Alternative hosts of *Spongospora subterranea* f. sp. *subterranea* in southern Africa

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Abstract

Spongospora subterranea f. sp. *subterranea* (Sss) is an obligate parasite that can only multiply within the living tissues of a host plant. To determine the host status of Sss in some of the crops and weeds commonly found in southern African fields, two greenhouse experiments were carried out. These experiments used both qPCR and light microscope examination to study the development of Sss in the roots of weeds and crops grown in inoculated soil. Zoosporangia and sporosori were observed microscopically in weed species *Acanthospermum hispidum, Ageratum conyzoides, Bidens pilosa, Cleome monophylla*, *Datura stramonium*, *Euphorbia heterophylla, Oxalis latifolia, Physalis angulata*, *Setaria pumila* and *Tagetes minuta,* whilst *Ipomea plebia, Setaria verticillata* and *Galinsoga parviflora* were non-hosts of Sss. Root galls were only observed on *D. stramonium* roots. The crops *Allium cepa*, *Solanum lycopersicum*, *Zea mays, Avena sativa* and *Triticum aestivum* were both zoosporangial and sporosorial hosts, whilst *Phaseolus vulgaris* and *Brassica juncea* were sporosorial hosts only. This study has added three new families to namely Oxalidaceae, Euphorbiaceae and Coppardaceae to the hosts of Sss. Two zoosporangial hosts were identified, namely *Avena strigosa* and *Glycine max.* These crops would be ideal to be included as trap crops in rotations with potatoes. The highest quantity of Sss DNA in weed species was recorded in the roots of *P. angulata, N. physalodes* and *A. conyzoides,* whilst in cultivated crops the highest quantity of Sss DNA was recorded in *Brassica juncea* followed by *Avena strigosa.* The study has shown the importance of weeds and some crops used in rotation with potatoes as alternative hosts of Sss and their potential to increase Sss inoculum level in the soil.

Keywords: alternative hosts, powdery scab, sporosori, zoosporangia

Introduction

The causal agent of powdery scab on potato is a soil-borne plasmodiophorid, *Spongospora subterranea* (Wallroth) Lagerheim f. sp. *subterranea* Tomlinson (Sss). The disease produces unsightly blemishes on the tuber surface, causing serious reductions in tuber quality and marketability (Karling 1968). In addition to this disease, Sss also causes root hyperplasia, giving rise to galls; and infects root hairs, which decreases root function, resulting in decreased absorption

of nutrients and water (Falloon et al. 2016). The pathogen is also a vector for the potato mop-top virus, which leads to growth reduction as well as internal tuber necrosis (Jones and Harrison 1969).

Spongospora subterranea f. sp. *subterranea* is an obligate parasite that can only multiply within the living tissues of a susceptible host plant (Walsh et al. 1996; Merz et al. 2005; Qu and Christ 2006). It belongs to the plasmodiophorids, which produce resting spores and zoospores (Falloon 2008). Plasmodiophorids fall under the phytomyxids, where large scale host shifts have been reported between closely related obligate biotrophic species (Neuhauser et al. 2014). Recently, phytomyxids have been reported to be able to readily adapt to a wide diversity of new hosts because they have retained the ability to covertly infect alternative hosts (Neuhauser et al. 2014), thereby threatening the productivity of key species in terrestrial and marine environments alike, via host shift speciation.

The life cycle of Sss has been described (Braselton 1995; Harrison et al. 1997; Merz 2008; Balendres et al. 2016) and this has helped in understanding of the biology of the pathogen. There are two major phases in the life cycle of a plasmodiophorid (Braselton 1995; Merz 2008; Kageyama and Asano 2009; Schwelm et al. 2015). These are the sporangial (primary) and sporogenic (cytogenous/secondary) phases. Merz (2008) described the sporangial phase as a stage where numerous secondary zoospores are formed in compartments within thin-walled zoosporangia. This is then followed by the secondary phase in which Sss produces sporosori, which are the resting structures of the pathogen. Resting spores germinate under favourable conditions to produce primary zoospores (Harrison et al. 1997).

Spongospora subterranea f. sp. *subterranea* has a wide host range, which includes plants belonging to the following families: Alliaceae, Aizoaceae, Amaranthaceae, Apiaceae, Asteraceae, Boraginaceae, Brassiccaceae, Caryophyllaceae, Chenopodiaceae, Coniferae, Convolvulaceae, Cyperaceae, Fabaceae, Lamiaceae, Papaveraceae, Plantaginaceae, Poaceae, Polygonaceae, Ranunculaceae, Resedaceae, and Solanaceae (Jones and Harrison 1972; Andersen et al. 2002; Qu and Christ 2006; Nitzan et al. 2009; Shah et al. 2010; Arcila et al. 2013). Cultivated crops that are hosts of Sss include maize, onion, tomato, oat, yellow mustard, oilseed radish, and rapeseed (Qu and Christ 2006; Arcila et al. 2013).

There are different classes of hosts of Sss; namely type 1, type 2 and trapping hosts (Arcila et al. 2013). Type 1 hosts are sporosorial hosts only, whilst type 2 are both zoosporangial and sporosorial hosts of Sss. The third group are the zoosporangial hosts which are also called “trapping hosts”. These zoosporangial hosts prevent the completion of the life cycle of Sss, consequently leading to a reduction in the inoculum level in the soil (Harrison et al. 1997; Qu and Christ 2006). Trapping hosts play an important role in the integrated management of the diseases caused by Sss. It is necessary to keep fields free of the type 1 and 2 hosts of the pathogen even during periods when the fields are left fallow.

Alternative hosts of Sss provide a means of survival for Sss over long periods of time (Falloon 2008). Thus far, no studies have been conducted to assess the susceptibility to Sss of crops and weeds commonly found in potato fields in southern Africa. Therefore, the objective of the study was to determine the Sss host status of selected cultivated crops and weeds commonly found in potato fields in southern Africa.

2 Materials and methods

2.1 Study site

Two greenhouse experiments were carried out, with the first one being done at University of Pretoria (South Africa) and repeated in Zimbabwe at the Scientific and Industrial Research and Development Centre, while and the second experiment was carried out at the University of Pretoria only.

2.2 Weeds and crop plants used in the study

Fourteen weeds commonly found in southern African potato fields were used in the first experiment (Table 1), whilst 12 field crops were used in the second experiment (Table 1).

Table 1. Weeds and field crops used in used in the first and second *Spongospora subterranea* f. sp. *subterranea* host range experiment

Botanical name	Common English name	Family
Acanthospermum hispidum	Upright star bur	Asteraceae
Ageratum conyzoides	Billy goat weed	Asteraceae
Allium cepa	Onion	Amarylidaceae
Avena sativa	White oat	Poaceae
Avena strigosa	Black oat	Poaceae
Bidens pilosa	Black jack	Asteraceae
Brassica juncea	Indian mustard	Brassicaceae
Brassica oleracea	Cabbage	Brassicaceae
Cleome monophylla	Spindlepod	Cappardaceae
Datura stramonium	Jimsonweed	Solanaceae
Daucus carota	Carrot	Apiaceae
Euphorbia heterophylla	Wild poinsettia	Euphorbiaceae
Galinsoga parviflora	Gallant soldier	Asteraceae
Glycine max	Soybean	Leguminosae
Ipomoea plebia	Sabi morning glory	Convolvulaceae
Nicandra physalodes	Apple of Peru	Solanaceae
Oxalis latifolia	Purple garden sorrel	Oxalidaceae
Physalis angulata	Wild gooseberry	Solanaceae
Raphanus raphanistrum	Oilseed radish	Brassicaceae
Secale cereale	Rye	Poaceae
Setaria pumila	Annual Timothy grass	Poaceae
Setaria verticillata	Burgrass	Poaceae
Sorghum bicolor	Grazing sorghum	Poaceae
Tagetes minuta	Mexican marigold	Asteraceae
Triticum aestivum	Wheat	Poaceae
Zea mays	Maize	Poaceae

2.3 Planting, management and sampling of the plants

2.3.1. First experiment (weed species)

Weed seeds were germinated in steam pasteurised sandy soil. At three leaf stage, they were transferred to plastic containers with Merz nutrient solution (Merz 1989). The Merz nutrient solution was composed of 710 mg calcium nitrate, 505 mg potassium nitrate, 492 mg magnesium sulphate, 272 mg potassium phosphate, 20 mg Fe EDTA and 1 ml of Hoagland solution in one liter of distilled water. The plastic potting containers were wrapped in aluminum foil and placed in a growth chamber where temperature was maintained at 20 °C with 16 hours of light. A Randomized Complete Block Design (RCBD) was used with three replicates per weed species. Sixteen plants per replicate were used, where eight were inoculated, whilst the other eight were not and served as the negative control. Each container was watered to saturation point at planting and thereafter every second day to maintain constant soil moisture. The experiment was repeated.

2.3.2. Second experiment (field crops)

Crop seeds were germinated in steam pasteurised sandy-loam soil, in plastic pots with a top diameter of 22 cm, a base diameter of 17 cm and height of 21 cm. Throughout the trial the greenhouse compartment temperature was maintained at 22 °C with 16 hours of light. The experiment was laid out as a RCBD with three replicates of each crop. Twelve plants were used per replicate, where six were inoculated with Sss and six remained uninoculated to serve as negative controls. Each pot was watered with 1080 ml of water on the day of planting to achieve saturation point and furthermore watered with an additional 150 ml every morning. All watering times and quantities were adjusted to the crops' growth stages and kept consistent by using an automated sprinkler system. This procedure ensured consistency amongst the crops and in the repeat trial.

2.4 Inoculum preparation and inoculation

Spongospora subterranea f. sp. *subterranea* inoculum was prepared by scraping peels off heavily scabbed potato tubers. An Sss AgriStrip test (BIOREBA AG, Switzerland) was performed on the ground scabby peels to confirm identity of the pathogen. After confirming Sss to be the causal agent of the tuber lesions, peels were air dried in envelopes at room temperature. The peels were then ground using a mortar and pestle. The powder obtained was sieved through a 0.5 mm sieve and suspended in sterile distilled water. A heamocytometer mounted on a light microscope was used to determine the concentration of the inoculum suspension. The suspension was adjusted to a final concentration of 1.0×10^4 sporosori per 1 ml of distilled water. When the seedlings reached the three leaf stage, they were inoculated with Sss at an equivalent of 500 000 sporosori/gram of soil or per ml or Merz Solution. Sterile water without sporosori was added to the control plants.

2.5 Root infection assessment

The first root infection assessment in the weed experiment was done three days after inoculation with Sss. The remaining plants were then transferred at 15 days after inoculation into pots (20 cm top diameter x 15 cm base diameter x 16 cm height) containing sterilized loamy soil and allowed to grow to maturity for three to four months depending on the crop. The second and third root assessments were done at transfer (15 days post inoculation), and 45 days post inoculation, respectively.

In the crop experiment, the plants were harvested 12 weeks after planting. For both experiments, the plants were carefully removed from the soil and the roots were then gently rinsed twice with sterile distilled water. After rinsing, five roots from each plant were chosen randomly, removed using a sterile scalpel and tweezers and placed in 1.5 ml Eppendorf tubes. These subsamples were used for microscopy. In addition to this, a further five roots per plant were removed, macerated in liquid nitrogen using a pestle and mortar, and placed into BioReba Bashing beads lysis tubes for DNA extraction.

Root infection was assessed by examination under a light microscope, using a method modified from Merz (1989). Roots were destained with destaining solution (ethanol/chloral hydrate/H_2O - 1:1:1 w/w/w) for 10 minutes, stained with a staining solution (3% formaldehyde, 3.5% phenol, 6% lactic acid, 87.2% ethanol/water and 0.3% water blue; all w/w) for 5 minutes and then fixed with lactic acid for 5 minutes (Merz 1989). Root samples were then examined microscopically at 100x magnification. Compound microscope Zeiss 195-041847 (Germany) was used in South Africa whilst Optika B-293PLi (Italy) was used in Zimbabwe. Zoosporangia infection was rated using a modification of the following scale: 0 = no zoosporangia; 1 = only a few zoosporangia on roots; 2 = 3–5 roots with zoosporangia; 3 = 6–9 roots with zoosporangia, moderate infection; 4 = >10 roots with zoosporangia, heavy infection (Merz 1989).

The observations that were recorded from the microscopy were based on first examining the negative control roots of each species. This was done to establish a point with which to compare infected roots. All observations were compared to images of known Sss infected roots from published articles (Merz 1997; Iftikhar et al. 2002).

2.5.1 DNA extraction and real time PCR

DNA was extracted from 0.5 g root tissue of each sample using a ZR Soil Microbe DNA kit™ (Zymo Research Corp, USA) according to the manufacturer's instructions. Real time PCR was done at the University of Pretoria (South Africa). Standards for qPCR were prepared using DNA extracted from suspensions with known sporosori concentrations (1, 10, 100, 1 000 and 10 000 and 100 000 sporosori per 1 ml of distilled water, respectively) as described by van de Graaf et al. (2003). The DNA was diluted with TE buffer to obtain a dilution series of DNA equivalent to 0.01, 0.1, 1, 10 and 100 sporosori per µl, which was then subsequently used to obtain a standard curve for estimating the concentration of the unknown samples. The tissue DNA was quantified using a ThermoScientific PikoReal qPCR machine using the protocol described by van de Graaf et al. (2003). Quantification of the Sss DNA in the roots of the plants was done at 15 and 45 days after inoculation.

2.6 Root galling assessment

Root gall severity scoring was done at 45 days after inoculation using the rating scale of van de Graaf et al. (2007), where 0 = no root galls, 1 = one or two root galls, 2 = several galls, mostly small (< 2 mm in diameter), 3 = many galls, some > 2 mm in diameter, and 4 = most major roots with galls, some or all > 4 mm in diameter.

3 Data analysis

Analysis of variance (ANOVA) on the quantity of Sss DNA in the roots of the various plants was calculated using GenStat® (Payne 2015). Mean separations were done using Fischer's protected LSD (5%).

4 Results

4.1 Root infection assessment

Both zoosporangia and sporosori were present in the roots of the weed species *A. hispidum*, *A. conyzoides, B. pilosa, C. monophylla, D. stramonium*, *E. heterophylla, N. physalodes, O. latifolia, P. angulata*, and *T. minuta,* and the crops *A. cepa*, *A. sativa*, *T. aestivum* and *Z. mays,* making these type 2 hosts (Tables 3 and 4). Only sporosori were observed in the roots of *S. pumila* and *B. juncea* making these species type 1 hosts (Tables 2). The only two crops containing zoosporangia alone were *A. strigosa* and *G. max,* indicating that these plants could be used as trapping hosts.

4.2. Quantification of Sss in the roots

Physalis angulata was recorded to have the highest concentration of Sss DNA both at 15 and 45 days after inoculation. This was followed by *N. physalodes*. The other weed species that recorded significantly ($p<0.05$) high concentrations of Sss DNA were *A. conyzoides*, *A. hispidum*, *E. heterophylla* and *O. latifolia*. Significantly ($p<0.05$) low Sss DNA concentrations were recorded in roots of *I. plebia*, *S. verticillata* and *G. parviflora* (Fig 1). The quantity of Sss DNA in roots of various crop species at 45 days after inoculation is shown in Figure 2. *Brassica juncea* recorded the highest concentration of Sss DNA followed by *A. strigose*, whilst *A. cepa* had the lowest Sss DNA concentration.

4.3 Root galling

Root galls were observed on the roots of *D. stramonium* only.

Table 2. Host status of *Spongospora subterranea* f. sp. *subterranea* in weeds and field crops, based on the presence or absence of sporosori and / or zoosporangia in roots

Botanical name	Common English name	Family	*Zoosporangia	*Sporosori	Host type	*Root galls
Acanthospermum hispidum	Upright star bur	Asteraceae	+	+	Type 2	-
Ageratum conyzoides	Billy goat weed	Asteraceae	+	+	Type 2	-
Allium cepa	Onion	Amarylidaceae	+	+	Type 2	
Avena sativa	White oat	Poaceae	+	+	Type 2	
Avena strigosa	Black oat	Poaceae	+	-	Trapping	
Bidens pilosa	Black jack	Asteraceae	+	+	Type 2	-
Brassica juncea	Indian mustard	Brassicaceae	-	+	Type 1	
Brassica oleracea	Cabbage	Brassicaceae	-	-	Non host	
Cleome monophylla	Spindlepod	Coppardaceae	+	+	Type 2	-
Datura stramonium	Jimsonweed	Solanaceae	+	+	Type 2	+
Daucus carota	Carrot	Apiaceae	-	-	Non host	
Euphorbia heterophylla	Wild poinsettia	Euphorbiaceae	+	+	Type 2	-
Galinsoga parviflora	Gallant soldier	Asteraceae	-	-	Non host	-
Glycine max	Soybean	Leguminosae	+	-	Trapping	
Ipomoea plebia	Sabi morning glory	Convolvulaceae	-	-	Non host	-
Nicandra physalodes	Apple of Peru	Solanaceae	+	+	Type 2	-
Oxalis latifolia	Purple garden sorrel	Oxalidaceae	+	+	Type 2	-
Physalis angulata	Wild gooseberry	Solanaceae	+	+	Type 2	-
Raphanus raphanistrum	Oilseed radish	Brassicaceae	-	-	Non host	
Secale cereale	Rye	Poaceae	-	-	Non host	
Seteria pumila	Annual Timothy grass	Poaceae	-	+	Type 1	-
Seteria verticillata	Burgrass	Poaceae	-	-	Non host	-
Sorghum bicolor	Grazing sorghum	Poaceae	-	-	Non host	
Tagetes minuta	Mexican marigold	Asteraceae	+	+	Type 2	-
Triticum aestivum	Wheat	Poaceae	+	+	Type 2	
Zea mays	Maize	Poaceae	+	+	Type 2	

*+ stands for positive or present whilst – stands for negative or absent.

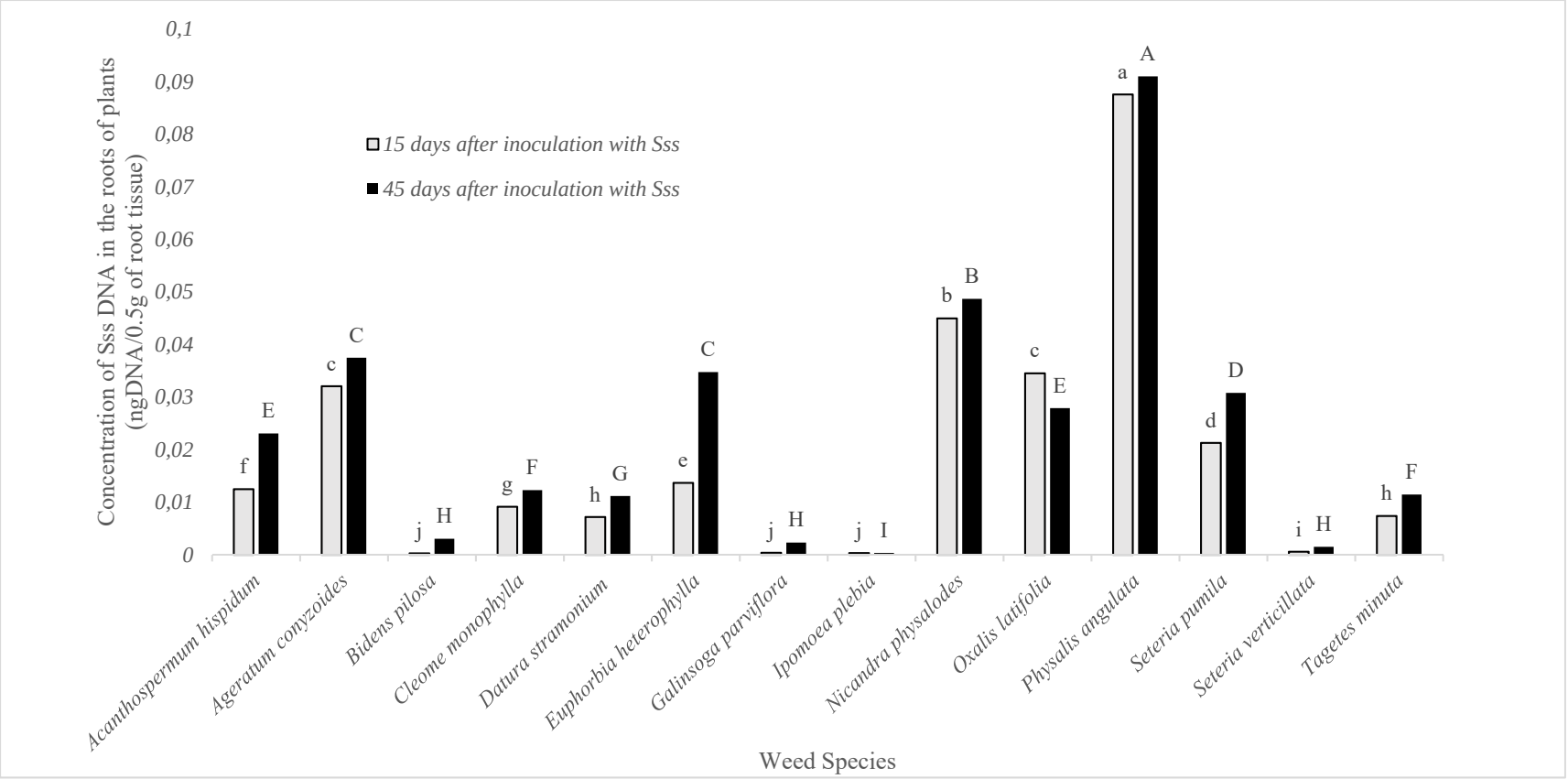

Figure 1. Quantity of *Spongospora subterranea* f. sp. *subterranea* DNA (ng DNA/0.5 g of root tissue) in the roots of various weed species at 15 and 45 days after inoculation. Bars with different letters indicate significant differences at $p<0.05$.

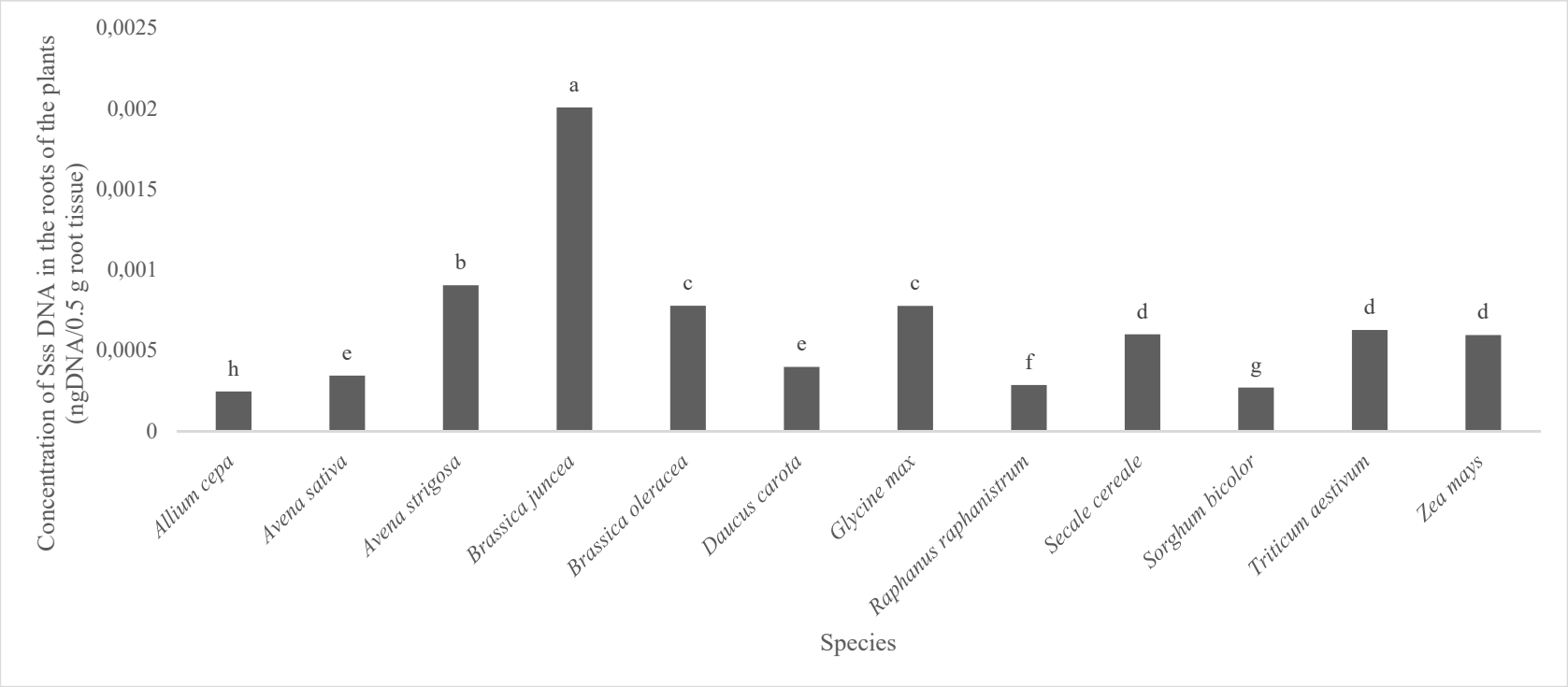

Figure 2. Quantity of *Spongospora subterranea* f. sp. *subterranea* DNA (ng DNA/0.5g of root tissue) in the roots of various crop species at 45 days after inoculation. Bars with different letters indicate significant differences at $p<0.05$.

5 Discussion

Spongospora subterranea f. sp. *subterranea* has been reported to have a wide host range (Jones and Harrison 1972; Andersen et al. 2002; Qu and Christ 2006; Nitzan et al. 2009; Shah et al. 2010; Arcila et al. 2013; Tsror et al. 2019). These hosts belong to the Alliaceae, Aizoaceae, Amaranthaceae, Apiaceae, Asteraceae, Boraginaceae, Brassiccacea, Caryophyllaceae, Chenopodiaceae, Coniferae, Convolvulaceae, Cyperaceae, Fabaceae, Lamiaceae, Malvaceae, Papaveraceae, Plantaginaceae, Poaceae, Polygonaceae, Ranunculaceae, Resedaceae, Solanaceae and Zygophyllaceae families. This study added three families to the list of host families of Sss, namely Oxalidaceae, Euphorbiaceae and Coppardaceae. Therefore, there is a great possibility that some hosts of Sss, such as weeds, might aid in the persistence and increase of Sss in the soil if they are not controlled.

The Solanaceae species tested in this study, namely *D. stramonium*, *N. physalodes* and *P. angulata* were all both zoosporangial and sporosorial hosts of Sss. *Physalis angulata* and *N. physalodes* have not previously been reported as hosts of Sss whilst *D. stramonium* has previously been reported to be a host of Sss (Qu and Christ 2006; Arcila et al. 2013). However, an earlier study by Qu and Christ (2006) reported *D. stramonium* to be a zoosporangial host only, but Arcila et al. (2013) found it to be both a zoosporangial and sporosorial host of Sss. The current study corroborates with what was reported by Arcila et al. (2013) since sporosori were also found in the roots of *D. stramonium,* making it a type 2 host. *Spongospora subterranea* f. sp. *subterranea* DNA quantification results showed a significantly ($p<0.05$) lower Sss DNA quantity in the roots of *D. stramonium* at 15 and 45 days after inoculation compared to other hosts in the Solanaceae family such as *P. angulata* and *N. physalodes.* The differences in terms of the quantity of Sss DNA amongst these Solanaceae hosts could indicate variation in susceptibility of these species to Sss. Differential responses in susceptibility to Sss have also been reported in potato varieties (Falloon et al. 2003; Nitzan et al. 2008; Maldonado et al. 2013; Falloon et al. 2015). It has been suggested that host resistance to diseases caused by Sss is likely to be influenced by different host genetic, biochemical or morphological factors (Falloon et al. 2015).

The majority of Asteraceae species tested in this study, namely *A. hispidum*, *A. conyzoides*, *B. pilosa* and *T. minuta* were both zoosporangial and sporosorial hosts, whilst *G. parviflora* was a considered to be a non-host of Sss, due to the absence of pathogen structures in the roots. Asteraceae family members such as *P. nepalense* and *P. clandestinum* have previously been reported to be hosts of Sss (Betancur et al. 2011). This is the first time that *A. hispidum*, *A. conyzoides*, *B. pilosa* and *T. minuta* are reported to be hosts of Sss. *Acanthospermum hispidum* recorded the highest Sss DNA quantity followed by *A. conyzoides* whilst *T. minuta* and *B. pilosa* recorded the lowest.

Five of the eight Poaceae species tested in this study namely *A. sativa*, *A. strigosa*, *T. aestivum*, *Z. mays* and *S. pumila* were hosts of Sss, whilst *S. verticillata*, *S. cereale*, *S. bicolor* were non-hosts. *Zea mays* has been reported to be a host of Sss (Arcila et al. 2013) but previous studies by Iftikhar and Ahmad (2005) reported it to be only a zoosporangial host of Sss. Qu and Christ (2006) did not find zoosporangia or sporosori in the roots of *Z. mays*. The Sss DNA quantity in the roots of *Z. mays* was very low compared to the other Poaceae species used in the study. This study also corroborates with what was reported by Qu and Christ (2006) that *A. sativa* is both a zoosporangial and sporosorial host of Sss whilst *S. bicolor* is a non-host. Qu and Christ (2006) reported that *S.*

cereale is a zoosporangial host of Sss, whilst it was shown to be a non-host in this study. *Seteria pumila* was found to be a sporosorial host of Sss, making it a type 1 host.

Low to medium quantities of Sss DNA were detected by qPCR in the roots of weeds and crops considered in this study to be the non-hosts, based on the absence of pathogen structures in the roots. These include *G. parviflora*, *I. plebian, S. verticillata, B. oleracea, D. carota, R, raphanistrum, S. cereale* and *S. bicolor*. Tsror et al. (2019) however, reported *S. verticillata* to be a host of Sss. In their artificial inoculation experiment only one out of six plants were positive for Sss using microscopy, while two out of five were positive when tested with qPCR. The low Sss DNA quantities detected in the roots of *G. parviflora*, *I. plebia, S. verticillata, B. oleracea, D. carota, R, raphanistrum, S. cereale* and *S. bicolor* in this study might therefore indicate that these plants could act as hosts of Sss, even though no Sss structures were observed under light microscopy. Alternatively, the amplification of DNA extracted from the roots of these plants might have been due to Sss sporosori which remained attached to the exterior surface of roots after washing. The experiments should be repeated to confirm results.

In contrast to type I or II hosts, trapping crops could be used in crop rotation with a susceptible host, such as potatoes, as they may reduce inoculum levels in the soil. This is an important aspect of an integrated disease management programme (Harrison et al. 1997; Arcila et al. 2013; Tsror et al. 2019). Trap plants such as soybean and black oats would be ideal in a crop rotation system due to their ability to obstruct the completion of the life cycle of the pathogen, thus preventing the production of secondary zoosporangia or sporosori (Qu and Christ 2006). The inability of non-host plants to become infected by Sss could be due to basal resistance, cell wall rigidity, poor contact of pathogen with host root or the production of phytoalexins by the host plant roots, preventing the pathogen from infecting or invading the root cells (Lipka et al. 2008).

Root galls were found on the roots of tomato and *D. stramonium* whilst the other plants tested in this study did not produce galls. The absence of root galls in Sss hosts, despite the presence of zoosporangia in the root, has previously been reported by Qu and Christ (2006), van de Graaf et al. (2007), Arcila et al. (2013) and Tsror et al. (2019), where many species of plants were inoculated with different sporosori concentrations, and became infected but did not develop root galls.

Spongospora subterranea f. sp. *subterranea* has been reported to persist in the soil for more than ten years (Falloon 2008) and alternative hosts of Sss could be partly responsible for the persistence of the pathogen. Studies by Burnett (1991) and van de Graaf et al. (2005) concluded that soil inoculum concentration of Sss did not significantly affect the incidence or severity of either tuber infection or powdery scab symptoms at maturity. Van der Graaf et al. (2005) further states that once infection has been established in the potato roots, secondary zoospores released from zoosporangia greatly reduce the importance of soil inoculum. This is the first study on the alternative weed and crop hosts of Sss in South Africa, and the results presented here will assist in development of an effective management strategy for Sss. Management or control of alternative hosts of Sss, both weeds and cultivated plants, and selection of the correct crops in a rotation cycle can assist in lowering Sss inoculum in the soil.

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References

Andersen, B.A.B., Nicolaisen, M., Nielsen, S.L. (2002). Alternative hosts for potato mop-top virus, genus Pomovirus and its vector *Spongospora subterranea* f. sp. s*ubterranea*. *Potato Research*, 45, 37-43.

Arcila, A.I.M., González Jaimes, E.P., Amaya, Z., María, C., Cotes Torres, J.M. (2013). Alternate hosts of *Spongospora subterranea* f. sp. *subterranea* identification in Colombia by bioassay. *Revista Faculty Nacional de Agronomía, Medellín*, 66(2), 6987-6998.

Arcila, I.M.A., Jaimes, E.P.G., Amaya, C.M.Z., Torres, J.M.C. (2013). Alternate hosts of *Spongospora subterranea* f. sp. *subterranea* identification in Colombia by bioassay. *Revista Faculty Nacional de Agronomia, Medellin*, 66(2), 6987-6998.

Balendres, M.A., Tegg, R.S., Wilson, C.R. (2016). Key events in pathogenesis of *Spongospora* diseases in potato: a review. *Australasian Plant Pathology*, 45, 229-240.

Betancur, G.J., Arcila, A.I., Zuluaga, A.C., Gonzalez, J.E. (2011). Identification of alternate *Spongospora subterranea* (Wallroth) *Lagerheim* f. sp. *subterranea* hosts in natural conditions in La Union and Santa Rosa de Osos municipalities. pp 68 In Proceedings of XXX Colombian Congress and XVI Latin American Phytopathology Congress. Bogota.

Braselton, J.P. (1995). Current status of the Plasmodiophorids. *Critical Reviews in Microbiology*, 21, 263-275.

Bulman, S.R., Marshall, J.W. (1998). Detection of *Spongospora subterranea* in potato tuber lesions using the polymerase chain reaction (PCR). *Plant Pathology*, 47(6), 759-766.

Burnett, F. (1991). The biology and control of powdery scab (*Spongospora subterranea*) of potatoes. Aberdeen, UK: University of Aberdeen, PhD Thesis.

Falloon, R.E. (2008). Control of powdery scab of potato: Towards an integrated disease management. *American Journal of Potato Research*, 85, 253-260.

Falloon, R.E., Genet, R.A., Wallace, A.R., Butler, R.C. (2003). Susceptibility of potato (*Solanum tuberosum*) cultivars to powdery scab (caused by *Spongospora subterranea* f. sp. *subterranea*), and relationship between tuber and root infection. *Australasian Plant Pathology*, 32, 377-385.

Falloon, R.E., Merz, U., Butler, R.C., Curtin, D., Lister, R.A., Thomas, S.M. (2015). Root infection of potato by *Spongospora subterranea*: knowledge review and evidence for decreased plant productivity. *Plant Pathology*, 65(3), 422-434.

Harrison, J.G., Searle, R.J., Williams, N.A. (1997). Powdery scab of potato – a review. *Plant Pathology*, 46, 1-25.

Iftikhar, S., Ahmad, I. (2005). Alternate hosts of *Spongospora subterranea* f. sp. *subterranea,* the causal organism of powdery scab of potato. Abstracts of papers presented at the 88th Annual Meeting of the Potato Association of America. Scottsbluff, Nebraska, August 8-12, 2004. *American Journal of Potato Research*, 82(1), 57-97.

Jones, R. A. C., & Harrison, B. D. (1969). The behaviour of potato mop-top virus in soil, and evidence for its transmission by *Spongospora subterranea* (Wallr.) Lagerh. *Annals of Applied Biology*, 63(1), 1-17.

Jones, R.A.C & Harrison, B.D. (1972). Ecological studies on potato mop-top virus in soil in Scotland. *Annals of Applied Biology*, 71, 47-57.

Kageyama, K., Asano, T. (2009). Life cycle of *Plasmodiophora brassicae*. *Journal of Plant Growth Regulation*, 28, 203-211.

Karling, J. S. (1968). The Plasmodiophorales. *The Plasmodiophorales*. (2nd edition).

Lipka, U., Fuchs, R., Lipka, V. (2008). Arabidopsis non-host resistance to powdery mildews. *Current Opinion in Plant Biology*, 11(4), 404-411.

Maldonado, H.M.L., Falloon, R.E., Butler, R.C., Conner, A.J., Bulman, S.R. (2013). *Spongospora subterranea* root infection assessed in two potato cultivars differing in susceptibility to tuber powdery scab. *Plant Pathology*, 62, 1089-1096.

Mäkäräinen, E., Rita, H., Teperi, E., Valkonen, P.T. (1994). Resistance to *Spongospora subterranea* in tuber-bearing and non-tuber-bearing *Solanum* spp. *Potato Research*, 37(2), 123-127.

Merz, U. (2008). Powdery scab of potato - occurrence, life cycle and epidemiology. *American Journal of Potato Research,* 85, 241-246.

Merz, U. (1992). Observations on swimming pattern and morphology of secondary zoospores of *Spongospora subterranea*. *Plant Pathology*, 41, 490-494.

Merz, U. (1989). Infectivity, inoculum density and germination of *Spongospora subterranea* resting spores: a solution-culture test system. *European Plant Protection Organization Bulletin*, 19, 585-92.

Merz, U., Walsh, J.A., Bouchek-Mechiche, K., Oberhansli, T.H., Bitterlin, W. (2005). Improved immunological detection of *Spongospora subterranea*. *European Journal of Plant Pathology*, 110, 71-77.

Neuhauser, S., Kirchmair, M., Bulman, S., Bass, D. (2014). Cross-kingdom host shifts of phytomyxid parasites. *BMC Evolutionary Biology*, 14, 33.

Nitzan, N., Boydston, R., Batchelor, D., Cosslin, J., Hamlin, L., Brown, C. (2009). Hairy nightshade is an alternative host of *Spongospora subterranea*, the potato powdery scab pathogen. *American Journal of Potato Research*, 86, 297-303.

Nitzan, N., Cummings, T.F., Johnson, D.A., Miller, J.S., Batchelor, D.L., Olsen, C., Quick, R.A., Brown, C. (2008). Resistance to root galling caused by the powdery scab pathogen *Spongospora subterranea* in potato. *Plant Disease*, 92, 1643-1649.

Payne, R.W. (2014) (Ed.) Introduction to GenStat® *for Windows*™ (18th Edition), VSN International, Hemel Hempstead, Hertfordshire, UK. © VSN International.

Qu, X., Christ, B. (2006). The host range of *Spongospora subterranea* f. sp. *subterranea* in the United States. *American Journal of Potato Research*, 83, 343-347.

Schwelm, A., Fogelqvist, J., Knaust, A., Jülke, S., Lilja, T., Bonilla-Rosso, G., Karlsson, M., Shevchenko, A., Dhandapani, V., Choi, S.R., Kim, H.G., Park, J.Y., Lim, Y.P., Müller, J.L., Dixelius, C. (2015). The *Plasmodiophora brassicae* genome reveals insights in its life cycle and ancestry of chitin synthesis. *Scientific Reports*, 5, 1-14.

Shah, F.A., Falloon, R.E., Bulman, S.R. (2010). Nightshade weeds (*Solanum* spp.) confirmed as hosts of the potato pathogens *Melodogyne fallax* and *Spongospora subterranea* f. sp. *subterranea*. *Australasian Plant Pathology*, 39**,** 492-498.

Sparrow, L.A., Rettke, M., Corkrey, S.R. (2015). Eight years of annual monitoring of DNA of soil-borne potato pathogens in farm soils in south eastern Australia. *Australasian Plant Pathology*, 44, 191-203.

Walsh, J.A., Merz, U., Harrison, J.G. (1996). Serological detection of sporosori of *Spongospora subterranea* and quantification in soil**.** *Plant Pathology*, 45, 884-895.

van de Graaf, P., Lees, A.K., Cullen, D.W., Duncan, J.M. (2003). Detection and quantification of *Spongospora subterranea* in soil, water and plant tissue samples using real time PCR. *European Journal of Plant Pathology*, 109, 589-597.

van de Graaf, P., Lees, A.K., Wale, S.J., Duncan J.M. (2005). Effect of soil inoculum level and environmental factors on potato powdery scab caused by *Spongospora subterranea*. *Plant Pathology*, 54, 22-28.

van de Graaf, P., Wale, S.J., Lees, A.K. (2007). Factors affecting the incidence and severity of *Spongospora subterranea* infection and galling in potato roots. *Plant Pathology*, 56, 1005-1013.