POWDERY SCAB OF POTATOES IN ALBERTA: MOLECULAR DIAGNOSTICS AND FUNGICIDE EFFICACY

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DEDICATION

This dissertation is dedicated to my beloved parents, who instilled in me the virtues of perseverance and commitment and relentlessly encouraged me to strive for excellence.

I also dedicate this thesis to the potato growers of Alberta, the steadfast stewards of the land whose tireless labour, grit, and unyielding resolve in the face of adversity have not only fed communities but also fueled my determination throughout this research. It is my earnest hope that this work contributes meaningfully to the advancement of the potato industry and offers tools to ease the path forward for those who labour at its core. Thank you all for shaping both this journey and the future of sustainable agriculture.

ABSTRACT

Powdery scab, caused by the soil-borne pathogen *Spongospora subterranea* f. sp. *subterranea*, forms root gall and tuber lesions in potatoes and vectors the Potato mop-top virus (PMTV) that causes spraing in tubers. Seven field sites with a history of powdery scab were selected, and the pathogen's presence in soil was confirmed by using the molecular diagnostics targeting the ITS region (ITS1-5.8S-ITS2). BLAST analysis showed 99-100% similarity with *S. subterranea* f. sp. *subterranea* in the NCBI database. Field trials over three years (2022, 2023, and 2024) evaluated the efficacy of five Syngenta-designed fungicide treatments: A21008A, Allegro (low, medium, and high doses), and A24367B. Three cultivars, Shepody, Russet Burbank, and Lady Claire, were planted in naturally infested fields. Root galls were assessed during the growing season, and tubers were evaluated for disease severity and yield in August-September. In 2023, Allegro was most effective in reducing root galls, followed by A24367B, whereas A21008A performed poorly at root gall reduction but was most effective in reducing tuber lesions. All treatments significantly suppressed the disease in Lady Claire. In the 2024 trials, none of the treatments suppressed galls in Russet Burbank, whereas all but Allegro-low significantly reduced galls in Lady Claire. No significant differences in total tuber yield were observed across treatments or cultivars. These findings highlight that treatment efficacy varies depending on the potato cultivar and the type of disease symptoms (root galls vs. tuber lesions). In bioassay, conducted by growing plants in the presence of controlled quantities of *S. subterranea*, visible symptoms of powdery scab were observed when the soil contained more than 15 cystosori per gram of soil. This underscores the need for targeted management strategies to control powdery scab in potato crops effectively.

STATEMENT OF ETHICAL AI

AI tools like ChatGPT, Grammarly and Quillbot were used in this dissertation to enhance sentence clarity and improve readability. These tools were used solely for linguistic refinement and did not contribute to the generation of research data, graphs, or images. The contents of this dissertation were reviewed thoroughly and edited to ensure accuracy, and the author takes full responsibility for the final work presented here.

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*In the name of **Allah Almighty**, the merciful, the beneficent, all praises (belong) to **Allah** alone, the Cherisher and Sustainer of the world. He is the First, He is the Last, He is the hidden, and He is all knowing (**Al-Quran)**. It is one of His infinite benedictions that He bestowed upon me with the potential and ability to complete the present research program and to make a meek contribution to the deep oceans of knowledge already existing.” وما توفيقي الا بالله” (and verily my success is only by Allah) (**Al-Quran, 11:88**).*

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LIST OF ABBREVIATIONS

PMTV	Potato Mop-Top Virus
RNA	Ribonucleic Acid
PGA	Potato Growers of Alberta
PCR	Polymerase Chain Reaction
LAMP	Loop-Mediated Isothermal Amplification
CP	Coat Protein
ORFs	Open Reading Frames
TEM	Transmission Electron Microscopy
ELISA	Enzyme-Linked Immunosorbent Assay
DAS-ELISA	Double Antibody Sandwich ELISA
DNA	Deoxyribonucleic Acid
ITS	Internal Transcribed Spacer
RFLP	Restriction Fragment Length Polymorphism
EDTA	Ethylenediaminetetraacetic Acid
PP-RCBD	Paired Plot - Randomized Complete Block Design
IFAP	In-Furrow-At-Planting
DAP	Days-After-Planting
RT-PCR	Reverse Transcribed Polymerase Chain Reaction
CTAB	Cetyltrimethylammonium Bromide
UV	Ultraviolet
TBE	Tris Borate EDTA
RO	Reverse Osmosis
MUSCLE	Multiple Sequence Comparison by Log-Expectation
MCL	Maximum Composite Likelihood
NJ	Neighbour Joining
DSI	Disease Severity Index
BLAST	Basic Local Alignment Search Tool
NCBI	National Center for Biotechnology Information
OSBP	Oxysterol Binding Protein
BPC	British Potato Council
SSPGA	Saskatchewan Seed Potato Growers Association

CHAPTER 1: INTRODUCTION

Potatoes are cultivated in every Canadian province, playing a vital role in agriculture. In Alberta, potato production is a vital component of the agriculture sector, with the province being one of the country's leading producers of potatoes. However, potato cultivation faces numerous challenges with production (Kroschel *et al.,* 2020), particularly due to diseases such as powdery scab, caused by a soil-borne, obligate pathogen *Spongospora subterranea* f. sp. *subterranea*. Powdery scab is characterized by the formation of scab lesions on tubers and galls on roots that carry the resting spores of *S. subterranea*. As a result of lesions on the tuber surfaces, powdery scab has a negative cosmetic impact due to the blemished appearance of the tubers. It primarily decreases their value in fresh market industries and causes problems for French fry and chip production in Alberta, as they require deeper peeling to remove surface lesions (Falloon *et al.,* 2016). The disease is particularly problematic in cool, wet soil conditions, which are common in many potato-growing regions of Alberta. *Spongospora subterranea* is also the only known carrier of the Potato mop-top virus (PMTV) (Kirk, 2008). It is a pathogenic plant virus with a single-stranded RNA genome. PMTV causes internal tuber damage (tuber flesh discoloration), known as spraing, making potatoes unmarketable and leading to significant economic losses in stored potatoes (Adolf *et al.,* 2020). The dual threat of powdery scab and PMTV underscores the need for effective management strategies to safeguard potato production.

The economic impact of powdery scab on potato yields in Alberta remains understudied, despite its growing incidence. The first open discussion about powdery scab was held at the 2006 PGA (Potato Growers of Alberta) annual meeting due to an increase in awareness of the incidence of the disease in Southern Alberta (Ron-Howard, 2006). The disease progresses rapidly, with even small amounts of inoculum causing symptoms (Falloon, 2009). Effective control measures are needed as processing industries may reject infected potatoes due to quality concerns (Falloon *et al.,* 2016). The long-term survival of *S. subterranea* resting spores makes disease control particularly challenging, necessitating early pathogen detection to advise management strategies for potato growers (Falloon, 2009; Yellareddygari *et al.,* 2018). Molecular detection methods such as conventional PCR (Strydom *et al.,* 2024), quantitative PCR (qPCR) (Simango *et al.,* 2020), loop-mediated isothermal amplification (LAMP) (Jiang *et al.,* 2023) and immunological-based techniques (ELISA) (Merz *et al.,* 2005) have become essential for diagnosing and quantifying *S. subterranea* infections with high sensitivity and specificity. These methods enable early pathogen detection in soil, roots and tubers, aiding in risk assessment and decision-making for disease management (Qu *et al.,* 2006; Nakayama *et al.,* 2007; Tegg *et al.,* 2016). Additionally, bioassays, including bait plant assays and soil inoculation studies (Alaryan *et al.,* 2023), further complement molecular diagnostics by assessing pathogen viability and disease potential under different environmental conditions (Balendres *et al.,* 2024).

Effective powdery scab management relies on integrating molecular diagnostics with practical disease control measures. Cultural practices such as crop rotation, seed selection (Harrison *et al.,* 1997), soil amendments (Wright *et al.,* 2021; Strydom *et al.,* 2024), irrigation management (Falloon, 2009), and the use of resistant cultivars (Bittara *et al.,* 2016) play a crucial role in suppressing the powdery scab incidence. However, despite these management strategies, no method has been proven universally effective for managing the disease (Merz and Falloon, 2009; Falloon *et al.,* 2016). Researchers have been trying to utilize different chemicals to manage the powdery scab for many years. Very few have shown strong or positive results in reducing disease severity and increasing the marketable yield (Houser and Davidson, 2010; Zeng *et al.,* 2020). Common fungicides such as fluazinam, flusulfamide, or cymoxanil have shown efficacy, but with inconsistent results that may be due to variations in the application method, soil inoculum level, cultivar susceptibility, or soil texture and structure (Bittara *et al.,* 2016; Houser and Davidson, 2010).

Given the persistent challenge of powdery scab in commercial potato production, this research aims to improve disease detection and management by integrating molecular diagnostics, bioassays, and novel field-based chemical control strategies. By assessing the relationship between *S. subterranea* population levels and disease severity, establishing predictive threshold levels, and evaluating the presence of PMTV in infected tubers, this study will contribute to a deeper understanding of pathogen epidemiology. Additionally, the evaluation of fungicide efficacy and phytotoxicity will provide valuable insights into the discovery and optimization of chemical control strategies. The findings of this research will help potato growers implement targeted and effective disease management practices, ultimately supporting sustainable potato production in Alberta and beyond.

CHAPTER 2: REVIEW OF LITERATURE

2.1. Potato production in Canada

Potato (*Solanum tuberosum* subsp. *tuberosum*) is an herbaceous annual crop belonging to the nightshade family (Solanaceae). It is the fifth-largest primary agricultural crop in Canada, following canola, wheat, soybean and corn, generating approximately $2 billion in farm cash receipts in 2023 (Agriculture and Agri-Food Canada, 2023/2024). Potato varieties are selected to target specific markets. Commonly cultivated potato cultivars grown in Canada include Russet Burbank, Shepody, Ranger Russet, CalWhite, Umatilla Russet, Superior, Russet Norkotah, Chieftain, Yukon Gold, Norland, Ranger Russet, Goldrush, Sangre and Umatilla Russet (Agriculture and Agri-Food Canada, 2017).

The export value of potatoes and potato products increased by 8% from 2022/2023, exceeding 4.6 billion in 2023/2024. Moreover, potatoes represent the predominant vegetable crop cultivated in Canada, comprising 29% of total vegetable revenue and 17% of overall horticultural revenue. In 2023, the total cultivation area of potatoes in Canada was 156,642 ha, with an annual production of 5,842,315 tons. Among the potato-growing provinces, Alberta contributed 24.7% to potato production in 2024 and maintained its position as the largest potato producer in Canada, followed by Manitoba (21.6%) and Prince Edward Island (20.4%) (Statistics Canada, 2024). In addition, Alberta is at the top in the export of seed potatoes. Alberta exported 64.8% with 50,668 metric tons of potatoes (Agriculture and Agri-Food Canada, 2023/2024).

2.2. Introduction to Powdery scab

Potato production is affected by numerous pathogens, including fungi, viruses, nematodes, phytoplasmas and bacteria. Over 100 diseases have been identified, each causing different levels of damage to potato crops (Stevenson *et al.,* 2001; EFSA Panel on

Plant Health (PLH) *et al.,* 2020). Powdery scab, caused by the plasmodiophorid, obligate pathogen *Spongospora subterranea* (Wallroth) Lagerheim f. sp. *subterranea* J.A. Tomlinson (Santala *et al.,* 2010), is a significant disease affecting potato production worldwide (Merz and Faloon, 2009; Wilson, 2016; Balendres *et al.,* 2016b). The pathogen primarily causes blemishes on potato tubers, which reduces tuber quality and, consequently, affects potato marketability. It is also the only known vector for the Potato mop-top virus (PMTV), further exacerbating the economic impact of the disease (Merz and Faloon, 2009).

2.3. Taxonomy and nomenclature of *S. subterranea.*

Kingdom	Protista
Phylum	Cercozoa
Subphylum	Endomyxa
Class	Phytomyxea
Order	Plasmodiophorida
Family	Plasmodiophoraceae
Genus	*Spongospora*
Species	*subterranea*
Forma Speciales	*subterranea*

European farmers had recognized powdery scab long before it was scientifically described. In Germany, it was commonly known as “Kartoffelräude.” This term was already in use prior to Wallroth’s 1842 publication, in which he described the disease as “Knollenbrand” and identified its causal organism as *Erysibe subterranea* (Melhus, 1913). The disease was later reported in England in 1846, and by 1886, it had also been observed in Sweden and Norway. In those regions, botanist J. Brunchorst conducted research on the disease and referred to its causal organism as *Spongospora solani*. Following J. Brunchorst's report, N.G. von Lagerheim proposed the name *Spongospora subterranea* in 1892. It was not until 1909 that T. Johnson provided evidence linking these earlier identifications, confirming the organism's name as *Spongospora subterranea* (Melhus, 1913; Kunkel, 1915).

2.4. Disease symptoms

2.4.1 Powdery scab

Spongospora subterranea can infect all underground parts of a potato plant, including stolons, tubers and roots. The symptoms of the disease remain undetectable for three or more weeks post-infection (Bhattacharyya and Raj, 1981; Johnson and Miliczky, 1993). Once it invades the plant roots, the pathogen triggers the host cells to divide and enlarge abnormally (hyperplasia and hypertrophy), resulting in visible gall symptoms. Root galls may become visible as early as four to five weeks after planting, with infected root hairs, epidermis, and stolons developing small, wart-like galls. These galls can range from 1.5 to 11 mm in diameter and change in colour from white to brown or black as they mature (Bittara *et al.,* 2018).

Early disease symptoms, visible to the naked eye, appear as small, raised purplish-brown pustules, most commonly observed at the distal end of immature tubers (Osborn, 1911; Lawrence and McKenzie, 1981). Later, these pustules dry and break open the periderm (Morse, 1914; Lawrence and McKenzie, 1981), leaving circular to oval, small, scabby blisters containing a brown powder consisting of a mass of clustered cystosori (spore balls) and appropriately known as powdery scab (Butler and Jones, 1949; Whitehead *et al.,* 1953; Sprau, 1953). Over time, while each scab lesion typically starts as a small, round spot less than 10 mm across, the blisters can burst and gradually join with nearby lesions, forming larger, irregular patches on the surface. The individual lesions range in size from 0.5 to 2 mm or more in diameter and frequently have raised borders as they mature (Harrison *et al.,* 1997; Christ, 2001; Johnson and Thomas, 2015). Scabs typically affect the outer tissues of potato tubers; however, in some cases, they may penetrate deeper, compromising a significant portion of the tuber (Whitehead *et al.,* 1953).

Powdery scab symptoms can be mistaken for those of common scab, caused by *Streptomyces scabies* or potato wart disease, caused by *Synchytrium endobioticum* (Obidiegwu, 2014). Nevertheless, only powdery scab lesions produce abundant cystosori that can be examined under a microscope (Bouchek-Mechiche and Wale, 2014).

2.4.2. Potato mop-top virus

Spongospora. subterranea is not only the causal agent of powdery scab but is also the sole known vector responsible for transmitting potato mop-top virus (PTV; genus Pomovirus, family Virgaviridae) (Tenorio *et al.,* 2006; Carnegie *et al.,* 2010; Robinson *et al.,* 2018; Anglin *et al.,* 2024). PMTV has been identified across many major potato-growing regions, and its continued emergence highlights the increasing concern it poses to crop health and management (Xu and Gray, 2020; Frampton *et al.,* 2022). The pathogen's resting spores can survive in the soil for extended periods, remaining infective. When conditions are favourable, these spores germinate, releasing zoospores that may carry PMTV (Kirk, 2008; Santala *et al.,* 2010; Yellareddygari *et al.,* 2018). As these motile spores invade plant roots or developing tubers, they introduce the virus directly into plant tissues (Jones and Harrison, 1969; Arif *et al.,* 1994; Kirk, 2008). This long-term soil persistence of infectious resting spores makes management through crop rotation largely ineffective (Jones and Harrison, 1972; Kirk, 2008). Soil infested with PMTV and stored under cold conditions for up to 15 years has been shown to remain capable of infecting plants (Santala *et al.,* 2010; Beuch *et al.,* 2014; Domfeh *et al.,* 2015).

PMTV typically causes spraing, a symptom marked by necrotic arcs and brown flecks within the tubers (Sandgren *et al.,* 2002; Santala *et al.,* 2010). The virus spreads throughout the plant, moving from infected roots and stolons into the stems and leaves, where it can lead to visible foliar symptoms. These often include chlorotic blotches or distinct V-shaped markings on the leaves, along with overall plant stunting, commonly referred to as 'mop top' (Harrison and Reavy, 2002; Tenorio *et al.,* 2006). However, these foliar symptoms are rarely observed in Southern Alberta.

Unlike its vector, PMTV has a fairly limited host range, primarily infecting plants from just three families, namely, Solanaceae, Tetragoniaceae and Chenopodiaceae (Brunt *et al.,* 1990; Anderson *et al.,* 2002). Some of the species susceptible to PMTV include *Tetragonia tetragonioides, Nicotiana tabacum, Chenopodium amaranticolor*, *Nicotiana benthamiana, Chenopodium album*, *Nicotiana debneyi*, and *Solanum nigrum* (Jones and Harrison, 1969; Harrison and Jones, 1970; Jones and Harrison, 1972; Arif *et al.,* 1995; Andersen *et al.,* 2002).

In recent years, PMTV has become more economically relevant, particularly as a pathogen affecting seed potatoes in North America, including Canada (Xu *et al.,* 2004), as well as in Poland (Budziszewska *et al.,* 2010), and the United States (Lambert *et al.,* 2003; Xu *et al.,* 2004; David *et al.,* 2010; Crosslin, 2011; Whitworth and Crosslin, 2013). This increased attention came in the wake of a notable outbreak during the 2001-2002 growing seasons in North America (Lambert *et al.,* 2003; Xu *et al.,* 2004), prompting renewed efforts toward managing its spread (Tenorio *et al.,* 2006).

2.5. Biology and life cycle of *S. subterranea*

Powdery scab is a polycyclic disease (Powelson and Rowe, 2008; Johnson and Thomas, 2015) caused by the soil-borne pathogen *S. subterranea*, which forms resting spores within cystosori. These resting spores can persist in the soil for several years, complicating disease management (Qu and Christ, 2004). The life cycle of *S. subterranea* includes biflagellate, uninucleate primary zoospores (n), which emerge from the resting spores. The primary zoospores swim using two unequal anterior whiplash-type flagella (Alexopoulos, 1962), infect root hairs, and form an uninucleate plasmodium that multiplies and develops into a multinucleate plasmodium, eventually producing thin-walled zoosporangia containing new identical secondary zoospores (Merz, 2008). Rain and irrigation water provide the conditions for zoospore dissemination and infection (Adams *et al.,* 1987; Johnson and Thomas, 2015). These secondary zoospores can swim only short distances in the moist soil and reinfect roots or initiate tuber infections, leading to the galls on roots and the characteristic powdery lesions on tuber skins (Harrison *et al.,* 1997; Gau *et al.,* 2013). Zoospores released from the pustules on tubers, galls on roots, and stolons may reinfect the host and repeat the life cycle as long as environmental conditions remain favourable (Harrison *et al.,* 1997).

2.6. Biology and life cycle of PMTV

PMTV can be passed from infected tubers to the next generations of plants, although the efficiency of transmission can vary (Valkonen, 2007). PMTV is composed of three single-stranded, positive-sense RNA segments (Gil *et al.,* 2016). The first segment, RNA1 (6.043 kb), encodes the RNA-dependent RNA polymerase (RdRp), a key enzyme required for replicating the viral genome (Kamal *et al.,* 2024). RNA2 (4.134 kb) carries the coat protein (CP) gene, which is essential for virus assembly and movement (Savenkov *et* *al.,* 1999; Kamal *et al.,* 2024). The third segment, RNA3, contains four open reading frames (ORFs), including the triple gene block (TGB1, TGB2, TGB3), which are involved in both local and systemic virus movement. The fourth ORF encodes a small 8k cysteine-rich protein believed to enhance viral virulence and suppress the plant's RNA silencing defences (Savenkov *et al.,* 2003; Samuilova *et al.,* 2013; Kamal *et al.,* 2024). The coordinated action of the TGB proteins is critical for the virus to move between cells and spread throughout the plant (Zamyatnin *et al.,* 2004; Cowan *et al.,* 2012; Haupt *et al.,* 2005; Tilsner *et al.,* 2010).

In infested soils, the resting spores of *S. subterranea* act as a reservoir for PMTV. When conditions are favourable, these spores release motile zoospores that carry the virus and infect host plants (Kirk, 2008). When potato roots encounter soil containing *S. subterranea* zoospores, the pathogen infects root tissues, stolons, young shoots, and tubers, introducing PMTV into the host cells (Hims and Preece, 1975). Once inside the potato plant, PMTV moves systemically through the vascular system, infecting stems, stolons and tubers. Infected tubers may show characteristic symptoms such as necrotic arcs, spraing, or internal discoloration (Sandgren, 1995; Abbas and Madadi, 2016). These symptoms are influenced by environmental conditions and cultivar susceptibility.

PMTV can persist in infected seed tubers, leading to the next cycle of infection when these tubers are planted. When PMTV-infected tubers are used as seed potatoes, the virus moves systemically to the leaves of potato plants. The foliar symptoms include V-shaped patterns with yellow blotches and severe shortening of internodes (Carnegie *et al.,* 2011). The virus can also be transmitted to new plants and young tubers. However, this transmission is limited, with only about 30-50% of progeny tubers becoming infected (Carnegie *et al.,* 2010; Davey *et al.,* 2014; Kirk, 2008). Therefore, while seed transmission contributes to the spread of PMTV, the primary mode of transmission remains through the soil-borne vector, *S. subterranea*. Limited transmission can also lead to the elimination of the virus from potato crops after a few generations when the tubers are planted in soil free of PMTV and *S. subterranea* (Calvert, 1968; Torrance *et al.,* 1999; Kirk, 2008; Davey *et al.,* 2014). When infected tubers are grown, secondary symptoms develop. Secondary symptoms include chevron-shaped yellow blotches and bunched upper leaves with rolled and wavy margins (Kurppa, 1989). Tubers may exhibit deep cracks, reticulations, blotchy or freckled surface markings and severe distortions (Harrison and Jones, 1970; Abbas and Madadi, 2016).

2.7. Impact of powdery scab and PMTV

Powdery scab poses significant challenges to potato production, impacting both market quality and crop yield. The disease primarily manifests as lesions on infected potato tubers, which are the most prominent damage caused by the pathogen, making this a challenge for the fresh produce marketing industry. The processing industry requires deeper peeling operations if the tubers are infected with powdery scab blemishes, causing more waste and less profit (Wale, 2000a).

In New Zealand, the cosmetic damage caused by powdery scab impacts the marketability of potatoes, particularly for the export of seed tubers. Seed producers face substantial financial losses due to infected seed lots being rejected when infection levels exceed the low tolerance thresholds (Falloon, 2008). In addition to cosmetic losses, infected seeds can spread the disease to healthy soils, reducing crop yields by reducing sprouts and decreasing the size and weight of tubers per plant (Falloon, 2008; Merz, 2008; Houser and Davidson, 2010). In severe cases, root galls can release cystosori, causing young plants to wilt and die (Lawrence and McKenzie, 1981).

Lesions on the tubers act as weak points in the skin, facilitating increased gas exchange. This can result in higher shrinkage and weight loss during storage, while also serving as entry points for secondary pathogens (Delahaut and Stevenson, 2009). The infected tubers are particularly susceptible to diseases such as late blight (*Phytophthora infestans*) (Dorojkin, 1936; Beregovoy, 1939; Schultz, 1952; Bonde, 1955), pink rot (*Phytophthora erythroseptica*) (Diriwächter and Parbery, 1991), dry rot (*Fusarium caeruleum*) (Foister *et al.,* 1952), and other rots (*Colletotrichum atramentarium*) (Wade, 1949).

Different studies on powdery scab have shown that the *S. subterranea* infection disrupts water (as documented by Hernandez Maldonado *et al.,* 2013) and nutrient (such as phosphorus-15% and potassium-11%) uptake because of the formation of galls (Balendres *et al.,* 2024). The plants use less water, and produce less dry matter, smaller shoots and fewer leaves, leading to a significant decline in overall production (Falloon *et al.,* 2004, 2005a; Lister *et al.,* 2004; Merz, 2008; Shah *et al.,* 2014). Early symptoms can lead to tuber deformities, inhibiting their growth and significantly reducing marketable yield (Dorojkin, 1936; Mol and Ormel, 1946; Sprau, 1953). Nielsen and Larsen (2004) documented that inoculating tomato plants with *S. subterranea* reduced the plant shoot and root mass.

Severely blemished tubers may require extra grading to remove infected tubers that may be discarded, or may not be harvested at all (Wale, 1987; Harrison *et al.,* 1997). Losses from powdery scab-infected tubers have been significant, with reports indicating up to 50% of potatoes deemed unmarketable in Australia (Hughes, 1980), and in some cases, entire harvests have been affected in Venezuela (Garcia *et al.,* 2004). However, in North America and especially in Canada, no significant losses have been reported even though powdery scab is reported to be present in various potato-growing areas. While specific distribution data is limited, the disease has been documented in multiple provinces, indicating its presence in several key potato areas. However, comprehensive, up-to-date information detailing its prevalence in all potato-growing regions of Canada is not readily available.

S. subterranea is currently recognized as the only known vector responsible for transmitting PMTV (Jones and Harrison, 1969). PMTV can lead to significant yield losses, especially in susceptible potato cultivars (Domfeh *et al.,* 2015b; Yellareddygari *et al.,* 2018). The virus is notably persistent, remaining infectious for over 18 years within cystosori even in the absence of a host plant (Calvert, 1968), which makes managing its spread particularly difficult. Yield losses of 57% and 67%, as well as the reduction in tuber numbers by 46% and 57%, have been reported in one of four assessed cultivars in two growing seasons, respectively (Carnegie *et al.,* 2010; Falloon *et al.,* 2024). In contrast, however, Nielsen and Molgaard (1997) and Yellareddygari et al. (2018) concluded that PMTV had little or no effect on potato crop productivity. Disease outbreaks are difficult to predict as symptoms can vary widely from year to year in the same cultivar and field (Sandgren, 1995; Latvala-Kilby *et al.,* 2009).

In Canada, PMTV is emerging as a significant concern for potato producers due to its impact on tuber quality. Although infected tubers may appear symptom-free and be considered marketable at harvest, the spraing symptoms often become visible later during storage. This delayed symptom development creates serious challenges for both the fresh market and processing industries, as affected tubers may be rejected by processors for French fry and chip production.

2.8. History of powdery scab in North America

Powdery scab was first identified in North America in 1913 when a Dominion Botanist of Canada, "Güssow," received tuber samples from various localities in Canada that were affected by powdery scab (Morse, 1914). He stated, “a disease well known in Europe but as far as I was able to ascertain hitherto not reported as established on any part of the continent of North America”. In the USA, ‘Morse’ reported powdery scab for the first time based on tuber samples received from Nebraska and Massachusetts. He emphasized its severity by stating that “powdery scab is without doubt the most serious disease with which the Maine potato growers have ever had to contend” (Morse, 1914). In 1914 and 1915, for the first time, ‘Melhus’ expanded the understanding of the disease, including the first documentation of root gall formation as a symptom of *S. subterranea* infection. His publications included data from the states of Maine, Connecticut, New York, Florida, Oregon, Washington and Minnesota.

Powdery scab likely spread to Canada via the trade of infected seed potatoes. It is mostly associated with regions that have cool and moist soils, ideal for the pathogen’s survival and proliferation. The earliest documented evidence of powdery scab in Canada dates back to the mid-1920s. In 1925, a Canadian plant disease survey report mentioned the presence of powdery scab in Prince Edward Island, Nova Scotia and Quebec, with very few cases reported (Canadian Phytopathological Society, 1925). In 1932, powdery scab was reported for the first time in British Columbia (Canadian Phytopathological Society, 1932). In 1974, Lawrence published a report detailing how to distinguish between common scab and powdery scab based on their symptoms (Lawrence, 1974). Later in 2004, the PGA (Potato Growers of Alberta) held their first open discussion on powdery scab during their annual meeting, prompted by a noticeable rise in its incidence in Southern Alberta (Ron-Howard, 2006).

2.9. Geographical occurrence of *S. subterranea*

Although powdery scab is commonly associated with temperate potato-producing regions, it is not confined to cool, wet climates. The disease can also thrive in warm, dry areas, often due to irrigation (Wale, 2000). Irrigation provides the moisture required for the pathogen to develop and can significantly lower soil temperature, especially when bore water is used. For instance, severe infections have been reported in sandy soils within semi-arid regions of Australia, where center-pivot irrigation is commonly used (de Boer, 2000).

Powdery scab has increasingly been documented through numerous new "first reports", suggesting that the disease may have previously gone unrecognized or underestimated in many regions (Merz, 1999). Evidence also indicates that the disease is spreading to countries and areas where it was not previously observed (Merz and Falloon, 2009). *S. subterranea*, the causative agent of powdery scab, has been reported on every continent (Merz, 2008; Merz and Falloon, 2009) and is now regarded as having a global distribution in regions where potatoes are cultivated (Fig. 2.1) (Balendres *et al.,* 2024).

Powdery scab was first documented in Europe in 1842 by Wallroth (Harrison *et al.,* 1997). However, it was not widely recognized until the mid-20th century, when more countries began reporting its presence and associated challenges. Kole (1954) noted its growing significance in the Netherlands, and researchers later suggested that the disease may have been previously overlooked or underestimated (Merz, 1999). Over time, its economic impact became evident across several European regions, including the United Kingdom (Hide, 1981; Wale, 2000), Switzerland (Winter and Winiger, 1983; Blum and Merz, 1993; Merz, 2000), Italy (Tuttobene, 1986), France (Andrivon *et al.,* 2000), Germany (Stachewicz and Enzian, 2002), Netherlands (Van de Haar, 2000), and Turkey (Eraslan and Turhan, 1989).

Additionally, the disease has been reported in most major potato-growing regions worldwide. Examples include Peru (Torres *et al.,* 1995) in South America (Clausen *et al.,* 2005; De Nazareno and Boschetto, 2002; Montero-Astúa *et al.,* 2002), Costa Rica (Montera-Astua *et al.,* 2002) in Central America and North America (Christ, 2001; Carling, 1996; Draper *et al.,* 1997), Japan (Nakayama *et al.,* 2007) in East Asia, India (Bhattacharya *et al.,* 1985), Pakistan (Ahmad *et al.,* 1996), and Korea (Kim *et al.,* 2003) in Asia, as well as Australia (de Boer, 2000) and New Zealand (Braithwaite *et al.,* 1994) in Australasia and Malta (Porta-Puglia and Milfsud, 2006).

Currently, in many countries, factors such as the intensification of potato production (Wright *et al.,* 2021), an increase in the use of susceptible cultivars (UCIMP, 2019), more frequent use of irrigation (Wale, 2000; Sinton *et al.,* 2022; Strydom *et al.,* 2024), and neglect of prevention measures are all contributing to the greater incidence of powdery scab than has previously occurred (Balendres *et al.,* 2024).

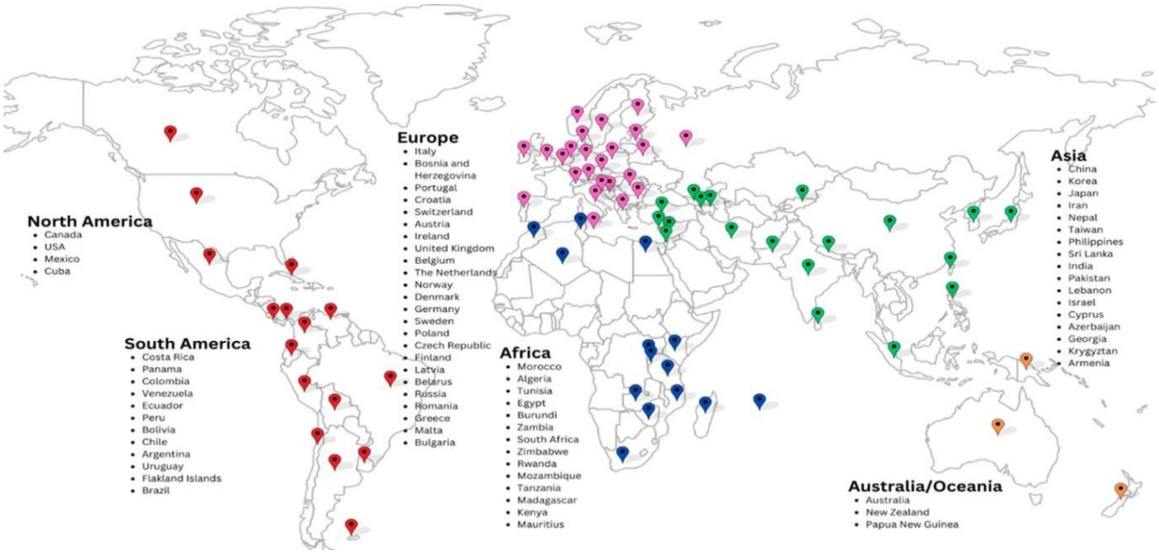

Figure 2.1. The geographical distribution of *S. subterranea* f. sp. *subterranea* as observed and detected on tomato or potato plants (CABI/EPPO, 2012; Strydom *et al.,* 2024).

2.10. Host range of *S. subterranea*

Spongospora subterranea has a well-characterized biology and life cycle that are critical for understanding disease management (Braselton, 1995; Harrison *et al.,* 1997; Merz, 2008; Balendres *et al.,* 2006; Simango *et al.,* 2020). The pathogen undergoes two main phases: the sporangial (primary) phase and the sporogenic (secondary) phase (Braselton, 1995; Merz, 2008; Kageyama and Asano, 2009; Schwelm *et al.,* 2015). During the sporangial phase, numerous secondary zoospores develop within thin-walled zoosporangia, which then initiate infection. The sporogenic phase results in the production of resting structures called cystosori, which enable long-term survival in the soil. Under favourable environmental conditions, these resting spores germinate to release primary zoospores, restarting the infection cycle (Harrison *et al.,* 1997; Simango *et al.,* 2020).

S. subterranea is notable for its broad host range, infecting nearly 90 plant species across 28 families, primarily as root infections (Balendres *et al.,* 2024). These host families include Aizoaceae, Apiaceae, Amaranthaceae, Asteraceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Coniferae, Cyperaceae, Fabaceae, Geraniaceae, Lamiaceae, Papaveraceae, Plantaginaceae, Poaceae, Polygonaceae, Ranunculaceae, Resedaceae, Rubiaceae, Solanaceae and Urticaceae (Qu and Christ, 2006; Merz and Falloon, 2009; Nitzan *et al.,* 2009). Among these, the Solanaceae family contains the greatest number of susceptible species with galls containing cystosori observed only in *Solanum tuberosum* (potato), *Solanum lycopersicon* (Qu and Christ, 2006), *Solanum nigrum* (Shah *et al.,* 2010), and *Solanum physalifolium* (Nitzan *et al.,* 2009; Shah *et al.,* 2010).

Host interactions with *S. subterranea* have been classified into three groups: type 1 hosts, which support only cystosori formation; type 2 hosts, which support both zoosporangial and sporosorial stages; and trapping hosts, which allow initial infection but inhibit completion of the pathogen's life cycle, thus reducing soil inoculum (Harrison *et al.,* 1997; Qu and Christ, 2006; Arcila *et al.,* 2013; Simango *et al.,* 2020). The use of trapping hosts is a valuable component of integrated disease management strategies aimed at lowering pathogen populations in infested soils.

2.11. Pathogen detection

Most seed potato certification and grading programs primarily assess tubers based on visible powdery scab symptoms (Tegg *et al.,* 2016), but this approach alone is insufficient to stop the unintentional spread of *S. subterranea* or the production of infected progeny tubers. Therefore, having a reliable and accurate detection method is essential not only to confirm suspected cases of powdery scab but also to identify contaminated seed lots and help prevent introducing the pathogen into clean fields (Falloon 2008; Hernandez Maldonado *et al.,* 2015).

Diagnosis of powdery scab based on visual symptoms is usually not very easy. Mature common scab lesions (Lapwood, 1973) are often confused with powdery scab, leading to misidentifications (De-Haan and Van-Den-Bovenkamp, 2005). Powdery scab can be detected through various methods, including visual identification of scabby lesions on the tuber surfaces or the presence of galls on root hairs. Other morphological tools, such as microscopic examination, plant bait or bioassays, are also used. Additionally, advanced molecular and serological techniques like conventional PCR, qPCR, ELISA and LAMP can provide precise detection.

2.11.1. Morphological identification

The pathogenic structures of *S. subterranea,* such as bi-flagellated zoospores, zoosporangia, resting spores and cystosori, can be observed in infected plant root tissues through light microscopy or TEM (transmission electron microscopy). (Merz, 1992; Falloon *et al.,* 2011; Arcila *et al.,* 2013). Zoospores of *S. subterranea* have a distinctive appearance, with two flagella of different lengths (Merz, 1992), and exhibit a unique swimming behaviour, making them a useful diagnostic feature (Amponsah *et al.,* 2022). However, relying solely on this method has its challenges as it demands the expertise of a skilled diagnostician to distinguish these structures accurately. Infected tissue often contains elements that resemble *S. subterranea* but originate from other pathogens, i.e., common scab, or the host itself (Hernandez Maldonado *et al.,* 2013). Combining morphological analysis with additional diagnostic tools helps improve the accuracy and reliability of identifying *S. subterranea*.

2.11.1.1. Bioassay

The tomato seedling bioassay is an effective method for detecting *S. subterranea* in soil. Tomato seedlings are particularly suitable for this purpose because they grow quickly, with root hairs forming within 2-3 weeks (Merz, 1989; Alaryan *et al.,* 2023). This method specifically identifies viable *S. subterranea* inoculum (Merz and Faloon, 2009) and is most reliable when paired with polymerase chain reaction (PCR) for confirmation (Nakayama *et al.,* 2007)

The seeds are planted in sterilized soil or growth media, a nutrient solution (Hoagland solution commonly) is provided, and the roots are exposed to the inoculum for at least one week. Roots are then stained with trypan blue/acid fuchsin and observed under a microscope for the presence of *S. subterranea* structures, i.e. cystosori, plasmodium, and zoosporangia (Balendres *et al.,* 2024). The bioassay method has certain drawbacks, primarily its slow pace, as it typically takes up to two weeks to complete. It relies on a detailed visual evaluation of disease severity in the root of the bait plants, which can be time-consuming and somewhat subjective. This process also requires a significant level of expertise to identify root hairs infected by *S. subterranea* using staining and molecular techniques. Despite these limitations, the bioassay is sensitive, capable of detecting as few as 10 cystosori or even fewer. However, when dealing with high concentrations of cystosori in the soil, only small soil samples can be analyzed effectively (Merz, 1993; Wale *et al.,* 1993).

2.11.1.2. Immunological-based techniques

For detecting and quantifying *S. subterranea* inoculum on seed tubers, the ELISA (Enzyme-Linked Immunosorbent Assay) technique was used to develop various methods based on monoclonal or polyclonal antisera to cystosori (Harrison *et al.,* 1993; Walsh *et al.,* 1996; Merz *et al.,* 2005). A plate-trapped polyclonal antigen form of ELISA (Harrison *et al.,* 1993; Walsh *et al.,* 1996), a DAS (Double Antibody Sandwich) ELISA using a monoclonal antibody (Merz *et al.,* 2005a,b; Montero-Astua *et al.,* 2008), and the *S. subterranea* Agristrip® developed by BIOREBA AG (Reinach, Switzerland) can be used for the detection of *S. subterranea* resting spores (Merz and Falloon, 2009; Bouchek-Mechiche *et al.,* 2011; Strydom *et al.,* 2024). These antibody-based methods are user-friendly, rapid and have high enough sensitivity (as few as one to ten cystosori/ml solution) and adequate specificity such that misdiagnoses are rare (Bouchek-Mechiche *et al.,* 2011; Strydom *et al.,* 2024).

2.11.2. Molecular identification

2.11.2.1. LAMP

A recent development in the detection of *S. subterranea* is the Loop-Mediated Isothermal Amplification (LAMP) method, introduced by Jiang et al. (2023). LAMP offers a quick, straightforward, and highly sensitive diagnostic approach, capable of detecting as little as 2 picograms of the pathogen's DNA. This technique is both cost-effective and reliable, making it ideal for identifying powdery scab infections (Jiang *et al.,* 2023; Strydom *et al.,* 2024).

The method includes two outer primers (F3 and B3) and two inner primers (F2 + F1c, and B2 + B1c), targeting six specific regions within a DNA fragment with several hundred base pairs. Additional loop primers may also be included to speed up the reaction (Negamine *et al.,* 2002; Jiang *et al.,* 2023). Unlike traditional amplification techniques, LAMP operates at a constant temperature, eliminating the need for complex thermocyclers; a simple water bath suffices.

One of the most practical aspects of LAMP is its ease of result interpretation. A successful reaction can be identified visually through a colour change in the reaction tube, without any technical or instrumental analysis.

2.11.2.2. Nucleic acid-based detection

In the mega-diverse fungal kingdom, for the identification of the species, different approaches based on DNA sequences have been established (Hibbett, 1992; Bridge *et al.,* 2005; Hibbett *et al.,* 2011; Hibbett *et al.,* 2013; Taylor *et al.,* 2013). Molecular phylogenetics based on DNA has supported and overturned some of the previous schemes for classification (Inderbitzin *et al.,* 2006; Runa *et al.,* 2009; Pryor *et al.,* 2009; Wang *et al.,* 2011; Alves *et al.,* 2013; Woudenberg *et al.,* 2013; Lawrence *et al.,* 2012, 2013, 2014).

DNA barcoding is known as the characterization and classification of eukaryotic organisms that depend on standardized genetic markers of DNA sequences (Hebert *et al.,* 2003; Meyer and Paulay, 2005; Schoch *et al.,* 2012; Schindel and Miller, 2005). Originally, DNA barcoding was designed to identify animal species (Hebert *et al.,* 2003). Species identification is done through small, consistent regions of DNA ranging between 400 and

800 base pairs (Kress *et al.,* 2012). While the logic of DNA barcoding, relying on the barcode gap where interspecific variation exceeds intraspecific variation, has proven effective for many groups, its application to Spongospora requires additional consideration. Although molecular identification methods for fungi advanced significantly about twenty years ago with the development of ribosomal operon primers (White *et al.,* 1990), *S. subterranea* is not a true fungus but a member of plasmodiophorids, a group of obligate biotrophic protists within the Cercozoa. This phylogenetic distinction may affect marker choice and the evolutionary rate of barcode regions, potentially influencing the resolution and reliability of specific species-level identification.

2.11.2.3. Conventional PCR

The DNA of *S. subterranea* in soil, water, tuber and plant tissues can be detected using a polymerase chain reaction (PCR) assay. The development of specific primers for *S. subterranea* has significantly enhanced the sensitivity of pathogen detection in both soil and plant samples, surpassing traditional immunological techniques such as ELISA (Bulman and Marshall, 1998; Bell *et al.,* 1999; Van De Graaf *et al.,* 2003; Merz and Falloon, 2009). PCR can detect even small quantities of DNA in a sample (Okubara *et al.,* 2005). Research indicates that PCR is capable of detecting as few as one to ten cystosori per gram of soil (Bell *et al.,* 1999).

2.11.2.4. Quantitative PCR

Quantitative PCR (qPCR), also known as real-time PCR (RT-PCR), has advanced beyond conventional PCR by enabling both the amplification and quantification of targeted DNA. This technique has been widely applied to detect *S. subterranea* in various sample types, including naturally infested soil, asymptomatic host plants, infected potato tubers and roots (van de Graaf *et al.,* 2003; Ward *et al.,* 2004; Wright *et al.,* 2012; Brierly *et al.,* 2013; Malik *et al.,* 2019; Simango *et al.,* 2020). Compared to traditional PCR, qPCR offers superior sensitivity, detecting *S. subterranea* even at low concentrations, including cases of tuber contaminations without visible symptoms (McCartney *et al.,* 2003; van de Graaf *et al.,* 2003). It also delivers faster results, demonstrates greater reference to sample contaminants and requires less technical expertise. These advantages make qPCR an essential tool in the management of *S. subterranea*, aiding in risk assessment and control strategies for powdery scab (Bell *et al.,* 1999; McCartney *et al.,* 2003; Ward *et al.,* 2004; Qu and Christ, 2006; Nakayama *et al.,* 2007; Tegg *et al.,* 2016).

2.11.2.5. Phylogenetics of *S. subterranea*

Bulman and Marshall (1998) and Bell et al. (1999) were among the first to genetically characterize *S. subterranea* by sequencing the ribosomal internal transcribed spacer (ITS) regions of the pathogen's DNA. Their work identified two distinct genetic groups, referred to as Type I and Type II. A subsequent study by Qu and Christ (2004) confirmed the existence of these two groups but noted differences from the earlier findings by Bulman and Marshall. Specifically, they observed that North American *S subterranea* samples were predominantly classified as Type II. Laster-Osorio et al. (2012) expanded on this classification by proposing a third group, Type III, based on an analysis of 127 ITS rDNA sequences from samples collected across four provinces in Colombia.

Further research by Qu and Christ (2006) utilized RFLP markers to examine North American isolates, revealing two distinct genetic clusters. One cluster included isolates from western North America, while the other comprised isolates from the eastern part of the continent. These findings underscore the genetic variation of the pathogen across different regions and emphasize the need for a broader global study. Such research could benefit from using diverse genetic tools, including microsatellite markers (as suggested by Dobrowolski *et al.,* 2003) or RFLP markers (Qu and Christ, 2006).

Interestingly, studies like that of Gau et al. (2015) have highlighted the greater genetic diversity in South American populations of *S. subterranea*, suggesting that this region may represent the pathogen's center of origin.

2.12. Management of powdery scab

Managing powdery scab caused by *S. subterranea* is challenging due to the pathogen's highly effective survival strategies, i.e., the three-layered resting spore wall of cystosori (Falloon, 2009). The difficulty in controlling powdery scab lies in the pathogen's biphasic lifecycle, including both short-lived and long-lived propagules (Merz, 2008). These propagules enable the pathogen to persist in the soil and limit the effectiveness of available control measures (Falloon, 2009). Currently, there is no single method to control the disease completely (Merz, 1993; Iftikhar *et al.,* 2007). However, adopting an integrated disease management approach that combines various strategies can help significantly reduce the risk of disease outbreaks (Harrison *et al.,* 1997). These strategies include cultural practices, chemical control and cultivar resistance.

2.12.1. Cultural practices

2.12.1.1. Seed selection and planting

Disease avoidance using clean seeds in clean soil represents the best method of disease prevention (Merz, 2000). Powdery scab is one of the factors considered during seed certification, as the use of certified seed tubers helps prevent the spread of seed-borne inoculum to other fields or regions (Harrison *et al.,* 1997). However, once the *S. subterranea* is established in the soil, management of the disease becomes difficult.

Susceptible cultivars play a significant role in the increase of inoculum pressure (Hernandez Maldonado *et al.,* 2013; Sparrow *et al.,* 2015).

Delaying planting to reduce the time during which potato plants are grown in disease-favourable cool soil temperatures may reduce the severity of powdery scab due to disease escape (Christ and Weinder, 1988; Johnson and Miliczky, 1993; Johnson and Cummings, 2015).

2.12.1.2. Suppressive soils

Soil suppression refers to the natural ability of soil to reduce or prevent plant diseases. Soils with disease-suppressive properties are classified into "general" and "specific" categories (Waller *et al.,* 2002; Wright *et al.,* 2021). General soil suppression occurs naturally and is influenced by factors such as soil chemistry, the physical properties of soil and the overall activity of the soil microbiota (Waller *et al.,* 2002; Cook, 2014; Schlatter *et al.,* 2017; Wright *et al.,* 2021). This broad-spectrum protection defends against a wide variety of soilborne pathogens but cannot be transferred between fields or soil if a small amount is mixed into a large one. However, improving the population, diversity, and activity of soil microorganisms can enhance the soil's effectiveness (Baker and Cool, 1974; Cook and Baker, 1983; Waller *et al.,* 2002; Cook, 2014; Schlatter *et al.,* 2017). In contrast, specific suppressive soils rely on the antagonistic actions of certain microbial groups or individual microorganisms to protect plants from specific pathogens (Wright *et al.,* 2021). These mechanisms are precise and targeted, offering an additional layer of protection for susceptible host plants. For example, suppression of fungal diseases through suppressive soils has been documented for several diseases including *Verticillium dahliae*, *Rhizoctonia solani* (Wiseman *et al.,* 1996), *Fusarium oxysporum* (Alabouvette *et al.,* 1993; Siegel-Hertz *et al.,* 2018), *Streptomyces scabies* (Menzies, 1959), *Pythium splendens* (Kao *et al.,* 1983), *Pythium ultimum* (Martin *et al.,* 1986), *Thielaviopsis basicola* (Stutz *et al.,* 1986), *Heterodera avenae* (Kerry, 1988), *Phytophthora cinnamomic* (Ko *et al.,* 1989), *Criconemella xenoplax* (Kluepfel *et al.,* 1993), *Phytophthora infestans* (Andrivon, 1994), *Meloidogyne spp.* (Weibelzahl-Fulton *et al.,* 1996), *H. schachtii* (Westphal *et al.,* 1999), *Ralstonia solanacearum* (Shiomi *et al.,* 1999), *Aphanomyces euteiches* (Persson *et al.,* 1999), *Plasmodiophora brassicae* (Murakami *et al.,* 2000). A well-known example of natural soil suppressiveness is take-all decline (TAD), which tends to develop after repeated wheat or barley cropping where the take-all disease has previously occurred. This phenomenon is mainly driven by the increase of certain beneficial Pseudomonas bacteria in the soil that produce the antibiotic 2, 4-diacetylphloroglucinol (2, 4-DAPG), which builds up in the root zone and helps suppress the disease (Kwak and Weller, 2013).

Furthermore, a recent study conducted by Wright et al. (2021) in New Zealand revealed that the disease-suppressive characteristics of soils, including microbial activities, were locally specific. These characteristics effectively reduced *S. subterranea* populations in the tested soils. The study emphasized the importance of pre-planting molecular analyses to detect Spongospora DNA and assessing detailed soil physicochemical characteristics. Such approaches could help develop environmentally sustainable strategies for managing powdery scab and mitigating its impact on potato crops (Hao and Ashley, 2021; Wright *et al.,* 2021; Strydom *et al.,* 2024).

2.12.1.3. Irrigation and soil moisture management

Cool and damp soil conditions promote the occurrence and severity of powdery scab in potato crops. Excessive soil moisture indirectly contributes to disease severity by altering soil conditions (Falloon, 2009). For instance, flooding depletes oxygen while increasing CO_2 levels, which can enhance the survival and activity of *S. subterranea*

(Harrison, 1997; Fiers *et al.,* 2011). These conditions may also influence the viability of resting spores and zoospores, as well as their ability to infect potato roots and stolons. Zoospores require free moisture for movement toward the host plant roots (Merz, 1992; Strydom *et al.,* 2024). Infection is most likely to occur at cooler soil temperatures (9-17°C), which favours the release of zoospores (Van de Graaf *et al.,* 2005, 2007; Shah *et al.,* 2012; Strydom *et al.,* 2024). Therefore, managing/manipulating soil conditions during crop growth can help minimize disease development (Falloon, 2009).

To prevent water retention and waterlogging, growers should focus on improving drainage (Hughes, 1980; Strydom *et al.,* 2024). Overworking the soil can create a fine tilth that retains excessive moisture, while frequent use of heavy machinery or farm traffic on wet fields may lead to compaction, further increasing water retention and reducing beneficial microbial activity (Wale, 2000; Sinton *et al.,* 2022; Strydom *et al.,* 2024). Proper irrigation management is also essential (Taylor *et al.,* 1986; Tuncer, 2002; van de Graaf *et al.,* 2007; Falloon, 2009; Shah *et al.,* 2014). Withholding irrigation during early crop growth when soil temperatures are cooler may suppress zoospore release from resting spores, reducing the chances of infection (Falloon, 2009). Similarly, delaying planting until the soil is warmer can help avoid the peak infection window for *S. subterranea* (Kirkham, 1986; Falloon, 2009). Research has shown that withholding irrigation for a month during the tuber setting period can reduce disease severity by 75% (Taylor *et al.,* 1986; Strydom *et al.,* 2024). However, this approach may also negatively impact tuber quality and yield (Shock *et al.,* 1992). Instead of eliminating irrigation entirely, growers can apply reduced amounts of water to meet soil moisture requirements without creating favourable conditions for the disease (Wale, 2000; Tuncer, 2002). Utilizing real-time soil moisture monitoring tools can support better irrigation decisions, allowing for adjustments that balance disease management with optimal crop growth (Whelan and Mulcahy, 2017).

2.12.2. Crop management practices

2.12.2.1. Chemical control

Managing soilborne pathogens such as *S. subterranea* through chemical application poses significant challenges due to soil properties, which can reduce fungicide effectiveness and the high inoculum levels often present (Wallace *et al.,* 1995). Despite these difficulties, several agrochemical treatments have shown a reduction in powdery scab incidence. These approaches typically involve targeting seed tubers or soil either before or at the time of planting to mitigate the disease's impact (O'Brien and Milroy, 2017). A study done by Braithwaite et al. (1994) assessed 25 fungicide treatments applied to heavily infected tubers before planting. Results indicated that some treatments significantly reduced the proportion of diseased tubers from an initial 95% at planting to an average of 29% in the subsequent crop, compared to untreated controls, where disease levels only declined to 70%. However, in their study, certain treatments showed phytotoxic effects as well (O'Brien and Milroy, 2017).

Falloon et al. (1996) examined the application of chemicals either as seed treatments or as in-furrow-at-planting applications. Fungicide groups such as dithiocarbamate (mancozeb), pyridinamine (fluazinam) and chlorophenol (dichlorophen-Na) were effective when used as seed treatments, lowering the incidence of powdery scab while enhancing the yield up to 36%. Fluazinam and flusulfamide have proven effective for managing powdery scab in infested soils and are registered for this purpose in New Zealand and USA. Specifically, fluazinam reduced powdery scab prevalence and increased the marketable yield of cultivars such as "Ruba" by 55% and "Agria" by up to 140% (O'Brien and Milroy, 2017). In the UK, fluazinam has been granted special authorization for use as a soil treatment in seed potato production, even though this specific application is not included on the product's standard label. Under this off-label approval, it can be applied at a maximum rate of 3 liters per hectare (Hilton *et al.,* 2007).

In 2006, Ron Howard highlighted a study conducted by Jill Thomson and Doug Waterer in 2005 at the University of Saskatchewan. The research evaluated the effectiveness of six fungicides: Allegro (40% fluazinam), Tuberseal (16% mancozeb), Dithane (75% mancozeb), Ranman (34.5% cyazofamid), Blinix in-furrow and Blinix at hilling (8.5% Rhamnolipid Biosurfactant) with an untreated control against powdery scab in a naturally infested field site. Among all the tested treatments, Ranman stood out as the most effective in significantly reducing the powdery scab impact on tubers and roots.

Cyazofamid, a fungicide traditionally used to control late blight, has recently shown considerable effectiveness in managing *S. subterranea* on both roots and tubers (Thomson *et al.,* 2006). Similarly, stable bleaching powder (calcium hypochlorite) ($Ca(OCl)_2$), calcium hydroxide ($Ca(OH)_2$), and calcium chloride ($CaCl_2$) applied to soil successfully decreased powdery scab incidence and severity in field trials conducted in Pakistan's Kaghan Valley, a key seed tuber-producing region (Hamidullah *et al.,* 2002).

Several studies have indicated that adjustment of soil chemical content or application of different nutrients can reduce powdery scab expression in potato crops. Soil application of sulfur (McCreary, 1967) or salts of zinc (Wale, 1987) has been shown to reduce the disease. Boron applied as sodium tetraborate reduced gall formation in potato plants when used at non-phytotoxic rates (Falloon *et al.,* 2001; Merz and Falloon, 2009).

Specific compounds in root exudates have been identified that stimulate resting spore germination and zoospore attraction toward host plant tissue (Balenders *et al.,* 2016a, 2018; Lekota *et al.,* 2020; Amponash *et al.,* 2023; Strydom *et al.,* 2024). Hoagland solution (a standard hydroponic nutrient solution) stimulates *S. subterranea* zoospore release in the absence of a host (Merz, 1997; Amponash *et al.,* 2023). Iron chelates, such as Fe (Ferric)-EDTA (ethylenediaminetetraacetic acid), have been reported to suppress powdery scab incidence (Strydom *et al.,* 2024). Research done by Balendres et al. (2018) found that certain chemical solutions, including those containing Fe-EDTA, could stimulate the premature/early release of zoospores from resting spores. This early release potentially reduces the number of viable resting spores in the soil, thereby decreasing the pathogen's inoculum density (Balendres *et al.,* 2018). By lowering the inoculum levels, the likelihood of infection in subsequent potato crops is diminished, offering a potential strategy for managing powdery scab (Strydom *et al.,* 2024).

2.12.2.2. Cultivar resistance

The most economical and environmentally friendly method to manage powdery scab is by developing disease-resistant cultivars. This can be achieved by introducing immunity genes from wild Solanum species, such as *S. curtilobum*, *S. andigenum* and *S. acaule* (Black, 1947; Boyd, 1951), which naturally possess resistance to the pathogen (Black, 1947; Boyd, 1951; Mäkäräinen *et al.,* 1994). Although several potato cultivars with different levels of resistance to *S. subterranea* have been identified (Falloon *et al.,* 2003), no cultivar is known to be completely tolerant to powdery scab (Table 2.1) (Falloon, 2008; Hernandez-Maldonado *et al.,* 2013; Bittara *et al.,* 2016).

Resistance varies from very resistant to highly susceptible (Falloon, 2008), which categorizes more resistant cultivars as showing fewer galls and fewer zoospores in their roots. However, the correlation is not tight, and exceptions exist (Falloon, 2003). A study that evaluated 99 potato cultivars for their resistance to powdery scab found most cultivars classified as “resistant” to tuber blemishes and somewhat resistant to root infection, though this relationship might not hold true in all conditions (Falloon *et al.,* 2003). In contrast, Zink et al. (2004) reported that tuber resistance does not necessarily correlate with root resistance. Additionally, potato cultivars with smooth skin are generally more prone to lesion development compared to those with russet skin (Miller, 2001).

Resistance in potatoes to root infection by *S. subterranea* has been assessed in several studies. Harrison et al. (1997) stated that all cultivars are susceptible (Sprau, 1953, 1966; Noll, 1963; Hide, 1981; Gans *et al.,* 1987; De-Boer, 1991) in his review on powdery scab. A greenhouse study by Falloon et al. (2003) found a strong positive correlation between zoosporangium infection of root cells and root gall formation in 15 potato cultivars. However, some cultivars showed exceptions; highly susceptible cultivars did not always display severe root galls, and the scab-resistant cultivar developed severe root hyperplasia. This suggests that susceptibility to root galls and scab lesions can vary independently among cultivars (Falloon *et al.,* 2016).

Houser and Davidson (2010) found variability in the susceptibility of potato cultivars to *S. subterranea*, noting differences in root galls and tuber lesions observed in greenhouse and field trials. For instance, Russet Burbank exhibited moderate susceptibility to root galls and resistance to tuber blemishes, while Atlantic displayed severe root galls with moderate tuber blemishes (Merz *et al.,* 2012; Falloon *et al.,* 2016). Most cultivars are highly susceptible to powdery scab, as out of 513 potato genotypes tested by Bhattacharya et al. (1985), only 13 showed high tolerance, though none were immune to powdery scab, and 397 were highly susceptible (Harrison *et al.,* 1997). A recent study conducted in North Dakota and Minnesota evaluated common North American potato cultivars. It ranked them based on their susceptibility to powdery scab, including root gall formation and tuber scabbing (Table 2.1) (Bittara *et al.,* 2016).

Table 2.1. Powdery scab and root gall formation susceptibility ranking of commonly known potato cultivars in North America.

		Root gall formation			
		Very Resistant	**Moderately Resistant**	**Moderately Susceptible**	**Very Susceptible**
Tuber scab formation	**Very Resistant**	Alturas Atlantic Bannock Russet Dakota Jewel Dakota Russet Dakota Trailblazer Dark Red Norland Karu Ranger Russet Russet Norkotah Yukon Gold	Alpine Russet	Russet Burbank Umatilla Russet Yagana	Snowden

	Moderately Resistant	Colorado Rose	Dakota Crisp Red Norland		Lamoka Nicolet
	Moderately Susceptible		Dakota Ruby Viking		Red Pontiac
	Very Susceptible			Dakota Pearl Red LaSoda	Ivory Crisp Kennebec Shepody

Resistance to powdery scab has been considered correlated with tuber periderm thickness (Strydom *et al.,* 2024). Compared to smooth-skinned cultivars, russet-skinned cultivars are reported to be more resistant (Houser and Davidson, 2010). According to Bone (1955), the susceptibility of the Kennebec cultivar to powdery scab lesions is due to its inability to form a thick cork layer beneath the developing blemishes. Furthermore, disease resistance in potatoes is believed to be controlled by multiple genes and inherited additively (Falloon *et al.,* 2003; Merz *et al.,* 2012). Later in the crop cycle, resistance mechanisms may function directly at the infection site (Falloon *et al.,* 2003). Additionally, the defence mechanisms active during the infection stage may differ in their effectiveness against the pathogen's sporosorus stage (Hernandez Maldonado *et al.,* 2013).

HYPOTHESES

1. The severity of powdery scab disease in commercial potato fields in Alberta is correlated with the population levels of *S. subterranea* cystosori in the field.
2. The establishment of region-specific threshold levels for the initial inoculum of a pathogen, using soil detection and bioassay methods, will accurately predict the onset and severity of disease in the potato crop, allowing for targeted and effective disease management.
3. *S. subterranea* affected tubers may exhibit the presence of Potato mop-top virus.
4. The effectiveness of different fungicides in managing powdery scab will vary, making some of them more effective in disease suppression. Moreover, their active ingredients differ in their phytotoxicity profiles, which will influence potato yields.

OBJECTIVES

This research project focused on the molecular detection of *S. subterranea* DNA from field soil, roots, and tubers where root galls and/or powdery scab lesions were observed, and quantification of *S. subterranea* propagules in the soil to provide producers with data on field selection and disease management. We also evaluated two different active ingredients (fungicides) for controlling powdery scab of potatoes under regional environmental conditions. This broad goal was achieved by accomplishing the following objectives:

1. Evaluation of the presence and population levels of *S. subterranea* in a minimum of 10 commercial potato fields suspected to have powdery scab.
2. Morphological and molecular characterization of *S. subterranea* from collected potato samples.

3. Establishment of region-specific threshold(s) for the initial inoculum of the pathogen at which disease can be problematic.

4. Evaluation of the *S. subterranea* affected tubers and/or roots for the presence of PMTV.

5. Evaluation of the efficacy of commercially available and new fungicides against powdery scab, as well as the phytotoxicity of these fungicides toward potato plants.

The experimental work in this study was conducted for three consecutive years, from 2022 to 2024, in different commercial potato fields of Alberta.

CHAPTER 3: MATERIALS AND METHODS

3.1. Soil sample collection

During the trials conducted in 2022, 2023 and 2024 (April-September), a total of 22 potato fields suspected of the presence of powdery scab in Southern Alberta were sampled. Composite soil samples of 5 to 7 cores up to 30 cm deep were collected from each field to assess the presence and quantity of *S. subterranea* in the soil. Each area was geotagged. The collected soil samples were dried in a conventional oven (Fisher ISOTEMP® OVEN, 200 series, 230F), hand mixed well and stored at 4°C until evaluated for the presence and quantity of powdery scab using different molecular diagnostic techniques.

3.2. Field selection

Fungicide efficacy trials were established in commercial fields with a history of powdery scab. Since *S. subterranea* is a long-lived obligate parasite, these fields were considered to have powdery scab disease potential. In 2022, all Southern Alberta potato fields were planted early, making it difficult to collect pre-plant soil samples. Therefore, a field in Central Alberta was selected that year. In 2023 and 2024, fields in Southern Alberta were selected because higher disease pressure was reported. In total, one field in 2022, four fields in 2023 and two fields in 2024 were selected based on the anecdotal history of powdery scab from the growers and local agronomists. Pre-plant soil samples were collected from these fields to confirm the presence of powdery scab using molecular techniques, i.e., PCR/qPCR.

3.3. Planting and fungicide applications

Each trial experiment was arranged in a paired plot-randomized complete block design (PP-RCBD) (Fig. 3.1). This design was particularly suited for this study because it allows for direct comparison between treatments within each pair, thereby minimizing the impact of uneven disease distribution in the field and improving the reliability of treatment comparisons. Each treatment pair consisted of 2 to 4 adjacent treatment rows and the adjacent untreated check rows. The rows were positioned within a commercial field that had been prepared for potato production. Treatment rows were 5 to 6 m long and were manually opened using garden hoes. The row spacing was 86 to 90 cm to match each grower's planter. Treatments were applied using electric backpack sprayers equipped with flat-fan nozzles, delivering the products in an 8-inch-wide band along the open furrow at label or manufacturer-recommended rates (Table 3.1). The seed tubers were cut and pre-treated with fungicides by the growers. The average seed piece size was determined by weighing 100 seed pieces before they were planted. The seed pieces were planted with an in-row spacing of 30 cm, ensuring consistent plant-to-plant distance. Fertilizer (Table 3.2) was applied over the seed, if needed, by using an electric backpack sprayer. The rows were manually closed by using garden rakes.

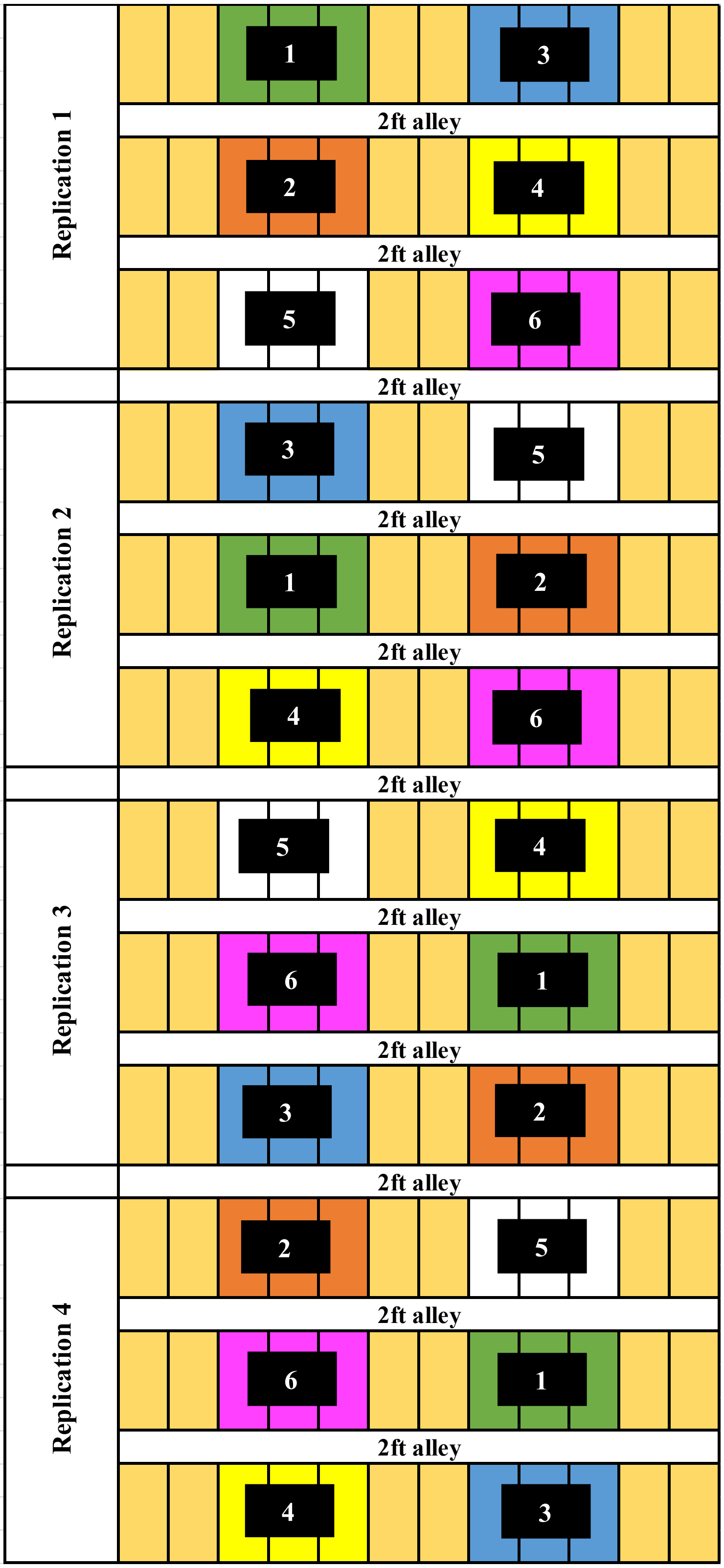

Figure 3.1. A representative paired-plot randomized complete block design (PP-RCBD) followed for the powdery scab trials.

Table 3.1. Treatments, their active ingredients and application rates.

	Treatment	Active ingredient	g/L	g ai/ha	L/ha
1.	Water 2023/EDTA 2024	Iron + EDTA	2	-	-
2.	A21008A	oxathiapiprolin	200	70	0.35
3.	Allegro-low	fluazinam	500	875	1.75
4.	Allegro-medium	fluazinam	500	1400	2.8
5.	Allegro-high	fluazinam	500	1750	4.5
6.	A24367B	oxathiapiprolin + fluazinam	18.9 + 379.4 Total = 398.3	70 + 1400 Total = 1470	3.69

Table 3.2. Fields, cultivars and agronomic practices followed in powdery scab trials.

Fields	Variety	Row spacing (inches)	Seed size (grams)	IFAP* Fertilizer
CA - 2022	Orchestra	34	N/A	Not applied at planting
SA1 - 2023	Shepody	34	65	10-34-0 + humic acid + zinc (28 gallons per acre)
SA2 - 2023	Russet Burbank	34	58	10-34-0 (20 gallons per acre)
SA3 - 2023	Russet Burbank	36	58	10-34-0
SA4 - 2023	Lady Claire	36	59	10-34-0
SA5 - 2024	Russet Burbank	34	58.5	10-34-0
SA6 - 2024	Lady Claire	36	67.6	10-34-0

***IFAP** = In-furrow at planting; 10-34-0 (N-P-K) = liquid ammonium polyphosphate fertilizer

3.4. Stand count, phytotoxicity and disease progress

Beginning 60 days after planting (60 DAP), the fields were visited regularly to collect in-season data, control weeds, observe phytotoxicity effects of treatments on plants and monitor the development of the disease. A brief visual assessment was performed in early July to check for potential treatment-related effects. Stand counts were taken post-emergence, and treated plots were compared with untreated controls for any visible signs of phytotoxicity such as stunting, chlorosis, or abnormal leaf development. Roots from untreated check rows were inspected for initial galls, and tubers were inspected for scab symptoms. Except for the field CA-2022, small galls were visible on the roots of all varieties by the end of July to early August in both 2023 and 2024. Roots with galls were collected in Ziplock bags and brought to the lab for further analysis, i.e., staining and molecular analysis.

3.5. Root and tuber sample collection for disease evaluation

Root and tuber samples were collected each year in August and September for the evaluation of the presence of powdery scab (Table 3.3). Root systems of plants were dug carefully by hand with a potato fork or spade to expose, but not to dislodge, root galls. Roots from 9 plants and 27 tubers from the same plants were collected from replicates of each treatment and untreated check rows, with each sample bagged separately. To preserve their condition, the root samples were stored in a cooler with ice packs and then held at 10 to 15°C until evaluated. Tubers were collected in sandbags and stored at 15°C until evaluated for powdery scab lesions.

Table 3.3. Powdery scab sample collection dates.

Fields	Sample collection dates
2022 – CA, Orchestra	September 16, 2022
2023 – SA1, Shepody	August 8-10, 2023
2023 – SA2, Russet Burbank	August 26,28, 2023
2023 – SA4, Russet Burbank	August 29, 2023
2023 – SA3, Lady Claire	September 8, 2023
2024 – SA5, Russet Burbank	August 21, 2024
2024 – SA6, Lady Claire	August 28, 2024

3.6. Harvesting for yield analysis

Between late August and early September of 2022 and 2023, yield samples were harvested by hand from a 3m section of the central row(s) within the treatment rows for the yield assessment (Table 3.4). Tubers were placed in sandbags and temporarily stored in a cold room at 15°C to dissipate field heat. The temperature was then gradually lowered to the desired storage level, and tubers were graded into size categories as described below (section no. 3.8). Yield samples were not collected in 2024 because no yield effect was observed in the samples collected in 2022 or 2023.

Table 3.4. Powdery scab trials harvesting dates.

Fields	Sample harvesting dates
CA - 2022, Orchestra	Sep 17, 2022
SA1 - 2023, Shepody	Aug 30, 2023
SA2 - 2023, Russet Burbank	Sep 9, 2023
SA3 - 2023, Russet Burbank	Sep 9, 2023
SA4 - 2023, Lady Claire	Sep 4, 2023

3.7. Disease evaluation

Root samples were evaluated for the presence of powdery scab galls. After carefully washing the roots, the number of galls per plant was counted, and a gall severity index was developed to standardize assessments and facilitate easier comparison across samples. Each plant was then classified into one of five gall index categories based on the number of galls on the major roots: 0 = no galls; 1 = 1-5 galls; 2 = 6-10 galls, 3 = 11-15 galls; 4 = ≥ 16 most major roots with galls (Fig. 3.2) (Merz *et al.,* 2012).

Figure 3.2. Root gall severity rating scale for powdery scab.

All tubers were hand-washed under tap water to remove soil and assessed visually for powdery scab lesions by following the slightly modified scab rating scale as 0 = no visible disease on tuber surface, 1 (1-2% tuber surface covered with lesions), 2 (2.1-5% tuber surface covered with lesions), 3 (5.1-10% tuber surface covered with lesions), 4 (10.1-25% tuber surface covered with lesions), 5 (25.1-50% or more tuber surface covered with lesions) (Fig. 3.3) (Merz, 2000).

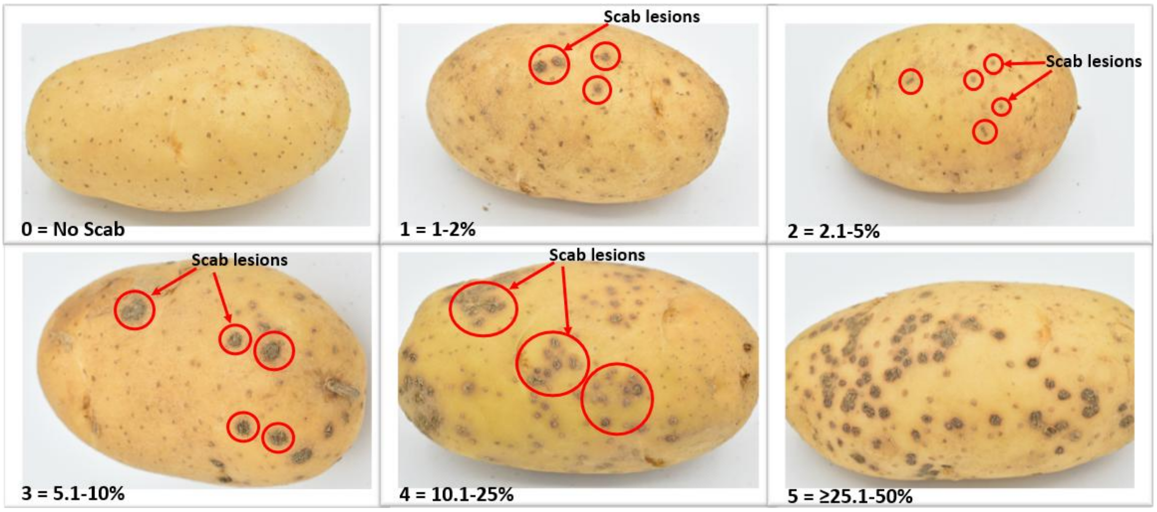

Figure 3.3. Scab severity rating scale for powdery scab affected tubers.

3.8. Yield assessment

Harvested potatoes were graded according to potato production specifications, taking into account their end use, to assess the impact of powdery scab on yield. The chipping potato tubers were graded into size categories for total and marketable yield (< 48mm, 48-88mm, >88mm, and deformed). Potatoes measuring between 48 and 88 mm in size and free from deformities were classified as marketable.

French fry potatoes were graded into size categories by weight (<113g, 113 to 170g, 170 to 284g, over 284g, and deformed). Tubers over 113g and free from deformities were considered marketable. Specific gravity was measured on a 20-tuber sample from each replicate using the weight in the air over weight in the water method.

3.9. PMTV assessment

In the 2023 field trials, after the yield assessment, 25 tubers from each bag of SA2-Russet Burbank and SA4-Lady Claire cultivars, only from untreated rows, were returned to storage at 15ºC for post-harvest evaluation of PMTV. In February (approximately 150 days after harvesting), 10 potatoes were cut longitudinally to observe for any PMTV spraing symptoms. The remaining 15 tubers were returned to storage and reexamined in April 2024 (approximately 240 days after harvesting) for the presence of spraing symptoms.

In the 2024 field trials, a similar assessment was followed. Tubers from SA5-Russet Burbank and SA6-Lady Claire cultivars were collected from untreated check rows of each of the four replications per trial. A total of 25 tubers per cultivar per replication were bagged and stored at 15ºC until PMTV assessments. Similar to 2023, ten tubers were examined for PMTV spraing symptoms in February 2024, and the remaining 15 tubers were reexamined in April 2024. Symptomatic tubers from both time points were tested using RNA extraction and RT-PCR techniques as described below in sections 3.12.2 and 3.13.

3.10. Statistical analysis

3.10.1 Disease severity assessment

Disease severity was analyzed using R software to perform a paired t-test. This analysis compared treatment values (Trt_Value) to untreated control values (UTC_Value) under specified parameters. While conducting the R analyses, the significance level (α) was set at 0.05, indicating a 5% risk of rejecting the null hypothesis when it is true. A two-tailed t-test was executed to determine whether there was a statistically significant difference in the means of Trt_Value and UTC_Value in either direction (greater than or less than). The output from this analysis included the calculated t-statistics, degrees of freedom, and the p-value. A p-value of less than or equal to the alpha level (<0.05) indicated a statistically significant difference between treatment and control groups.

3.10.2. Yield data analysis

Yield data was analyzed statistically using single variant ANOVA and Tukey's Multiple Comparison Test (SPSS; $\rho \leq 0.05$).

3.11. Morphological identification

The morphological identification of *S. subterranea* in potato roots and galls was done by using the Acid Fuchsin staining method. Four lateral root segments with galls, each 20 mm in length, were randomly selected from the field samples. The roots were thoroughly washed to remove any attached soil. The roots were cut into 2-3 cm sections and placed in a beaker containing a solution of 50 ml of water and 20 ml of 5.25% sodium hypochlorite (bleach). They were incubated in this solution for further removal of soil residues. After four minutes of incubation, the roots were rinsed and soaked in tap water for 15 minutes to wash away any residual bleach.

A staining solution was prepared by mixing 30 ml of Acid Fuchsin (C.I. 42685, Matheson Coleman and Bell) stain with 30 ml of water. The root sections were immersed in the stain. The mixture was heated in a microwave for 30-40 seconds until it reached a boil. The roots were then allowed to cool to room temperature, after which they were rinsed with tap water to remove excessive stain.

For preservation, the roots were transferred into a beaker containing 20-30 ml of glycerin. A few drops of 5M sodium chloride (NaCl) were added to enhance the staining contrast. The roots were rested at room temperature for one to two days in ambient light conditions. Finally, the roots were examined under a Zeiss Axioskope 40 microscope at 40X magnification, equipped with an Axiocam 208 camera (Carl Zeiss, Jena, Germany) to observe the presence of *S. subterranea* cystosori and zoospores and to capture micrographs. Size measurements were performed using the ZEN blue 3.1 imaging software (Carl Zeiss).

3.12. Molecular analysis

3.12.1. *S. subterranea* DNA extraction

Total DNA was extracted from the soil samples using the PuroSPIN™ Soil Genomic DNA Purification Kit (LUNA Nanotech, ON, Canada) by following the manufacturer's instructions.

3.12.2. PMTV RNA extraction

3.12.2.1. CTAB method

RNA from tubers showing spraing symptoms and roots showing powdery scab galls was extracted by following the CTAB protocol 1 ($CTAB_1$) method as described by Carpinetti et al. (2021). 100mg of tuber tissue was frozen in liquid nitrogen and ground into a fine powder using a pestle and mortar. Then, 900µl of extraction buffer preheated at 65°C was added to the powdered tissue, mixed well, and transferred to a 2 ml Eppendorf tube. The tube was shaken vigorously several times and incubated at 65ºC for 10 minutes to help break down cell structures. After incubation, an equal volume of chloroform: isoamyl alcohol (24:1, v/v) was added to the mixture. The tube was shaken again and centrifuged at 10,000×g for 10 minutes at 4ºC to separate phases. The supernatant was carefully transferred to a new 1.5 ml Eppendorf tube. The supernatant was re-extracted by adding 65µl of chloroform: isoamyl alcohol, followed by centrifugation at 25,000×g for 30 minutes at 4°C. RNA was precipitated by adding 0.5 volume of LiCl and incubating at 4ºC overnight. The RNA was then collected by centrifuging the tube at 25,000×g for 30 minutes at 4°C. The pellet was washed three times with 75% ethanol (v/v), each followed by centrifugation at 10,000×g for 20 minutes at 4°C. Once the pellet was washed and the ethanol removed, it was air dried at room temperature, dissolved in 30µl of RNase-free water and stored at -80^{o}C for further analysis.

3.12.2.2. BIOREBA potato DNA/RNA extraction kit

BIOREBA potato DNA/RNA extraction kit (Art. No. 810003) was also used to extract total RNA from PMTV-affected potato tissues following the manufacturer's instructions. The extracted RNA was immediately stored at -80^{o}C until further use (cDNA synthesis and RT-PCR, section no. 3.13).

3.12.3. DNA/RNA quantification

BioNanodrop was used to determine the concentration and quality of each DNA and RNA sample at 260/280 nm absorbance with the exchange of optical density of 1=50µg/ml.

3.12.4. PCR amplification

DNA amplification was performed using primers targeting the Internal Transcribed Spacer (ITS) region of *S. subterranea* using primer pairs Spo1/Spo2 (372 bps) and Spo8/Spo9 (390 bps) (Bulman and Marshall, 1998). The details of the primer sequences and their programs are given below (Table 3.5). PCR amplifications were carried out in a total of 25µl reactions. Each reaction contained 6.8µl of master mix (AllTaq Master Mix Kit, Quantabio, Cat. No. / ID: 203144), 1µl of each forward and reverse primer, 3µl of sample DNA template and 14.2 µl of nuclease-free water. Amplifications were carried out in a thermocycler (Eppendorf^{TM} Mastercycler^{TM} Nexus X2).

Table 3.5. Primers used for the detection of *S. subterranea* f. sp. *subterranea* (Sss).

Sss primers	**ITS Sss primer sequences (5’-3’)**	**PCR program**				**Reference**
		Denaturation temperature	**Annealing temperature**	**Cycles**	**Extension temperature**	
Spo1	ATTGTCTGTTGAAGGGTG	94°C for 30 seconds	53°C for 30 seconds	35	72°C for 45s	Bulman and Marshall, 1998
Spo2	GGTTAGAGACGAATCAGAA					
Spo8	CTGGGTGCGATTGTCTGTTG		60°C for 30 seconds			
Spo9	CACGCCAATGGTTAGAGACG					

3.13. RT-PCR

Reverse Transcribed Polymerase Chain Reaction (RT-PCR) was performed on the stored RNA extracted from PMTV-affected tissues (tubers and roots). Thermo Scientific RevertAid First Strand cDNA synthesis kit (Thermo Scientific^{TM}, Cat. No. / ID: K1621) was used to synthesize cDNA from the total RNA samples by following the manufacturer's instructions.

cDNA amplification was performed by using specific primers targeting different gene regions. C819/H360 (460bp) (MacKenzie, 1996; Xu *et al.,* 2004) and pmt1/pmt2 (553bp) (Xu *et al.,* 2004) were used to target the "coat protein" region, while pmtF/pmtR (417bp) (Xu *et al.,* 2004) were used to target the "RNA2" region of the PMTV. The details of the primer sequences and their programs are given below (Table 3.6). PCR amplification conditions were optimized, and final amplifications were carried out in a total of 25µl reactions. Each reaction contained 6.8µl of master mix (AllTaq Master Mix Kit, Quantabio, Cat. No. / ID: 203144), 1 µl of each forward and reverse primer, 3 µl of sample cDNA template and 14.2 µl of nuclease-free water. Amplifications were carried out in a thermocycler (Eppendorf^{TM} Mastercycler^{TM} Nexus X2).

Table 3.6. PMTV-specific primers, used for the detection of PMTV.

PMTV primers	**PMTV primer sequences (5'-3')**	**PCR program**			**Cycles**	**References**
		Denaturation temperature	**Annealing temperature**	**Extension temperature**		
C819	CTATGCACCAGCCCAGCGTAACC	95°C for 1 minute	64°C for 1 minute	72°C for 1 minute	40	MacKenzie, 1996; Xu et al. (2004)
H360	CATGAAGGCTGCCGTGAGGAAGT					
pmtF4	CAGCAACCACAAACAGACAGG					Xu et al. (2004)
pmtR4	AAGCCACTAACAAAACATACTGC					
pmt1	TCTCGGATACCAC CCT TGGA					
pmt2	CTATGCACCAGCCCAGCGT					

3.14. Agarose gel electrophoresis

Amplified PCR products (*S. subterranea* and PMTV) were analyzed using horizontal gel electrophoresis (Nanopac-300). For this purpose, 1.0% (w/v) agarose gel (Thermo Scientific, USA) and 0.5X Tris Borate EDTA (TBE) buffer were used by staining with gel green. Gel electrophoresis was done at 110 V for 60 minutes. The amplified PCR products of targeted gene regions were stained with Gel Green (100 µg/ml) (Fisher Biotech) and compared with a 1kb DNA ladder (Thermo Scientific^{TM}, Cat. No. R0611). The DNA fragments were visualized under UV light using the Gel Doc XR + System with Image Lab^{TM} software (Bio-Rad, USA).

3.15. Phylogenetic analysis

PCR products from seven different trial sites were sent to Azenta Life Sciences (South Plainfield, N.J., USA) for DNA sequencing. The ITS region of *S. subterranea* was targeted for phylogenetic analysis due to its utility in distinguishing among plasmodiophorid taxa. The resulting nucleotide sequences were assembled and refined for subsequent analysis. Reference sequences of *S. subterranea* and other closely related plasmodiophorids were obtained from the NCBI GenBank database, including representative sequences from *Plasmodiophora brassicae*, *Polymyxa graminis*, *Spongospora subterranea* f. sp. *nasturtii* and previously identified Type I and Type II isolates of *Spongospora subterranea* f. sp. *subterranea* from different geographical locations.

Phylogenetic relationships were inferred using the Neighbour-Joining (NJ) method (Saitou and Nei, 1987) in MEGA 11 (Tamura *et al.,* 2021). Sequence alignments were performed using the MUSCLE (Multiple Sequence Comparison by Log-Expectation) algorithm. The final dataset consisted of 32 nucleotide sequences with 155 positions in the final alignment. The evolutionary distances used to generate the tree were computed using the Maximum Composite Likelihood (MCL) method (Tamura *et al.,* 2004), with branch lengths expressed as the number of base substitutions per site. Rate variation among sites was modelled using a gamma distribution with a shape parameter of 5. The robustness of tree topology was assessed using a bootstrap test with 1000 replicates. The tree was rooted with the *Colletotrichum coccodes* sequence as an outgroup.

3.16. Quantification of *S. subterranea*

Real-time quantitative PCR (qPCR) analyses of DNA extracted from soil samples collected from the seven trial potato fields (CA, SA1-SA6) were performed in Hard-Shell 96-well PCR plates using CFX Opus 96 Real-Time PCR systems (Bio-Rad, USA). For each sample, 1µl template DNA was added to a 24 µl reaction consisting of 12.5 µl perfeCTa qPCR Thoughmix (Quantabio, USA), 9.6 µl nuclease-free water, 0.75 µl each of the primers, targeting and amplifying a 434 bp ITS gene region, SsF (5'-GTCGGTTCTACCGGCAGACC-3') and SsR (5'-GCACGCCAATGGTTAGAGACG-3') at a concentration of 10 µM (Qu *et al.,* 2006). The thermal cycle protocol for PCR amplification was as follows: initial denaturation at 95°C for 2 minutes, followed by 30 cycles of 94°C for 30 seconds, 60°C for 1 minute, 72°C for 1 minute, and a final extension of 10 minutes at 72°C.

The real-time PCR assay included a series of standards containing varying amounts of *S. subterranea* DNA to ensure accurate quantification. The DNA was extracted from a known number of cystosori, following the method outlined by Bell et al. (1999). This DNA was then diluted with nuclease-free water to create a series of dilutions equivalent to 50,000, 5000, 500, 50, 5, 0.5 and 0.05 cystosori per 500 µl.

For each unknown sample, the *Cq* (quantification cycle) value was calculated by the CFX Manager™ software. Additionally, the PCR assay always included a non-template control containing no DNA to detect any possible contamination or non-specific amplification. All samples, including the positive and negative controls, were tested in triplicate to ensure reliability and accuracy in the results.

DNA for qPCR was extracted from three independent sub-samples from each field soil sample to determine the variability in *Cq* values between sub-samples.

3.16.1. Generating the standard curve

To analyze the qPCR data, the *Cq* values for the standard values were plotted on the y-axis against the log-transformed concentrations of the standards on the x-axis. A scatter plot was created, and the linear regression trendline was added to the plot to fit the data. The regression equation and the R-squared (R^2) values were displayed directly on the chart to indicate the equation of the line and the goodness of fit for the model. The equation used to calculate the standard curve is as follows:

$$Cq = m \times (log10[Concentration]) + b$$

where 'm' represents the slope, and 'b' represents the y-intercept. To calculate the concentration for unknown samples, the equation was rearranged as follows:

$$Concentration = 10\text{^}\frac{(Cq - b)}{m}$$

The R^2 value of the standard curve was examined to ensure it was close to 1, indicating a strong linear relationship between *Cq* values and log-transformed concentrations. Additional graphs were generated to visually compare the calculated concentrations of the unknown samples against the standard curve, highlighting any deviations that could affect assay performance (not shown).

3.17. Bioassay for the establishment of the disease threshold level

To identify the infection threshold of powdery scab, tissue-cultured plantlets of the Russet Burbank cultivar were screened for *S. subterranea* before planting by using molecular detection tools, i.e., DNA extraction and PCR. Twelve pots were filled with a 3:1:1 mixture of coconut coir, vermiculite and perlite with a tablespoon of Osmocote (14:14:14, slow-release) fertilizer. Pots were placed in separate saucers to prevent cross-contamination and labelled according to inoculum concentration.

Four concentrations of *S. subterranea* cystosori (0, 1, 15, and 150 cystosori/g soil) were used. The initial spore suspension was obtained from a soil sample of a known powdery scab-infested field. A hemocytometer was used to count the number of cystosori (1,680,000) in a 120 ml homogenized stock spore suspension, under the light microscope. From the stock spore suspension, serial dilutions of 0, 100, 1000 and 10,000 spores/ml were prepared using the equation $C_1V_1 = C_2V_2$. The corresponding inoculum volumes of stock suspension used were: 0.714 ml for 10,000 spores/ml, 0.07 ml for 1000 spores/ml, 1 ml for 100 spores/ml and 0 ml for the control (water only). These inoculum dilutions were mixed into the prepared potting mixture before transplanting plantlets. Plants were grown under controlled conditions in a plant growth cabinet set to a daytime temperature of 22°C (14h) and nighttime temperature of 12°C (10h). Soil moisture was maintained by watering with reverse osmosis (RO) water every two days or as needed.

Root samples were collected 14, 28, and 42 days after planting. Plants were carefully uprooted, rinsed with tap water to remove the attached soil, cut into small sections, and placed in labelled Falcon tubes, stored at -20°C, for molecular analysis.

Molecular analysis of the collected root samples was done using DNA extraction and PCR techniques, and *S. subterranea*-specific primers (Spo8/Spo9) were used (Table

3.5). Gel electrophoresis (Nanopac-300) was performed to analyze the PCR products, and the gel was observed using the Gel Doc XR+ System with Image Lab^{TM} software (Bio-Rad, USA) (section 3.14).

Microscopic analysis of the root samples included the staining of root samples using the Acid Fuchsin (C.I. 42685, Matheson Coleman and Bell) stain method. The stained root samples were observed under a Zeiss Axioskope 40 microscope at 40X magnification, equipped with an Axiocam 208 camera (Carl Zeiss, Jena, Germany), for the presence of *S. subterranea* cystosori.

CHAPTER 4: RESULTS

4.1. Disease severity

4.1.1. Effect of fungicides against powdery scab

In 2022, no disease was observed on the roots and tuber samples.

Gall severity and treatment efficacy varied across fields and cultivars during the 2023 field trials. In the SA1-Shepody field, A21008A appeared to result in the lowest gall severity based on the numerical values, and this difference was not statistically significant. In contrast, treatments Allegro-low, Allegro-high and A24367B significantly reduced gall severity compared to their adjacent untreated checks, despite having slightly higher numerical values than A21008A. In the SA2-Russet Burbank field, although Allegro-high had the lowest numerical value, no treatments showed statistically significant differences. In the SA3-Russet Burbank field, Allegro-medium resulted in the lowest gall severity, and the reduction was statistically significant. This was closely followed by A24367B. In the SA4-Lady Claire field, Allegro-low had the numerically lowest gall severity, but Allegro-medium was the only treatment showing a statistically significant reduction (Figure 4.1).

In the 2024 field trials, Allegro-high had the numerically lowest values in the SA5-Russet Burbank field, but none of the treatments showed statistically significant differences in reducing the gall severity. In the SA6-Lady Claire field, all treatments except Allegro-medium resulted in statistically significant reductions in gall severity (Figure 4.2).

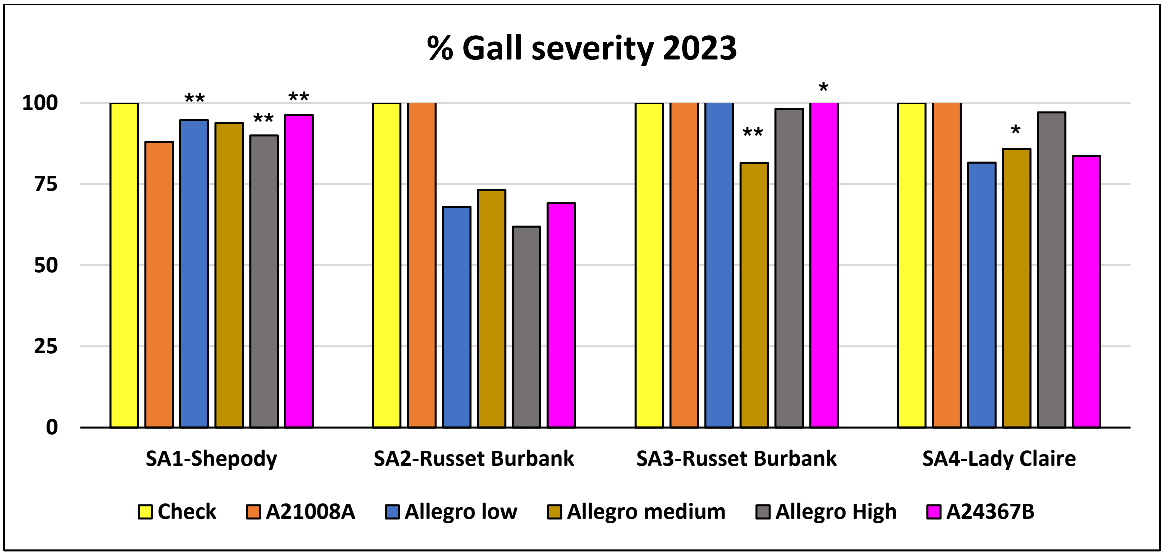

Figure 4.1. Percent gall severity of powdery scab across five treatments in four field trials in 2023. Treatment values are expressed relative to their adjacent untreated check values (Trt/Check×100), with the 100% check bar included as a visual reference. Asterisks indicate statistical significance compared to the check (**= ρ < 0.05; *ρ = < 0.1).

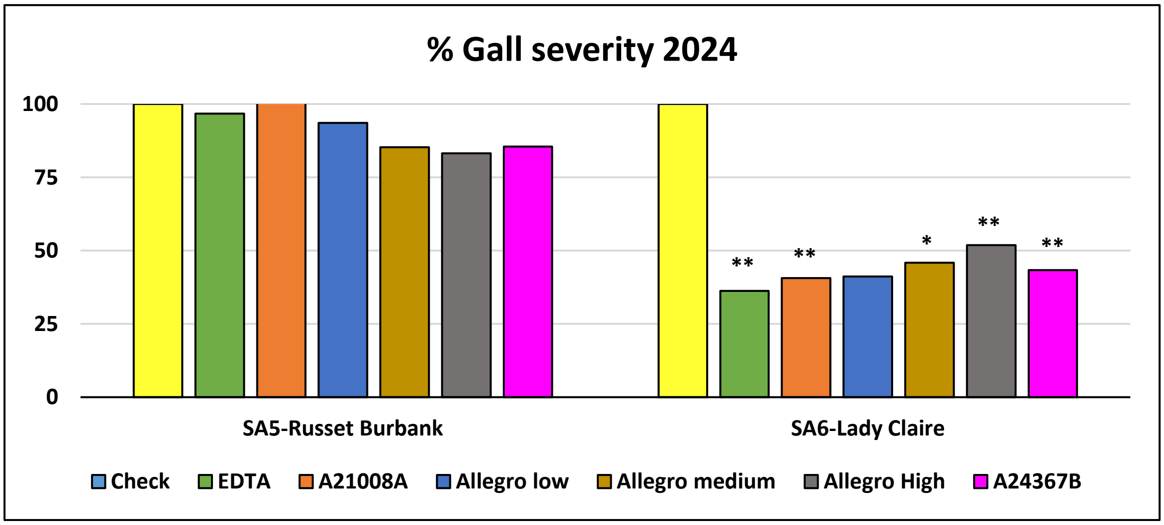

Figure 4.2. Percent gall severity of powdery scab across six treatments in four field trials in 2024. Treatment values are expressed relative to their adjacent untreated check values (Trt/Check×100), with the 100% check bar included as a visual reference. Asterisks indicate statistical significance compared to the check (**= $\rho < 0.05$; *ρ = < 0.1).

For scab suppression, in the 2023 field trials, none of the treatments in SA1-Shepody and SA2-Russet Burbank fields significantly reduced scab severity. However, A24367B exhibited the lowest numerical scab severity in both fields. In the SA3-Russet

Burbank field, the difference was not statistically significant by any of the treatments. In contrast, the SA4-Lady Claire field demonstrated a significant treatment response: Allegro-low had the lowest scab severity and was statistically significant, with all other treatments also showing significant suppression (Figure 4.3).

In the 2024 field trials, the SA5-Russet Burbank field exhibited mixed outcomes. EDTA and A21008A were among the lowest in scab severity and were statistically significant. Allegro-low and Allegro-high had numerically low values but were not statistically significant. Interestingly, A24367B, though not among the numerically lowest, showed a statistically significant scab reduction. In the SA6-Lady Claire field, EDTA, A21008A, Allegro-low, and A24367B showed lower mean scab values, but none of them were statistically significant. However, Allegro-high, despite not being numerically lowest, exhibited statistically significant scab suppression (Figure 4.4).

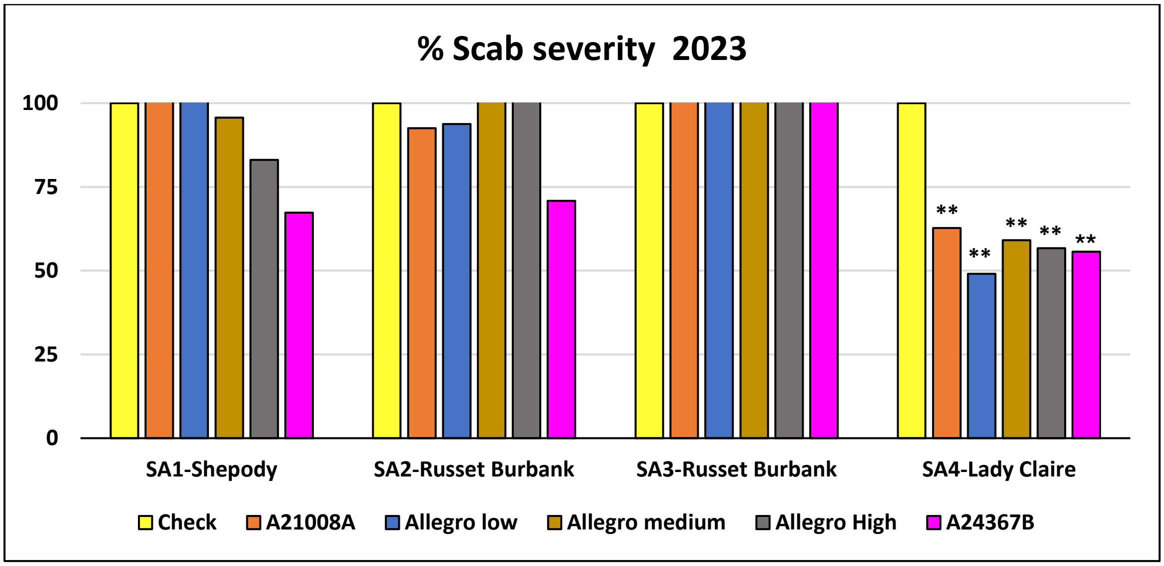

Figure 4.3. Percent scab severity of powdery scab across five treatments in four field trials in 2023. Treatment values are expressed relative to their respective untreated check values (Trt/Check×100), with the 100% check bar included as a visual reference. Asterisks indicate statistical significance compared to the check (**= $\rho < 0.05$; *$\rho = < 0.1$).

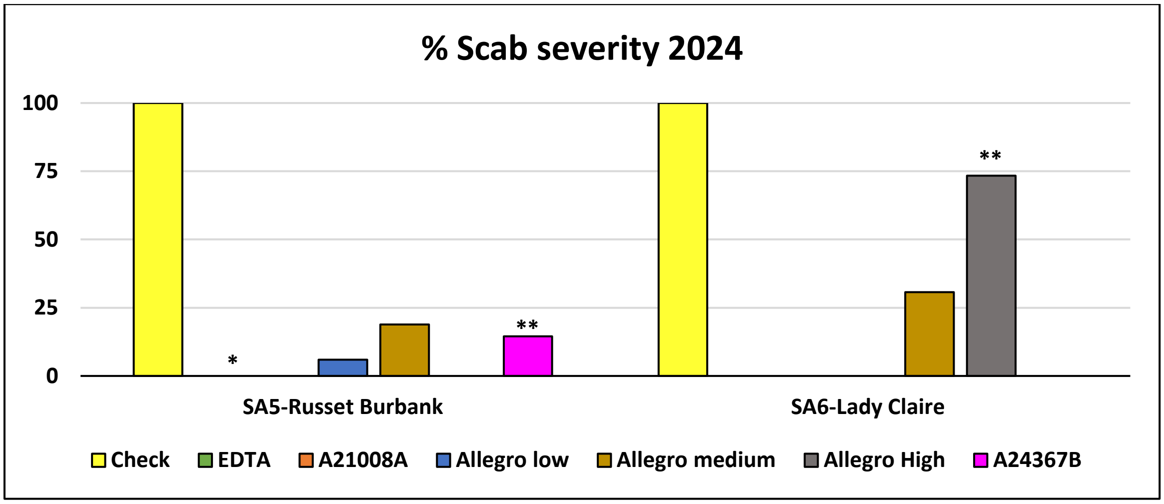

Figure 4.4. Percent scab severity of powdery scab across six treatments in four field trials in 2024. Treatment values are expressed relative to their respective untreated check values (Trt/Check×100), with the 100% check bar included as a visual reference. Asterisks indicate statistical significance compared to the check (**= $\rho < 0.05$; *$\rho = < 0.1$).

4.2. Morphological assessment

4.2.1. *S. subterranea*

Staining with acid fuchsin made the individual zoospores within the cystosori visible. Microscopic examination of ruptured galls and cystosori showed clusters of spherical to slightly irregular-shaped resting spore balls (cystosori) measuring approximately 4.5 to 5.5 μm in diameter (Figures 4.5 and 4.6).

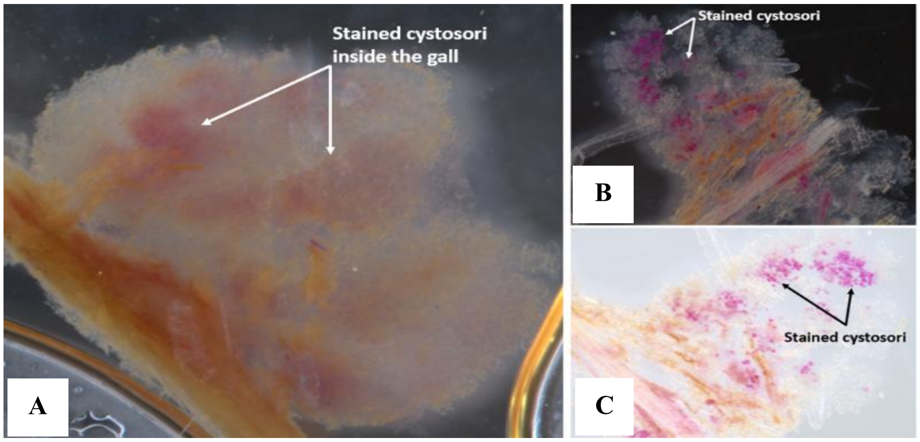

Figure 4.5. Powdery scab cystosori of *S. subterranea* observed under a stereo microscope. **(A)** Stained root gall showing internal cystosori; **(B-C),** released cystosori (stained) upon gall rupture. Gall sizes ranged from 0.5-10 mm.

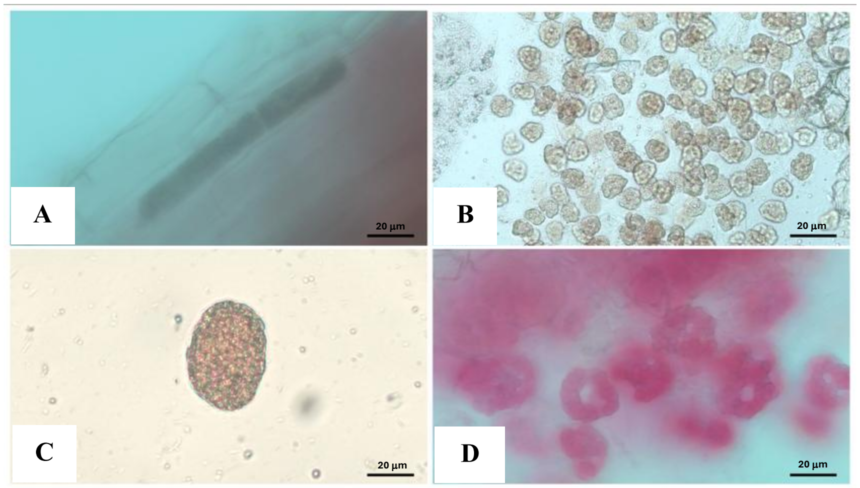

Figure 4.6. Light microscopic observation of *S. subterranea* within an infected potato root sample. **(A)** Plasmodium of *S. subterranea* inside a root cell. **(B-C)** Unstained cystosori appear light to dark brown. **(D)** Stained cystosori with visible zoospores inside, observed under a compound light microscope. Scale bars: 20 µm.

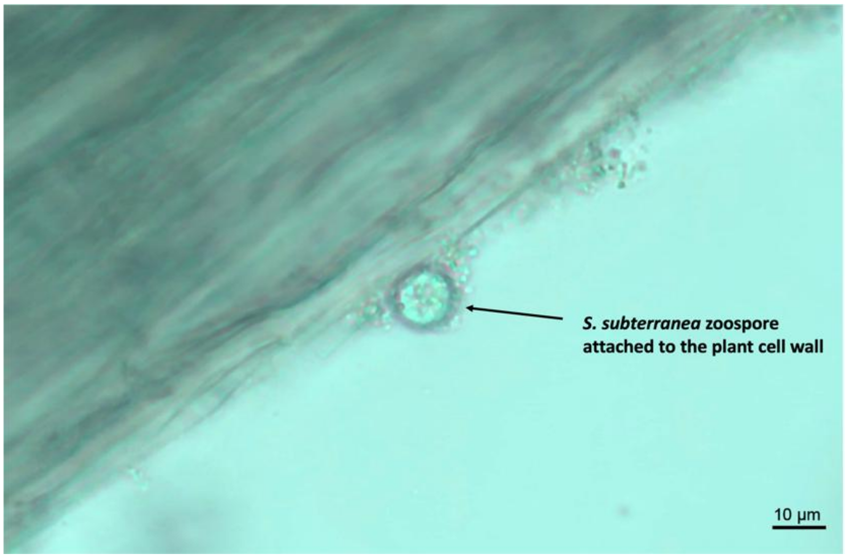

Figure 4.7. Microscopic observation of *S. subterranea* zoospore attached to the root epidermal cell wall (indicated by arrow) before encystment. Scale bar: 10 µm.

4.2.2. PMTV

In both 2024 and 2025, tubers stored at 15°C were examined for PMTV spraing symptoms at two time points. In February 2024, 10 out of 25 stored tubers of Russet Burbank and Lady Claire cultivars from each replication (40 tubers in total) were cut longitudinally; however, no spraing symptoms were observed. The remaining 15 tubers per bag were returned to storage and reexamination in April 2024, where 1 to 3 tubers from both cultivars displayed arc-shaped spraing symptoms (Figure 4.8).

In February 2025, a similar assessment was conducted on Russet Burbank and Lady Claire cultivar tubers, with 10 out of 25 tubers per replication (40 tubers in total) examined for PMTV symptoms. Unlike in 2024, where no symptoms were observed at this stage, 3 to 4 tubers from the Russet Burbank cultivar examined in 2025 exhibited arc-shaped spraing symptoms, indicating an earlier onset of detectable infection. From the Lady Claire cultivar, no examined tubers showed any spraing signs. The remaining 15 tubers per bag were returned to storage for continued observation.

Upon examining the remaining 15 tubers from each cultivar in April 2025, the results were similar to those observed in February 2025. Only Russet Burbank tubers showed spraing symptoms, with 1 to 2 symptomatic tubers found per bag. No Lady Claire tubers were found to exhibit spraing symptoms.

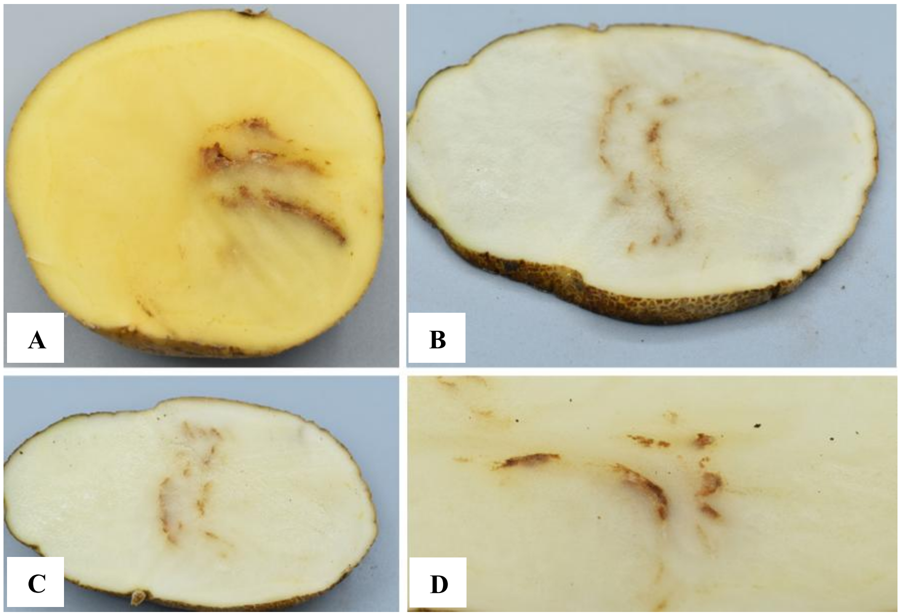

Figure 4.8. **(A)** Lady Claire and **(B-D)** Russet Burbank tubers harvested in 2023 showing spraing (arc-shaped, light to dark brown corky/necrotic streaks) caused by PMTV.

4.3. Molecular identification

4.3.1. *S. subterranea*

4.3.1.1. PCR detection

The ITS gene was targeted as the primary barcode for molecular identification of *S. subterranea* associated with powdery scab. For the amplification of the ITS region of rDNA, two different pairs of *S. subterranea*-specific primers were used. The primer set of Spo1 and Spo2 amplified DNA fragments of 372 bp (Figure 4.9), whereas the primer set of Spo8 and Spo9 yielded bands of 390 bp (Figure 4.10).

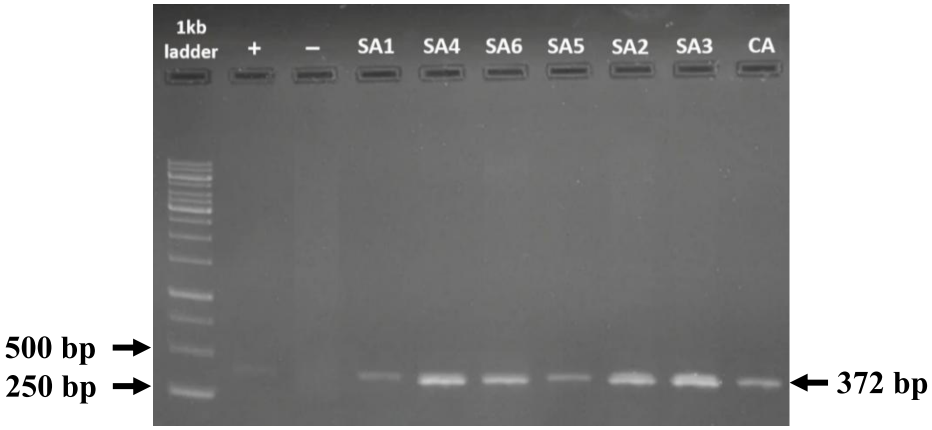

Figure 4.9. PCR products amplified using *S. subterranea*-specific primers Spo1/Spo2. **Lane 1:** 1 kb DNA ladder; **Lane 2:** positive control (*S. subterranea* genomic DNA previously confirmed by sequencing); **Lane 3:** negative control (non-template control); **Lanes 4-10:** soil DNA extracts from the seven selected fields (SA1-CA). The arrow (right) points to the predicted PCR products of ~372 bp.

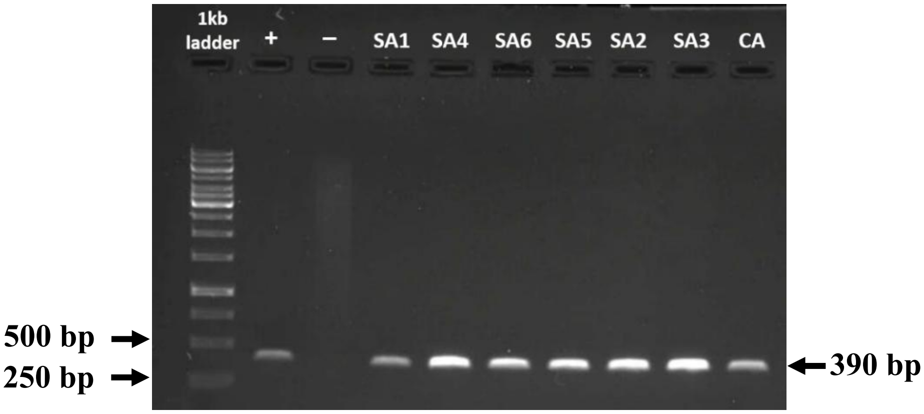

Figure 4.10: PCR products amplified using *S. subterranea*-specific primers Spo8/Spo9. Lane 1: 1 kb DNA ladder; Lane 2: positive control (*S. subterranea* genomic DNA previously confirmed by sequencing); Lane 3: negative control (non-template control); Lanes 4-10: soil DNA extracts from the seven selected fields (SA1-CA). The arrow (right) points to the predicted PCR products of ~390 bp.

The internal transcribed spacer region of isolates was amplified and sequenced commercially (Azenta, Massachusetts, United States) by using dideoxy-nucleotide chain termination sequencing.

4.3.2. PMTV detection

PMTV was detected in both 2023 and 2024 symptomatic tuber samples, using RT-PCR with PMTV-specific primers targeting different viral gene regions. The primer set of C819/H360 amplified DNA fragments of ~460 bp targeting the "coat protein" region, while pmtF/pmtR, amplified DNA fragments of ~417 bp targeting the "RNA2" region of the PMTV (Figures 4.11 and 4.12).

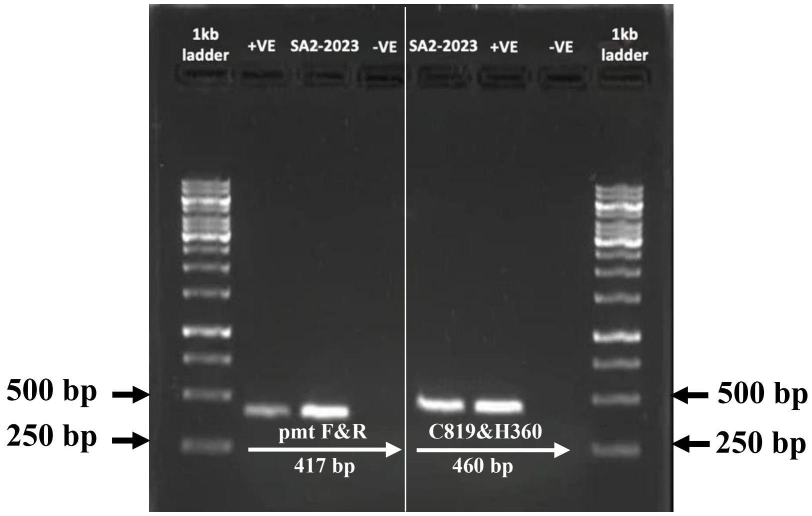

Figure 4.11. PCR analysis of the 2023 PMTV samples using PMTV-specific primers pmtF/pmtR (left, ~417 bp) and C819/H360 (right, ~460 bp). **Lanes:** 1kb DNA ladder; **+ve:** positive control (PMTV genomic DNA previously confirmed by sequencing); **-ve:** negative control (non-template control); **PMTV:** Spraing affected sample.

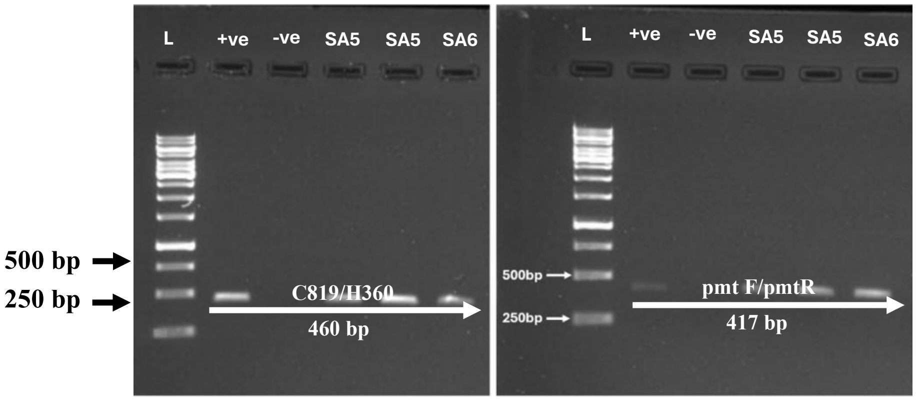

Figure 4.12. PCR analysis of the 2024 PMTV samples, evaluated using PMTV-specific primers C819/H360 (left, ~460 bp) and pmtF/pmtR (right, ~417 bp). Lanes: **L**, 1kb DNA ladder; **+ve**, positive control; **-ve**, negative control; **A-C:** Spraing affected samples.

4.4. BLAST analysis

An initial comparison of the obtained sequences of *S. subterranea* isolates was conducted using the BLAST (Basic Local Alignment Search Tool) function against sequences available in the NCBI (National Center for Biotechnology Information) database. The percentage of similarity between the obtained sequences and the available sequences ranged from 98 to 100%, confirming that the obtained ITS sequences belonged to *S. subterranea* f. sp. *subterranea*. The sequences were subsequently deposited in the NCBI GenBank for accession numbers.

Similarly, the obtained sequences of PMTV isolates were compared with sequences available in the NCBI database using BLAST. The similarity percentage between the obtained sequences and available sequences also ranged from 98% to 100%, confirming that the CP (coat protein) and RNA2 sequences belonged to PMTV. These sequences were also deposited to the NCBI GenBank for accession numbers.

4.5. Phylogenetic analysis of the ITS region of *S. subterranea*

The phylogenetic analysis revealed that all newly sequenced *S. subterranea* isolates from Alberta, including CA, SA1-SA6, form a strongly supported monophyletic clade, indicating a close genetic relationship and low intraspecific variation among them (Figure 4.13). These Alberta isolates showed 99-100% sequence identity with previously reported *S. subterranea* isolates from diverse regions such as South Africa (KU182475.1, KU182474.1, KU182458.1, KU182460.1, KU182472.1), Sri Lanka (MT116436.1), New Zealand (AF102819.1, AF102820.1), USA (AY604171.1), United Kingdom (AF104308.1, Y12829.1), Ireland (AF305697.1), Switzerland (KF018378.1) and Greece (KF208654.1).

S. subterranea f. sp. *nasturtii* forms a separate, well-supported clade from *S. subterranea* f. sp. *subterranea*. This indicates that host specialization has driven genetic divergence, reinforcing the hypothesis that different formae speciales of *S. subterranea* may have evolved independently to infect distinct plant species. *Plasmodiophora brassicae* (club root pathogen) and *Polymyxa graminis* isolates form distinct clusters but are evolutionarily distant from *S. subterranea* f. sp. *subterranea*. *Colletotrichum coccodes* (MK531998.1) served as an outgroup, confirming the tree’s robustness in distinguishing plamodiophorid pathogens from unrelated fungi. The distinct placement of *S. subterranea*

f. sp. *nasturtii* highlights its genetic separation from *S. subterranea* f. sp. *subterranea,* supporting the hypothesis of host specificity.

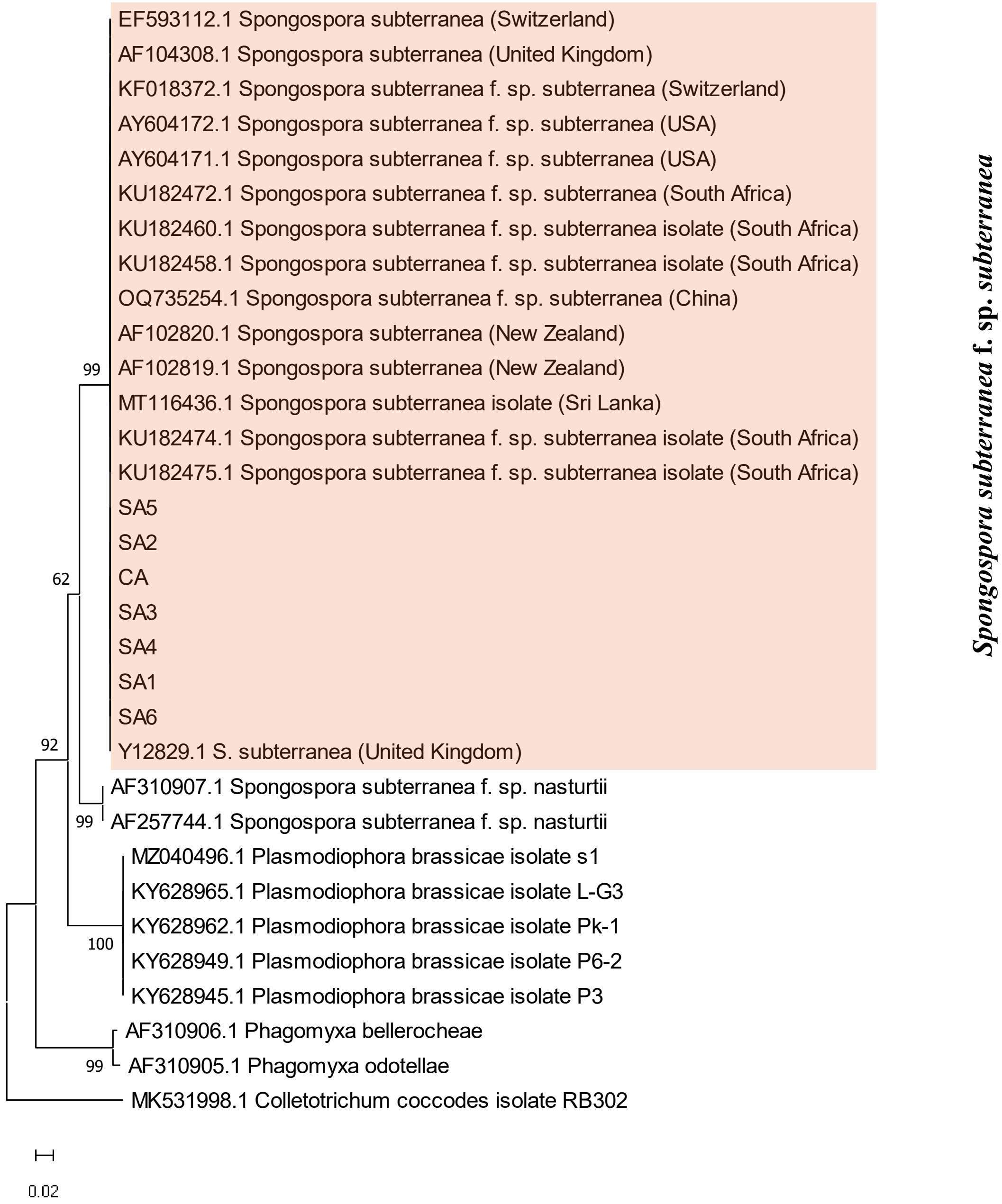

Figure 4.13. The phylogenetic tree of *S. subterranea* f. sp. *subterranea* and other plasmodiophorids based on the ITS dataset comprising 32 nucleotide sequences. Isolates are represented by accession numbers and names. Branches with more than 50% bootstrap support are shown at the nodes. Highlighted area shows the *S. subterranea* f. sp. *subterranea* isolates from the present study aligning with the other reference sequences of *S. subterranea* f. sp. *subterranea.*

4.6. qPCR analysis of the collected soil samples

The qPCR analysis of soil sub-samples revealed substantial variation in *Cq* values, indicating an uneven distribution of *S. subterranea* inoculum within the soil (Table 4.1). While the standard curve allowed for estimating inoculum levels in unknown samples, the results showed limited consistency in *Cq* values across repeated extractions from the same soil sample. In some instances, *Cq* values were lower, suggesting a higher pathogen concentration, while in other extractions from the same sample, *Cq* values were higher, indicating a lower detected inoculum level. This resulted in a wide range of estimated pathogen quantities, reflecting the heterogeneous nature of inoculum distribution within soil matrices.

Table 4.1. Estimated range of *S. subterranea* cystosori per gram of soil derived from three separate DNA extractions and qPCR amplifications.

Soil samples	Variety	Cystosori per gram of soil
2022 – CA	Orchestra	1-15
2023 – SA1	Shepody	260-1437
2023 – SA2	Russet Burbank	140-529
2023 – SA3	Russet Burbank	15582-33589
2023 – SA4	Lady Claire	658-77097
2024 – SA5	Russet Burbank	47-339
2024 – SA6	Lady Claire	209-3360

4.7. Yield data analysis

Harvested tuber samples from 2022 and 2023 field trials provided information about scab disease severity and the effect of fungicide treatments on yield and tuber size distribution in each field. For the 2022 trial with the Orchestra cultivar, post-harvest analysis revealed no signs of powdery scab symptoms on tuber or root samples, despite collected soil samples testing positive for *S. subterranea* DNA. Since the trial was conducted in a seed production field with the cultivar Orchestra, the size distribution of the harvested tubers was also evaluated. The analysis showed no significant differences in total yield or marketable yield among treatments (Table 4.2). However, some significant differences were observed in the small-size category, where the yield of smaller potatoes tended to decrease as the overall yield increased.

For 2023 field trials, in the SA1 field with the Shepody cultivar, differences were observed only in the yield of tubers over 284 g (10 ounces). Allegro-low and Allegro-high produced more large tubers compared to treatments A21008A, Allegro-medium, and A24367B, but their yields were not significantly different from the control (water). Additionally, A24367B yielded fewer large tubers than all treatments except Allegro-medium. However, these differences did not impact the overall marketable yield (Table 4.3).

In the SA2 field with the Russet Burbank cultivar, no significant differences were found in any yield parameters across treatments (Table 4.4). Similarly, no significant differences were observed in any yield parameters in the SA4 field with the Lady Claire cultivar (Table 4.6).

In the SA3 field with the Russet Burbank cultivar, there was a significant difference in the yield of small tubers (culls). Specifically, Allegro-high had a lower yield of small potatoes compared to A21008A, while the other treatments did not show significant differences (Table 4.5).

Overall, while some treatments affected certain yield categories, these differences did not significantly impact the total marketable yield.

Furthermore, no visible signs of phytotoxicity such as chlorosis, stunting, or leaf deformation were observed in any of the treated plots during the 2022-2024 growing seasons.

Table 4.2. Yield (ton/ac) and size distribution of tubers across different treatments for the field CA with the Orchestra cultivar.

2022 – CA-Orchestra				
Treatments	**Yield (ton/ac)**	**Marketable Yield**	**Under 48mm**	**48 to 88 mm**
Control	14.7	0.8	0.8 ab	13.9
A21008A	14.6	1.3	1.3 a	13.3
Allegro-Low	15.5	0.7	0.7 b	14.9
Allegro-Medium	16.5	0.5	0.5 b	16
Allegro-High	18.46	0.9	0.9 ab	17.6
A24367B	18.16	0.9	0.9 ab	17.2

Table 4.3. Yield (ton/ac) and quality parameters of potato tubers across different treatments for the field SA1 with the Shepody cultivar.

2023 – SA1-Shepody								
Treatments	**Yield (ton/ac)**	**Marketable Yield**	**Mean Tuber Size (oz)**	**SG**	**< 4 oz**	**4 to 10 oz**	**> 10 oz**	**Deformed**
Control	19.4	16.2	5.85 ab	1.089	2.9	9.3	6.9 ab	0.1
A21008A	18.2	15.3	5.65 ab	1.090	2.8	9.7	5.5 bc	0.0
Allegro-Low	19.3	17.6	6.82 a	1.088	1.6	9.6	7.9 a	0.0
Allegro-Medium	16.4	14.2	5.75 ab	1.087	2.2	9.9	4.3 cd	0.0
Allegro-High	19.9	17.4	6.42 a	1.090	2.4	8.8	8.6 a	0.0
A24367B	16.0	14.2	5.05 b	1.087	2.8	10.5	2.8 d	0.0

Table 4.4. Yield (ton/ac) and quality parameters of potato tubers across different treatments for the field SA2 with the Russet Burbank cultivar.

2023 - SA2-Russet Burbank								
Treatments	**Yield (ton/ac)**	**Marketable Yield**	**Mean Tuber Size (oz)**	**SG**	**< 4 oz**	**4 to 10 oz**	**> 10 oz**	**Deformed**
Control	25.2	19.9	4.7	1.087	4.8 a	15.0	4.9	0.1
A21008A	24.7	17.6	4.6	1.085	5.5 a	14.0	4.5	0.2
Allegro-Low	22.5	17.2	4.9	1.087	5 a	12.8	4.3	0.4
Allegro-Medium	24.0	17.9	5.2	1.086	4.6 a	12.2	5.6	0.6
Allegro-High	24.3	17.1	5.0	1.084	5.4 a	12.1	5.0	0.7
A24367B	24.2	19.0	4.7	1.085	5.0 a	12.8	6.1	1.4

Table 4.6. Yield (ton/ac) and size distribution of tubers across different treatments for the field SA4 with Lady Claire cultivar.

2023 – SA4-Lady Claire						
Treatments	**Yield (ton/ac)**	**Marketable Yield**	**SG**	**Under 48mm**	**48 to 88 mm**	**> 88 mm**
Control	25.0	17.8	1.097	7.2 a	17.8	0.0
A21008A	25.1	19.5	1.098	4.8 a	19.5	0.9
Allegro-Low	26.0	20.2	1.100	5.6 a	20.2	0.2
Allegro-Medium	24.1	19.4	1.098	4.7 a	19.4	0.0
Allegro-High	26.1	19.9	1.097	5.7 a	19.9	0.5
A24367B	22.8	17.6	1.096	5.3 a	17.6	0.0

Table 4.5. Yield and quality parameters of potato tubers across different treatments for the field SA3 with the Russet Burbank cultivar.

2023 – SA3-Russet Burbank								
Treatments	**Yield (ton/ac)**	**Marketable Yield**	**Mean Tuber Size (oz)**	**SG**	**< 4 oz**	**4 to 10 oz**	**> 10 oz**	**Deformed**
Control	24.7	16.9	4.3	1.084	6.9 ab	12.3	4.6	0.7
A21008A	24.5	16.3	4.2	1.082	7.6 a	14.5	2.7	0.4
Allegro-Low	25.9	18.0	4.3	1.084	7.0 ab	14.7	4.3	0.8
Allegro-Medium	24.6	18.1	4.6	1.083	6.1 ab	14.5	4.6	0.4
Allegro-High	22.5	17.7	4.3	1.083	4.2 a	12.7	4.9	0.5
A24367B	24.1	16.0	4.1	1.082	6.6 ab	12.6	4.4	0.4

4.8. Bioassay

S. subterranea DNA was detected in root samples as early as two weeks after the inoculation. At 0 cystosori/g soil, no *S. subterranea* DNA was detected in any of the samples at 2, 4, and 6 weeks, confirming the absence of infection in the control plants. In contrast, *S. subterranea* DNA was detected at all time periods (2, 4, and 6 weeks) in treatments with 1, 15, and 150 cystosori/g soil, indicating the presence of the pathogen across all inoculated concentrations and time intervals (Table 4.7).

Table 4.7. PCR detection of *S. subterranea* DNA in Russet Burbank roots at different inoculum concentrations (0, 1, 15, and 150 cystosori/g soil) over time (2, 4, and 6 weeks after planting).

		Cystosori concentration per gram of soil			
		0	**1**	**15**	**150**
Time (Weeks)	**2**	-	+	+	+
	4	-	+	+	+
	6	-	+	+	+

CHAPTER 5: DISCUSSION

Powdery scab of potato, caused by the plasmodiophorid pathogen *S. subterranea* f. sp. *subterranea*, is an important quality-limiting disease in many temperate areas of the world where potato crops are intensively grown for human consumption (Falloon *et al.,* 2003; Merz and Falloon, 2008; Balendres *et al.,* 2016b; Wilson, 2016). Soil-borne inoculum is the major and most important factor for the development of powdery scab (Nakayama *et al.,* 2007; Merz, 2008; Brierley *et al.,* 2013). The current study hypothesized that the severity of powdery scab in commercial potato fields in Alberta is associated with the population levels of *S. subterranea* cystosori present in the soil. Our findings support this hypothesis as powdery scab was detected in all 22 sampled fields, the majority of which were located in Southern Alberta. While disease symptoms on tubers and roots were not strictly proportional to inoculum levels, fields with soil populations exceeding roughly 150 cystosori per gram were more likely to show visible symptoms of powdery scab. This pattern suggests that higher inoculum levels may increase the risk of symptom expression; however, other factors, such as cultivar susceptibility and environmental conditions, also play a significant role in disease development.

The detection of powdery scab in all tested fields indicates that *S. subterranea* is well established in these fields. It suggests that *S. subterranea* cystosori populations exceed levels commonly associated with symptom development, particularly when cystosori counts were above 150 cystosori per gram, indicating that inoculum levels in these fields are often sufficient to initiate infection and sustain disease development. Historical reports indicate that powdery scab has been a persistent and growing concern in Western Canada. According to the report submitted by Ron Howard (2006) to the Potato Growers of Alberta (PGA), the incidence and severity of powdery scab have been rising since 2000, with multiple outbreaks recorded in Alberta, Saskatchewan, and Manitoba. Notably, galls associated with powdery scab were already observed in Southern Alberta, and by 2004, local agronomists identified significant disease problems in the region. The affected areas included locations from North of Brooks to South of Grassy Lake, as well as regions surrounding Nobleford and Lethbridge (PGARA, 2008). These findings highlight that powdery scab has been present in Southern Alberta for many years, and as expected, all the fields sampled in the current study were found to be positive for powdery scab.

The persistence and expansion of powdery scab in Alberta may be attributed to historical limitations in disease diagnostics and management strategies. Adding to the complexity is the fact that powdery scab and common scab produce nearly identical symptoms, making accurate diagnosis challenging (De-Haan and Van-Den-Bovenkamp, 2005). As *S. subterranea* is an obligate parasite, studying the pathogen poses significant challenges, further complicating the study and advancement of control efforts. This study was therefore designed to verify the presence, quantify the pathogen population, and explore potential management strategies for powdery scab in Alberta's commercial potato fields.

Researchers have been trying to utilize different chemicals to manage powdery scab for many years, and very few have shown strong or consistent efficacy in reducing disease severity and increasing marketable yield (Houser and Davidson, 2010; Al-Mughrabi, 2025). Continuous regulatory withdrawal of effective soil chemicals and limited investment in developing and registering new chemistries have contributed to the lack of availability of fungicides for powdery scab management (Strydom *et al.,* 2024; Al-Mughrabi, 2025). Currently, there are no highly effective chemical treatments registered for powdery scab control in Canada, with available materials often having limited efficacy due to the robustness of the pathogen resting spores, uneven distribution of inoculum, and soil properties (Braithwaite *et al.,* 1994). Registered fungicides applied to the soil before or during the growing season can, however, provide some protection against disease through inhibition of resting spore germination or killing of zoospores before root or tuber infection (Falloon *et al.,* 1996; de Boer, 2000; Falloon *et al.,* 2008; Thangavel *et al.,* 2015; Simango and van der Waals, 2017). The primary objective of this study was to evaluate the efficacy of different fungicides in reducing the severity of powdery scab. The present study reports the efficacy of five different in-furrow chemical treatments to mitigate powdery scab in potatoes through seven field trials conducted from 2022 to 2024 in Alberta commercial potato fields previously reported to be infested with the pathogen.

The first treatment, A21008A, contained oxathiapiprolin, (1-(4-{4-[5-(2,6-difluorophenyl)-4,5-dihydro-3-isoxazolyl]-2-thiazolyl}-1-piperidinyl)-2-[5-methyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl] ethenone (Miao *et al.,* 2016), as an active ingredient. The molecular target of oxathiapiprolin is the oxysterol-binding protein (OSBP) (Cohen, 2020). It is a novel fungicide known for its high efficacy against various oomycete genera, including Phytophthora and Pythium, which cause disease in many crops, including cucumbers and peppers (Cohen, 2015; Cohen *et al.,* 2018a; Miao *et al.,* 2018; Cohen *et al.,* 2018b; Ji and Csinos, 2015; Olaya *et al.,* 2016; Cohen *et al.,* 2018c; Belisle *et al.,* 2017; Gray *et al.,* 2018).

A study done by Miao et al. 2016 demonstrated that oxathiapiprolin exhibited strong inhibitory activity against a range of agriculturally important plant pathogenic oomycetes, including *Phytophthora spp.*, *Peronophythora litchii*, *Plasmopara viticola*, *Pseudoperonospora cubensis*, *Peronospora parasitica*, and *Pythium ultimum*. The Miao et al. (2016) study demonstrated that oxathiapiprolin can suppress zoospore release and motility at relatively higher concentrations (>1 μg mL^{-1}), but for some pathogens like *P. cubensis*, low concentrations (4.10×10^{-4} μg mL^{-1}) showed suppression as well. Other studies have also indicated that, in addition to suppressing mycelial growth, oxathiapiprolin can also significantly inhibit the formation of sporangia and zoospore germination in *Phytophthora capsica* (Ji and Csinos, 2015).

To the best of our knowledge, the efficacy of oxathiapiprolin against plasmodiophorids, especially powdery scab, has not been previously reported. In the present study, the treatment A21008A, containing oxathiapiprolin as a single active ingredient at a concentration of 70 g ai/ha, showed inconsistent effects in suppressing root gall and scab from both 2023 and 2024 field trials. In 2023 field trials, A21008A showed significant scab suppression only in the SA4-Lady Claire field, while in 2024 trials, it showed significant gall suppression only in the SA6-Lady Claire field. Some possible factors may account for this lack of efficacy. Firstly, oxathiapiprolin may require a higher dosage or multiple applications to be effective, depending on the level of disease pressure and environmental conditions. As indicated by other studies, oxathiapiprolin is effective against different pathogens at different concentrations (high to low) (Miao *et al.,* 2016; Keinath, 2022). The high disease pressure or significant pre-existing soil inoculum levels of *S. subterranea* might have overwhelmed the potential of any single treatment. Furthermore, oxysterol-binding proteins are known to be present in oomycetes but have not yet been documented in *S. subterranea*. As *S. subterranea* is a plasmodiophorid with distinct biology, it is likely that oxathiapiprolin does not affect its zoospores. Furthermore, oxathiapiprolin may be more effective when used in combination with other fungicides that have different modes of action, thereby enhancing their overall efficacy.

Different studies have indicated that conventional fungicides, including fluazinam, applied as seed treatments or in-furrow soil treatments often provide partial but not complete control of powdery scab (Braithwaite *et al.,* 1994; Falloon *et al.,* 1996; Hamidullah *et al.,* 2002; Liu *et al.,* 2021; Thomson *et al.,* 2006; Falloon *et al.,* 2009; Thangavel *et al.,* 2015; Simango and Van der Waals, 2017). This study evaluated three different concentrations of fluazinam, including Allegro-low (875 g ai/ha), Allegro-medium (1400 g ai/ha) and Allegro-high (1750 g ai/ha). Fluazinam [3-chloro-N-(3-chloro-5-trifluoromethyl-2pyridyl)-α, α, α-trifluoro-2,6-dinitro-*p*-toluidine] is a protective fungicide classified under the phenyl-pyridinamines group (Kalamarakis *et al.,* 2000; Qu *et al.,* 2018). The mode of action of fluazinam is to interrupt the fungal cell's energy production by uncoupling mitochondrial oxidative phosphorylation (Mao *et al.,* 201; Qu *et al.,* 2018). While fluazinam is currently registered in Canada for controlling late blight in potatoes via foliar application, it is not approved for powdery scab management. However, it is registered as a soil-applied treatment for controlling powdery scab in other countries such as the USA, New Zealand and Australia. This suggests that there are no major concerns regarding crop safety or environmental impact associated with this method of application. Additionally, it implies that the manufacturer (Syngenta) might be open to expanding the label to include soil applications for powdery scab management (SSPGA, 2014).

The results of this study revealed that Allegro-medium (fluazinam at 1400 g ai/ha) provided the most consistent suppression of gall and scab incidence across all seven field trials. Allegro-high (fluazinam at 1750 g ai/ha) followed closely, while Allegro-low (fluazinam 875 g ai/ha) had the least impact. However, none of the fluazinam treatments achieved complete control of the disease during the 2023 and 2024 field trials. These findings align with previous studies indicating that fluazinam can reduce the incidence of powdery scab severity but does not provide total control. Falloon (2008) reported that fluazinam applied to infected seed tubers before planting in uncontaminated soil effectively suppressed the disease, but it was not completely eradicated (Merz and Falloon, 2009). Also, De-Boer (2000) found that fluazinam had no significant effect on the incidence or severity of powdery scab in progeny tubers when planted in contaminated soil. In New Zealand, fluazinam is registered for reducing powdery scab in infested soil (Falloon, 2008). Similarly, a 2014 report by the Saskatchewan Seed Potato Growers Association (SSPGA) highlighted that fluazinam applied in-furrow and to the hill before hilling provided some level of protection but did not result in disease control.

Additional insights into fluazinam's effectiveness were reported in a study funded by the British Potato Council (BPC) (807/211, 2002), which investigated control options for powdery scab. The findings suggested that fluazinam treatments were more effective at reducing disease severity than eliminating the seed-borne inoculum of *S. subterranea*. The efficacy of fluazinam appeared to be greater when soil-borne inoculum was the primary infection source rather than seed-borne inoculum (Brierley and Less, 2008). This supports the observations from the current study where fluazinam treatments in-furrow-at-planting reduced disease severity but did not achieve suppression, particularly in heavily infested soils.

Additionally, this study examined the efficacy of an alternative treatment, A24367B, a combination of oxathiapiprolin (70 g ai/ha) and fluazinam (1400 g ai/ha). The results showed that A24367B achieved suppression of gall formation and scab levels similar to Allegro-medium. This finding suggests that while fluazinam alone at 1400 g ai/ha is effective to a certain degree, combining it with oxathiapiprolin does not necessarily provide additional benefits beyond what fluazinam alone achieves. Given that oxathiapiprolin is known to target oomycetes rather than *S. subterranea*, its lack of additional impact in this mixture suggests that fluazinam remains the primary active ingredient driving disease suppression in this case. However, further investigation may be needed to determine if oxathiapiprolin offers any indirect benefits, such as influencing soil microbiota or interacting with fluazinam in a way that affects disease progression.

Overall, these findings reinforce that fluazinam can be a useful tool in powdery scab management, but complete disease control remains elusive. The partial suppression observed suggests that repeated application, either within a season or across successive potato crops, may be necessary to gradually reduce the soilborne inoculum. Implementing such treatments over multiple years, especially in fields with a known history of disease, could contribute to long-term management outcomes. The exploration of combination treatment such as A24367B further underscores the potential of integrated strategies, as combining active ingredients may not only improve efficacy against powdery scab but also offer broader protection against other soilborne diseases like Sclerotinia blight (Smith *et al.,* 1992), Late blight (Schepers *et al.,* 2018; Cohen *et al.,* 2021) and pink rot (Syngenta Canada, 2025). Also, when used alongside resistant potato varieties, these combination products could provide a more effective and sustainable approach to disease suppression, enhancing both immediate control and future crop resilience.

Potato plants infected with *S. subterranea* not only suffer from quality deterioration, but the pathogen can also hinder plant growth by disrupting root function, ultimately weakening the host plant (Falloon *et al.,* 2004; Lister *et al.,* 2004; Al-Mughrabi, 2025). In this study, there was no noticeable/significant reduction in plant growth or vigour due to root galls. This could be attributed to the absence of early infections on roots or stolons, likely due to dry weather conditions in Southern Alberta during the initial stages of crop development, which were unfavourable for disease establishment. Soil moisture plays a crucial role in the development of powdery scab, as increased moisture levels help lower soil temperature around the root system. This, in turn, facilitates disease progression by aiding the zoospore's movement toward host plant tissues (Strydom *et al.,* 2024; Al-Mughrabi, 2025). The absence of these favourable conditions early in the season may have limited the severity of root infections in this study.

Yield data was collected only from 2022 and 2023 field trials, providing insights into the impact of fungicides on overall yield and tuber size distribution. The CA-2022 trials with the Orchestra cultivar showed no visible powdery scab symptoms on tubers or roots, despite the field testing positive for *S. subterranea* DNA. Yield analysis revealed no significant differences in total or marketable yield between treatments. qPCR analysis of the CA-2022 field indicated very high *Cq* values as compared to other trial fields of 2023 and 2024, suggesting minimal pathogen DNA levels and, consequently, low disease pressure. This low inoculum load, combined with the moderate resistance of the Orchestra cultivar, likely contributed to the absence of disease symptoms. The moderately resistant cultivar may have further limited the pathogen's ability to infect or proliferate, even under conditions where the pathogen was detectable.

The SA1 field with the Shepody cultivar did not show any significant disease impact on the total marketable yield across treatments, even though the Shepody is reported to be highly susceptible to powdery scab in previous studies (Nitzan *et al.,* 2008; Johnson and Thomson, 2015). However, differences in tuber size distribution were noted, particularly in the yield of large tubers (>284g or 10 ounces). These findings align closely with the field study conducted by Johnson and Thomas (2015), who observed that potato cultivars such as Shepody and Umatilla Russet were not negatively affected by powdery scab root galling in three of four years of trials. Despite severe root galling on Shepody, affecting more than 30% of the root area, no yield reduction was detected. This supports the interpretation that the absence of yield reductions in the current study may be due to a similar cultivar response under field conditions, where physiological compensation or root system redundancy may mitigate the effects of root galling. The presence of larger tubers in some treatments suggests that certain management strategies may have supported plant vigor, allowing Shepody to allocate resources toward producing high-grade tubers, even in the presence of disease.

Johnson and Thomas (2015) also noted that while a yield impact was expected due to root galling, it was not observed, likely due to a complex interplay of cultivar tolerance, environmental conditions, and disease severity. Their findings and the present study differ from those in other regions, such as Colombia (Gilchrist *et al.,* 2011) and Australia (Shah *et al.,* 2012), where root galling did correspond with yield reductions, suggesting that environmental and agronomic context play a critical role in determining the impact of powdery scab on yield. Johnson and Thomas (2015) concluded that trying to control root galls only might not be effective in typical field conditions. Even when symptoms look severe, they don't always lead to a drop in yield, especially in cultivars like Shepody, which can still perform well when growing conditions are good.

In the SA2 field planted with the Russet Burbank cultivar and the SA4 field with Lady Claire, no significant differences in any yield parameters were observed across treatments. These findings are consistent with previous research highlighting the role of cultivar characteristics, particularly skin type and susceptibility, to powdery scab development. Nitzen et al. (2008) noted that while Russet Burbank is susceptible to root gall formation, it tends to develop fewer tuber lesions due to its russet skin, which acts as a physical barrier limiting lesion expansion. This characteristic helps maintain tuber quality even in infected fields, reducing the likelihood of yield or marketability loss. Moreover, they suggested that such cultivars could potentially limit *S. subterranea* inoculum buildup over time, offering both short- and long-term benefits to growers.

Similarly, Lady Claire, a yellow-skinned cultivar (GOC, 2023), has also been associated with low disease severity in both roots and tubers. Bittara et al. (2016) found that genotypes with russet or yellow skin often exhibit lower disease indices (<0.05), likely due to similar physical resistance mechanisms. This aligns with the current study, where neither Lady Claire nor Russet Burbank showed notable treatment effects on yield, suggesting that inherent cultivar resistance or tolerance may have mitigated the disease's potential impact.

In contrast to the SA2-Russet Burbank field, the SA3-Lady Claire field exhibited significant differences in the yield of small tubers (culls), with treatment 5 producing fewer culls than treatment 2. However, no significant effects were seen on the total marketable yield. This suggests that while overall productivity was maintained, certain treatments may have influenced tuber size distribution.

While certain treatments influence specific yield traits, these variations did not substantially affect the total marketable yield. Also, no phytotoxicity effects were observed following the application of any fungicide treatments, indicating that the products used were not detrimental to plant health. This is a positive outcome as it confirms that the fungicides tested can be applied without compromising plant vigour or development under the field conditions provided in the present study. This aligns with the previous studies on both fluazinam and oxathiapiprolin, which have shown low or negligible phytotoxicity across plant species when applied at recommended rates. Studies done by Younes et al. (2020) found that although pepper and eggplant exhibited a temporary decline in plant length shortly after the application of fluazinam, plants quickly recovered and ultimately showed enhanced physiological traits and yield. This suggests that the fluazinam may trigger early plant defence mechanisms without lasting phytotoxic effects (Younes *et al.,* 2020). Similarly, for oxathiapiprolin, research evaluating oxathiapiprolin as a seed treatment for soybeans reported no adverse effects, but rather improved emergence and vigour of seedlings as compared to non-treated controls, with no signs of phytotoxicity involved (Hegstad *et al.,* 2021).

Furthermore, the ability of potatoes to maintain productivity despite the high disease pressures observed in the trial fields highlights the effectiveness of Alberta growers's agronomic practices. The province benefits from a well-developed irrigation system that ensures consistent water availability, even during the dry years, which helps reduce crop stress and supports healthy plant growth even under disease pressure. Additionally, the timely and balanced applications of fertilizers ensure that plants receive essential nutrients during critical growth stages, enhancing their resilience and ability to compensate for root damage caused by *S. subterranea*. These integrated practices not only help sustain tuber development but also mitigate the visible impact of powdery scab on marketable yield, allowing commercial production to remain viable even in infested fields.

To further verify the presence and the population level of *S. subterranea*, this study employed molecular techniques. For the detection of *S. subterranea*, different techniques were utilized, including the conventional PCR, Loop-mediated isothermal amplification (LAMP) (Jiang *et al.,* 2023), and BIOREBA Sss AgriStrip (Art. No. 111181). Conventional PCR was used to confirm the presence of *S. subterranea* in soil samples by amplifying pathogen-specific DNA regions. The PCR amplification provided a qualitative assessment, identifying fields where *S. subterranea* was present. Visualization of PCR products through gel and sequencing of those PCR products confirmed the successful detection of *S. subterranea* in multiple samples, further supporting field observations of disease prevalence. To quantify the population of *S. subterranea* in the collected soil samples from the trial fields, the qPCR technique was used.

Several studies have explored the connection between soil-borne inoculum levels and disease development, aiming to assess disease risk (Merz, 1993; Nakayama *et al.,* 2007; Qu *et al.,* 2006). One of the key challenges in managing *S. subterranea* is its ability to survive in the soil through resting spores formed in tuber lesions and root galls. Additionally, the pathogen produces both short and long-lasting propagules, further contributing to increasing soil infestation (Brierley *et al.,* 2009; Merz, 2008). Previous studies show that as soil inoculum level increases, powdery scab severity also rises, but this relationship is less clear at lower inoculum levels. Shah et al. (2012) reported that when the inoculum ranged between 25-300 cystosori per gram of soil, disease severity remained similar (~10% surface coverage), indicating that even low inoculum levels can still cause disease. Likewise, Van de Graaf et al. (2005) found no significant variation in disease severity or incidence when inoculum levels ranged between 5 and 50 cystosori per gram of soil.

The results from this study, by testing soil samples from seven trial fields using the qPCR technique and *S. subterranea*-specific primers, further reinforce these findings. Each soil sample, representing a homogenized composite of 5-7 soil cores per field (biological replicate), was tested multiple times through independent DNA extractions. Each DNA extraction was subjected to tri-replicate qPCR reactions (technical replicates). Variations in cystosori counts were observed across different samples. This variability suggests an uneven distribution of inoculum in the soil and potential limitations in current quantification methods. Field studies have attempted to correlate pre-planting inoculum levels with the expression of disease. Qu et al. (2006) reported an association between inoculum levels (136 to 14,500 cystosori/g soil) and disease incidence in four fields, while Brierley et al. (2012) observed increasing powdery scab incidence in 113 fields when inoculum exceeded 10 cystosori/g soil. Variability in cystosori levels within subsamples suggests uneven distribution, which may explain inconsistencies in large-scale studies. In the present study, all fields sampled in 2023 and 2024 (SA1- SA6), planted with Russet Burbank and Lady Claire cultivars, exhibited visible symptoms when soil inoculum was detected to be above 100 cystosori per gram of soil as determined by qPCR analysis. In contrast, the 2022 field (CA-Orchestra) found to have approximately 15 cystosori per gram of soil, showed no visible disease symptoms despite the presence of *S. subterranea* DNA being confirmed through conventional PCR. These findings indicate that cystosori concentration and cultivar resistance play key roles in disease expression, supporting the development of a qPCR-based soil testing protocol for predicting powdery scab risk. Such a tool can help Canadian potato growers make informed decisions on cultivar selection and field management to reduce disease impact.

The bioassay results of this study demonstrated that molecular methods could detect *S. subterranea* in the roots of Russet Burbank tissue culture plants within two weeks of inoculation, even at an inoculum level as low as one cystosori per gram of soil, despite the absence of visible symptoms. This aligns with the findings of Nakayama et al. (2007), where even low inoculum levels could still contribute significantly to disease severity due to the infection potential of resting spores. Additionally, this supports Brierley et al. (2012), who observed increased disease incidence with rising inoculum levels but also suggest that even below their arbitrary threshold of 10 cystosori per gram, disease can still develop rapidly under favourable conditions. Given the bioassay results of the current study, where even a single cystosori per gram of soil was detectable and potentially sufficient to initiate the disease (Nakayama *et al.,* 2007), it suggests that under optimal conditions, even minimal inoculum can lead to substantial disease outbreaks, supporting the idea that infection potential may be a more critical factor than absolute inoculum counts.

Molecular identification of *S. subterranea* using species-specific primers was an effective method for confirming the presence of powdery scab. Suspected isolates were identified using these primers, followed by DNA sequencing. The ITS1-5.8S-ITS2 region of the isolates was used to construct phylogenetic relationships. *S. subterranea* is a phytomaxid species within the plasmodiophorid group parasitizing green plants (Bulman *et al.,* 2001; Balendres *et al.,* 2016; Neuhauser *et al.,* 2014). Bulman and Marshall (1998) and Bell et al. (1999) started the genetic characterization of *S. subterranea* by sequencing the ribosomal transcribed spacer (ITS) regions of the pathogen's DNA across the isolates from Australia, New Zealand, Scotland, Peru and the Netherlands, leading to the identification of two *S. subterranea* groups: Type I and Type II (Balendres *et al.,* 2016). Their research found that South American isolates were uniformly Type I, whereas Type II predominated in Australasia and North America.

Phylogenetic analysis of *S. subterranea* isolates from Alberta provides meaningful insights into the genetic structure and potential origins of these populations. The close clustering of all newly sequenced Alberta isolates (CA, SA1-SA6) into a strongly supported monophyletic clade, coupled with short branch lengths, suggests limited genetic diversity and a likely recent common ancestor. This genetic uniformity may reflect localized spread from a single or a few introductions rather than multiple independent introductions.

The present data also reinforces the genetic separation between *S. subterranea* f. sp. *subterranea*, the cause of powdery scab and *S. subterranea* f. sp. *nasturtii*, the cause of crook rot of watercress (Tomlinson, 1958; Balendres *et al.,* 2016). As indicated in previous studies (Down *et al.,* 2002), the two formae specialis formed distinct clades, supporting the proposal by Dick (2001) to elevate *S. subterranea* f. sp. *nasturtii* to species rank based on both host specificity and morphological characters. The separation between these groups was robust in the phylogeny of this study, affirming the evolutionary divergence between these pathotypes.

In the phylogenetic analysis of the present study, *S. subterranea* formed a genetically distinct group with other plasmodiophorid species, such as *Plasmodiophora brassicae* and *Phagomyxa spp.* within the phylum Cercozoa. This supports previous research, including studies done by Cavalier-Smith and Chao (1997), Bulman et al. (2001), and Down et al. (2002), which showed that plasmodiophorids are closely related to other groups like chlorarachneans, euglyphids and sarcomonads. These findings back the classification proposed by Cavalier-Smith (1998), which placed plasmodiophorids within Cercozoa, a group that includes various amoeboid protists.

In conclusion, this study confirms that the Alberta isolates from the present study are genetically consistent with previously published global sequences, highlighting their close evolutionary relationships with isolates from other regions. The observed genetic structure emphasizes the importance of understanding pathogen diversity when developing effective management and guiding future resistant breeding strategies for disease control.

PMTV is believed to have originated in the Andean region of South America, as suggested by several earlier studies (Hinostroza and French, 1972; Salazar and Jones, 1975; Xu *et al.,* 2004; Tenorio *et al.,* 2006). Since then, the virus has spread globally and is now reported in numerous potato growing regions, including Europe, Asia and both North and South America (Calvert and Harrison, 1996; Crosslin, 2011; David *et al.,* 2010; Harrison *et al.,* 1997; Lambert *et al.,* 2003; Latvala-Kilby *et al.,* 2009; Mallik and Gudmestad, 2015, Wale, 2000; Whitworth and Crosslin, 2013; Xu *et al.,* 2004; Domfeh *et al.,* 2015). In North America, the virus was first confirmed by Xu et al. (2004), who reported PMTV in both the United States and Canada, as the virus had previously been thought to be absent from commercial potato production.

PMTV transmission is closely linked to its vector, *S. subterranea*, the causal agent of powdery scab. The virus can persist for extended periods in the soil, surviving in the long-lived resting cystosori of the vector, with viability reported for over 18 years even in the absence of a host plant (Calvert, 1968; Arif *et al.,* 2014). Despite this strong association, not all tubers showing powdery scab symptoms are necessarily infected with PMTV. Arif et al. 2014 highlighted that the presence of surface lesions or pustules alone does not guarantee PMTV infection. Tubers showing external scab symptoms but lacking internal browning or arc-shaped necrosis often tested negative for PMTV using both ELISA and PCR detection methods (Arif *et al.,* 1994).

In the present study, RT-PCR was used to detect PMTV due to its high sensitivity (Arif *et al.,* 1994; Mumford *et al.,* 2000; Rantanen *et al.,* 1999; Torrance *et al.,* 1993; Xu *et al.,* 2004; Kalischuk *et al.,* 2013), which is particularly important when the virus is present at low concentrations or unevenly distributed within host tissues (Arif *et al.,* 1994; Sokmen *et al.,* 1998; Xu *et al.,* 2004). Two different genomic regions, coat protein (CP) and RNA 2, were targeted because these regions are known to be highly conserved (Scott *et al.,* 1994; Reavy *et al.,* 1998; Mayo *et al.,* 1996; Xu *et al.,* 2004).

The studies done by Xu et al. (2004) reported a challenge of uneven viral distribution, which complicated the confirmation of test results. Their surveillance across North America found PMTV in 4.3% of potato consignments, many of which showed no visual symptoms at the time of testing. These findings align with the present study where tubers from the field suspected to have powdery scab were stored, and no visible signs of spraing were observed when initially examined in February 2024. However, when the same tubers were reassessed in April 2024, 1 to 3 tubers out of 25 examined tubers showed arc-shaped spraing symptoms, suggesting that the virus had been present earlier but only appeared visually after extended storage time with favourable conditions. Interestingly, an earlier onset of PMTV symptoms was observed in the following year. In February 2025, 3 to 4 tubers out of 15 tested tubers already showed visible arc-shaped spraing during initial observation. This progression may reflect changes in viral load or environmental conditions facilitating the symptom expression. These findings support the previous studies that PMTV may remain asymptomatic for extended periods but can eventually cause visible symptoms under certain environmental or physiological conditions (Xu *et al.,* 2004; Domfeh *et al.,* 2015). Not all the fields that tested positive for powdery scab in this study were found to harbour PMTV, highlighting that the presence of the vector does not always correlate with virus infection (Arif *et al.,* 2014).

In Alberta, there is less current concern about powdery scab, but PMTV is a growing concern among growers because of its emerging presence in Alberta and its ability to persist in soil via its vector *S. subterranea* and its potential impact on tuber quality and marketability. PMTV was first reported in Alberta in 2016 by Kalischuk et al. (2016). In the present study, the proportion of tubers that showed spraing symptoms (1 to 3 Russet Burbank and Lady Claire tubers in 2024, 3 to 4 Russet Burbank tubers in 2025 and none from Lady Claire tubers, out of 25 tubers in total for each cultivar) suggests that the virus is present in Southern Alberta but in a small proportion of the tubers much like erratic distribution of PMTV reported in previous (Xu *et al.,* 2004; Arif *et al.,* 2014). Kalischuk et al. (2016) found no PMTV detection in asymptomatic tubers, which aligns with the present study, where Lady Claire and Russet Burbank tubers showing no symptoms of PMTV were tested with RT-PCR and found negative for PMTV.

The relatively low proportion of tubers showing PMTV spraing symptoms in this study, ranging from 4% to 16% depending on the time of storage and year, suggests that while the virus is present in Southern Alberta, its incidence may currently be limited or under-detected. However, this low detection rate should not be interpreted as an indication of minimal risk. As demonstrated in previous studies, PMTV often exists in asymptomatic tubers or expresses symptoms only under physiological or environmental conditions (Xu *et al.,* 2004; Arif *et al.,* 2014). The erratic distribution of the virus within tubers and between lots further complicates accurate detection. Therefore, while our findings provide preliminary evidence of PMTV in at least three of Southern Alberta's commercial potato fields, they also underscore the need for expanded regional surveillance and long-term epidemiological studies. Comprehensive monitoring, combined with the development of effective diagnostic tools and management strategies, will be critical in preventing the further spread and establishment of PMTV in the region.

CHAPTER 6: CONCLUSION

The current study provides a comprehensive investigation into the occurrence, detection, management, and genetic characterization of *S. subterranea* f. sp. *subterranea* (Sss), the causal agent of powdery scab, across multiple field sites in Southern Alberta. Through the integration of conventional field assessment, molecular diagnostics, fungicide efficacy trials, and phylogenetic analysis, this work enhances our understanding of the pathogen's distribution, biology and impact in a regional context. The pathogen was confirmed in all tested field soils using molecular tools, confirming its distribution in Alberta's potato-growing regions. Genetic characterization of Alberta isolates (CA, SA1-SA6) showed 99-100% identity to previously identified *S. subterranea* isolates around the globe, indicating that the Alberta isolates are not genetically distinct and likely share a common global lineage. The use of molecular diagnostic tools, particularly qPCR, enhanced the sensitivity and reliability of detecting *S. subterranea* in both soil and plant samples. qPCR also enabled the quantification of inoculum levels, providing a reliable method for assessing disease risk and guiding management decisions. Disease severity was associated with soil inoculum levels, with symptoms becoming more visible when cystosori were detected in high numbers per gram of soil. This association suggests that higher concentrations of *S. subterranea* cystosori in soil could be a strong indicator of increased disease risk. This supports the potential of using soil inoculum quantification as a predictive tool or disease risk assessment. However, disease expression in the field is also influenced by cultivar susceptibility, such as in tolerant cultivars, even high inoculum levels may not result in severe symptoms. Therefore, both soil pathogen load and cultivar selection must be considered together when evaluating disease risk and developing effective management strategies. In addition to detecting the *S. subterranea*, molecular assays also revealed the presence of PMTV in three out of seven tested fields. This suggests that PMTV is not uniformly present in all *S. subterranea*-infected fields, highlighting its variability in virus distribution across sites. This variability highlights the need for routine monitoring and early diagnostics, given PMTV's latent nature and its dependence on *S. subterranea* for transmission. Fungicide trials demonstrated that fluazinam was moderately effective at reducing disease severity when applied at medium concentrations, although complete disease control was not achieved. A24367B, a combination product, showed broader potential activity and may be useful in managing additional soilborne pathogens such as *Pythium spp.* and *P. erythroseptica*. However, the limited efficacy of fungicide alone underscores the need for integrated disease management strategies that reduce pathogen pressure while maintaining environmental sustainability. Furthermore, no phytotoxicity effects were observed in any of the treated plots, indicating good crop safety under field conditions.

In summary, this work advances the understanding of *S. subterranea* in Alberta by combining field-based and molecular approaches. It highlights the importance of accurate detection, targeted chemical applications, pathogen diversity analysis, and the development of integrated management approaches. Collectively, these findings provide a foundation for future research and practical tools that can help mitigate the impact of powdery scab on potato production in the region

CHAPTER 7: FUTURE RESEARCH DIRECTIONS

In this study, inoculum levels above 15 cystosori per g of soil were found to be closely associated with disease expression, emphasizing the importance of accurate and representative soil sampling. Despite this, the study also identified a gap in standardized soil sampling. More precise guidelines are needed to determine optimal sample numbers, location and processing to estimate inoculum levels at the field scale reliably. This will improve diagnostic accuracy and enhance decision-making in disease risk forecasting.

Moving forward, several key areas require further research. One potential area involves the study of suppressive soils, which naturally inhibit disease development. Identifying the biological or physicochemical properties responsible for this suppression could lead to novel, environmentally sustainable management strategies. In addition, there is a need for further research on plant pathogen signaling pathways, particularly involving chelating agents like Fe-EDTA. These compounds may disrupt zoospore's behaviour in the absence of a host and offer a potential means of preventing early infection stages.

Research into the role of non-host crops and weed species in the persistence and spread of *S. subterranea* is also required. Understanding whether these plants can support the survival or spread of *S. subterranea* will provide critical insights for crop rotation planning and sanitation practices. Additionally, the identification and breeding of potato cultivars with resistance or tolerance to powdery scab remain essential for long-term disease management. Resistant varieties, combined with improved diagnostics and field monitoring, offer a sustainable path forward for reducing the impact of this disease in Alberta.

Given the vector relationship with *S. subterranea*, parallel surveillance of PMTV and improvement of virus-specific detection tools should remain part of integrated disease management, especially in seed production systems.

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Appendix 1: Site-specific experimental designs

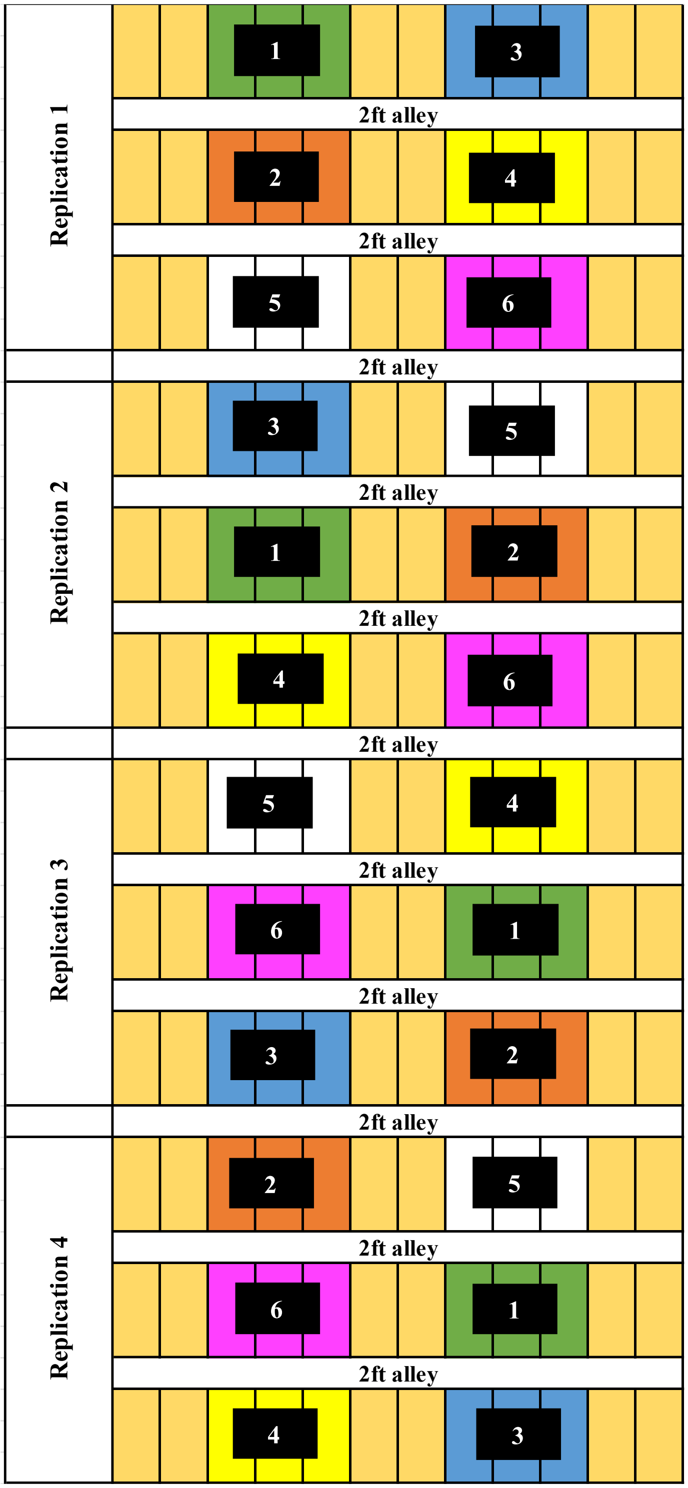

Figure A1.1: RCBD paired-plot design followed for the field trial of SA1-Shepody-2023.

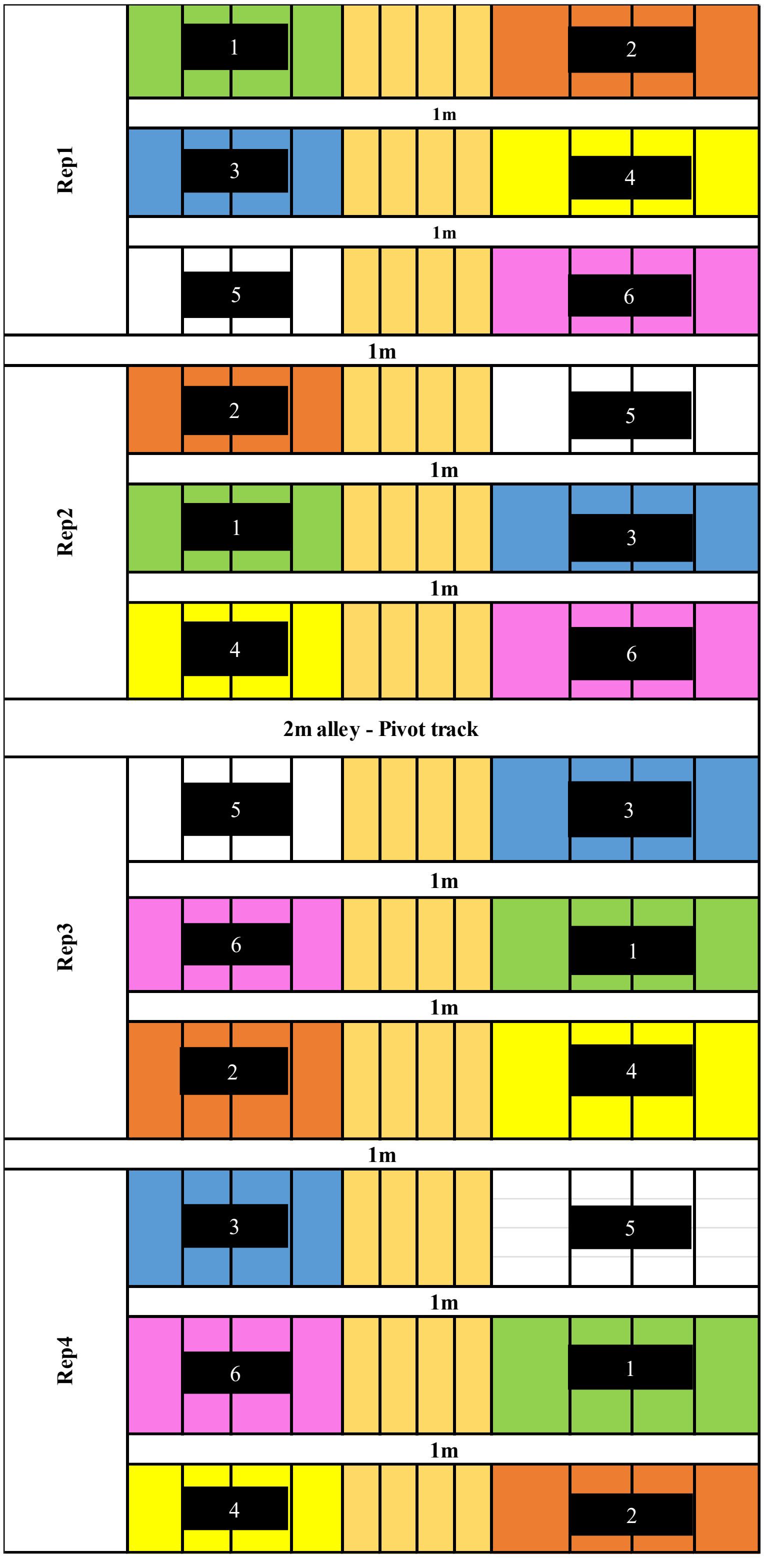

Figure A1.2: RCBD paired-plot design followed for the field trial of SA2-Russet Burbank-2023 and SA5-Russet Burbank-2024.

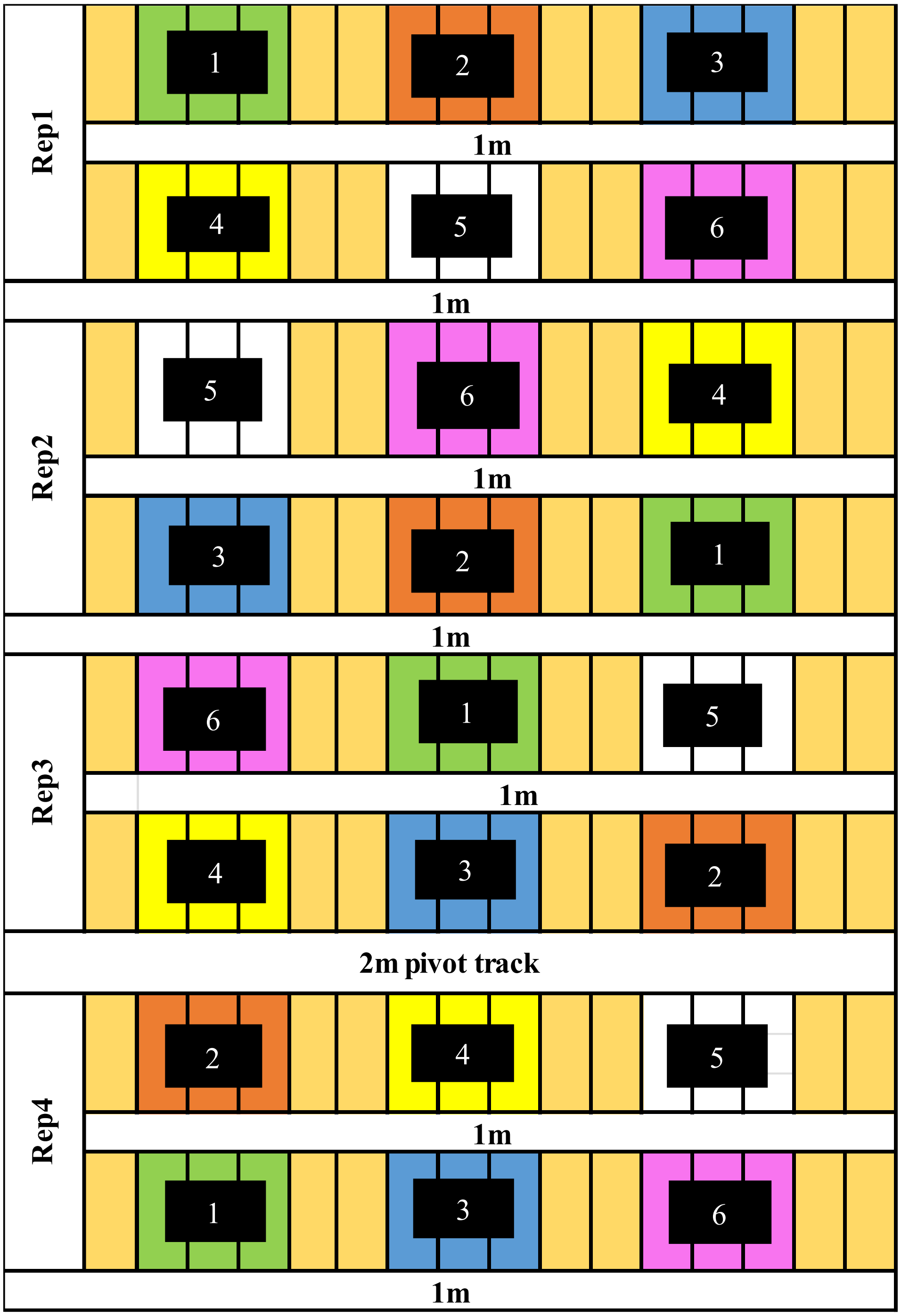

Figure A1.3: RCBD paired-plot design followed for the field trial of SA3-Russet Burbank-2023.

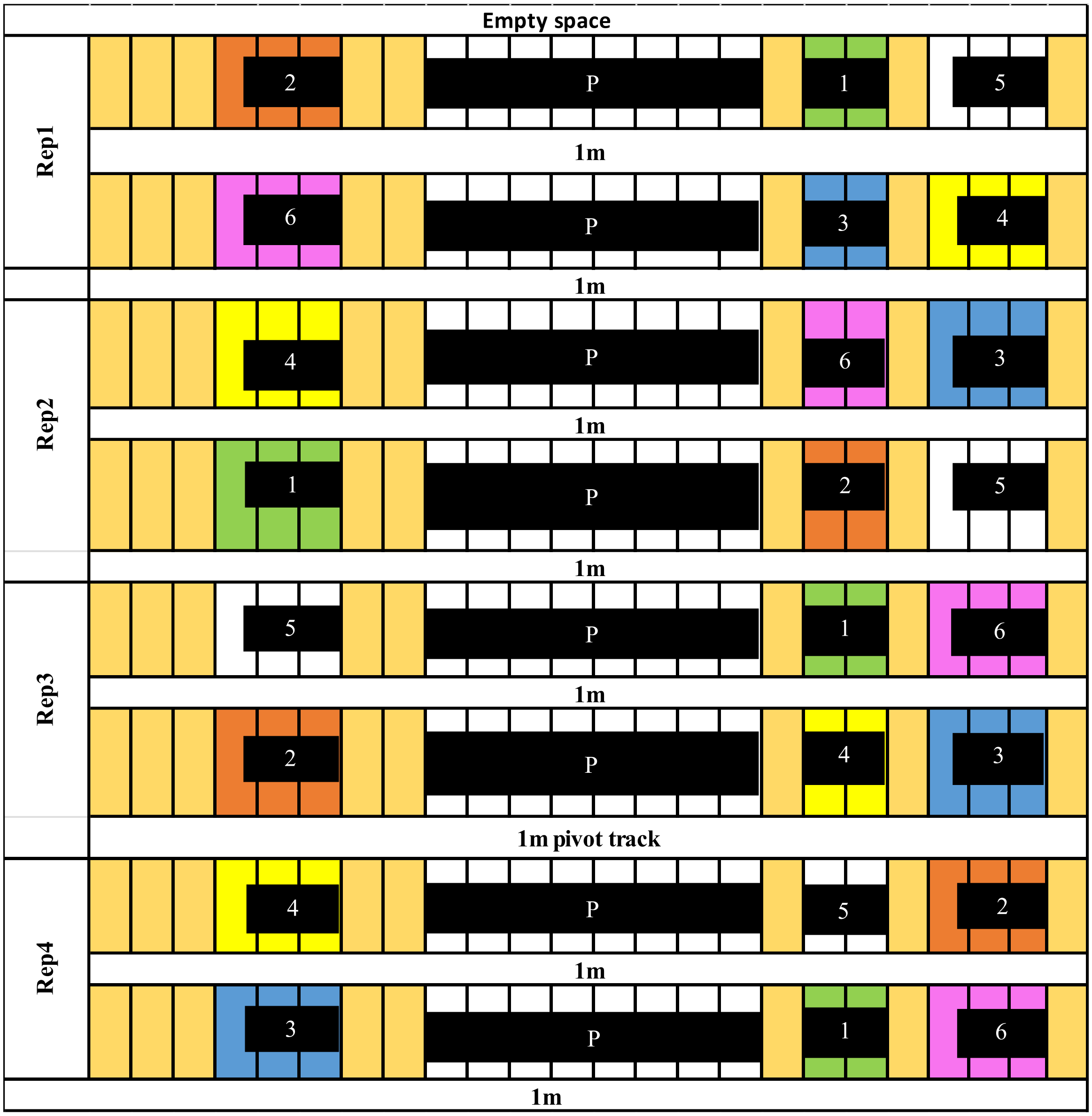

Figure A1.4: RCBD paired-plot design followed for the field trial of SA4-Lady Claire-2023.

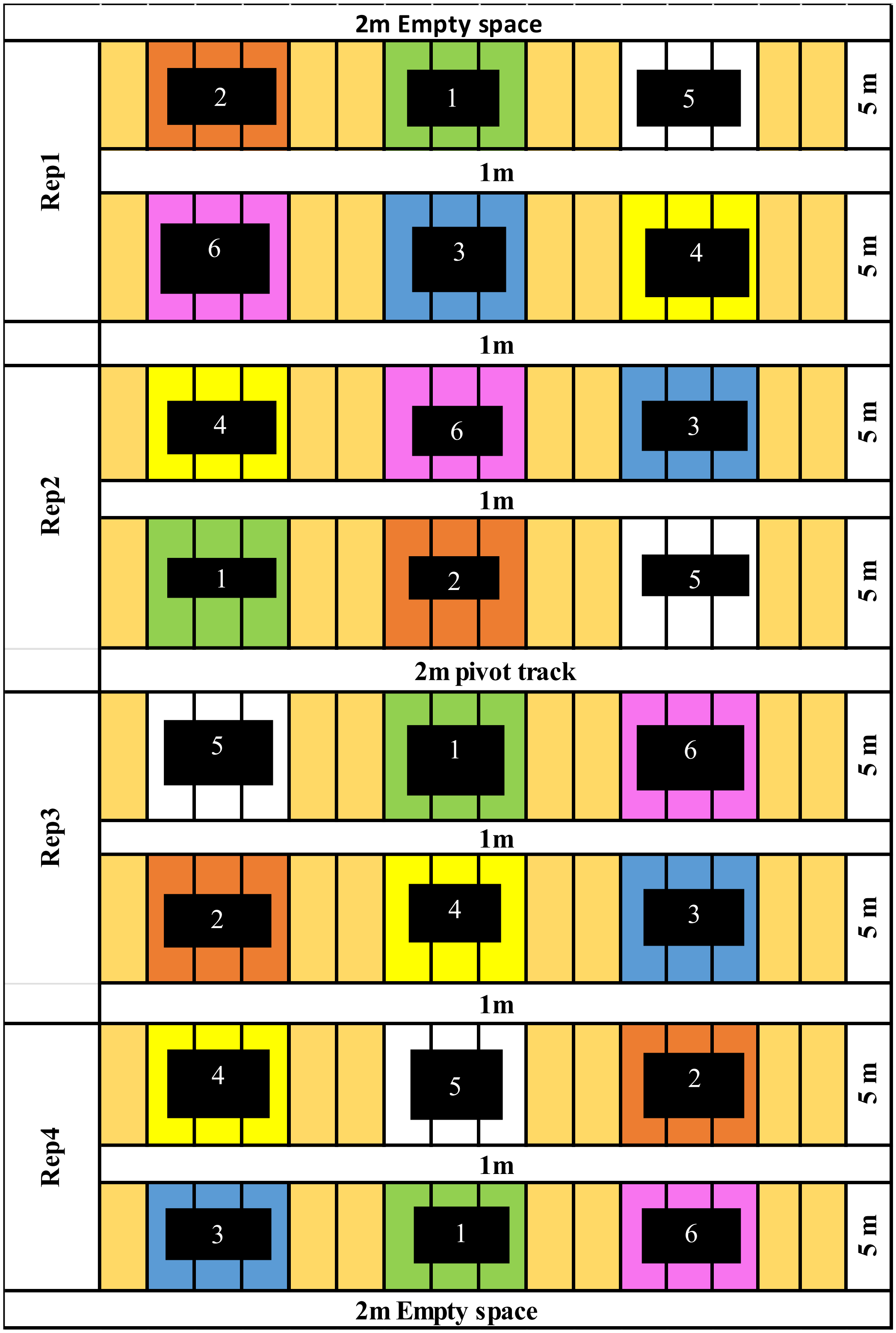

Figure A1.5: RCBD paired-plot design followed for the field trial of SA1-Shepody-2023.

Appendix 2: Galls and Scab severity Index for 2023 and 2024 field trials

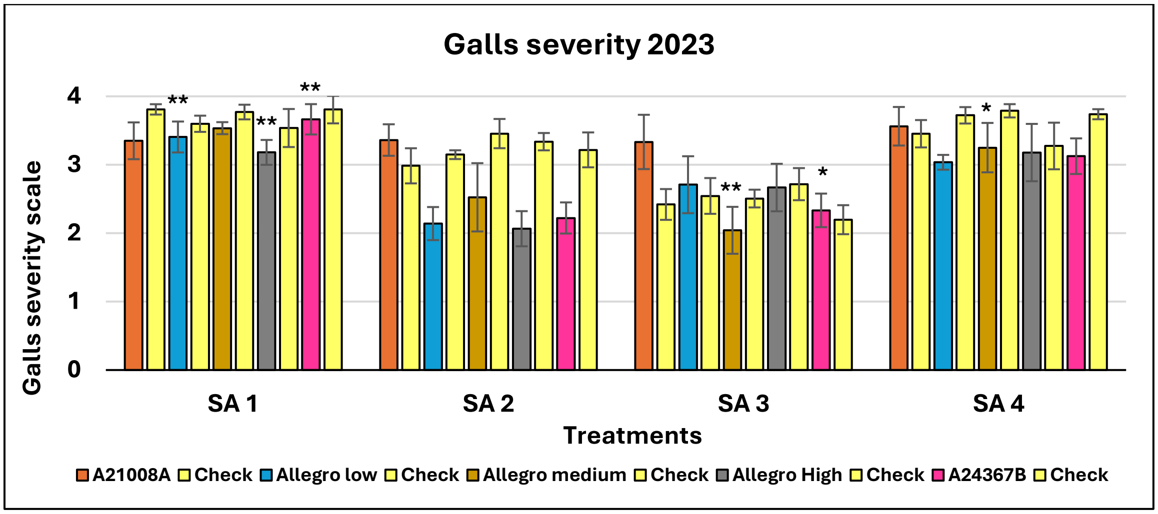

Figure A2.1: Effect of different treatments on gall severity in tuber samples collected in 2023, measured following a disease severity scale of 0-4. Coloured bars represent different treatments, while light yellow bars indicate the untreated checks as controls adjacent to each treatment. Error bars represent the standard error of the mean. Asterisks indicate statistical significance compared to the check (**= ρ < 0.05; *ρ = < 0.1).

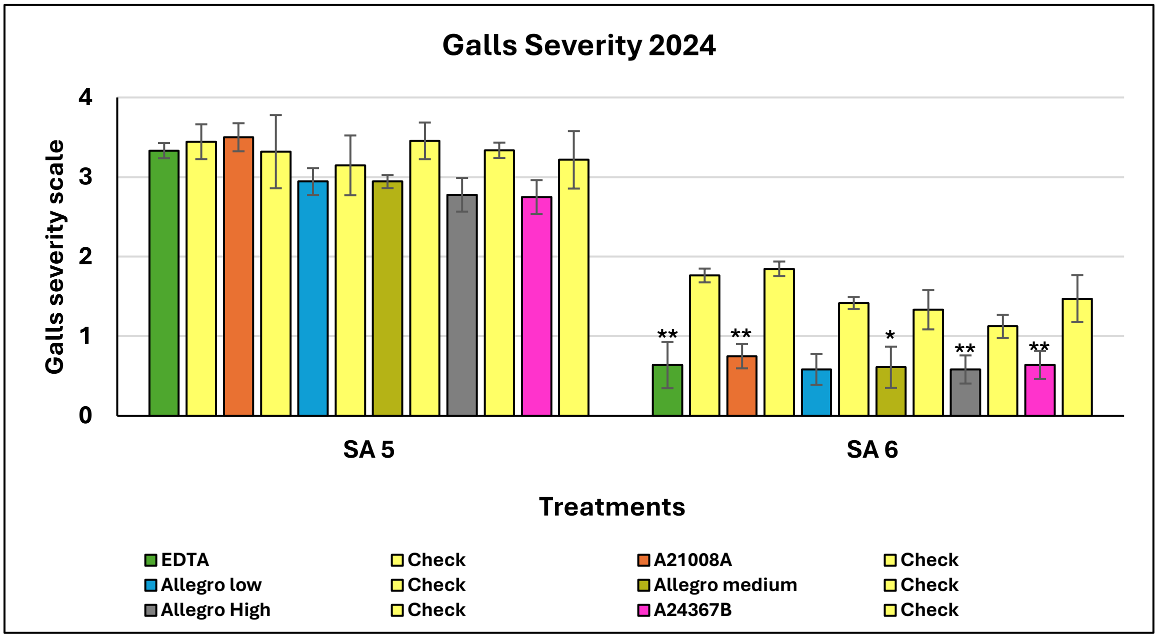

Figure A2.2: Effect of different treatments on gall severity in tuber samples collected in 2023, measured following a disease severity scale of 0-4. Coloured bars represent different treatments, while light yellow bars indicate the untreated checks as controls adjacent to each treatment. Error bars represent the standard error of the mean. Asterisks indicate statistical significance compared to the check (**= ρ < 0.05; *ρ = < 0.1).

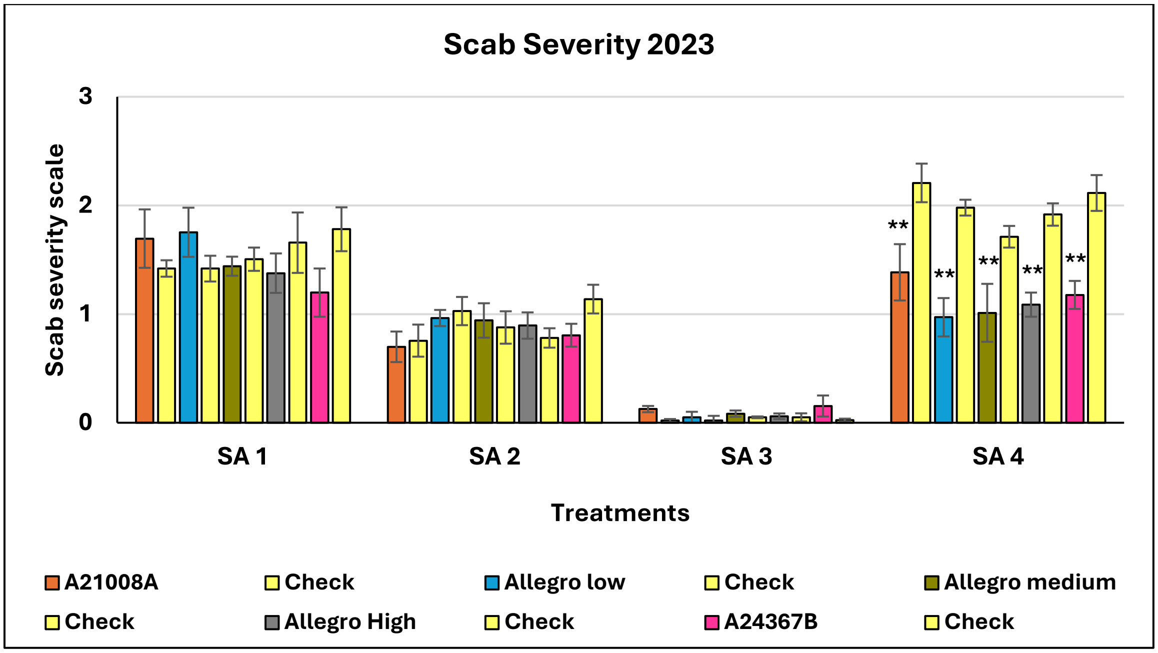

Figure A2.3: Effect of different treatments on scab severity in tuber samples collected in 2023, measured following a disease severity scale of 0-5. Coloured bars represent different treatments, while light yellow bars indicate the untreated checks as controls adjacent to each treatment. Error bars represent the standard error of the mean. Asterisks indicate statistical significance compared to the check (**= $\rho < 0.05$; *$\rho = < 0.1$).

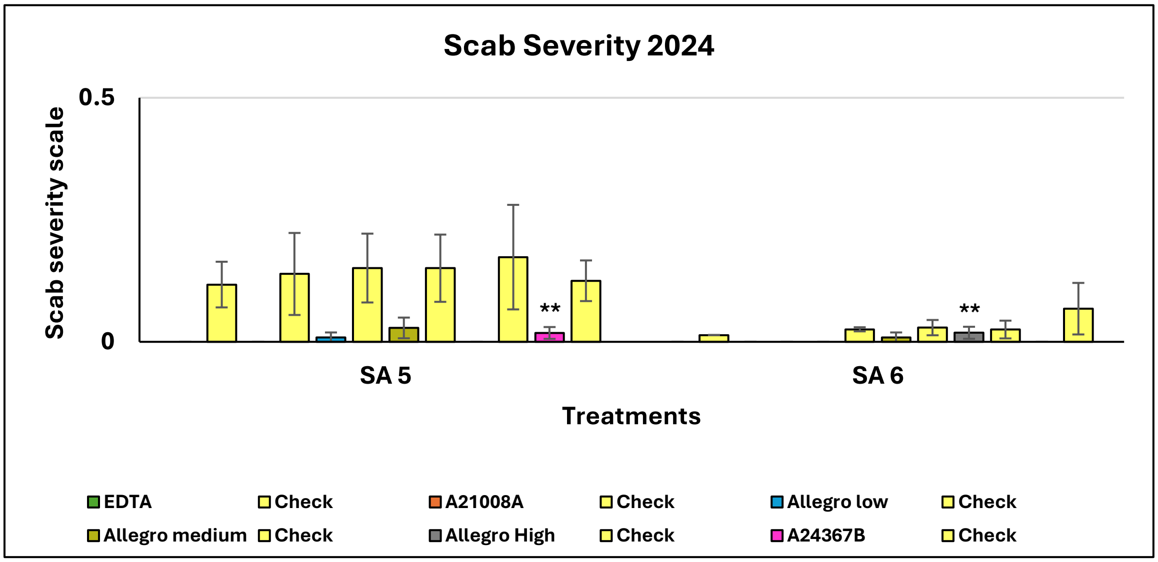

Figure A2.4: Effect of different treatments on scab severity in tuber samples collected in 2024, measured following a disease severity scale of 0-5. Coloured bars represent different treatments, while light yellow bars indicate the untreated checks as controls adjacent to each treatment. Error bars represent the standard error of the mean. Asterisks indicate statistical significance compared to the check (**= $\rho < 0.05$; *$\rho = < 0.1$).

Appendix 3: Alternative powdery scab detection methods

In this study, alternative *S. subterranea* detection tools like LMAP and the Sss strip method were tested to evaluate their potential applicability under our laboratory conditions. LAMP was considered for its rapid and sensitive detection capabilities without the need for a sophisticated thermocycler, making it suitable for on-site or resource-limited diagnostics. The Sss AgriStrip® method is a user-friendly immunochromatographic test designed for the quick and visual identification of *S. subterranea* antigens in root or tuber tissues.

LAMP

The loop-mediated isothermal amplification (LAMP) method was used for the detection of *S. subterranea* DNA from plant and soil samples due to its high specificity, reliability and rapid detection capability. The protocol followed the optimized conditions outlined by Jiang et al. (2023), using primers specifically designed for *S. subterranea*. Reactions were performed using the SuperScript^{TM} IV RT-LAMP Master Mix (Cat. No. A51802). Each LAMP reaction had a total volume of 25 µl and included 1 µl of DNA template. Primer concentrations and other components were prepared according to the master mix guidelines. The assay was performed at a constant temperature of 60°C for 50 minutes using a heat block. Results were assessed visually and documented through photographs.

Table A3.1: Primers used in the LAMP method.

LAMP primers	Primer sequences (5’-3’)	References
F3	GGTTCCCACAACGATGAAGA	(Jiang *et al.,* 2023); (Balendres *et al.,* 2024)
B3	CTTTCAAGCCATGGACCGA	
FIP (F1c+F2)	CGAAAGCGCAACTTGCGTTCAA + GCAGCGAAATGCGATACGT	
BIP (B1c+F2)	AGCATGCCTCTTTGAGTGTCGG + CCAGAGCTCATAGTCCCCTT	
Loop F	TCACTGAATTCTGCAATTCGC	
Loop B	CTATTCTCCCGGAAACGCCCT	

The LAMP assay successfully amplified *S. subterranea* DNA from selected soil samples, with positive reactions indicated by a distinct colour change from pink to yellow, visible to the naked eye. The assay included a positive control containing previously confirmed *S. subterranea* genomic DNA (tube 1, positive), which produced a yellow colour, and a non-template negative control (tube 2, negative), which remained pink, confirming assay specificity.

Among the tested samples, tube 3 (soil samples from the trial field CA-2022) and tube 4 (commercial potting mix) showed no colour change and remained pink, indicating negative results for *S. subterranea*. In contrast, tubes 5 and 6 (soil samples from the trial fields SA1-2023 and SA5-2024) showed a distinct orange-yellowish colour, confirming the presence of *S. subterranea* DNA.

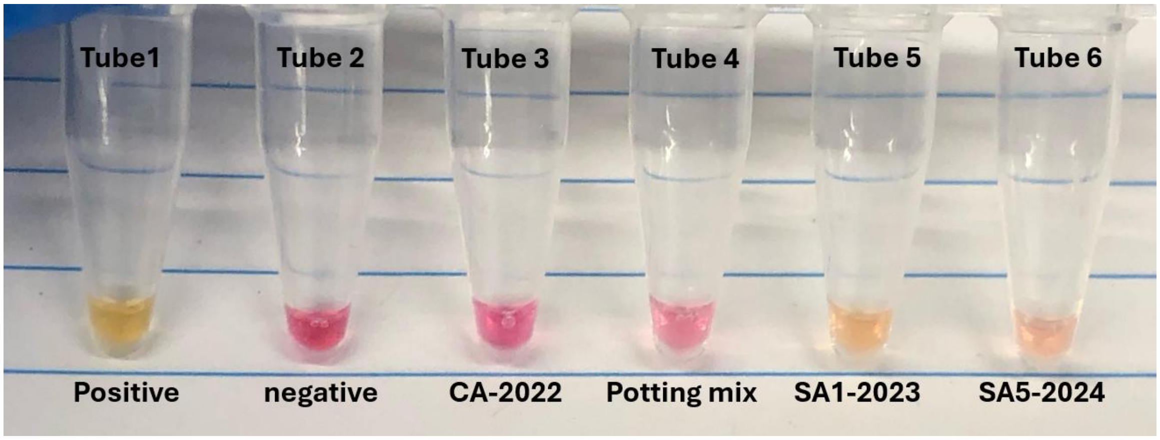

Figure A3.1: Visual results of the LAMP assay for *S. subterranea* detection using colorimetric endpoint. Tubes 1 and 2 represent the positive and negative controls, respectively. Tubes 3 and 4 (soil samples from the trial field CA-2022 and potting mix) remained pink, indicating negative results. Tubes 5 and 6 (soil samples from the trial fields SA1-2023 and SA5-2024) showed a colour change to an orange-yellowish colour, indicating positive amplification and the presence of *S. subterranea* DNA.

Sss AgriStrip method

The “Sss AgriStrip” (Art. No. 111181), developed by BIOREBA AG (Reinach, Switzerland), was also tested for the detection of *S. subterranea* cystosori in tuber and root samples. The test followed the manufacturer’s protocol and utilized a straightforward immunoassay with single-use strips.

The Sss AgriStrip test effectively detected *S. subterranea* DNA in tuber and root samples from the SA2-Russet Burbank field suspected of having powdery scab. Positive samples were indicated by the appearance of two distinct bands on the strip, one control band and one test band, within the manufacturer’s recommended incubation time. In contrast, negative samples displayed only a single control band, confirming the absence of *S. subterranea.*

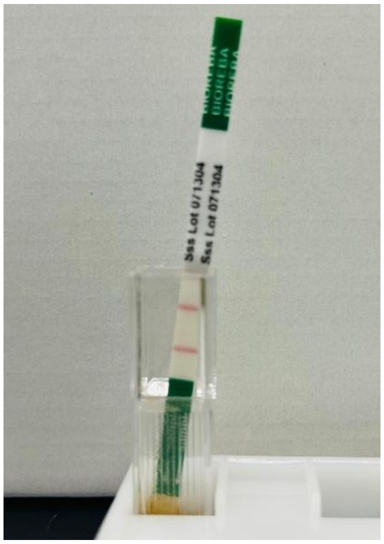

Figure A3.2: An Sss AgriStrip showing two visible bands: the upper band is the control line, confirming the test functioned correctly, and the lower band is the test line, indicating a positive result for the presence of *S. subterranea* DNA in the sample.