



# Detection and eradication of *Spongospora subterranea* in mini-tuber production tunnels

**Authors:**

Jessica Wright<sup>1</sup>  
Alison K. Lees<sup>2</sup>  
Jacquie E. van der Waals<sup>1</sup>

**Affiliations:**

<sup>1</sup>Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa

<sup>2</sup>The James Hutton Institute, Invergowrie, Dundee, United Kingdom

**Correspondence to:**

Jacquie van der Waals

**Email:**

jacquie.vdwaals@up.ac.za

**Postal address:**

Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, South Africa

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Powdery scab, a root and tuber disease caused by the pathogen *Spongospora subterranea* f.sp. *subterranea* (*Sss*), poses a major problem to potato producers worldwide because it affects potato quality. Inoculum can be seed-borne or originate from contaminated growing media or contaminated equipment. During 2006, a potato mini-tuber production facility in Ceres in the Western Cape Province of South Africa had an outbreak of powdery scab. The purpose of this study was to detect *Sss* in the production facility and identify the source or sources of contamination so that corrective measures could be taken to eradicate the pathogen. Swab samples were taken from numerous points in the facility in 2009 and *Sss*-specific primers (*Sps1* and *Sps2*) were used in a polymerase chain reaction to detect *Sss*. Of 11 surfaces tested, 6 were positive for *Sss*. A second set of swab samples was taken after efforts were made to eradicate the pathogen through improved facility hygiene measures to determine whether these corrective measures were efficient. Corrective measures resulted in a disease-free harvest from 2009 onwards. This novel study has value for the mini-tuber industry as production tunnels can be tested for the presence of *Sss* and other pathogens before planting to ensure that, where suitable control measures are available, disease-free mini-tubers are produced.

## Introduction

Potato (*Solanum tuberosum* L.) is one of the most important food crops, both in developed and developing countries. Over the past two decades, potato production has more than doubled in developing countries.<sup>1,2,3</sup> Thus, there is an increasing demand for seed tubers that are true-to-type, disease-free and high yielding.<sup>4,5,6</sup> In potato seed-tuber production, each cycle of newly produced tubers are progeny of plants that developed from previously planted seed-tubers. These are commonly known as potato seed-tuber generations. As potato seed-tubers are commercially produced in the field, each generation accumulates, and further transmits viral, fungal or bacteriological disease-causing agents to the next generation. Hence, as the age of the generations increases, the plant's production potential tends to decline.<sup>1</sup> To reduce this problem growers use tissue culture multiplication, also known as mini-tuber technology.<sup>3,7</sup> In this system, potato seed tubers are first multiplied *in vitro* via nodal cuttings in tissue culture and then in the field giving rise to true-to-type and disease-free plant materials.<sup>3,7</sup>

A disease of major concern to potato producers globally, including in South Africa, is powdery scab. This disease is caused by the obligate, plasmodiophorid pathogen *Spongospora subterranea* (Wallroth) Lagerheim f.sp. *subterranea* Tomlinson (*Sss*).<sup>8</sup> The most common visible symptoms of powdery scab on tubers include purple-brown pimple-like swellings or necrotic spots, usually first observed at the rose end of the tubers, and the development of root galls.<sup>9,10,11</sup> As the swellings increase in size, the epidermis ruptures.<sup>12</sup> Mature lesions become hollow and filled with a powdery mass of sporosori (aggregates of resting spores), which can survive in growing media and soil for many years.<sup>11,13</sup> When conditions are favourable,<sup>14</sup> that is, when temperature is between 12 °C and 17 °C and free water is present, the resting spores (within the sporosori) release zoospores, which infect new host tissues.<sup>11</sup> Powdery scab diminishes potato quality and marketability, which results in significant economic losses to growers.<sup>14,15,16,17</sup>

Infected seed tubers play an important role in the dissemination of *Sss* to areas where the disease was not previously present.<sup>18</sup> Sporosori of the scab pathogen can, however, also be transmitted in infested growing media and/or on contaminated farm equipment.<sup>19,20,21</sup> Thus, the most reliable management strategy for controlling powdery scab is to ensure the use of only pathogen-free seed tubers, the use of pathogen-free growing media and strict farm and production area hygiene.<sup>11</sup>

A number of techniques are currently used for the detection and quantification of *Sss*, including enzyme-linked immunosorbent assay (ELISA) systems, bioassays, conventional polymerase chain reaction (PCR) techniques and real-time PCR techniques.<sup>22,23</sup> PCR can be used to detect *Sss* in infected potato roots and tubers, in infected symptomless host plant roots and in infested



field soils as this technique is highly specific, relatively fast and reliable.<sup>15,16,24,25</sup> PCR is of immense value in powdery scab disease management because it can be used to identify sources of powdery scab contamination.

Starting in 2006, a potato mini-tuber production facility in Ceres (Western Cape Province, South Africa) experienced a series of outbreaks of powdery scab. These outbreaks were the first report of powdery scab in this facility and no other diseases had previously been recorded from this facility. Several possible sources of inoculum were considered at the time, including contamination from the surrounding environment, the source of plant material used, water, workers and the growing media. Potatoes or other potential *Sss* hosts are not produced near the mini-tuber facility, nor have they been produced in the area in the past. The only agriculture that occurs within the surrounding area is fruit production. The fruit is predominantly citrus and fertilised with organic fertiliser and not compost. No animal farms are located in the immediate vicinity of the mini-tuber production facility.

Despite attempts at removing possible sources of pathogen contamination, by changing the growth medium, using chemically treated borehole water, placing copper sulphate foot baths at the entrance to the tunnels, installing new drippers and using new crates, powdery scab outbreaks continued in the following 2 years. Before planting in 2009, the management personnel of the tunnels requested help in determining the source or sources of powdery scab inoculum. The aim of this study was thus to trace the presence of *Spongospora subterranea* f.sp. *subterranea* in the mini-tuber production tunnel and identify potential sources of contamination, so that corrective measures could be taken to eradicate the pathogen.

## Materials and methods

### Sampling

Two sets of swab samples were taken from the potato production facility in Ceres. The first set of sampling was done in August 2009 and the second set in October 2009, after various measures were taken to eradicate the pathogen, based on the findings of the first sampling.

A number of swab samples (Transwab<sup>®</sup>, Medical Wire and Equipment, Corsham, Wiltshire, England) were taken from various surfaces throughout the production facility in Ceres. These surfaces included the concrete floor of the tunnels, the wooden bridge over the run-off channel, the run-off channel, water troughs, drain pipes, shade net, wet wall, the tunnel frame, the entrance floor to the tunnel, the outside wash bath and cleaned crates. Swab samples were transported in a cooler box and processed the following day.

### Sample analysis

DNA was extracted from each of the swab samples using the ZR Soil Microbe DNA kit<sup>™</sup> (Zymo Research Corporation,

Irvine, CA, USA). PCR was carried out in a final volume of 25  $\mu$ L containing 0.5  $\mu$ L of dNTPs (250  $\mu$ M of each dATP, dTTP, dGTP and dCTP) (Bioline, London, UK), 2.5  $\mu$ L PCR reaction buffer (16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 67 mM Tris-HCL at pH 8.8) (Bioline), 0.25  $\mu$ L *Taq* polymerase (1 U) (Bioline), 1.25  $\mu$ L MgCl<sub>2</sub> (2.5 mM) (Bioline) and 0.25  $\mu$ L (0.5  $\mu$ M) *Spongospora subterranea* specific primers, *Sps1* (5'-CCT GGG TGC GAT TGT CTG TT-3') and *Sps2* (5'-CAC GCC AAT GGT TAG AGA CG-3'),<sup>15</sup> which were designed to yield an amplification product of 391 base pairs. DNA template (5  $\mu$ L) was added to each reaction.

A thermocycler and 200- $\mu$ L thin-walled PCR tubes were used in the PCR process. The thermal profiles were initial denaturation at 95 °C for 2 min, followed by 35 cycles of melting (95 °C for 20 s), annealing (64 °C for 25 s) and extension (72 °C for 50 s) with a final cycle at 72 °C for 10 min. Amplified DNA, as well as positive and negative controls, were subjected to electrophoresis on 1% agarose gel, stained with ethidium bromide and run alongside standard size markers (HyperLadder II, Bioline). The results were viewed under ultraviolet illumination. PCR reactions were repeated twice.

## Results

### Sampling

A total of 40 swab samples were taken during August 2009. After various measures were taken to eradicate the pathogen, a second set of 40 swab samples were taken during October 2009. Both sets of samples were collected from the same sites (Table 1).

### Sample analysis

Of the 11 surfaces sampled in August, 2009, 6 tested positive for *Sss*. These six were the tunnel floor, the run-off channel, water troughs, drain pipes, the entrance floor and cleaned crates (Table 1).

Based on our findings from the first sampling, corrective measures were taken prior to the 2009 production period to eradicate the inoculum. These measures included the laying of a new concrete floor, new drippers, new pipes, use

**TABLE 1:** Presence of *Spongospora subterranea* on swab samples taken in mini-tuber production tunnels before corrective measures were taken.

Sample taken from	Number of samples taken	Samples positive for <i>S. subterranea</i>
Tunnel floor	10	4
Wooden bridge	1	0
Run-off channel	3	3
Water troughs	6	1
Drain pipes	6	1
Shade net	2	0
Wet wall	3	0
Tunnel frame	1	0
Entrance floor	2	1
Outside water bath	3	0
Cleaned crates	3	1



of new growing medium, new crates and sterilisation of all equipment and surfaces, including the outside water bath.

PCR analysis of the swab samples taken after the corrective measures were carried out indicated that *Sss* inoculum had been eradicated from the production tunnel.

## Discussion

The occurrence of powdery scab in this South African mini-tuber production facility was of great concern as the planting material used in this mini-tuber production facility was from a certified laboratory and good phytosanitary measures were followed, indicating that the *Sss* contamination was most likely introduced through the imported coconut peat. Coconut peat is organic in origin, which increases the possibility that it may harbour pathogens.<sup>6</sup> Hanafi<sup>26</sup> recently found that the sanitary quality of coconut peat is lower than previously presumed, because of its organic origin. Rolot and Seutin<sup>6</sup> and Hanafi<sup>26</sup> observed common scab (*Streptomyces scabies* (Thaxter) Lambert & Loria), powdery scab (*Spongospora subterranea*) and even soft rots (*Pectobacterium* and *Dickeya* spp.) in mini-tuber production facilities as a result of contaminated organic growing media. Supporting the hypothesis that *Sss* contamination was most likely introduced through the imported coconut peat is the fact that no previous outbreaks of powdery scab had ever been reported at this facility until the use of coconut peat. However, after the outbreak of powdery scab in 2006, the mini-tuber facility destroyed the coconut peat, so PCR tests could not be conducted on the peat to conclusively prove that it was infested with *Sss* sporosori.

Ideal conditions for powdery scab development include high moisture and low temperature (12 °C – 17 °C).<sup>4,11,23</sup> During the production of mini-tubers the temperature in the tunnels is maintained at 15 °C – 18 °C,<sup>2</sup> thus favouring the development of powdery scab. As a result of the polycyclic nature of powdery scab,<sup>27,28,29</sup> zoospores continue infecting roots and new zoosporangia develop in roots until the environmental conditions are no longer favourable.<sup>11,29</sup>

The results of this study confirm that the sporosori of *Sss* were able to survive in the tunnel in the absence of a host, from one season to the next.<sup>14</sup> The sporosori that remained in the run-off channels, water troughs and the drain pipes were thus the most likely sources of primary inoculum that led to the re-occurrence of disease in the 2007 and 2008 seasons following the use of coconut peat as a growing medium. Corrective measures taken to eliminate the pathogen from the mini-tuber production tunnel resulted in a disease-free harvest from 2009 onwards.

This is a novel study that has significant potential for the potato industry, particularly the mini-tuber industry, as surfaces and equipment in production tunnels can be tested for the presence of *Sss* before planting, allowing ample time for the application of corrective measures if and where necessary. Producers should nonetheless ensure that

growing material is pathogen free to prevent introduction of the powdery scab pathogen into tunnels. This study is the first in which swabs have been used for the detection of *Sss*; this method can be adapted and used to detect other potato pathogens in tunnels before planting to ensure that disease-free mini-tubers are produced and sold to specialist growers for later generation of high-quality seed tubers in the field.

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## Competing interests

We declare that we have no financial or personal relationships which may have inappropriately influenced us in writing this paper.

## Authors' contributions

J.E.v.d.W. was the project leader; J.W. was the student and performed the experiments; and A.K.L. cosupervised the research and made conceptual contributions.

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