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Establishing the Host Status of Various Cover and Rotation Crop Species to the Soil-Borne Pathogen, *Spongospora subterranea* f. sp. *subterranea,* in South Africa

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Abstract

Spongospora subterranea f. sp. *subterranea* (Sss) is the causal agent of the potato tuber disease, powdery scab. Sss infects a wide range of species, often used for cover cropping or as rotation crops. These alternative hosts are classified into several host types based on the presence of specific Sss pathogenic structures within their roots. True hosts produce sporosori that could contribute to the soil inoculum, whilst trap crops prevent Sss from completing its life cycle. This study investigated the Sss host status of various crops using a combination of bioassays and microscopic root assessments for pathogen detection, and molecular techniques for infection confirmation. Sss DNA was detected in all 26 species evaluated, and these were subsequently classified as alternative hosts of Sss. Most species assessed were identified as true hosts. *Brassica alba*, *Chloris gayana*, and *Cucurbita moschata* were the only species in which only the zoosporangial life cycle phase was noted and were classified as potential trap crops. Several species identified in the literature as trap crops and non-hosts were reclassified based on the results of this study. Additional research and field trials are required to determine the importance of the host status of rotation crops on Sss soil inoculum.

Keywords Plasmodiophorid · Powdery scab · Root galling · Sporosori · Zoosporangium

Introduction

Spongospora subterranea (Wallroth) Lagerheim f. sp. *subterranea* Tomlinson (Sss) is an obligate, soil-borne plant pathogen (Harrison et al. 1997; Neuhauser et al. 2014). Sss is responsible for three different diseases, all occurring in potato

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(*Solanum tuberosum* L.) crops. Powdery scab, an unsightly tuber blemish disease, is the most well-known and devastating of these (Falloon 2008). Severe outbreaks of powdery scab can significantly reduce the quality and marketability of infected potato tubers (van der Waals 2015a; Wilson 2016). The other diseases, root gall formation (hyperplasia) and 'zoosporangia' root infection, occur in the root systems of host plants (Hernandez Maldonado et al. 2013; Thangavel et al. 2015). Both diseases can disrupt the root functionality of infected roots by inhibiting water and nutrient absorption (Gilchrist et al. 2011; Shah et al. 2012; Johnson and Cummings 2015), which could subsequently reduce plant growth and tuber yield (Falloon et al. 2016).

The Sss life cycle contains two distinct phases: the zoosporangial (primary) and sporogenic (secondary) phases (Braselton 1995; Balendres et al. 2016b). The zoosporangial phase entails secondary zoospore production and release from host plant root tissue, whilst the sporogenic phase completes the pathogen's life cycle by forming resting spores (Merz 2008). These thick-walled resting spores aggregate into clumps (sporosori), the survival structures that allow for long-term dormancy and persistence in the soil (Braselton 1995; Balendres et al. 2016b). The resting spores are stimulated to release primary mobile bi-flagellated zoospores under favourable soil conditions and when triggered by specific chemical compounds in root exudates (Merz 1989; Balendres et al. 2016a; 2017a; 2018). Zoospores are the pathogenic structures responsible for host root and tuber tissue infection (Merz 1997).

Sss has been documented to have an extensive host range, which includes a diverse array of species belonging to more than 30 botanical families (Jones and Harrison 1969; 1972; Andersen et al. 2002; Shah et al. 2010; Arcila et al. 2013; Clark et al. 2018; Simango et al. 2020; Tsror et al. 2020; Alaryan et al. 2023). These alternative hosts include numerous rotation or cover crop species and weeds that occur frequently in potato fields (Andersen et al. 2002; Simango et al. 2020; Tsror et al. 2020). In the past, root gall formation was mainly associated with plants in the Solanaceae family, but various studies have reported sporosori and root gall development in non-solanaceous species (Qu and Christ 2006; Nitzan et al. 2009; Simango et al. 2020). It has been suggested that Sss root galls form due to rapid, mass sporosorus production in the infected root cells and that their development signals the completion of the pathogen's life cycle, as is the case with the closely related plasmodiophorid pathogen, *Plasmodiophora brassicae* Woronin (Kageyama and Asano 2009; Merz and Falloon 2009). However, the root galls induced by Sss infection do not always contain visible sporosori (Qu and Christ 2006).

The host species of Sss can be classified into different host types based on the pathogenic structures observed within their roots (Arcila et al. 2013). Non-host plants are not susceptible to infection, and no signs of the pathogen are visible within their root cells. Type I hosts exhibit only sporosori when examined, whilst Type II hosts display all life stages (zoosporangia and sporosori). Finally, in 'trap crops' only zoosporangia develop within their roots. This prevents the life cycle from being completed, as no new resting spores are produced (Arcila et al. 2013; Simango et al. 2020). Establishing the Sss host status of a species is accomplished through a combination of biological assays, microscopic root assessments, and molecular analysis of the inoculated root tissue to confirm pathogen presence and successful infection (Merz 1992; Tsror et al. 2020). The Sss pathogenic structures can be difficult to

identify and differentiate morphologically. Variation in the observed morphology of sporosori and zoosporangia can be quite substantial between different species and when compared to the morphological characteristics described in literature (Arcila et al. 2013; Falloon et al. 2016; Balendres et al. 2016b). The variation in the shape and size of these structures is most likely host-dependent and determined by the unique interactions that occur between Sss and each host species (Arcila et al. 2013).

The Sss true host species (Types I and II) have the potential to produce sporosori within their roots, which can contribute to the soil pathogen inoculum levels when the plant material decomposes (Arcila et al. 2013; Alaryan et al. 2023). If potato growers cultivate these true host species, it can result in more severe powdery scab disease in subsequent potato crops by increasing the inoculum between potato growing seasons (Clark et al. 2018; Alaryan et al. 2023). It is essential to characterise the full host range of Sss to better understand its epidemiology and, more importantly, to make informed decisions regarding rotation crop selection in a potato cropping system.

Due to the lack of effective control measures for Sss diseases and soil inoculum, the employment of a an integrated pest management strategy (IPM) is advised (Strydom et al. 2024). An IPM strategy consists of various control measures implemented at different periods during and between potato growing seasons. Crop rotation and cover cropping are some of these components and have been recommended as good management tools for soil-borne pathogens (Wright et al. 2015). Crop species classified as Sss non-hosts or trap crops should preferably be included in a crop rotation scheme (Strydom et al. 2024). At the same time, the cultivation of Types I or II hosts is discouraged as this could result in the build-up of inoculum in potato fields. Utilising trap crops for inoculum management is effective for several soil-borne pathogens (Murakamia et al. 2000). Radish (*Raphanus sativus* var. *longipinnatus*) substantially reduced *Plasmodiophora brassicae* resting spore concentrations when cultivated in infested soil (Murakamia et al. 2000). Indian mustard (*Brassica juncea* L.) was reported to decrease powdery scab incidence by 40% when used as a green manure, due to the unique biofumigation properties many Brassica crops possess (Larkin and Griffin 2007). Other crops, including oilseed radish (*Raphanus sativus*), perennial ryegrass (*Lolium perenne*), white mustard (*Brassica alba* L.), and winter rapeseed (*Brassica napus. napus*), have been recorded to reduce powdery scab disease when cultivated in highly infested soils (Larkin and Griffin 2007; Larkin and Lynch 2018). Various studies have identified potential Sss trap crop species using previously mentioned assessment techniques (Arcila et al. 2013; Simango et al. 2020; Tsror et al. 2020). The sole use of crop rotation and trap crops for Sss management is insufficient due to the durability of Sss resting spores, which can still be viable after 5 years of dormancy (de Boer 2000; Balendres et al. 2016b). However, they are important components of an IPM strategy to control Sss.

This study aimed to determine the Sss host status of 26 selected rotation and cover crop species often cultivated by South African potato growers. A combination of artificially inoculated biological assays (hydroponic system and greenhouse pot trial), microscopic root assessments for pathogen identification, and conventional and quantitative polymerase chain reaction (PCR) analyses for infection confirmation was used to determine the host status of each species. This study is

a continuation of previous research projects that focused on characterising the Sss host range in South Africa (Simango et al. 2020). Additional crop species were evaluated for their Sss host status, and several species were reassessed to confirm their status and substantiate the results or claims made by Simango et al. (2020).

Materials and Methods

Cover and Rotation Crop Seed Collection

The 26 species included in this study (Table 2) belong to six different plant families, all of which contain members that have been confirmed as alternative hosts of Sss in previous research studies (Jones and Harrison 1972; Qu & Christ 2006; Arcila et al. 2013; Tsror et al. 2020). Tomato is a proven Type II host of Sss (Simango et al. 2020), and cultivars 'Moneymaker' and 'Rio Grande' were included in this experiment as the positive control for successful Sss root infection comparisons. The seed of these crops was obtained from a quality and trusted cover crop and vegetable seed provider based in South Africa.

Preparation of Pathogen Inoculum

The pathogen inoculum used in this study was obtained from potato tubers with powdery scab sourced from different locations in South Africa. The presence of Sss in lesions was confirmed using an Agristrip test kit (Bioreba AG, Reinach, Switzerland). Powdery scab lesions were peeled from the surface of heavily infected tubers using a sterilised scalpel, and the peels were air-dried at 30 °C for 72 h. The dried peels were then ground into a fine powder using a sterilised mortar and pestle. The powder was passed through mesh sieves (200 µm, 100 µm, 75 µm, and 35 µm). A small quantity (100 mg) of this inoculum was suspended in 10 ml of sterile distilled water. The suspension was viewed using a haemocytometer under a compound microscope (×40), and a final inoculum concentration of 1×10^5 sporosori/ml of Merz nutrient solution (Merz 1989) was prepared. The solution consisted of 710 mg calcium nitrate, 505 mg potassium nitrate, 492 mg magnesium sulphate, 272 mg potassium phosphate, 20 mg Fe (Ferric)- EDTA (ethylenediaminetetraacetic acid), and 1 ml of Hoagland solution (Sigma-Aldrich, MA, USA) in 1 l of sterile distilled water. The inoculum suspension was incubated at 18 ℃ in the dark for 48 h before artificial inoculation to stimulate primary zoospore release from the resting spores (Balendres et al. 2018).

Seed Germination

The cover crop seeds were germinated in sterilised plastic seedling trays containing pasteurised vermiculite. The growth medium was kept moist by regular spraying with sterile distilled water. The seedling trays were placed in a growth chamber at 18 ℃ (12 h light; 12 h dark), the optimal temperature for Sss root infection and disease

development (van de Graaf et al. 2005; 2007). Once the seedlings reached the three-leaf stage, they were carefully transplanted into 120-ml bioassay containers.

Seedling Transplant and Artificial Inoculation

Each bioassay container (120 ml) received three seedlings of the same species, with six containers per species (five replicates and one uninoculated control). Each container was filled with Merz nutrient solution (diluted five-fold for the bioassay), covered with a thin piece of polyethylene foam, and wrapped in tin foil to minimise light penetration. An incision was created in the foam through which seedlings were inserted to suspend the root system in the solution. The containers were kept in a growth chamber at the same parameters used for seed germination. After 7 days, the 'treated' seedlings were inoculated using a plastic syringe with 10 mℓ of the pre-conditioned Sss sporosori suspension (1×10^5 sporosori/ml solution). The untreated control seedlings received 10 ml of full-strength Merz nutrient solution.

Root Assessments

Two weeks after inoculation, root segments (±20 mm in size) were sampled from the crown root region of five plants per species (one from each inoculated replicate) and from the untreated control. The root sections were examined microscopically for the presence of the Sss structures. Additional root tissue (at least 150 mg) was used for conventional and real-time PCR to confirm infection and quantify Sss DNA.

Greenhouse Pot Trial

After the first root sampling event, the remaining cover crop seedlings were transferred to their respective 2-l plastic pots (21 cm top diameter, 13 cm bottom diameter, and a height of 20 cm) containing sterile (autoclaved) vermiculite. Each pot was watered with 200 ml of the diluted Merz nutrient solution, and the treated pots were inoculated with 20 ml of the inoculum suspension (1×10^5 sporosori/ml nutrient solution). The pot trials were conducted in a greenhouse compartment, and pots were arranged in a Randomised Complete Block Design. The plants were kept at 18 ℃ (±2 ℃), under ambient light conditions, and watered with distilled H_2O every second day until saturation, to promote Sss infection and pathogenesis.

Harvest and Final Root Assessments

Plants were harvested 2 months after inoculation. The root systems were triple-washed with distilled water to remove sporosori or vermiculite from the roots' surface. Each root system was visually examined for root galls using a magnifying glass (×2.5 magnification). The incidence of root gall formation was rated according to the presence (+) or absence (-) of galls. Root samples were again taken for microscopy, PCR, and real-time PCR analysis to detect Sss structures, confirm infection,

and quantify the Sss DNA concentration. The bioassay and greenhouse pot trials were repeated to ensure the reliability of the results.

Microscopic Root Observations

Microscopic root assessments were conducted at 2- and 10-week post inoculation of the plants. The protocol used for root staining and microscopic examination was developed by Merz (1989). This method consisted of using several different solutions (Table 1). Firstly, the freshly cut roots were washed under running tap water to remove any potential surface contaminants. Solutions A and B were heated in a warm bath (60 ℃). The root samples were submerged in separate glass beakers containing 30 ml of solution A for 10 min whilst continuously agitated to de-stain the plant tissue. The root samples were transferred to other glass beakers with 30 ml of solutions B and C for 5 min. If a successful infection occurred, these solutions stained any potential pathogenic Sss structures within the root epidermal cells and hairs. The stained root sections were fixed to a microscope slide with lactic acid for 5 min at room temperature and then covered with a coverslip.

The roots were microscopically examined at × 100 magnification using a high-resolution light microscope to obtain images of the pathogenic structures. The roots sampled from the positive control plants (tomato) were examined to determine if successful infection had occurred. The structures detected in the roots of inoculated plants were compared with the corresponding uninoculated samples to differentiate pathogen structures from naturally occurring root organelles. The observed microscopic images were compared to images of established infected roots from host plants obtained from published articles for accurate identification of the pathogen structures and reliable host classification (Merz 1997; Nitzan et al. 2007; Arcila et al. 2013; Falloon et al. 2016; Balendres et al. 2017). The presence of plasmodia, zoosporangia, and sporosori in any of the replicate tissue samples indicated successful infection of the roots by Sss and was used to determine the host status of each crop species.

DNA Extraction and Conventional Polymerase Chain Reaction Analysis

The root tissue samples taken at the 2- and 10-week harvest periods were used for DNA extraction and conventional PCR analysis. Before the extractions, the plant material was carefully washed with sterile distilled water to remove any surface

Table 1 The components of the different solutions used during root staining (Merz 1989)

Solution	Components
A	Ethanol, distilled H_2O (l), and chloral hydrate (1:1;1 v/v/v ratio)
B	3.5 % phenol, 6 % lactic acid, 3 % formaldehyde and 87.2 % ethanol-H2O (at a 1:1 v/v ratio)
C	Lactophenol blue solution
D	Lactic acid

contaminants and allowed to air dry in a growth chamber (30 ℃) for 3 days. The root tissue was then ground into a fine powder using liquid nitrogen with a sterile mortar and pestle. A sample (≤ 150 mg) was used for DNA extraction with the Zymo Research Quick-DNA Plant/Seed Miniprep Kit® (Zymo Research Corp®, USA) and the manufacturer's instructions were followed accordingly.

The PCR assays were run in a 2720 Thermal Cycler (Applied Biosystems) using an optimised version of the protocol described by Bulman and Marshall (1998). The final PCR reaction volume of 50 µl consisted of 25 µl DNA Taq® 2 × Master mix with standard buffer (New England Biolabs, USA), 0.5 µl of each Sss specific primer Sp01 (5′-ATT GTC TGT TGA AGG GTG-3′), and Sp02 (5′-GGT TAG AGA CGA ATC AGA A-3′) (Bulman and Marshall 1998), 20 µl nuclease-free H_2O, and 4 µl of the extracted DNA template. Initial denaturation at 94 °C (2 min) was followed by 35 subsequent cycles of melting (94 °C) for 30 s, annealing (56 °C) for 30 s, extension phase (72 °C) for 45 s, and finally a cycle of 72 °C for 7 min. The amplified PCR products were run on a 1% agarose gel stained with Roti-safe Gel stain, and a 100 bp molecular ladder was used. A positive control (Sss inoculum DNA) and a negative control (nuclease-free H_2O) were also run on each gel.

Quantitative PCR Analysis

Real-time (qPCR) analysis was conducted on the 10-week post-inoculation root tissue DNA samples. This molecular detection technique was implemented due to its specificity and high sensitivity for detecting low quantities of the target DNA, ensuring accurate pathogen DNA quantification and reliable host status allocation.

The QuantaBio Q real-time PCR Cycler (Quantabio®, Massachusetts, USA) was used for the qPCR assays. The species-specific primers described in the primer design and qPCR protocol of van de Graaf et al. (2003) were used. The forward (SsTQF1(5′-CCG GCA GAC CCA AAA CC-3′)) and reverse (SsTQR1 (5′-CGG GCG TCA CCC TTC A-3′)) primers were used for Sss DNA amplification whilst the probe (SsTQP1 (5′-CAG ACA ATC GCA CCC AGG TTC TCA TG-3′)), that was labelled at the 5′ end with a fluorescent reporter dye 6-carboxyfluorescein (FAM) and at the 3′ end with quencher dye 6-carboxy-tetramethyl-rodamine (TAMRA), was used for DNA quantification (van de Graaf et al. 2003). The qPCR master mix reaction protocol and thermocycling conditions recommended by the Luna® Universal Probe qPCR Master Mix (New England Biolabs® Inc., Massachusetts, USA) manual were optimised and followed. Each 10 µl qPCR master mix reaction included 5 µl Luna® universal probe qPCR master mix (1X), 3 µl template DNA, 1 µl sterile nuclease-free H_2O, 0.4 µl SsTQF1 (10 µM), 0.4 µl SsTQR1 (10 µM), and 0.2 µl of the SsTQP1 (10 µM).

Each qPCR assay included the following: a non-template control (nuclease-free H_2O instead of DNA template), a negative control (DNA sample prepared with sdH_2O instead of root tissue), two positive control samples (DNA template from ground Sss infected potato peels), and two replicates of all seven Sss DNA standards (10^{10} to 10^3 copies/µl).

Past studies have used DNA extracted from inoculum samples whose sporosori concentrations were determined via haemocytometer counts to prepare the DNA standards (van de Graaf 2003; Mallik et al. 2019). This is a very inaccurate method, as a single Sss sporosorus can contain 155 to 1526 resting spores (Falloon et al. 2011). The obligate nature of Sss makes it almost impossible to obtain pure cultures of the pathogen required for molecular analysis. In this study, a PCR product obtained by extracting the DNA from infected potato peels was sequenced. This sequence was run through the BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and a match was found (Accession number: AY604172.1, percentage similarity: 100%). This sequence was used to create a synthetic DNA sequence (372 base pairs) transformed into a plasmid (PUC-57). This transformed plasmid was inoculated into *Escherichia coli* bacteria, which were then cultured. The plasmid DNA was isolated from the bacterial cells using a Plasmid DNA extraction kit. A conventional PCR was conducted on the plasmid DNA samples to isolate and amplify the linear Sss DNA. The Sss DNA concentration (copy number/µl) of the PCR product was determined using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, USA). This value was used to prepare a 10^{10} Sss DNA copies/µl sample (50 µl) by adding the appropriate amount of sterile nuclease-free H_2O to the PCR tube. A dilution series of Sss DNA (10^9–10^3 copies/µl) was then created by pipetting 5 µl of the previous standard's DNA into a 200-µl PCR tube with 45 µl of H_2O. Two replications of each standard were included in every assay to create a standard curve from which the concentration of Sss DNA was determined within the samples. If the characteristics of the standard curve were outside the range that is considered acceptable (Efficiency = 90–110%, $R^2 > 0.95$) for a valid assay, the run was repeated. The qPCR thermocycling conditions included an initial denaturation step of 1 min at 95 ℃, followed by 40 cycles of denaturation (15 s at 95 °C) and extension (30 s at 60 °C).

Samples with a quantification cycle (Cq) value higher than that of the non-template control samples were considered not infected/Sss-free (0 copies/µl). An average concentration for each treatment combination was calculated. The final Sss DNA concentrations were converted from the number of Sss plasmid DNA copies/µl to Sss DNA pg/g of root tissue using the following formulae:

$$Sss\ DNA\ (ng/\mu l) = Copy\ number/\mu l \times 4.077051 \times 10^{-10} (Amount\ of\ Sss\ DNA\ (ng)\ in\ 1\ copy\ number)$$

$$Sss\ DNA\ (pg/g\ of\ root\ tissue) = Sss\ DNA\ (ng/\mu l) \times 10^3 \times 6.67\ (150\ mg\ of\ root\ tissue\ was\ used\ for\ DNA\ extraction)$$

Statistical Data Analyses

The qPCR analyses data gathered from these trials were subjected to an appropriate analysis of variance using the statistical software GenStat® (VSN International, 2018) or SAS software (Version 9.4; SAS Institute Inc., Cary, USA). The treatment means were compared using Fisher's protected least significance difference *t*-test at a 5% level of significance.

Results

The Sss host status screening results for the 26 different crop species are presented in Table 2. The data collected from each trial's 2- and 10-week harvest periods were pooled and presented as a single result, except for the root gall inspection and qPCR analysis results, which only represent the final harvest period (Table 2). The data for the microscopy and PCR results were pooled for the following reasons: The pathogen was considered present within the roots of a species if a single Sss structure was identified via microscopy in any single replicate at either of the two harvest periods and either of the two trials; A crop species was determined to be infected by Sss if any replicate's root tissue DNA samples obtained a positive PCR sample. Infections were confirmed and quantified using qPCR analysis. The Sss DNA concentration for all the replicate samples, from a single species, was combined, and an average Sss DNA concentration was provided in the 'qPCR' column. The combined microscopic and molecular analysis results from both trials were considered when assigning a host type to each species.

There was a significant difference ($p < 0.05$) in the Sss DNA concentration observed in the root tissue samples during the first and second trial repetitions. *Crotalaria juncea* was the only species with Sss root DNA concentrations significantly higher than both *S. lycopersicum* (tomato) cultivars during the first trial (Table 2). This was 1.77 pg Sss DNA/g of root tissue compared to 0.2673 pg ('Money maker') and 0.0067 pg ('Rio Grande'), respectively. The only species with a DNA concentration not significantly lower than *C. juncea* were *B. napus* and *S. cereale*. The *S. cereale* DNA concentration was significantly higher than the positive control ('Rio Grande') as well as numerous other species. *Raphanus sativus var. longipinnatus*, *C. pepo*, and *B. juncea* had the lowest average DNA concentrations in trial 1. The root infection levels were considerably lower during the second trial for most species. The 'Rio Grande' *S. lycopersicum* cultivar had significantly higher root infection levels than all other species in the second trial. Several species (*H. annuus, B. napus*, and *P. glaucum*) had substantially higher Sss root concentrations than the other species, while some (*B. alba*, *B. nigra*, *R. sativus*, *E. tef*, and *F. esculentum*) had no recorded infections during this specific trial. During both trials, some species produced insufficient root tissue (due to seedling die-off), and DNA extraction and molecular analysis could not be performed. This is indicated by 'NA' in Table 2. No Sss DNA was detected in the root of any negative control samples.

Non-host species are not susceptible to infection by Sss; thus, no pathogen structures would be observed within their roots during microscopy, and the PCR and qPCR results would be negative. Based on the above-mentioned criteria, no species were classified as non-hosts during this study. No Sss structures were observed within *Arachis hypogaea* (peanut) roots, but Sss was detected in the root tissue samples in qPCR assays. This species could not confidently be classified as a non-host for this reason. Several species had negative PCR results during the first trial, and most did during the second trial (Table 2). Sss DNA was amplified in root tissue DNA samples from 16 species when using conventional PCR. However, the results from the qPCR analysis indicated some amplification of Sss

Table 2 The host type status of different crop species to *Spongospora subterranea* f. sp. *subterranea* based on the presence (+) or absence (-) of zoosporangia (Z) and sporosori (S), incidence of root galls (RG) and detection (PCR) and quantification (qPCR) of Sss in root tissue

Family	Scientific name	Common name (cultivar)	Trial 1					Trial 2					Host type
			[a]Z	[a]S	[d]RG	[b]PCR	[c]qPCR	[a]Z	[a]S	[d]RG	[b]PCR	[c]qPCR	
Amaranthaceae	*Beta vulgaris* subsp. *vulgaris*	Beetroot	+	+	0	-	0.028	+	+	0	-	0.003*	Type 2
	Helianthus annuus L.	Sunflower	+	+	0	+	0.153	-	-	0	-	0.018*	Type 2
Brassicaceae	*Brassica alba* L.	White mustard	+	-	0	+	0.169	-	-	0	-	0	Trap crop
	Brassica juncea L.	Brown mustard*	+	+	0	-	0.009	+	+	0	-	NA	Type 2
	Brassica napus L.	Forage rape	+	+	0	+	0.872	+	+	0	-	0.0187	Type 2
	Brassica nigra L.	Black mustard	+	+	3/5	+	0.347	-	-	0	-	0	Type 2
	Raphanus sativus	Tillage radish	+	+	0	-	0.064	-	-	0	-	0	Type 2
	Raphanus sativus var. *longipinnatus*	Japanese radish	+	+	0	-	0.003	-	-	0	-	0.0067*	Type 2
Cucurbitaceae	*Cucurbita moschata*	Butternut squash	+	-	0	-	0.012	-	-	0	-	0.0047*	Trap crop
	Cucurbita pepo L.	Pumpkin	->	+	0	-	0.008	+	+	0	-	0.0013*	Type 2
Fabaceae	*Arachis hypogaea* L.	Peanut	-	-	0	-	0.0173	-	-	0	-	NA	NA
	Crotalaria juncea L.	Sunn hemp	-	+	0	+	1.77*	-	+	1/5	-	0.0133*	Type 1
	Medicago sativa	Lucerne (Icon)	-	+	5/5	+	0.1167	-	+	3/5	-	0.008*	Type 1
	Lupinus angustifolius	Bitter lupins	+	+	0	-	0.036	-	-	0	-	0.0027*	Type 2
	Pisum sativum L.	Forage pea (Aragorn)	+	+	0	+	0.1347	-	+	0	-	0.0007*	Type 2
	Vicia sativa L.	Common vetch	+	+	0	+	0.29	+	+	0	-	0.0033*	Type 2
	Vigna unguiculata (L.) Walp.	Cowpea (IT 18)	+	+	0	+	0.177	-	-	0	-	0.002*	Type 2

Table 2 (continued)

Family	Scientific name	Common name (cultivar)	Trial 1					Trial 2					Host type
			[a]Z	[a]S	[d]RG	[b]PCR	[c]qPCR	[a]Z	[a]S	[d]RG	[b]PCR	[c]qPCR	
Poaceae	*Avena strigosa* Schreb.	Black oats*	-	+	0	-	0.0327	-	-	0	-	0.0004*	Type 1
	Avena sativa	Forage oats	-	-	0	-	NA	+	+	0	-	0.0001*	Type 2
	Chloris gayana Kunth	Rhodes grass (Katambora)	+	-	0	+	0.2	+	-	0	-	0.0001*	Trap crop
	Eragrostis tef	Teff (SA Brown)	+	+	0	+	0.0453	-	-	0	-	0	Type 2
	Hordeum vulgare L.	Barley	+	+	0	-	0.0047	+	+	0	-	0.0019*	Type 2
	Pennisetum glaucum (L.) R. Br.	Pearl millet	+	-	0	+	0.1153	+	+	0	-	0.0045*	Type 2
	Secale cereale L.	Stooling rye (Winter grazer)*	+	+	0	+	1.01*	-	+	0	-	0.0022*	Type 2
	Sorghum bicolor (L.) Moench	Forage sorghum (Supergraze 1000)*	-	+	0	+	0.0413	-	-	0	-	0.0023*	Type 1
Polygonaceae	*Fagopyrum esculentum* Moench	Buckwheat	+	+	0	+	0.118	-	-	0	-	0	Type 2
Solanaceae	*Solanum lycopersicum* L.	Tomato (Moneymaker)	+	+	0	+	0.2673	-	-	0	-	NA	Type 2
		(Rio Grande)	+	+	0	-	0.0067	-	-	0	+	0.0390	Type 2

*Crop species that were evaluated by Simango et al. (2020) and were reassessed in these trials.

[b]+ is for a positive *Spongospora subterranea* PCR result, while - indicates the absence of Sss within the sample.

[c]Sss DNA in root tissue (pg/g of root tissue). 0 indicates the absence of detection. A sample with a Cq value higher than that of the negative control sample was determined to be uninfected. NA indicates sample not tested. * indicates that the species had an average Sss DNA concentration significantly different ($p < 0.05$) from one of the positive control samples (*S. lycopersicum* Rio Grande).

[d]The number of plants out of the total number on which Sss-induced root galls were present.

DNA in the root tissue from all 28 species (including tomato) in one or both trials. Thus, all species were assumed to have been successfully infected and classified as hosts of Sss. The very low concentrations of Sss DNA recorded in the roots of most of these species may explain detection by qPCR but not by PCR. Sss pathogenic structures (zoosporangia and sporosori) that resembled those in pictures from published scientific articles were observed in roots of all species (except *A. hypogaea*), and they can thus be labelled as hosts.

All but three of the evaluated crop species were true hosts (Type I or II) of Sss, showing the presence of only sporosori or both sporosori and zoosporangia in the roots. Four species (*A. strigosa*, *C. juncea*, *M. sativa*, and *S. bicolor*) were classified as Type I hosts. The remaining 20 (including both *S. lycopersicum* cultivars) were identified as Type II (Table 2). In both trials, sporosorus and zoosporangium development were recorded as early as 14 days post-initial inoculation. All the crop species evaluated from the botanical families amaranthaceae and polygonaceae resulted in sporosorus production.

Trapping crops exhibit only zoosporangia or plasmodia in the roots and are not regarded as 'complete' Sss hosts. *Brassica alba* (White mustard; Brassicaceae), *C. gayana* (Rhodes grass; Poaceae), and *C. moschata* (Butternut squash; Cucurbitaceae) were the only crop species containing only zoosporangia and were thus classified as potential trap crops (Table 2). These three species belong to different botanical families. Initially, *P. glaucum* was classified as a trap crop based on results from the first trial, but sporosori were observed during the second trial, subsequently classifying it as a Type II host.

Root gall formation was documented on the root systems of three crop species (*B. nigra*, *C. juncea,* and *M. sativa*) at the 10-week post inoculation harvest (Fig. 1H and I; Table 2). The highest incidence was recorded on the roots of *M. sativa* in

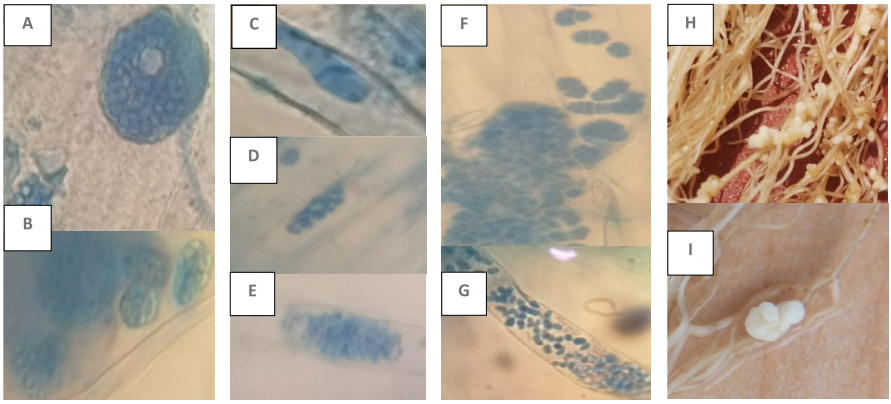

Fig. 1 *Spongospora subterranea* f. sp. *subterranea*-associated structures observed in the roots of evaluated crop species (**A-G**). Sss sporosori from the inoculum suspension observed at a magnification of 100x (**A**). Sporosori in the epidermal root cells of *Avena strigosa* (**B**). Plasmodium/immature zoosporangium in a root hair of *Brassica alba* (**C**). Zoosporangium/zoosporangia in the root hairs and root epidermal cells of *Pennisetum glaucum* (**D**), *Eragrostis tef* (**E**), *Solanum lycopersicum* (**F**), and *Vigna unguiculata* (**G**). Root galls on Medicago sativa (**H**) and Brassica nigra (**I**) roots

both trials (5/5, Trial 1 and 3/5, Trial 2). *Brassica nigra* had the second highest root gall formation incidence (3/5, Trial 1), whereas galls were only observed on one out of five *C. juncea* specimens in Trial 2. All galls observed were cream-coloured (< 3 mm in size) and similar in appearance to the surrounding healthy root tissue (Fig. 1I). None of these root galls contained visible sporosori when examined by microscopy, but galls from all three species were shown to be Sss-positive in PCR and qPCR assays in Trial 1.

The variation in morphology of Sss structures between the different crop species was evident (Fig. 1A–G). Mature sporosori (Fig. 1A) from powdery scab lesions on tubers are distinguishable from developing sporosori seen in the roots of *A. strigosa* epidermal root cells (Fig. 1B) at the 10-week harvest period. A developing zoosporangium (plasmodium) in the root hair of a *B. alba* plant can be observed in Fig. 1C. Sss zoosporangia morphology is dissimilar between distinct species, as can be seen by the various forms of zoosporangia in the roots of different species in Fig. 1D–G.

Discussion

Spongospora subterranea f. sp. *subterranea* is the soil-borne pathogen responsible for the devastating potato tuber disease, powdery scab (Jeger et al. 1996; Balendres et al. 2016b). This pathogen has a wide host range, which includes numerous crop species often rotated with potatoes (Simango et al. 2020; Tsror et al. 2020). Alternative host species of Sss can potentially allow the pathogen to complete its life cycle by the production of survival structures (sporosori) in their roots. This could increase inoculum levels in potato fields, resulting in a more severe powdery scab disease epidemic in the following potato crop (Arcila et al. 2013; Simango et al. 2020; Alaryan et al. 2023). It is thus essential to further establish the host range of Sss for improved understanding of its epidemiology, management purposes, and rotation crop selection. Pot trials were done to determine the host status of 26 selected crop species, often cultivated as rotation or cover crops in South African potato fields.

All crop species assessed in this study were shown to be Sss positive based on molecular analysis or microscopy of the root tissue (likely successfully infected) and thus hosts of some type. These results support observations made in other studies showing Sss has an extensive host range, and this expands our knowledge of the diversity of Sss hosts. Of the species tested, 23 are newly recorded hosts of Sss in South Africa, and 14 are first reports as Sss hosts globally (Jones and Harrison 1969; Qu and Christ 2006; Arcila et al. 2013; Simango et al. 2020; Tsror et al. 2020). These newly recorded hosts are *Brassica alba*, *Brassica nigra*, *Chloris gayana*, *Crotalaria juncea*, *Cucurbita moschata*, *Cucurbita pepo*, *Eragrostis tef*, *Helianthus annuus*, *Lupinus angustifolius*, *Pennisetum glaucum*, *Raphanus sativus* var. *longipinnatus*, *Sorghum bicolor*, *Vicia sativa* and *Vigna unguiculata*. They belong to five different botanical families (Amaranthaceae, Brassicaceae, Cucurbitaceae, Fabaceae, Poaceae), all of which contain species previously reported to be Sss hosts (Arcila et al. 2013; Simango et al. 2020). Thus, no new plant families have been added to the Sss host range list.

The majority of species (22/25) screened in this study were shown to be true Sss hosts. Inspection of inoculated *Arachis hypogaea* (peanut) roots and PCR assays yielded no signs of Sss. It is assumed that Sss successfully infected *A. hypogaea* (positive qPCR result), but no host-type allocation could be made due to the lack of pathogenic structures observed in roots. *Avena strigosa* (Fig. 1B), *Crotalaria juncea*, *Medicago sativa*, and *Sorghum bicolor* contained only sporosori within their infected roots during both trials and were thus classified as Type I hosts. The remaining species were classified as Type II hosts, as sporosori and zoosporangia were observed in their root hairs or epidermal cells. *Brassica alba* (white mustard), *Chloris gayana* (Rhodes grass), and *Cucurbita moschata* (butternut squash) were the only species where only the zoosporangial phase of the Sss life cycle was noted and were thus classified as potential trap crops. *Solanum lycopersicum* (tomato) is frequently used as the positive control in Sss host identification studies due to it being highly susceptible to infection by this pathogen (Jones and Harrison 1969; 1972; Qu and Christ 2006; Shah et al. 2010; Arcila et al. 2013; Clark et al. 2018; Tsror et al. 2020). Both tomato cultivars included in this study were successfully infected by Sss and allowed completion of the pathogen's life cycle during both trials; therefore, verifying the virulence of the inoculum source used.

The concentration of Sss DNA detected in roots by qPCR varied significantly ($p < 0.05$) between species and trials. Significantly higher Sss DNA concentrations were noted in most of the species in Trial 1 compared to Trial 2, indicating that the conditions during Trial 2 were probably less favourable for Sss pathogenesis than during Trial 1. Frequent load-shedding (scheduled electricity interruptions) is the most probable reason, as this affected the temperature in the greenhouse compartment. The highest Sss DNA concentrations in these greenhouse trials were detected in the roots of *C. juncea*, followed by *S. cereale* and *B. napus*. The positive control (*S. lycopersicum*; Rio Grande) species had the highest Sss DNA concentration during the second trial. This might indicate that, despite the sub-optimal temperatures during this trial, highly susceptible species were still infected. A few species, even though categorised as true hosts, had very low DNA concentrations in their roots. Sss might thus be able to complete its life cycle and produce sporosori within these species' roots when they are grown in a highly regulated and optimal environment, such as a greenhouse. The soil environment in a potato field is less conducive to Sss infection and disease development, and the same result may not be observed (Tsror et al. 2020).

Several species investigated in this study have been evaluated in past research projects. *Avena sativa* (Simango et al. 2020), *P. sativum* (Arcila et al. 2013), and *R. sativus* (Arcila et al. 2013; Tsror et al. 2020) had previously been classified as Type II hosts and this was confirmed in the present study. Simango et al. (2020) observed only sporosori (Type I) in *B. juncea* roots, but both stages of the Sss life cycle were observed in the present study. Numerous species have previously been identified as potential trap crops (Qu and Christ 2006; Simango et al. 2020). Some of these species were assessed in this trial with different results. Sporosori were observed in the epidermal root cells of *A. strigosa* (black oats), contradicting the trap crop designation made in another South African study (Simango et al. 2020). In the present study, *B. napus, S. cereale,* and *F. esculentum* were identified as true hosts (Type

II), whilst Qu and Christ (2006) had classified them as trap crops based on a 2-week post-inoculation assessment. *Avena sativa*, *H. vulgare*, *M. sativa*, *S. cereale*, and *S. bicolor* were previously classified as Sss non-hosts (Qu and Christ 2006; Simango et al. 2020; Tsror et al. 2020), but all exhibited sporosori and zoosporangia in their roots and were confirmed to be infected by Sss in the current study. Sporosori and zoosporangia were recorded in *A. hypogaea* by Tsror et al. (2020), but no pathogen structures were observed in these trials. The difference in host type allocations for the same species between studies could be due to many factors. These include different pathogen isolates (Qu and Christ 2004; Muzhinji and van der Waals 2019) or crop cultivars used in the various studies, and unfavourable trial conditions for disease development. Another reason could be that the specific root sections examined (microscopy) or tissue samples taken for molecular analysis were free from the pathogen or specific pathogenic structures. Too short a timeframe between pathogen inoculation and sampling/assessment could also result in differences in results between studies. Misidentification of the pathogen structures is also a potential reason for discrepancies, as differences in the morphology of Sss pathogenic structures make accurate identification difficult (Thapa et al. 2025). Accidental contamination of root or DNA samples, leading to false positives, is possible. All these factors could contribute to the over- or underestimation of the Sss host range.

This study supports the observations made by other authors (Merz et al. 2004; Arcila et al. 2013; Thangavel et al. 2015) that Sss infection and development of pathogenic structures (zoosporangia and sporosori) can occur 2 weeks post-inoculation. However, in some species, sporosorus development can take as long as 2 months (Arcila et al. 2013). Thangavel et al. (2015) demonstrated that potato roots are susceptible to infection by Sss throughout the plant's life cycle, which may also be the case with other non-potato crop species. Because the rate of Sss disease development might be host-dependent and the roots can be infected at any time, monitoring root infection at several intervals over longer periods when conducting host status trials is essential.

Root gall formation can be induced by Sss infection on the roots of some host plant species (Qu and Christ 2006; van de Graaf et al. 2007; Nitzan et al. 2009; Shah et al. 2010; Hernandez Maldonado et al. 2013; Johnson and Cummings 2015), including *S. lycopersicum* and *A. sativa* (Qu and Christ 2006), neither of which showed galls in the present study. Sss-induced root gall formation was, however, recorded on the root systems of *B. nigra*, *C. juncea*, and *M. sativa* for the first time. It has been hypothesised that Sss root galls form due to rapid sporosorus production in the infected root cells, as with the closely related *Plasmodiophora brassicae* (Kageyama and Asano 2009). This is not necessarily the case with Sss, as its root galls do not always contain viable or visible sporosori (Qu and Christ 2006). Root gall formation is not needed for Sss to complete its life cycle, as it has been shown that some alternative host species produce sporosori in low quantities within their root cells and root hairs (Arcila et al. 2013; Simango et al. 2020). These low levels prevent large visible galls from developing (Nitzan et al. 2009).

The Sss DNA concentrations recorded in root tissue in these trials varied, which could partly be due to the degree of susceptibility of each plant species to Sss infection. Numerous studies have been conducted in the last few decades to determine

the susceptibility of different potato cultivars to Sss root disease development (Merz et al. 2004; Nitzan et al. 2010; Hernandez Maldonado et al. 2013, 2015; van der Waals 2015b; Bittara et al. 2016). The results from these studies indicate that potato resistance to Sss infection is most likely polygenic and due to multiple resistance genes (Falloon et al. 2003; Genet et al. 2005; Balotf et al. 2021). Several plant polymers and root surface proteins have a role in pathogen attachment and encystment (Hardham and Suzaki 1986; Yu et al. 2023b). Also, the degree of susceptibility of a specific potato cultivar to Sss root infection is mainly determined by the ratio of zoospore attractant and inhibitory compounds in root exudates, the abundance of defence-associated metabolites, and the level of upregulation of important defence-related genes (Lekota et al. 2020; Amponsah et al. 2022; Balotf et al. 2022). It can be assumed that similar factors have a role in determining the susceptibility of alternative host plants to Sss root infection and, ultimately, the Sss host status of a species (Strydom et al. 2024). Those species classified as non-hosts have higher basal resistance or more effective defensive strategies, which Sss is not equipped to overcome (Kombrink and Schmelzer 2001). This can include structural defences like cell wall reinforcement (prevents penetration) or the production of antimicrobial compounds (phytoalexins). These resistance traits interfere with pathogen attachment, infection, establishment, and multiplication within the host plants (Kombrink and Schmelzer 2001; Amponsah et al. 2022; Yu et al. 2022). This could be why some Sss life stages (structures) can be completed (developed) within certain species but not others. Regarding true host species, the pathogen can evade the resistance mechanisms and modify the plant's metabolism, which allows its zoospores to infect the host plant and eventually develop more resting spores (Arcila et al. 2013). The susceptibility of each host, however, still determines the level of infection and the amount of new inoculum (sporosori) produced, as observed in the range of Sss DNA concentrations in different true host species in this study. Arcila et al. (2013) suggested that trap crops can only produce zoosporangia due to resistance-related genes in those species triggering a hypersensitive response (programmed cell death) around the site of infection, which then inhibits the pathogen from forming sporosori. This hypothesis has yet to be supported.

The morphology (size and shape) of Sss structures (zoosporangia and sporosori) can vary substantially between host plant species (Arcila et al. 2013), as can be seen in Fig. 1A and B. *Spongospora subterranea* f. sp. *subterranea* sporosori range in size from 18 to 100 µm in diameter (Falloon et al. 2006) and can contain between 155 and 1526 resting spores (Falloon et al. 2011). Mature Sss sporosori typically have a spongy or 'honeycomb' appearance (Fig. 1A) with a diverse range of shapes (irregular, spherical, elongated, oval) (Montero-Astúa et al. 2005; Arcila et al. 2013). The sporosori isolated from powdery scab lesions on tubers are usually larger than those obtained from Sss-induced root galls (Villegas et al. 2008). The morphological variation in Sss structures is mainly driven by the environment and the unique host–pathogen interactions occurring with each host species (Arcila et al. 2013). This host-dependent morphology is observed in the diversity of zoosporangia shapes, as seen in Fig. 1C–G. This can also be observed in the photos of Sss structures found in published articles (Nitzan et al. 2007; Arcila et al. 2013; Falloon et al.

2016; Balendres et al. 2016b). Each plant species assessed in this experiment had unique cell shapes and sizes, affecting the Sss zoosporangia morphology.

Crop rotation as an Sss disease management strategy has been investigated in the past to determine if it could limit inoculum accumulation and reduce the pathogen's ability to survive from one potato season to another (Harrison et al. 1997; Nitzan et al. 2007; Larkin 2008; Larkin and Lynch 2018). The indirect effect of crop rotation on Sss through enhancing the population of beneficial microbes and thus increasing the natural suppressiveness of the soil has also been assessed (Mazzola 2007; Wright et al. 2015, 2021). Earlier crop rotation-focused studies reported no significant decrease in the incidence or severity of powdery scab in the subsequent potato crop (de Boer 2000; Wale 2000). This was probably due to the combination of soil with long-lived (5 + years) Sss resting spores and short rotation cycles (de Boer 2000; Balendres et al. 2016b). The studies by Larkin and Griffin (2007) and Larkin and Lynch (2018) have, however, produced results in favour of this control method. Various species, including Brassica crops like brown mustard, white mustard, oilseed radish, and winter rapeseed, as well as some non-Brassica crops (perennial ryegrass and buckwheat), were shown to significantly reduce powdery scab disease in infested fields. White mustard, classified as a trap crop in the present study, has shown promising results in field studies (Larkin and Lynch 2018). Longer rotation cycles (3–5 years), growing tolerant potato cultivars, and rotation with Sss non-hosts have often been recommended as powdery scab management options (Harrison et al. 1997; Shah et al. 2010; Arcila et al. 2013; Simango et al. 2020; Tsror et al. 2020). Qu and Christ (2006) proposed that the Sss hosts classified as trapping crops could potentially be employed in the management of soil pathogen inoculum levels by stimulating resting spore germination but inhibiting life cycle completion (sporosori formation), thus, in theory, reducing the soil inoculum load in the field. The efficacy of this management strategy has been demonstrated in fields infested with other soil-borne pathogens. This includes a 71% reduction in *P.brassicae* soil inoculum levels after radish (*Raphanus sativus* var. *longipinnatus*) cultivation in infested fields (Murakamia et al. 2000). The crops identified as trap crops in this study can, therefore, perhaps lower Sss inoculum levels when included in a potato cropping system. The efficacy of trap crops in reducing Sss inoculum levels in the field has yet to be verified. It has also been recommended to potato growers that Sss hosts classified as true hosts should preferably not be included in crop rotation schemes or cover crop mixes cultivated in potato fields (Arcila et al. 2013; Simango et al. 2020; Tsror et al. 2020). Additionally, special emphasis should be given to removing and destroying common weed species and volunteer rotation or potato crops from the field, which may produce resting spores that can increase the Sss soil inoculum levels (de Boer 2000; Alaryan et al. 2023). Excluding true hosts would be ideal, but many rotation and cover crop species have been shown to fall in this category in the present study, which makes this management strategy impractical to potato growers.

The evaluation methods (artificially inoculated bioassays, microscopic root assessments, and molecular identification) used in identifying and classifying Sss alternative hosts in this study and others are rapid and effective but have shortcomings. Some

species had very low concentrations of pathogen DNA in this study, which could only be detected by the more accurate qPCR analysis and not the PCR assay. However, pathogenic structures were observed and resembled images in published articles (Nitzan et al. 2007; Arcila et al. 2013; Falloon et al. 2016; Balendres et al. 2016b), allowing host status allocations to be made. The PCR and microscopy results did not always correspond, so qPCR was implemented. The necessity of using multiple detection methods (morphological identification and sensitive molecular analyses) for confirming host status is not limited to Sss, but is also addressed in a review on the potato pathogen, *Phytophthora erythroseptica,* by Thapa et al. (2025). Trial repetition is also vital in ensuring accuracy, as minor variance in the trial can influence the results.

The importance of host type in determining Sss disease expression levels in subsequent potato crops is also questioned (Strydom et al. 2024). In the present study, *B. juncea* (Indian/brown mustard) was classified as a Type II host and should thus increase Sss soil inoculum and powdery scab disease, but was found to decrease Sss disease by 40% when grown in rotation with potatoes as a biofumigant (Larkin and Griffin 2007). Similar results have been observed with other true host species (*B. napus*, *F. esculentum*, and *R. sativus*) (Larkin and Griffin 2007; Larkin and Lynch 2018). qPCR assay results from our study indicate that some of the species evaluated and classed as true hosts had extremely low pathogen levels within their roots, such as *B. juncea*, *R. sativus* var. *longipinnatus*, and *C. pepo*. These alternative hosts may produce sporosori at quantities too low to significantly contribute to the soil pathogen inoculum levels, and as previously stated, species established as alternative hosts in greenhouse trials may be infected at lower rates in the field or not at all because the environment is less conducive to disease development (Clark et al. 2018; Tsror et al. 2020; Alaryan et al. 2023).

Conclusion

Future research should focus on expanding the list of crops and weed species evaluated for their host status to Sss. Additionally, long-term greenhouse and field trials must be conducted in artificially and naturally infested soils to determine the effect of different Sss host types, especially true hosts, on soil inoculum levels and Sss disease severity in subsequent potato crops. Some species might only be infected under high inoculum levels and controlled, optimal environmental conditions, but rarely in the field (Tsror et al. 2020). Meanwhile, potato growers should avoid cultivating highly susceptible cover crop species in contaminated fields (e.g. sunn hemp, stooling rye, and forage rape) until additional field-based studies have been conducted. If the cultivation of trap crops or non-host species is found to be effective at inoculum maintenance or reduction, it would be advised to include the three species identified in this study in rotation programmes. Trap crop cultivation would, however, not be completely effective as an Sss control strategy, as the relationship between the initial level of soil inoculum and the final incidence and severity of Sss-induced disease remains unclear. Hence, a continuous integrated management approach for Sss is advised, which should include crop rotation, trap crop cultivation, and adequate field sanitation, such as volunteer plant removal.

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Declarations

Conflict of interest The authors declare no competing interests.

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