhauser et al. 2014). Plasmodiophorids are the better known members of the group because they include a number of plant parasites causing economically significant diseases of crops including brassicas, potatoes, and grain crops (e.g. maize, rice, wheat, sorghum). The most studied species is the clubroot-causing *Plasmodiophora brassicae*, a parasite of crucifers which accounts for up to 10% loss of the worldwide production of *Brassica* crops. Other well-studied species include *Spongospora subterranea*, which causes powdery scab of potato and can serve as a vector for Potato Mop Top Virus. Non-pathogenic species such as *Polymyxa graminis* transmits economically important viruses to a number of grain plants while *Polymyxa betae* is the vector for beet necrotic yellow vein virus, the cause of sugar beet “rhizomania”.

**Life Cycle**

The life cycle (Fig 1) has been described in the excellent reviews of Harrison et al. (1997), (Merz 2008), and Balendres et al. (2016). In the asexual or zoosporangial phase (Fig 1 inner circle) the host plant is infected by haploid biflagellate zoospores that encyst on the root or tuber surfaces and penetrate the plant tissue. Within the plant the zoospores develop into multicellular plasmodia. After repeated cell divisions the plasmodia differentiate into zoosporangia from which haploid biflagellate secondary zoospores are released. These exit the sporangia through pores that extends through the sporangium walls and the root surfaces resulting in release directly into the soil. The secondary zoospores can initiate infections on other parts of the same plant such as tubers, or on adjacent plants. This provides the basis for multiple re-infection in a single growing season and thus the potential for very rapid inoculum build-up.

In the sexual phase (Fig 1 outer circle) thick walled resting spores each 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 Sss it 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
the same growers. Similar observations were reported by Gau et al. (2013). In some Andean regions of South America short day relatives of the widely grown long day potato species *Solanum tuberosum* ssp *tuberosum* are cultivated. These are *S. tuberosum* ssp. *andigena*, and *S. tuberosum phureja*. South American samples of *Sss* from *S.t. phureja* root galls formed one group, whilst samples from tuber lesions on *S.t. tuberosum* and *S.t. andigena* formed another group. Samples from the rest of the world formed a third group with a highly clonal structure.

Analysis of North American isolates with a suite of RFLP markers showed that they clustered into two groups, one included isolates originating from western North America., and the second included isolates originating from eastern North America (Qu and Christ 2006b).

The results of the different studies show that there is genotypic variation across geographical regions. They also highlight the need for a large scale study on a world wide population using multiple genetic loci such as microsatellite markers (Dobrowolski et al. 2003) or RFLP markers (Qu and Christ 2006b). The greater genetic diversity found in the South American population (Gau et al. 2015) suggests that this may be the centre of origin of the pathogen. Movement of potato propagules from South America needs to be appropriately regulated to minimize the risk of introducing new pathogen diversity into the world’s production regions.

**Current Control of Powdery Scab**

**Host resistance**

Although a number of potato cultivars with different levels of resistance to *Sss* have been identified (Falloon et al. 2003), no cultivar is known to be completely resistant to powdery scab (Falloon 2008; Hernandez Maldonado et al. 2013). Resistance varies from very resistant to very susceptible (quantitative) suggesting a polygenic basis (Falloon 2008). In general, more resistant cultivars have fewer galls and fewer zoospores in their roots although the correlation is
not tight and exceptions exist (Falloon et al. 2003). Hernandez Maldonado et al. (2013) compared Sss accumulation in roots and the numbers of galls that developed on a resistant and a susceptible potato cultivar and found that although there was little difference between the cultivars at early stages of infection there was increasing divergence as the disease progressed. This suggests that although the primary infection may be the same in both cultivars, secondary infection may be restricted in the resistant cultivar.

### Chemical control

There are a number of agro-chemicals that can be applied to seed tubers or to the soil, at or before planting, that have been shown to reduce powdery scab levels (reviewed in Falloon (2008)). Braithwaite et al. (1994) evaluated 25 fungicide treatments applied to severely infected tubers just before planting. Plant emergence was assessed, and at maturity tubers were harvested and scored for powdery scab infection. A number of the treatments reduced the proportion of diseased tubers from 95% at planting to an average of 29% in the subsequent crop compared with the untreated controls which declined to 70%. Some of the other treatments resulted in phytotoxic effects. Treatment of tubers with zinc sulfate and zinc oxide six weeks before planting also reduced infection in the subsequent crop compared with untreated controls.

A subsequent study evaluated chemicals applied either as tuber dressings or as in-furrow applications. The fungicides fluazinam, mancozeb, dichlorophen-\(\text{Na}\), were effective as tuber dressings of infected tubers, reducing the incidence of powdery scab and increasing the yield of tubers (kg/plot) by up to 36% (Falloon et al. 1996). The same study evaluated in-furrow treatments by planting uninfected tubers of cvs Rua and Agria into heavily infested soil. Treatments with fluazinam reduced powdery scab incidence, and increased yield of marketable 'Rua' tubers by up to 55%, and 'Agria' tubers by 140%. High rates of mancozeb also reduced incidence of the disease and increased marketable yield of 'Rua' by 34%, and of 'Agria' by 68%. In-furrow treatment with zinc oxide and foliar treatments with phosphorous acid did not control the disease.
Crop rotation

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

Larkin and Griffin (2007) evaluated the potential of rotation with brassica crops to decrease the level of pathogen inoculum in soil and so reduce disease severity in subsequent potato crops. Brassicas produce sulphur compounds, glucosinolates, which break down to isothiocyanates that are toxic to a wide range of phytopathogens. In a field trial, Indian mustard significantly reduced Sss inoculum levels in the soil as measured by a bioassay. Crops of rapeseed or yellow mustard were less effective. All three brassica species decreased the severity of powdery scab by 25-39% relative to a standard oats rotation. The brassicas also reduced the incidence of tubers with scabs by 19-40% with Indian mustard again being the most effective. Indian mustard produces high levels of glucosinolates, some of which convert to the most biologically active forms of isothiocyanates produced by brassicas (Charon and Sams 1999). In the same study, rotation with 'Lemtal' ryegrass led to similar reductions in disease severity and incidence of tubers with lesions as were observed after rotation with the brassicas. This suggests that factors other than the production of isothiocyanates may have been responsible for the reduction in incidence and severity.
As an alternative to direct pathogen inhibition, rotation crops may also affect pathogens indirectly by influencing changes in the soil microbial community. This may increase disease suppression by the soil. In a study to examine the effects of rotation systems on soil microbial communities, Larkin and Honeycutt (2006) demonstrated distinctive effects of specific rotation crops and cropping sequences on these communities. They also found higher populations of microorganisms that are generally beneficial to plants, such as *Pseudomonas* spp. and *Trichoderma* spp., after planting barley, canola, and sweet corn crops.

**Agronomic factors**

Agronomic factors, particularly soil nitrogen status and soil water content were found to affect the severity of disease (Shah et al. 2014). The incidence and/or severity of powdery scab were increased by nitrogen (nitrate and ammonium) applications. Nitrogen application resulted in a greater amount of Sss DNA in the soil and this effect was observed for two years after the trial. A positive correlation between soil Sss DNA and disease severity was observed by Brierley et al. (2013) and by Nakayama and Sayama (2013). However, Shah et al. (2012) reported that in their study there was no consistently strong relationship between the amount of inoculum at time of planting as measured by the number of sporosori per g soil and disease incidence or severity at harvest. Irrigation treatment also affected disease severity. An irrigation regime optimal for potato growth resulted in greater severity, but not greater incidence, of powdery scab than a constrained irrigation input (Shah et al. 2014). This is consistent with higher soil moisture content facilitating the movement of zoospores through the soil and increasing the chances of plant infection (Merz 2008; Balendres et al. 2016).

**Potential for Biocontrol**

Biocontrol, based on the use of an organism to restrict the ability of a pathogen to cause disease is an aspect of plant disease control that has received
much attention in recent years. This is fuelled by the emergence of fungicide resistance in pathogen populations, deregistration of fungicides, and growing concerns about the use of chemicals in food production. In contrast biocontrol is seen as a safe, non-toxic, renewable alternative. In some cases of biocontrol the level of control rivals that achieved with chemicals although a lack of consistency of control is often a concern.

Identifying biocontrol agents

There are few reports of studies aimed to identify potential biological control agents (BCAs) for Sss in potato. The most extensive study is that of Nakayama and Sayama (2013). They reported 54 – 70% suppression of disease development over three years by application of an isolate of Aspergillus versicolour to tubers. These values compared to 77 to 93% suppression by the synthetic fungicide fluazinam applied in furrow. While the BCA was less effective than the synthetic fungicide, the suppression was statistically significant.

Another approach was taken by Nielsen and Larsen (2004) who used tomato as a model to examine the efficacy of commercially available biocontrol products to reduce the infection of root hairs by primary zoospores of Sss. Each agent was assessed in two experiments. The commercially produced biocontrol products TRI 002 and Binab TF (both containing Trichoderma harzianum) gave a statistically significant reduction in root colonization in both experiments. By contrast the biocontrol product TRI 003 (also containing Trichoderma harzianum) gave no significant reduction in either experiment. The product FZB24, containing Bacillus subtilis, gave a reduced infection in only one of the two experiments. In each experiment, all plant growth parameters examined were markedly lower in the infected controls than the uninfected controls. While some of the control agents increased the plant growth parameters above that in the infected control, these increases were not consistent.

In the experiments of Nielsen and Larsen (2004) a very high inoculum concentration of the pathogen was used; much greater than could be expected in
field soil or in soil adhering to the surface of seed tubers (e.g. 0-148 sporosori/g of soil; (Brierley et al. 2013)). It is possible that more effective and more consistent control could be obtained with more typical disease pressures; biological control of *Plasmodiophora brassicae* has been found to be more effective at lower pathogen pressure (Narisawa et al. 2005; Peng et al. 2011). It was not known whether the BCAs tested by Nielsen and Larsen (2004) were inhibitory to *Sss*. In contrast, in the study by Nakayama and Sayama (2013) soil fungi were initially screened for inhibition of *Ss* infectivity using a bioassay.

Although research on biocontrol of *Sss* is limited, biocontrol of *Plasmodiophora brassicae*, has been studied more extensively. A number of studies have identified endophytes with the ability to suppress this pathogen. These include *Bacillus subtilis*, *Gliocladium catenulatum*, *Heteroconium chaetospira*, *Microbispora rosea ssp rosea*, *Streptomyces griseoruber*, *S. griseoviridis*, *S. lydicus*, *S. olivochromogenes* and *Trichoderma atroviride*, *T. harzianum* and *T. viride*. (Lahlali et al. 2014; Lee et al. 2008; Narisawa et al. 1998; Peng et al. 2014; Peng et al. 2011; Wang et al. 2012; Wang et al. 2011). In some studies a number of these potential biocontrol agents have shown levels of suppression of clubroot greater than 85% and approaching the level of effectiveness observed from synthetic fungicides.

The evidence from the limited studies that have been carried out suggests that developing biocontrol strategies for plasmodiophorid diseases is possible. However a problem common to all biocontrol strategies is the lack of consistency of disease reduction. Several researchers have reported that using mixtures of BCAs has increased the consistency of biocontrol across sites with different conditions. Slänger et al. (2001) in their investigation into postharvest dry rot of potato found that formulations of mixed BCAs performed more consistently across 32 storage environments varying in cultivar, washing procedure, temperature, harvest year, and storage time. Enhanced biocontrol using mixtures of BCAs has been reported for control of late blight in potato (Slänger et al. 2007), diseases of poplar (Gyenis et al. 2003), chilli (Muthukumar et al. 2011), and cucumber (Raupach and Klopper 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.)
2009), physical occlusion of the pathogen by occupying sites on root surfaces that the pathogen would use for entry (Blumenstein et al. 2015), and biofilm formation (Newman et al. 2008). A given BCA may use more than one mechanism of inhibition, and different BCAs may exert their effects at different times during the crop growing season. In vitro screening only identifies those BCAs acting through the production of antibiotics or cell lysis. Further, in the specific case of Sss, in vitro screening is not possible as the species is an obligate biotroph. Thus initial screening would need to employ in planta methods similar to those used by Nakayama and Sayama (2013).

In vitro inhibition or inhibition of disease development in greenhouse trials does not always translate to effective disease management in the field where weather, soil and biological variability are likely to be much greater. Ultimately, the only realistic evaluation is by field trials. Given the range of edaphic and environmental factors that can influence the effectiveness of BCAs (as noted in the following section), the number and location of evaluation sites needs to be selected to appropriately reflect the anticipated range of usage. As the production of potato expands geographically into Mediterranean, sub-tropical and even high altitude tropical areas, this aspect becomes more significant. Further, given that soil microbial communities can vary dramatically with soil type and land management, variation in these factors needs to be captured also.

Sss, impacts potato very early in crop development (Hughes 1980; Taylor et al. 1986). Screening methodology must reflect the need for protection to be established by the time of plant emergence. With a focus solely on reducing tuber symptoms, protection would need to persist to beyond tuber initiation; although longer protection would be preferable as root damage continues to occur beyond that stage (Hughes 1980), impacting host nutrient and water uptake.

Stability across production environments

Studies on the influence of environment on the efficacy of biological
control of soil-borne diseases demonstrate the influence of temperature (Jang et al. 2011; Landa et al. 2001; Landa et al. 2004; Schmidt et al. 2004), soil water status (Schmidt et al. 2004) and soil physical and chemical characteristics (Ownley et al. 2003) on the levels of control achieved. Given the increasingly diverse conditions and geographic locations under which potato is now produced (Birch et al. 2012; Devaux et al. 2014), this will be an important consideration.

No research has been reported that evaluates the influence of environmental variables (either soil or weather) on the effectiveness of BCAs in supressing Sss and little has been reported in relation to P. brassicae. Studying the control of P. brassicae by Heterconium chaetospira, Narisawa et al. (2005) found effective suppression under low to moderate moisture conditions but not at high water status. On the other hand, Peng et al. (2011) found that periods of dry soil impeded the effectiveness of a range of BCAs to varying degrees. Bacillus subtilis and the synthetic fungicide, fluazinam were particularly sensitive. Significantly, while the results of Zhou et al. (2014) showed variation in the effectiveness of bacterial isolates in suppressing P. brassicae in Chinese cabbage, the effectiveness of the synthetic fungicide also varied.

Effective formulation of BCA

The form in which a BCA is applied to a crop may affect its persistence and thus its effectiveness. Resistant propagules such as bacterial endospores, fungal conidia, chlamydospores or oospores are more persistent than vegetative bacterial cells or fungal hyphal fragments (Schisler et al. 2004). For spore-forming bacteria, yeasts, and fungi, the production system can be optimised for the production of spores. Fermentation environments and culture age influence the efficacy, stability and desiccation tolerance of many BCAs including fungi, yeasts, and bacteria (Schisler et al. 2004). The fungal biocontrol agent Trichoderma harzianum developed mycelium after four days at 28 °C and chlamydospores after 10 days in liquid medium (potato dextrose broth) at 28°C while on solid media (e.g., PDA, grains, wheat bran,) it produced conidia (Mishra
Persistence can be enhanced by mixing the BCA with additives that supply nutrients, protect it from desiccation, from UV, and antagonistic organisms, and increase its ability to stick to the surface of plants. The different types of additives were reviewed by Schisler et al. (2004). The addition of calcium carbonate to rice grain cultures of *Trichoderma martiale* stimulated conidia production and enhanced the persistence of the BCA in the field and during storage (Hanada et al. 2009). Formulation of strains of fluorescent *Pseudomonas* with talc has been reported to enhance efficacy in control of Fusarium wilt in banana (Saravanan et al. 2004) and tomato (Sarma et al. 2011). Inclusion of chitin in the growth medium for the production of chitinolytic strains of *Serratia* has been found to be effective and it is postulated that the chitin may also stimulate the growth of other chitinolytic species in rhizospheres which would help to prevent fungal infection (Kim et al. 2008).

Encapsulation of the BCA within a biodegradable matrix of protein (whey, poly-Lysine) polysaccharide (e.g., cellulose, alginites, chitosan, pectin), or other polymers such as lignin, protects the BCA from biotic and abiotic stress factors and promotes shelf life and persistence in the environment. Overall encapsulation results in greater disease control. The topic of encapsulation, including the types of materials and how to make capsules, is reviewed by Vemmer and Patel (2013).

Methods of application of BCA

A major factor in the efficacy of biocontrol agents is the method of application. BCAs can be applied as seed (tuber) dressings, as soil-drenches or as a foliar spray. For rhizospheric organisms that will encounter the pathogen before it enters the host plant, and prevents entry either by occlusion or killing the pathogen it is essential that the BCA is able to colonize root s as they develop.

For soil-borne diseases, soil drenches are most effective as they allow
extensive colonization of roots (Gossen et al. 2013). In-furrow applications or seed dressings are alternatives although they may not be as effective in providing high levels of inoculation. McLean et al. (2005) compared methods of introducing *Trichoderma atroviridae* as a BCA for *Sclerotium cepivorum* (white rot) in onions. In a glasshouse trial, they found that a pellet formulation maintained greater levels of the BCA in the soil compared to solid-substrate or seed-coating formulations. Pellets also gave more extensive colonization of root systems of the onion seedlings. In a subsequent field trial a pellet formulation resulted in a greater number of CFU/g of soil than a solid substrate form. The pellet formulation also gave a more persistent inoculum.

Timing of the application may also have an important impact. In a trial of suppression of the related clubroot pathogen *P. brassica* by the fungal BCA *H. chaetospira*, it was found that soil application of a granular formulation of the fungus reduced clubroot severity by > 80% relative to the control. However, applying *H. chaetospira* at seeding was much less effective than earlier soil application. More extensive root colonization by *H. chaetospira* resulted in greater suppression of *P. brassicae* infection and subsequent clubroot development (Lahlali et al. 2014) Thus, the greater suppression by the earlier application may have resulted from more extensive root colonization.

In the case of Sss, protection is required from about the time of plant emergence (Hughes 1980; Taylor et al. 1986). Thus, for any BCA to be effective, early colonization of the below ground plant parts will be critical.

*Incorporation into disease management systems*

Falloon (2008) reviewed the development of integrated management systems for Sss. There is currently no single management practice available that provides full and reliable control of the disease. Effective management relies on the coordinated use of a number of approaches. The main components listed by Falloon (2008) were crop rotation, field selection, resistant cultivars, pathogen-free planting material, appropriate pesticide use and sound crop management.
The use of biological control is compatible with the majority of these components; however compatibility with agro-chemicals (fungicides and or pesticides) is an obvious challenge.

Potato producers use a wide range of agro-chemicals as dressings for seed tubers, applied either before or after storage, or as in-furrow applications at plantings in bands up to 20 cm wide. Fungicides which are used against a range of possible pathogens, are a particular issue. These vary from broad spectrum treatments to products targeting specific disease organisms. Their mobility within potato plants also ranges from contact to fully systemic. This will be a major consideration for the incorporation of BCAs into the production system. Clearly, the significance will depend on the particular chemical in use and its mode of application on the one hand and the identity of the BCA and the mode of inoculation on the other. For example, in-furrow application of fungicides may interfere with the successful establishment of BCAs applied in-furrow or to the seed piece depending on the identity of the BCA and the fungicide applied. On the other hand broad-spectrum soil fumigants such as metam-sodium (sodium salt of methyl dithiocarbamate) are still in use in some regions as a means of reducing soil-borne diseases prior to planting potatoes. Although these treatments are broad spectrum (Xie et al. 2015), there is likely to be limited interference with BCAs applied to the seed potato or at planting as the fumigants are released from the soil some weeks prior to planting. However, questions have been raised regarding the impact of such fumigants on the size and structure of soil microbial communities (De Cal et al. 2005; Macalady et al. 1998). Significant alterations in community structure may have unforeseen consequences for the success of the BCA, the pathogen, or soil functions.

Approaches to combining biocontrol agents and agrochemicals for use against the target pathogen are important for IPM. Levels of disease suppression achieved by combining the control methods are typically equal or superior to the use of BCA alone (Deberdt et al. 2008; Hidalgo et al. 2003) (see discussion in Hanada et al. (2009)).
Given that potato is vegetatively propagated, introducing an endophyte into potato tubers via the parent seed crop may provide a level of protection from non-systemic agrochemicals. This may provide a novel method of combining biocontrol with seed-tuber dressings or in-furrow pesticide applications in an integrated system. If feasible, this approach would also provide a degree of protection for the BCA from environmental and soil variability, as well as providing the earliest possible introduction of the BCA which is important given the early impact of Sss on potato plants and tuber quality. A structured experimental investigation is required.

Is Successful Biological Control of Sss Possible?

The limited work available suggests that efforts to identify organisms capable of successfully suppressing disease development by Sss are likely to be successful. To achieve this, a high throughput in planta assay will be required for screening endophytic and rhizospheric organisms. The method described by Nakayama and Sayama (2013) could be used for this purpose as could the procedure of Nielsen and Larsen (2004). In this regard Andrea Ramirez et al. (2013) have described a stem cutting assay to screen cultivars for resistance to Sss that could be adapted to screen for disease control by microbial isolates. Another possibility described by Merz et al. (2004) is a procedure to screen potato cultivars for resistance using tissue culture plantlets.

However, identifying biological antagonists would appear to be the more straightforward aspect of the research. The greater challenge lies in developing a formulation and method of application that will provide adequate protection consistently across production systems and geographic locations within the intended range of use. Mixtures of BCAs may prove valuable in this respect. Importantly, the formulation and mode of use will need to allow integration with other disease control methods, especially the use of fungicides and pesticides.

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.

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Captions for Figures

Figure 1. Tentative life cycle of *Spongospora subterranea* f.sp. *subterranea*. The outer path represents sexual reproduction and the inner path represents asexual reproduction. (Reproduced from Merz (2008) with permission of Springer).
Fig 1: