STUDIES ON THE DEVELOPMENT AND MANAGEMENT OF POWDERY SCAB AND ROOT GALL FORMATION CAUSED BY *SPONGOSPORA SUBTERRANEA* ON POTATO (*SOLANUM TUBEROSUM* L.)

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Studies on the development and management of powdery scab and root gall formation caused by *Spongospora subterranea* on potato (*Solanum tuberosum* L.)

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

The biotroph protozoan *Spongospora subterranea* causes root gall formation and powdery scab on potato. Symptoms on tubers affect directly the quality and marketability of the harvested product while infection in roots are associated with yield reductions. Moreover, *S. subterranea* is the vector of the *Potato mop-top virus*. The management of the disease is difficult due to the limited number of current control options and requires the integration of control measures among which host resistance represents the most economically and long-term approach. This dissertation focuses on the evaluation of management strategies for the control of powdery scab and root gall formation. In the first study, a total of 43 potato cultivars and 80 advanced clones representing a range of skin types were assessed for their response to powdery scab and root gall formation in five field experiments. High levels of resistance was observed among genotypes against the development of disease on tubers and roots which accounted for 32.5% of the evaluated population. Resistance to powdery scab and root gall formation was shown to follow a continuum from very susceptible to very resistant. In the second study, the effect of chloropicrin soil fumigation on the soil populations of *S. subterranea* and the concomitant development of disease in roots and tubers was investigated in seven field trials. Results indicated a reduction of pathogen soil inoculum, which in most cases was accompanied by an increase in yield and disease on tubers and roots. These results were confirmed in controlled condition experiments in which the amount of pathogen DNA detected in roots increased with the fumigant rate. In the third study, reciprocal grafts between 'Shepody' (susceptible to powdery scab and root gall formation) and 'Dakota Trailblazer' (resistant) were prepared in order to assess the effect of grafting on the translocation of susceptibility factors affecting the infection and development of root galls. The amount of pathogen DNA increased,

as did the number of root galls, on graft combinations involving ‘Shepody’. The results presented in this dissertation highlight the importance of cultivar selection in the management of root gall formation and powdery scab on tubers.

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DEDICATION

To my family

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

CHAPTER I. LITERATURE REVIEW

The Potato

The origin of the cultivated potato (*Solanum tuberosum* L.) has been a matter of controversy with the Andes region and the lowlands of Chile as sources of two separate introduction events (Hosaka and Hanneman 1988). However, the Andean potato predominated in Europe in the 1700s, but declined with the subsequent introduction of Chilean germplasm (Ames and Spooner 2008). The process of dissemination of the potato in Europe was accompanied by a number of introduction events which represented the early breeding efforts before the late blight [*Phytophthora infestans* (Mont.) de Bary] epidemic of the 1840s (Rios et al. 2007). Regardless of these efforts, the limited number of introductions occurring in Europe resulted in an important species bottleneck, molding a narrow genetic base for potatoes outside of its origin center (Xu et al. 2011).

The introduction of the potato in Europe by the Spanish conquistadors was evidenced by local production taking place in Tenerife, Canary Islands, in 1565 (Ochoa 2001). Afterward, the potato made its way back to America. Following its introduction to England in 1590, the potato was brought to Bermuda in 1613, from where it reached the United States, being recorded for the first time in Virginia in 1622 (Glendinning 1983). Another early introduction event took place in Londonderry, New Hampshire, during 1719, with potato-seed shipments from Ireland (Brown 2010). The establishment of potatoes in United States was relatively slow, but reports show that by 1748, potatoes were commonly grown in Albany, NY (De Jong et al. 2011).

This early potato production was able, nevertheless, to start an incipient breeding program from which cultivars like the Howard were obtained, and later re-introduced to Great Britain in 1765 (Graham and Poai 1999). The reports of late blight in Ireland during the 1840s were followed by the first record of the disease in Philadelphia, PA, in 1843, and later in Utica, NY, in 1846 (Fry et al. 1993; Glendinning 1983). These discoveries stimulated the research for cultivar resistance among the genotypes available at the time, as well as, the introduction of new genotypes of *S. tuberosum* from South America, and later Central America (Plaisted and Hoopes 1989).

The "Potato Disease" as Late Blight was known soon after its discovery, caught the attention of the amateur botanist Rev. C. E. Goodrich from Utica, NY. In his first 1847 essay on the disease, C. E. Goodrich stated the hypothesis that late blight was the result of sudden weather alterations during critical periods of plant growth and the exhausted energy of the potato cultivars grown in Europe and the US (Heffron 1865). The first introduction by C. E. Goodrich of exotic potato germplasm from Colombia in 1849, was followed by the incorporation of cultivars assumed to be originally from Chile in 1850 and 1851 (Bethke et al. 2014; Heffron 1856). The 1851 shipment consisted of light red, red, and purple potatoes from which the cv. Rough Purple Chili was selected based on its earliness (Love 1999). During 1853, a seedling of 'Rough Purple Chili' was chosen under the name 'Garnet Chili'. This open-pollinated cultivar was released in 1857 and served as a seedling source from which the cv. Early Rose was selected in 1861 by A. Bresee of Vermont (Bethke et al. 2014; Plaisted and Hoopes 1989).

The production of potato cultivars in Europe and the US prior to the 1900's was in a broad sense, based on the cv. Early Rose and its seedlings. As a result, cultivars such as Busola, Imperator and Jubel were developed in Europe as well as their US counterparties 'Irish Cobbler'

and ‘Early Ohio’ (Plaisted and Hoopes 1989). Another important seedling of ‘Early Rose’ is the cv. Burbank selected by Luther Burbank in 1873. ‘Russet Burbank’ (syn. ‘Netted Gem’), a sport of ‘Burbank’, was introduced in the US market in 1902, and although its origin is unknown, the field performance (e.g. yield and tuber size) and organoleptic attributes of ‘Russet Burbank’ served to established important quality standards for the US potato industry (Bethke et al. 2014). In turn, ‘Russet Burbank’ became one the most prominent cultivars in the US (Bethke et al. 2014; Love 1999; Plaisted and Hoopes 1989).

A 1999 study of the pedigree of the 44 most prominent potato cultivars in the United States revealed a narrow genotype pool with 20 founding clones identified as contributing between two and 100% of their pedigrees. Founding clones consisted of four types: land races, immediate descendant of land races, USDA releases, and one derivative of *S. demissum* x *S. tuberosum*. A group of 12 founding clones, which included the cv. Busola, Chippewa, Daber, Earlaine, Imperator, Katahdin, Sutton's Flourball and Triumph, appeared in 61-100% of the studied potato pedigrees. The cultivars Garnet Chili and Early Rose were found in the ancestry of all 44 genotypes evaluated (Love 1999). The founding clone Germ. No. 3895-13 (*S. demissum* x *S. tuberosum*) is an example of the introduction of new gene pools from closely related *Solanum* species. Other examples of sources are *S. acaule*, *S. chacoense*, *S. phureja*, *S. stoloniferum*, *S. tuberosum* spp. *andigena* and *S. vernei*. Their incorporation into US breeding programs has contributed to the development of cultivars such as Atlantic, Calwhite, Goldrush and NorDonna (Bradshaw 2007; Love 1999).

Potato is the fourth most important food crop, after maize, rice and wheat, with 18.3 billion tonnes (t) produced in 2012. The United States is the fifth largest world producer, with 21 million t in 2012, and an estimated crop value of $3.74 billion. A breakdown of potato utilization

shows 25.6% used for table stock, 61.2% for processing, 5.1% for seed, and 8.1% others and shrinkage (USDA 2013). Potato production in North Dakota and Minnesota accounts for 10% of national production, with 1.1 and 0.9 MM t, respectively. During 2012, potato stocks by skin type (market class) consisted of 16% red-skinned, 16% white-skinned, 1% yellow-skinned, and 67% russet-skinned for North Dakota, and 8%, 4%, 1% and 87% for Minnesota, respectively.

North Dakota is the second largest potato seed producer with 12.4% of the total area for certification. During 2013, the most important potato cultivars by area planted were Russet Burbank (37.1%), Prospect (10.1%) and Norland (8.1%) for North Dakota, and Russet Burbank (55.3%), Norland (18.6%), and Umatilla Russet (5.5%) in Minnesota (USDA 2013).

Potato is one of the most consumed vegetables in the United States, especially among the young. Records on potato consumption showed a 60% increase from 1965-1997 (Cavadini et al. 2000). This increase was favored by a higher consumption of processed potatoes. In 1997, potato accounted for 50% of the total vegetable composition; processed potatoes represented 25% of the vegetable intake (Cavadini et al. 2000; Krebs-Smith et al. 1996).

The Disease

Origin and distribution

Powdery scab of potato is a disease with a relative short history. In Europe during the 19th century, potato growers were aware of the presence of powdery scab; in Germany for instance, the disease was called "Kartoffelräude" (Melhus 1914). However, it was not until 1841 when F. W. Wallroth, for the first time, described and recorded the occurrence of the disease under the name "Knollenbrand", and its etiologic agent as *Erysibe subterranea* (Melhus 1914). The first report of powdery scab in England was in 1846, and 1886 for Sweden and Norway, where the

botanist J. Brunchorst studied the disease and named its etiological agent as *Spongospora solani*. Later, in 1892, N. G. von Lagerheim proposed the name *Spongospora subterranea* after studying J. Brunchorst report. The evidence that connected these two organisms was brought by T. Johnson in 1909, who stated the name of the microorganism as *Spongospora subterranea* (Kunkel 1915; Melhus 1914).

In 1892, N. G. von Lagerheim described powdery scab as widely distributed in Quito, Ecuador, raising the question whether the disease was introduced or native to South America (Melhus 1914). Later, Lyman and Rogers (1915) found evidence of powdery scab growing on native *Solanum* species in regions from Peru where potato seed interchange had not been reported. Molecular data supports the theory of South America as the center of origin of *S. subterranea*, and similarly suggest Europe acted as a "bridgehead" from where the pathogen spread to other continents (Gau et al. 2013; Qu and Christ 2004). To date, powdery scab is found in most of the potato growing regions of the world, including hot and dry countries where farming is carried out at high altitudes, and with or without irrigation (Wale 2000).

Powdery scab was first reported in the United Stated in 1913 from potato seed shipments coming from The Netherlands, Belgium and Canada (Güssow 1913). However, soon after its discovery, the establishment of powdery scab in the US was confirmed as samples of scabbed tubers were collected from Florida, Connecticut, Maine, Massachusetts, Minnesota, Nebraska, New York, Oregon and Washington State (Melhus 1914; 1915; Morse 1913). To date, the reported distribution of powdery scab also includes Alaska, Idaho, California, Colorado, Montana, North Dakota, Pennsylvania and Wyoming (Carling 1996; Draper et al. 1997; Mohan et al. 1991; Qu and Christ 2004). In 1916, a new insight of the disease, consisting of the formation of root galls was reported for the first time in the US (Melhus et al. 1916).

Symptoms and effects

Spongospora subterranea is able to infect all underground organs of potato (i.e. stolons, tubers and roots) where the pathogen stimulates the enlargement and division of host cells leading to appearance of symptoms. When mature, these outgrowths are filled with masses of sporosori (syn. cystosori, spore balls) of the pathogen (Christ 2001). On tubers, the first symptoms are purplish brown lesions followed by small blisters than can occur as early as 43 days after planting (Christ and Weidner 1988); these blisters will burst and may coalesce with other lesions to increase the affected area (Figure 1.1A, 1.1B). Mature lesions may show raised borders and vary in size from 0.5 to 2 mm in diameter with visible spore masses from 75 days after planting; however, immature blisters may not burst until tubers are stored (Gans 2000; Kirkham 1986).

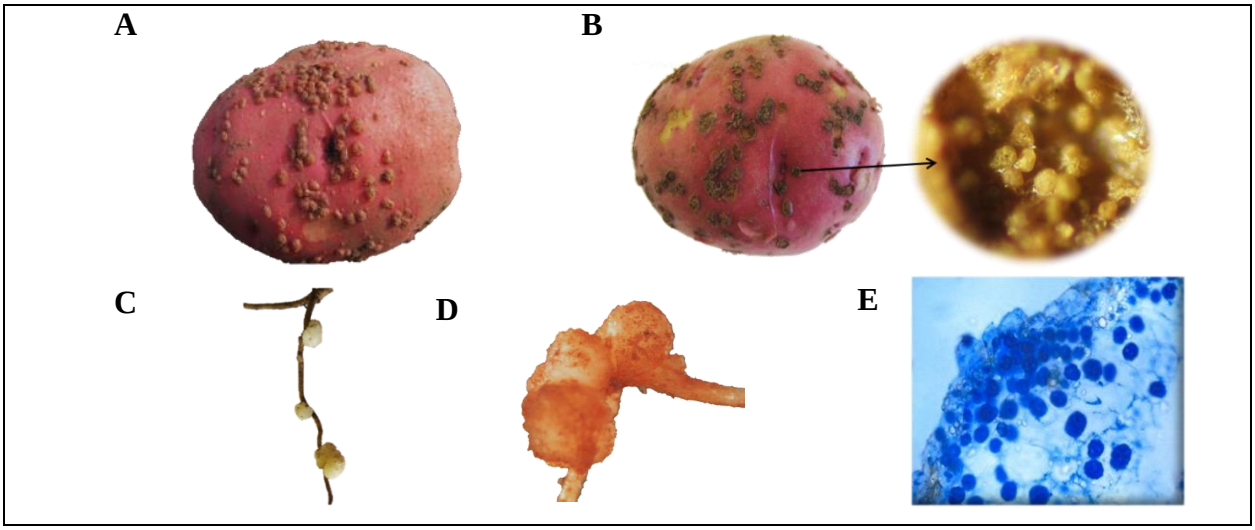

Figure 1.1. Symptoms and signs of *Spongospora subterranea* on tubers and roots. A: immature lesions (blisters) on cv. Red LaSoda; B: mature lesions and lesion close-up (50x) in cv. Red LaSoda; C: young root galls in cv. Lamoka; D: mature root gall in cv. Russet Burbank; E: root gall section cv. Russet Burbank (400x; Photographs F. Bittara).

The cankerous stage of powdery scab in favored by environmental conditions promoting reinfection cycles at the lesion site (Harrison et al. 1997). Roots of the potato plant develop young creamy white galls, which turn dark brown when mature (Figure 1.1C, 1.1D). Galls can develop throughout the growing season increasing the pathogen inoculum level in the soil as the galls decay (Falloon 2008; Schwarzel 2002). On tubers and roots, the incubation period for *S. subterranea* might be as short as three weeks (Kole 1954).

The main effect of powdery scab is cosmetic due to reduced quality of diseased tubers, thus reducing their value for either fresh or processing purposes. The production of certified tuber-seed is also affected by the disease, however the extent of the reduction will depend on certification tolerances which vary from country to country (Falloon 2008; Wale 2000). In the United States, tubers with >5% of affected area are subject to class downgrade, and tubers >25% are considered unmarketable (Houser and Davidson 2010; USDA 2014). Reports of losses due to unmarketable tubers can be as high as 50% in Australia (Hughes 1980) to 100% of the harvested product in Venezuela (García et al. 2004).

The powdery scab pathogen is also capable of infection of roots of susceptible cultivars. Root infection by *S. subterranea* has been reported to reduce root dry weight, water absorption and the intake of nutrients such as phosphorus (-15%) and potassium (-11%) (Falloon et al. 2005a; Lister et al. 2004; Shah et al. 2011). The detrimental effect of the infection by the pathogen on growth parameters including plant height and foliar dry weight has been reported on the susceptible cultivars Diacol Capiro (*S. tuberosum* spp. *andigena*) and Iwa (*S. tuberosum* spp. *tuberosum*) grown in infested soil and spiked potting mix, respectively (Gilchrist et al. 2011; Shah et al. 2011). The extent of the damage also included reduction of tuber yield and mean tuber weight. Conversely, on the resistant cv. Gladiator no effect on root or shoot growth was

observed; however, water use by this cultivar was reduced 26% compared to the non-inoculated control (Hernandez Maldonado et al. 2013).

The extent of the damage due to the infection by *S. subterranea* is likely to interact with the potato cultivar (Shah et al. 2011). In contrast, field studies using the cultivars Shepody and Umatilla Russet showed no detrimental effect on either tuber yield or mean tuber weight due to the development of symptoms on roots and tubers (Johnson and Cumming 2015). Although no association was observed between disease development and total yield, the severity of powdery scab on tubers was reported linearly related to marketable yield (Braightwaite et al. 1994). Additionally, preliminary data suggest a reduction in plant emergence and number of tubers when infected seed-tubers (10% infected area) were planted, however, evidence linking seed-borne inoculum level to yield parameters is scarce (Merz 2000).

The infection of host tissue by *S. subterranea* is described to cause necrosis due to cell disruption, facilitating the invasion of root and tuber pathogens, such as *Colletotrichum coccodes, Fusarium* spp., *Phytophthora* spp. and *Rhizoctonia solani* (Harrison et al. 1997; Kole 1954; Lister et al. 2004). The interaction among infecting microorganisms is likely to have a compound negative effect on the host. This might explain yield reduction reports on russet-skinned cultivars grown in the Columbia Basin (WA), where high disease pressure on roots has been reported (Brown et al. 2007; Johnson and Cumming 2015; Nitzan et al. 2008). Additionally, *S. subterranea* is the vector of the *Potato mop-top virus* (PMTV) (Jones and Harrison 1969); which can lead to losses up to 20% due to quality reduction by tubers showing necrosis symptoms (Nielsen and Nicolaisen 2000).

Causal Organism

Taxonomic classification and morphology

Spongospora subterranea (Wallroth) Lagerheim current taxonomic classification:

Super-group (Kingdom): Rhizaria Phylum: Cercozoa

Class: Phytomyxea Order: Plasmodiophorida

Family: Plasmodiophoridae Genus: *Spongospora*

Species: *Spongospora subterranea* [= *Spongospora subterranea* (Wallroth) Lagerheim f. sp. *subterranea* Tomlinson].

Phytomyxea comprises two orders (Plasmodiophorida and Phagomyxida), within which 12 genera and 44 species are included (Bulman and Braselton 2014; Neuhauser et al. 2010). Plasmodiophorids are defined as a monophyletic group sharing the following features: cruciform nuclear division, obligate intracellular parasitism, heterocont biflagellated zoospores and environmentally resistant resting spores (Braselton 1995; Bulman et al. 2001; Qu and Christ 2004). Although the phylogenetic relationships among members the Plasmodiophorida group is under development, members of this clade are reported as parasitic in higher plants and members of the Stramenopila group in which hypertrophy of host cells is usually observed. Their somatic stage is constituted by a plasmodium that develops within the host cell and leads to zoosporangia formation and subsequently zoospore release (Braselton 1995; Neuhauser et al. 2010). Two subgroups are recognized based on nuclear features: the Plasmidiophora group which is characterized by having small nuclei with a volume of approximately 14 μm^3; and the Sorosphaera group (which contains *S. subterranea*) characterized by nuclei volumes ranging between 23 to 32 μm^3. In addition to the nuclear features, these two groups differ in their host-

parasite barriers. The members of the Sorosphaera group have a single membrane barrier throughout the development of the sporogenic plasmodium, whereas the Plasmidiophora group is able to develop a membrane of five to seven layers (Braselton 1992).

The genus *Spongospora* includes 4 species: *S. campanulae*, *S. cotulae, S. nasturtii* and *S. subterranea*. Traditionally, *S. subterranea* has been divided into two *formae speciales* based on the similarity of their resting spores and host range (Neuhauser et al. 2010). However, it has been argued that host specificity, differences in sporangial states and habit, as well as, information generated from DNA sequences, provide enough support to ensure their placement into the species rank (i.e. *S. subterranea* and *S. nasturtii;* Dick 2001). Molecular evidence obtained from the analysis of the pathogen 5.8S rRNA gene, the Internal Transcribed Spacers (ITS), as well as, the *S. subterranea* actin gene have provided additional evidence to support species status (Bulman and Braselton 2014; Gau et al. 2013; Neuhauser et al. 2010; Qu and Christ 2004).

Among the somatic characteristics reported for *S. subterranea* are: sporosorus usually has the shape of a sponge, often hollow, or with many irregular and open channels, varying in size from 19-85 µm, hyaline to yellow, brown or green (Figure 1.2A). A single sporosorus is composed of about 700 resting spores, ranging from 160-1530 (Falloon et al. 2011). Resting spores are 3.5 to 5 µm in diameter with a three-layered cell wall. A host cell often contains more than one sporosorus (Kole 1954).

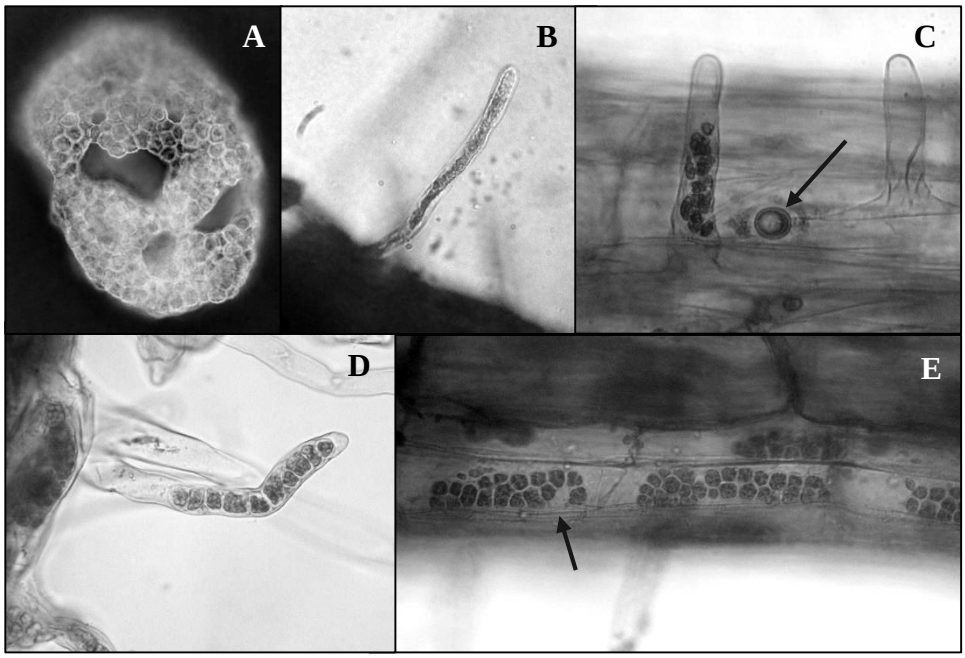

Figure 1.2. Somatic structures of *Spongospora subterranea*. A: sporosorus (spore ball); B: plasmodium; C: young zoosporangia in root hair and encysted zoospore (arrow); D: mature zoosporangia in root hair; E: discharged zoospores from mature zoosporangium (arrow; 400x; Light micrographs F. Bittara).

The zoosporangium (sporangial and sporogenic) is small, ovoid or spherical, or elongated, lobed and irregular, single or slightly grouped (Figure 1.2C-E). Each zoosporangium contains an even number of secondary zoospores. The zoospores are 2.5 to 4.6 µm in diameter and morphologically indistinguishable. Each zoospore has two anterior whiplash flagella of unequal length (Bulman and Braselton 2014; Falloon et al. 2011; Hutchinson and Kawchuk 1998, Merz 2008).

Life cycle

Under favorable moisture conditions, a single uninucleate zoospore (presumably haploid) germinates from a resting spore (Harrison et al. 1997). Resting spores are resistant to adverse environmental conditions and can remain viable for over 10 years (Braselton 2001; Merz 2008).

The factors controlling the primary release of zoospore are not fully understood, but alternating periods of dry and moist conditions, as well as, high (40ºC) and freezing temperatures have been reported to stimulate germination (Harrison et al. 1997; Kole 1954). Although the presence of a host plant or root exudates can stimulate zoospore germination, this does not seem to be a necessary requirement (Harrison et al. 1997; Merz 1989). The release of zoospores appear to occur in a staggered fashion with no evidence of dormancy (Falloon et al. 2011; Merz 1993).

The length of time a zoospore can swim before it encysts is influenced by temperature (Harrison et al. 1997). The primary zoospore will infect the epidermal cells of roots, shoots, stolons and tubers, initiating the sporangial phase of the pathogen (Braselton 1995; Harrison et al. 1997). Prior to infection, the zoospore encysts to produce a tubular structure (Rohr) in which a projectile-like body (Stachel) is formed. Zoospore infection occurs through the injection of the cyst content, enabled by the formation of an adhesorium (Braselton 1995; 2001). Successive cruciform divisions result in the formation of a multinucleated plasmodium; later on, a number of non-cruciform (non-meiotic) divisions take place to form an immature zoosporangium containing an even number of secondary zoospores (Harrison et al. 1997). Once released into the environment, secondary zoospores can re-infect the host tissue and develop a successive sporangial plasmodium, leading to numerous infection cycles of short-living propagules determining the polycyclic nature of the pathogen (van de Graaf 2000). However, secondary zoospores can also result in a sporogenic plasmodium (sporogenic phase), which undergoes cruciform division and meiosis, leading to resting spore formation.

The development of secondary plasmodia of *S. subterranea* takes place in the cortical cells of roots, and epidermal and sub-epidermal cells in tubers (Braselton 2001). The passive spread of the pathogen within host tissue is favored by the hyperplasia of cells, however, the

migration of sporangial zoosporangia has also been described (Bulman et al. 2011; Kole 1954). Infection leading to the formation of a sporogenic plasmodium is accompanied by the hyperplasia and hypertrophy of the infected cells, resulting in symptom development (Neuhauser et al. 2010). The conditions determining whether an infection will become sporangial or sporogenic are not known; furthermore, primary zoospores may also give rise to sporangial or sporogenic infections (Braselton 2001; Neuhauser et al. 2010).

The sexual reproduction of *S. subterranea,* as well as other Plasmodiophorids, is not fully understood and lacks of conclusive evidence. A tentative sexual cycle has been outlined based on reports on the *Plasmodiphora brassicae* life cycle (Merz 2008). During the sexual phase, two zoospore may fuse to form a dikaryon, which develops a binucleate plasmodium (sporogenic) that undergoes karyogamy, followed by meiosis resulting in the formation of resting spores.

Pathogen variation

The study of variability in *S. subterranea* has been influenced by the nature of the pathogen over time. Variation based in sporosori volume among isolates from 13 countries has been reported (Falloon et al. 2011). However, its biological significance is unknown, as the authors concluded that sporosori size was likely to be affected by environmental conditions, rather than the host. On the other hand, differences in the aggressiveness of *S. subterranea* isolates causing root infection were reported when the cv. Ditta was exposed to sporosori from Japan and the US, which resulted in higher infection than isolates from New Zealand and Switzerland (Merz et al. 2004). Similar results were observed among isolates collected from two potato growing regions of Japan (Nakayama et al. 2003).

The extent of the effect of differences in root infection among isolates on the formation of root galls needs further investigation. In field trials performed across five European countries (Denmark, France, Germany, Scotland and Switzerland), no evidence of a host x pathogen interaction was observed for either powdery scab or root gall formation (Merz et al. 2012). On the other hand, although differences in the degree of susceptibility to powdery scab of the cv. Kennebec grown under field conditions in Peru (moderately resistant) and New Zealand (very susceptible) has been reported, it has also been suggested that environmental conditions affecting the phenology of the host might influence the development of the disease (Falloon et al. 2003; Torres et al. 1995). Potato cultivars with a high *S. tuberosum* spp. *tuberosum* component are known to perform poorly at high altitudes (Rodriguez et al. 2009).

An evaluation of the molecular variation in *S. subterranea* has shown low diversity in areas where potato has been introduced compared to South America, the origin of potato. Preliminary reports addressed to study the variation of the pathogen ITS region showed no differences among isolates from Europe and Australasia, but these samples were different than a Peruvian isolate, which at the same time, was identical to a sample from Inverness, Scotland (Bulman and Marshall 1998). Further investigation confirmed the existence of two ribotypes of the pathogen which were identified as I and II. Differences among ribotypes ITS sequences consisted of varying nucleotide content, as well as deletions and insertions (Qu et al. 2000). The occurrence of a specific ribotype varied with geographical region. Ribotype I was found in South America, ribotype II in North America and Australasia, while both ribotypes were present in the British Islands (Qu and Christ 2004). In addition, the existence of ribotype II and the discovery of a third variant was recently reported in Colombia (Osorio et al. 2012).

Additional information on the molecular variability has supported these results, and similarly, added new insights in regard to the population biology of the pathogen. In a study of the genetic variation of *S. subterranea* across continents, higher levels of diversity were observed in four South American countries, compared to samples from 15 countries on other four continents (Gau et al. 2013). In this study, the presence of three *S. subterranea* genotype groups (A, B, C) were found to be associated with specific plant organs and hosts. Group A was present in root gall samples from *S. phureja* originating in Colombia. Group B was obtained from galls and tuber lesions isolated from either *S. tuberosum* spp. *tuberosum* or spp. *andigena* from Venezuela. A third group (C) was observed on European samples of roots and tuber lesions from *S. tuberosum* spp. *tuberosum* (Gau et al. 2015). When tested for pathogenicity, group C resulted in the greatest amount of infection, whereas group A had provided the lowest. Evidence of genotype shifting was observed, as most infected organs exhibited the genotype group C regardless of inoculum source. Genotype C was also found among South America populations of *S. subterranea* (Gau et al. 2015).

The study of global populations of *S. subterreanea* has also shown that US isolates had the lowest diversity across continents, suggesting a clonal population with some degree of variability (Gau et al. 2013; 2015). These results were in agreement with previous reports in which differences among, but not within, location of isolates from the US and Canada were observed (Qu and Christ 2006). In this study, two lineages were identified; the first included isolates from New Brunswick, Maine, Pennsylvania and Colorado, and the second composed of isolates from Idaho, California, North Dakota and Washington. Distribution was associated with trading routes within the country, and reinforces the possibility of a founder's effect on populations outside of South America (Gau et al. 2013).

Environmental Conditions Conducive for Disease Development

Soil water content

The influence of soil water content on the development of powdery scab has been recognized since early reports of the disease (Melhus 1914). Water content in the soil is essential for *S. subterranea* zoospores to move and reach host tissue; moreover, the timing and amount of water present in the soil will likely influence powdery scab occurrence and severity (Harrison et al. 1997; Merz and Falloon 2009). The availability of water in the soil during the first half of the growing season and its influence in the disease development has been described previously (Adams et al. 1987; Diriwätcher and Parbery 1991; Hughes 1980). A reduction in disease severity was observed after water was withheld during the tuber susceptibility period starting one week before tuber initiation and ended three weeks later (tuber initiation defined as >50% forming tubers > 5 mm in diameter; Taylor et al. 1986). Further evidence has supported this statement (Christ and Weidner 1988; van de Graaf et al. 2005).

The amount of water present in the soil is a determining factor for pathogen infection and subsequent stages of the disease. Water availability at levels ranging between -0.01 and -0.03 bars are reported to stimulate high levels of root infection (de Boer et al. 1985). Similarly, higher powdery scab incidence and severity were observed when plants were keep under constant soil moisture (-1 bar), than those grown under a fluctuating water regime (van de Graaf et al. 2005). However, when irrigation was applied at intervals of two weeks, the incidence of powdery scab was unaffected (Adams et al. 1987). Conversely, no significant differences between constant and fluctuating moisture regimes on the development of root galls were observed (van de Graaf et al. 2007).

It has been suggested that moisture conditions commonly found in, at least, growing fields in the United States are conducive enough for the development of powdery scab (Zink et al. 2004). Irrigation facilitates disease development in areas where temperature might not be conducive, or at altitudes in countries with hot weather (Harrison et al. 1997; Wale 2000). In experiments in which soil moisture was kept constant throughout the season, differences in the level of powdery scab were observed between two planting dates. Late planting (mid-June) coincided with higher soil temperatures (Christ and Weidner 1988). Therefore, the interaction between soil temperature and moisture influence the occurrence and severity among field and growing seasons (Christ 1989).

Soil temperature

In general, temperatures that are favorable for the growth and development of the potato crop are also encouraging for the development of the powdery scab or root gall formation. The ability of the pathogen to germinate and swim before infecting the host tissue is also affected by temperature (Harrison et al. 1997). A higher release of zoospores has been observed when incubating *S. subterranea* sporosori at temperatures between 15 and 25°C, while germination decreased at temperatures between five and 10 °C, and was completely inhibited at 30°C. Although zoospore germination is favored by temperatures ranging from five to 25°C, the development of powdery scab seems to be favored by a narrower range of temperature. Conducive temperatures for disease development range from 10 to 20°C, however the highest levels of disease severity were observed at 12°C and 17°C for powdery scab on tubers and root gall formation, respectively (de Boer et al. 1985; van de Graaf et al. 2005; 2007). In these studies disease on roots did not occur at nine degrees Celsius.

Soil texture and pH

The physical characteristics of the soil are responsible for the movement and interchange of water and gases available for the development of root and tubers. Soil texture influences the content and availability of free water for biochemical reactions (water activity), and at the same time, soil structure determines the living space for microorganisms (Harrison et al. 1997; Fiers et al. 2012). Although powdery scab and root gall formation are reported on a variety of soil textures, the prevalence of the disease seems to be higher in sandy and organic soils (van de Haar 2000). In Turkey, powdery scab is primarily a problem in sandy and sandy loam soils, where 43% of the potato production takes place (Tuncer 2002). A similar scenario was observed when soil samples from 122 commercial fields in the United Kingdom were tested for *S. subterranea*. The pathogen was detected in 75% of the samples, most of them soil of sandy texture (Brierley et al. 2009).

In contrast, in experiments in which sandy and clay loam soils were kept at the same temperature and soil matrix potential, clay soils yielded the highest levels of root infection (de Boer 2000). Conversely, fewer galls were reported on plants grown on clay soil compared to sandy and loam soils (van de Graaf et al. 2007). The amount of powdery scab, however, did not differ significantly among soil types, although on average higher disease severity was observed in the lighter soils (van de Graaf et al. 2005). Conditions of poor water drainage favor the opening of lenticels, as well as, encouraging the decrease of oxygen and enrichment of carbon dioxide levels; under these conditions, tuber infection is promoted even if tuber have reached maturity (Harrison et al. 1997).

It is not known whether the efficacy of a chemical treatment on the control of powdery scab is affected by the moisture holding capacity of the soil. Nevertheless, when fluazinam was

incorporated prior to planting, disease reduction was observed in clay loam soils, but not in sandy loams with low pH values (Boer et al. 2005). Alternatively, pH levels commonly found in arable soils (pH 5.4 - 7.1) do not appear to affect the intensity of root infection, or the development of symptoms on tubers and roots (Falloon et al. 2005b; Merz 1989; Kole 1954). Evidence of the effect of soil amendments used to reduce soil pH are scarce, but an increase in powdery scab severity was reported when lime was applied prior to planting (Garcia et al. 2004).

Source of inoculum and concentration

Spongospora subterranea is a seed-borne and tuber-borne pathogen. Disease can develop from disease free and asymptomatic tubers in infested soil, or from infected tubers planted in pathogen-free soil (Harrison et al. 2007). Inoculum borne on seeds is likely responsible for both short and long-distance spread of the disease (Brierley et al. 2012; Merz 2000; Merz and Falloon 2009). Mature lesions carry 8500 sporosori on average, each containing a number of resting spores (Bouchek-Méchiche et al. 2005; Falloon et al. 2011). Nevertheless, disease on roots can develop from symptomless tubers bearing *S. subterranea* sporosori when planted in pathogen-free soil (Tegg et al. 2015). On the susceptible potato cultivar Bintje, the amount of pathogen sporosori bore on tubers was ten times higher (100 - 500) than the less susceptible cultivar Nicola (Bouchek-Méchiche et al. 2005).

The transmission of *S. subterranea* to progeny tubers does not appear to be straightforward (Brierley et al. 2012). Sporosori of the pathogen have been associated with lenticels on tubers through which tuber infection by *S. subterranea* has been described (Brierley et al. 2012; Diriwächter and Parbery 1991). Despite the aspects related to pathogen transmission, the relationship between seed-borne inoculum level and the subsequent degree of disease

development, was better explained for the root phase of the disease, rather than the tuber phase (Braightwaite et al. 1994; Burnett 1991; Tegg et al. 2015). Another aspect of pathogen epidemiology, such as the presence of latent infections under suboptimal conditions for disease development, have been reported, but the extent of their contribution to disease development is not known (van de Graaf et al. 2005).

A number of studies focused on elucidating the relationship of the soil-borne inoculum and disease development have been published as part of an effort to estimate disease risk (Merz 1993; Nakayama et al. 2007; Qu et al. 2006). However, *S. subterranea* is able to persist in the soil via resting spores produced in tuber lesions and root galls, and at the same time, the production of both short and long lasting propagules can increase the soil infestation levels (Brierley et al. 2009; Merz 2008). In experiments conducted under controlled conditions in which *S. subterranea* inoculum ranged from zero to 100000 sporosori · ml soil^{-1}, a curvilinear relationship was observed between the severity of powdery scab and the spiked inoculum (Shah et al. 2012); however, no clear relationship was observed at inoculum levels lower than 1000 sporosori. At low inoculum levels (e.g. 25 - 300 sporosori · ml soil^{-1}) similar disease severity scores (10% surface coverage) were observed (Shah et al. 2012). Additionally, no significant differences in disease severity and incidence were reported when inoculum levels ranged from five to 50 sporosori · ml soil^{-1} (van de Graaf et al. 2005).

The relationship between levels of inoculum at pre-planting and subsequent disease expression has suggested an association between inoculum levels ranging from 136 - 14500 sporosori g · soil^{-1} and the disease incidence in four fields (Qu et al. 2006). However, when a larger number of fields (113) were monitored for disease occurrence, it was found that powdery scab incidence and the number of progeny crops developing the disease increased with the

inoculum level when an arbitrary threshold scale was used (0, <10 and >10 sporosori g · soil^{-1}; Brierley et al. 2012).

Conversely, other reports have described the initial amount of pathogen inoculum weakly or not related to the development of powdery scab on tubers, but associated to the level of inoculum at harvest time (Shah et al. 2014). In a set of field experiments across five European countries, no relationship between the index of powdery scab and the amount of inoculum at planting was found (Merz et al. 2012). Similar results were observed in Japan; however, although no association between disease severity and initial inoculum was found, a significant relationship between severity and infection potential (i.e. infecting propagules equivalents) was observed, accounting for about 60% of the total variation (Nakayama et al. 2007).

Despite the degree of association observed between disease expression and levels of inoculum at pre or post-planting, reports using molecular approaches have shown maximum inoculum levels of 148 sporosori g · soil^{-1} in the UK, 105 sporosori g · soil^{-1} in Japan and 14,400 sporosori g · soil^{-1} in the US (Brierley et al. 2012; Nakayama et al. 2007; Qu et al. 2006). Using a bioassay technique, inoculum levels > 500 sporosori g · soil^{-1} were reported in highly contaminated soils in Switzerland (Merz 1993). These results suggest that under sub-optimal field conditions, inoculum levels may be a significant factor in disease development but, under favorable conditions, a relatively low amount of inoculum can result in substantial disease development.

Hosts range

The range of hosts in which *S. subterranea* is able to infect is relatively broad. In the US, the pathogen is able to infect nine in ten plant families commonly found in cropping fields.

Families in which infection by *S. subterranea* has been observed include Amarantaceae, Asteraceae, Brassicaceae, Chenopodiaceae, Cyperaceae, Fabaceae, Poaceae, Polygonaceae and Solanaceae (Qu and Christ 2006). Similarly, observations of *S. subterranea* zoosporangia have been reported in roots of plants belonging to the families Rubeaceae, Geraniaceae, Urticaceae and Violaceae. Although root infection tested positive in ten of 14 crop plants and six of 12 weed species confronted by the pathogen, the number of species developing galls after four months of incubation was by far lower. Three plant species were able to form root galls (*Dactylis glomerate* L., *Solanum ptycanthum* Dun. and *Datura stramonium* L.), and another three, including two non-solanaceous crops, formed galls and sporosori (*Avena sativa* L., *Brassica campestris* L. and *Lycopersicon esculentum* Mill.) (Qu and Christ 2006). The formation and infectivity of *S. subterranea* sporosori produced in weeds has been reported for nightshades such as *Solanum nigrum* and *S. physalifolium* (Shah et al. 2010); the latter is described as *S. sarrachoides* elsewhere (Nitzan et al. 2009). Other crop species on which *S. subterranea* is able to infect are: sorghum (*Sorghum vulgare* L.), tobacco (*Nicotiana tobaccum* L.), pea (*Pisum sativum* L.), cauliflower (*Brassica oleracea* L.), radish (*Raphanus sativus* L.) and turnip (*Brassica rapa* L.) (Iftikhar and Ahmad 2005).

Management Approaches

The management of powdery scab is particularly difficult. The biphasic nature of the pathogen allows the production of short- and long-lived propagules limiting the efficacy of the strategies available (Merz 2008). Currently, there is not a single strategy that effectively controls powdery scab or root gall formation; therefore, an integration of approaches is essential for disease management (Falloon 2008).

Cultural practices

Disease avoidance using clean seed in clean soil represents the best method of disease prevention (Merz 2000). Avoidance of seed-borne inoculum using certified seed-tubers reduces the chances of spreading the disease to other locations inside or outside the cropping fields (Harrison et al. 1997). However, once *S. subterranea* is established in the soil, management of the disease becomes difficult. Inoculum of the pathogen can be produced on roots and tubers of the host, increasing the inoculum pressure, especially when susceptible cultivars are planted (Hernandez Maldonado et al. 2013; Sparrow et al. 2015).

Resting spores produced by the pathogen are resistant to environmental stresses and can remain viable for > 10 years (Braselton 1995). Fields in which potato has been grown once in at least five years are likely to remain at high risk of developing powdery scab (Sparrow et al. 2015). In such a case, seven years of crop rotation excluding potatoes, might be required to reduce the risk of developing the disease (Sparrow et al. 2015). The inoculum level of *S. subterranea* can also be influenced by year to year crop rotation. An increase of the pathogen infestation levels was observed after a potato/wheat rotation, but a potato/pea rotation resulted in lower infestation level (Shah et al. 2014). On the other hand, when *Datura stramonium* was planted prior to potatoes, a reduction in powdery scab severity was observed (White 1954). However, the effect of this rotation on the pathogen inoculum level was not investigated.

The growing conditions required for the development of the potato crop in soils with low capacity of water and nutrient retention are facilitated by the use of irrigation. The occurrence of powdery scab include irrigated light soils (≥ 33% sand), in which temperature and soil moisture are conducive for disease development (de Boer et al. 1985). In Turkey, where 45% of the potato production takes place on sandy soils, a significant reduction of powdery scab was achieved by

managing irrigation and levels of nitrogen fertilization (Tuncer 2002). In this study water withhold during tuber formation had little or no effect on tuber yield (de Boer 1985; Tuncer 2002). The incidence and severity of powdery scab was reported to increase when nitrogen rates of 200 - 400 Kg N · ha^{-1} were applied; the effect of the nitrogen rate on the disease was also influenced by the irrigation regime (Shah et al. 2014).

Other management approaches, such as selection of planting date and the improvement of the soil drainage capacity, have been described to influence the development of powdery scab and root gall formation (Christ 1989; Falloon 2008); however, further research on these topics is required.

Chemical management

The chemical control of *S. subterranea* has been based on two fundamental aspects: reduction of inoculum on the seed, and primary inoculum in the soil. Evaluation of chemical compound has also included the use of phosphoric acid applied to the foliage, but no effect on the control of powdery scab was reported (Falloon et al. 1996). Seed treatments based on formalin resulted in a reduction of the disease incidence of 18%, but phytotoxic effects were observed in the course of the experiments (Falloon et al. 1998). Seed treatment reported to reduce powdery scab on tubers included the use of quintozene and mercury, which have been banned due to their toxic and detrimental effects to the environment (Merz 2000; Nachmias and Krikun 1988). A significant reduction in the occurrence of powdery scab was described when infected seed was treated with a mixture of maneb and zinc oxide (Mazin™); however, differences were observed in two of 13 experiments (Braithwaite et al. 1994; Parker 1984). Seed treatment with fluazinam and mancozeb, alone and combined, as well as dichlorophen-Na and

dichlofluanid, resulted in an increase of marketable tubers of 36% for the susceptible cultivar Agria (Braithwaite et al. 1994; Falloon et al. 1996). Conversely, chemical compounds such as thiophanate methyl, mancozeb, cymoxanil and flutolamil when applied as seed treatment on the cultivar Atlantic resulted in no disease reduction (Christ 2004).

The use of chemicals aimed at reducing inoculum level and pathogen infectivity have shown inconsistent results. Furthermore, the efficacy of the treatment is suggested to vary upon the inoculum level (Burgess and Wale 1994; Falloon et al. 1996; Wale 2000). When incorporation of fluazinam, flusulfamide, mancozeb, cypronidil, dichlorophen-Na or sulfur to the soil prior to planting, powdery scab was reduced in 20%; however, disease pressure reported was relatively low (28%) (Genet et al. 1996). In addition, soil treatments using azoxystrobin, cymoxanil, dimethimorph, fluazinam, mancozeb, metalaxyl or propamocarb resulted in no significant differences on experiments using the susceptible cultivar Kennebec (Christ 2001). Conversely, when fluazinam was applied in-furrow, a significant disease reduction was observed (Davidson and Houser 2009). The use of fumigants, on the other hand, appear to yield more consistent results when applied at pre- or post-planting. The use of metam sodium applied through the irrigation water was reported to reduce powdery scab (Nachmias and Krikun 1988). Although no significant differences in disease reduction were observed when using chloropicrin, the treatment disease index was lower than the control (Hughes 1980). Additionally, reduction of powdery scab incidence was observed when using Telopic (61% 1,3-dichloropropene, 35% chloropicrin) and metam sodium in field experiments conducted in Israel over two consecutive years; disease reduction ranged from 70 - 90% (Tsror et al. 2009).

Host resistance

The use of host resistance against the pathogen *S. subterranea* represents a long term, cost-effective approach for disease management, and together with the environmental considerations, the most influencing factors for disease development (Johnson and Cummings, 2015). Genetic resistance is especially suitable on pathogen populations with low genetic diversity (Gau et al. 2013). In a study performed across five European countries, no evidence of genotype x pathogen interaction was observed, resulting in similar levels of disease development across locations (Merz et al. 2012). However, the use of host resistance can be affected by factors defining the market (Harrison et al. 1997).

There is no immunity to *S. subterranea* in potato as most cultivars available develop symptoms on tubers or roots (Falloon et al. 2003). The evaluation of resistance to the disease has been performed traditionally under field condition, but efforts for establishing greenhouse methods for disease evaluation have been reported (Baldwin et al. 2008; de Boer 1991; Falloon et al. 2003; Houser and Davidson 2010; Kirkham 1986; Merz et al. 2004; Torres et al. 1995; Wastie 1991; Wastie et al. 1988). Most of the reports are aimed at studying powdery scab on tubers and to a less extent root gall formation (Nitzan et al. 2008). The evaluation of both phases of the disease is of special interest since cultivars with a low degree of susceptibility in tubers may be susceptible to root gall formation, rendering disease on roots unsuitable as predictors of the tuber phase (Falloon et al. 2003).

The number of studies aimed at the evaluation of sources of resistance within the *Solanum* genus against *S. subterranea* is relatively low. However, *S. acaule* was reported to show resistance to pathogen infection, compared to other *Solanum* species including *S. sucrense* and *S. brevides* (Mäkäräinen et al. 1994). Similarly, among the genotypes reported to show

resistance to the formation of root galls in the Columbia Basin (WA), two characteristics were observed in common: resistant genotypes derived from the introgression of *S. bulbocastanum* and the resistant cultivar Summit Russet appeared more than once in their parental background (Nitzan et al. 2008).

Little is known about the mechanisms involved in resistance to powdery scab. As a general concept, russet-skinned cultivars are more resistant to powdery scab than smooth-skinned varieties (Houser and Davidson 2010). Furthermore, it has been suggested that resistance to the disease may be under control of multiple genes (Falloon et al. 2003; Merz et al. 2012) and inherited in an additive manner (Wastie 1991). In a recent study, a significant correlation between the development of powdery scab on tubers (disease index) and a storage protein (lipoxygenase) was observed; similarly, lipoxygenase was also related to the tuber skin type (Perla et al. 2014). However, it has been suggested that resistance mechanisms may act at the infection place later in the crop cycle (Falloon et al. 2003). Furthermore, the defense mechanisms involved in resistance to *S. subterranea* during the root infection (zoosporangium stage) might affect differently the sporosorus stage of the pathogen (root and tuber symptoms) (Hernandez Maldonado et al. 2013). Although root infection was reported to better explain disease development on tubers, exceptions were also noted (Falloon et al. 2003).

Literature Cited

Adams, M. J., Read, P. J., Lapwood, D. H., Cayley, G. R., and Hide, G. A. 1987. The effect of irrigation on powdery scab and other diseases of potatoes. Annals of Applied Biology 110:287-294.

Ames, M., and Spooner, D. M. 2008. DNA from herbarium specimens settles a controversy about origins of the European potato. American Journal of Botany 95:252-257.

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

Baldwin, S. J., Genet, R. A., Butler, R. C., and Jacobs, J. M. E. 2008. A greenhouse assay for powdery scab (*Spongospora subterranea* f. sp. *subterranea*) resistance in potato. Potato Research 51:163-173.

Bethke, P. C., Nassar, A. M. K., Kubow, S., Leclerc, Y. N., Xiu-Qing, L., Haroon, M., Molen, T., Bamberg, J., Martin, M., and Donnelly, D. J. 2014. History and origin of Russet Burbank (Netted Gem) a sport of Burbank. American Journal of Potato Research 91:594-609.

Bouchek-Méchiche, K., Montfort, F., and Andrivon, D. 2005. Assessing seed borne inoculum as a source for soil contamination by *S. subterranea* using nested PCR and real-time PCR. American Journal of Potato Research 82:59.

Bradshaw, J. E. 2007. Breeding potato as a major staple crop. Pages 277-332 in: Breeding major food staples. M. J. Kang and P. M. Priyadarshan, eds. Blackwell Publishing, Iowa, US.

Braithwaite, M., Falloon, R., Genet, R., Wallace, A., Fletcher, J., and Braam, W. 1994. Control of powdery scab of potatoes with chemical seed tuber treatments. New Zealand Journal of Crop and Horticultural Science 22:121-128.

Braselton, J. P. 1992. Ultrastructural karyology of *Spongospora subterranea*. Canadian Journal of Botany 70:1228-1233.

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

Braselton, J. P. 2001. Plasmodiophoromycota. Pages 81-91 in: The Mycota VII, Part A, Systematics and Evolution. D. J. McLaughlin, E. G. McLaughlin and P. A. Lemke, eds. Springer-Verlag, Berlin-Heidelberg.

Brierley, J. L., Stewart, J. A., and Lees, A. K. 2009. Quantifying potato pathogen DNA in soil. Applied Soil Ecology 41:234-238.

Brierley, J. L., Sullivan, L., Wale, S. J., Hilton, A. J., Kiezebrink, D. T., and Lees, A. K. 2012. Relationship between *Spongospora subterranea* f. sp. *subterranea* soil inoculum level, host resistance and powdery scab on potato tubers in the field. Plant Pathology 62:413-420.

Brown, C. R., Vandemark, G. J., Cummings, T., Batchelor, D., Miller, J., Olsen, C., and Johnson, D. 2007. Resistance to powdery scab in potato. Potato Progress 7:1-4.

Brown, C. 2010. History. Pages 2-4 in: Commercial Potato Production in North America. W. H. Bohl and S. B. Johnson, eds. Online: http://potatoassociation.org

Bulman, S. R. and Braselton, J. P. 2014. Rhizaria: Phytomyxea. Pages 99-112 in: The Mycota VII, Systematics and Evolution, Part A. 2nd ed. D. J. McLaughlin and J. W. Spatafora, eds. Springer-Verlag, Berlin-Heidelberg.

Bulman, S., Candy, J. M., Fiers, M., Lister, R., Conner, A. J., and Eady, C. C. 2011. Genomics of biotrophic, plant-infecting plasmodiophorids using in vitro dual cultures. Protist 162:449-461.

Bulman, S., Kühn, S., Marshal, J., and Schnepf, E. 2001. A phylogenetic analysis of the SSU rRNA from members of the Plasmodiophorida and Phagomyxida. Protist 152:43-51.

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

Burgess, P., and Wale, S. 1994. Development of an integrated control strategy for powdery scab of potatoes. Pages 301-306 in Brighton crop protection conference, pests and diseases, 1994, vol. 1. British Crop Protection Council, Farnham, UK.

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

Carling, D. E. 1996. First report of powdery scab of potatoes in Alaska. Plant Disease Note 806-01N. Plant Disease 80:1208.

Cavadini, C., Siega-Riz, A. N., Popkin, B. M. 2000. US adolescent food intake trends from 1965 to 1996. Western Journal of Medicine 173:378-383.

Christ, B. J. 1989. Effect of planting date and inoculum level on incidence and severity of powdery scab on potato. Potato Research 32:419-424.

Christ, B. J. 2001. Evaluation of in-furrow fungicide treatments for powdery scab control. Online: http://potatohealth.cas.psu.edu

Christ, B. J. 2001. Powdery scab. Pages 35-36 in: Compendium of Potato Diseases. 2nd ed. W. R. Stevenson, W. R. Loria, R. Franc, G. D. and D. P. Weingartner, eds. The American Phytopathological Society, St. Paul, MN, US.

Christ, B. J. 2004. Powdery Scab Workshop—Summary Notes. Online: http://www.uidaho.edu/ag/plantdisease/scabnote.html

Christ, B. J., and Weidner, R. J. 1988. Incidence and severity of powdery scab of potatoes in Pennsylvania. American Potato Journal 65:584-588.

Davidson, R. D., and Houser, A. 2009. Evaluation of fluazinam application methods in-furrow for control of powdery scab on potato. American Journal of Potato Research 86:140.

de Boer, R. F. 1991. Evaluation of potato cultivars in the greenhouse and field for resistance to powdery scab. Australian Journal of Experimental Agriculture 31:699-703.

de Boer, R. 2000. Research into the biology and control of powdery scab of potatoes in Australia. Pages 79-83 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees, eds. Aberdeen, Scotland.

de Boer, R. F., Crump, N. S., and Edwards, J. E. 2005. Powdery scab (*Spongospora subterranea*) of potatoes research in Australia. American Journal of Potato Research 82:64.

de Boer, R. F., Taylor, P. A., Flett, S. P., and Merriman, P. R. 1985. Effects of soil temperature, moisture and timing of irrigation on powdery scab of potatoes. Pages 197-198 in: Ecology and management of soilborne plant pathogens. Proceedings of section five of the fourth International Congress of Plant Pathology. C. A. Parker, A. D. Rovira, K. J. Moore, and P. T. W. Wong, eds. Melbourne, Australia.

De Jong, H., Sieczka, J. B., and De Jong, W. 2011. The complete book of potatoes: what every grower and gardener needs to know. Timber Press Inc., Portland, US.

Dick, M. 2001. Straminipilous fungi: systematics of the Peronosporomycetes including accounts of the marine straminipilous protists, the plasmodiophorids and similar organisms. Kluwer Academic Publisher, Dordrecht.

Diriwächter, G., and Parbery, D. G.1991. Infection of potato by *Spongospora subterranea*. Mycological Research 95:762-764.

Draper, M. A., Secor, G. A., and Gudmestad, N. C. 1997. First report of potato powdery scab, caused by *Spongospora subterranea* f. sp. *subterranea*, in North Dakota. Plant Disease 81:693.

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

Falloon, R. E., Curtin, D., Lister, R. A., and Butler, R. C. 2005a. Root function and growth of potato plants reduced by *Spongospora subterranea* infection. American Journal of Potato Research 82:68.

Falloon, R. E., Curtin, D., Lister, R. A., and Butler, R. C. 2005b. Different soil pHs have little effect on *Spongospora subterranea* infection of potato roots. American Journal of Potato Research 82:68-69.

Falloon, R., Genet, R., Nott, H., Wallace, A., Fletcher, J., and Braam, F. 1998. Formaldehyde dip for powdery scab. New Zealand Commercial Grower 53: 26-27.

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

Falloon, R., Merz, U., Lister, R. A., Wallace, A. R., and Hayes, S. P. 2011. Morphological enumeration of resting spores in sporosori of the plant pathogen *Spongospora subterranea*. Acta Protozoologica 50:121-132.

Falloon, R., Wallace, A., Braithwaite, M., Genet, R., Nott, H., Fletcher, J., and Braam, W. 1996. Assessment of seed tuber, in-furrow, and foliar chemical treatments for control of powdery scab (*Spongospora subterranea* f. sp. *subterranea*) of potato. New Zealand Journal of Crop and Horticultural Science 24:341-353.

Fiers, M., Edel-Hermann, V., Chatot, C., Le Hingrat, Y., Alabouvette, C., and Steinberg, C. 2012. Potato soil-borne diseases. A review. Agronomy for Sustainable Development 32: 93-132.

Fornier, N. 1997. Epidemiology of *Spongospora subterranea*, the cause of powdery scab of potatoes. University of Aberdeen, Aberdeen, UK.

Fry, W. E., Goodwin, S. B., Dyer, A. T., Matuszak, J. M., Drenth, A., Tooley, P. W., Sujkowski, L. S., Koh, Y. J., Cohen, B. A., Spielman, L. J., Deahl, K. L., Inglis, D. A., and Sandlan, K .P. 1993. Historical and recent migrations of *Phytophthora infestans*: chronology, pathways, and implications. Plant Disease 77:653-661.

García, R., Garnica, J., and Espinosa, Y. 2004. Estudio de epifitias en la roña de la papa (*Spongospora subterranea*) en el Estado Mérida, Venezuela. Revista Latinoamericana de la Papa, Suplemento especial, Marzo, Oral 40.

Gau, R. D., Merz, U., and Falloon, R. E. 2015. Infection risk potential of South American *Spongospora subterranea* f. sp. *subterranea* root gall and tuber lesion inoculum on potato (*Solanum tuberosum* ssp. *tuberosum*). American Journal of Potato Research 92:109-116.

Gau, R. D., Merz, U., Falloon, R. E., and Brunner, P. C. 2013. Global genetics and invasion history of the potato powdery scab pathogen, *Spongospora subterranea* f. sp. *subterranea*. PLoS ONE 8(6): e67944. DOI:10.1371/journal.pone.0067944

Genet, R., Falloon, R., Braithwaite, M., Wallace, A., Fletcher, J., Nott, H., and Braam, F. 1996. Disease resistance and chemical for control of powdery scab (*Spongospora subterranea*): progress toward integrated control in New Zealand. 13th Triennial Conference of the European Association for Potato Research. Abstracts of Conference Papers and Posters. Veldhoven, the Netherlands.

Gilchrist, E., Soler, J., Merz, U., and Reynaldi, S. 2011. Powdery scab effect on the potato *Solanum tuberosum* ssp. *andigena* growth and yield. Tropical Plant Pathology 36:350-355.

Glendinning, R. D. 1983. Potato introductions and breeding up to the early 20th century. New Pathologist 94:479-505.

Graham, H., and Poai, N. 1999. "Nga - Riwai Maori — Maori Potatoes". The Open Polytechnic of New Zealand. Working Paper No 2/99. Pp. 73.

Güssow, H. T. 1913. Powdery scab of potatoes, *Spongospora subterranea* (Wallr.) Johns. Phytopathology 3:18-19.

Harrison, J. G., Searle, R. J., and Williams, N. A. 1997. Powdery scab disease of potato - a review. Plant Pathology 46:1-25.

Heffron, D. S. 1865. Goodrich's seedling potatoes. Pages 36-39 in: The Gardener's Monthly and Horticultural Advertiser, vol. VII. W. G. P. Brinckloe ed. Philadelphia, US.

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

Hosaka, K., and Hanneman, R. 1988. The origin of the cultivated tetraploid potato based on chloroplast DNA. Theoretical and Applied Genetics 76:172-176.

Houser, A. J., and Davidson, R. 2010. Development of a greenhouse assay to evaluate potato germplasm for susceptibility to powdery scab. American Journal of Potato Research 87:285-289.

Hughes, I. K. 1980. Powdery scab (*Spongospora subterranea*) of potatoes in Queensland: occurrence, cultivar susceptibility, time of infection, effect of soil pH, chemical control. Australian Journal of Experimental Agriculture and Animal Husbandry 20:625-632.

Hutchison, L. J., and Kawchuk, L. M. 1998. *Spongospora subterranea* f. sp. *subterranea*. Canadian Journal of Plant Pathology 20:118-119.

Iftikhar, S., and Ahmad, I. 2005. Alternate hosts of *Spongospora subterranea* f. sp. *subterranea*, the causal organism of powdery scab of potato. American Journal of Potato Research 82:74-75.

Johnson, D. A., and Cummings, T. F. 2015. Effect of powdery scab root galls on yield of potato. Plant Disease 99:1396-1403.

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

Kirkham, R. P. 1986. Screening for resistance to powdery scab disease of potatoes. Australian Journal of Experimental Agriculture 26:245-247.

Kole, A. P. 1954. A contribution to the knowledge of *Spongospora subterranea* (Wallr.) Lagerh., the cause of powdery scab of potatoes. Tijdschrift over plantenziekten 60:1-65.

Krebs-Smith, S. M., Cook, D., Subar, A. F., Cleveland, L., Friday, J., Kahle, L. L.1996. Fruit and vegetable intakes of children and adolescents in the United States. Archives of Pediatrics and Adolescent Medicine 150:81-86.

Lagerheim, G. 1892. Remarks on the fungus of a potato scab (*Spongospora solani* Brunch). The Journal of Mycology 7:103-104.

Lister, R. A., Falloon, R. E., Curtin, D., and Butler, R. C. 2004. *Spongospora subterranea* reduces host (*Solanum tuberosum*) growth. Pages 135-136 in: Proceedings of the 3rd Australasian soilborne diseases symposium. K. M. Ophel Keller and B. H. Hall, eds. South Australian Research and Development Institute, Adelaide.

Love, S. 1999. Founding clones, major contributing ancestors, and exotic progenitors of prominent North American cultivar. American Journal of Potato Research 76: 263-272.

Lyman, G. R., and Rogers, J. T. 1915. The native habitat of *Spongospora subterranea*. Science 42:940-941.

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

Melhus, I. E. 1914. Powdery scab (*Spongospora subterranea*) of potatoes. Bulletin of the U. S. Department of Agriculture, No. 82. Pp. 1-16.

Melhus, I. E., Rosenbaum, J., and Schultz, E. S. 1916. *Spongospora subterranea* and *Phoma tuberosa* on Irish potato. Journal of Agricultural Research 7:213-253.

Merz, U. 1989. Infectivity, inoculum density and germination of *Spongospora subterranea* resting spores: a solution-culture test system. Bulletin OEPP 19:585-592.

Merz, U. 1993. Epidemiological aspects of powdery scab of potatoes caused by *Spongospora subterranea*. Pages 104-106 in: Proceedings of the 2nd symposium of the international working group on plant viruses with fungal vectors. Montreal, Canada.

Merz, U. 2000. Experiments on direct control and yield loss made in New Zealand. Pages 51-52 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees eds. Aberdeen, Scotland.

Merz, U. 2008. Powdery scab of potatoes - occurrence, life cycle and epidemiology. American Journal of Potato Research 85:239-246.

Merz, U., and Falloon, R. E. 2009. Review: powdery scab of potato - increased knowledge of pathogen biology and disease epidemiology for effective disease management. Potato Research 52:17-37.

Merz, U., Lees, A. K., Sullivan, L., Schwärzel, R., Hebeisen, T., Kirk, H. G., Bouchek-Mechiche, K., and Hofferbert, H. R. 2012. Powdery scab resistance in *Solanum tuberosum*: an assessment of cultivar x environment effect. Plant Pathology 61:29-36.

Merz, U., Martinez, V., and Schwaerzel, R. 2004. The potential for the rapid screening of potato cultivars (*Solanum tuberosum*) for resistance to powdery scab (*Spongospora subterranea*) using a laboratory bioassay. European Journal of Plant Pathology 110:71-77.

Merz, U., Walsh, J., Bouchek-Mechiche, K., Oberhänsli, T., and Bitterlin, W. 2005. Improved immunological detection of *Spongospora subterranea*. European Journal of Plant Pathology 111:371-379.

Mohan, S. K., Davis, J. R., and Hafez, S. L. 1991. Powdery scab of potato. Proceedings of the University of Idaho Winter Commodity Schools 1991, vol. 23. Pp. 242-244.

Morse, W. J. 1913. Powdery scab of potatoes in the United States. Science 38:61-62.

Nachmias, A., and Krikun, J. 1988. Etiology and control of powdery scab of potato in a semi-arid region of Israel. Phytoparasitica 16:33-38.

Nakayama, T., Horita, M., and Shimanuki, T. 2007. *Spongospora subterranea* soil contamination and its relationship to severity of powdery scab on potatoes. Journal of General Plant Pathology 73:229-234.

Nakayama, T., Merz, U., Nakagawa, A., Takehara, T., and Shimanuki, T. 2003. Differences in zoosporangial root infection of some potato varieties inoculated with Japanese and foreign field isolates of *Spongospora subterranea* f. sp. *subterranea*. Pages 115-118 in: Proceedings of the 5th symposium of the international working group on plant viruses with fungal vectors. C. Rush and U. Merz, eds. Zurich, Switzerland.

Neuhauser, S., Bulman, S., and Kirchmair, M. 2010. Plasmodiophorids: the challenge to understand soil-borne, obligate biotrophs with a multiphasic life cycle. Pages 51-78 in: Molecular Identification of Fungi. Y. Gherbawy and K. Voigt, eds. Springer-Verlag.

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

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

Ochoa, C. 2001. Universal gift. Page 208 in: The potato treasure of the Andes: from agriculture to culture. C. Graves, ed. Centro International de la Papa. Lima, Peru.

Osorio-Giraldo, I., Orozco-Valencia, M., Gutiérrez-Sánchez, P., González-Jaimes, P., and Marin-Montoya, M. 2012. Variabilidad genética de *Spongospora subterranea* f. sp. *subterranea* en Colombia. Bioagro 24:151-162.

Parker, A. 1984. Evaluation of chemical dip treatments for the control of powdery scab of potatoes. Tests of Agrochemicals and Cultivars Nº5 (Annals of Applied Biology 104, Suppl.). Pp. 62-63.

Perla, V., Jayanty, S. S., Holm, D. G., and Davidson, R. D. 2014. Relationship between tuber storage proteins and tuber powdery scab resistance in potato. American Journal of Potato Research 91:233-245.

Pitts, T. 2000. Summary of the session on present control measures. Pages 31-33 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees, eds. Aberdeen, Scotland.

Qu, X. S., and Christ, B. J. 2004. Genetic variation and phylogeny of *Spongospora subterranea* f. sp. *subterranea* based on ribosomal DNA sequence analysis. American Journal of Potato Research 81:385-394.

Qu, X. S., and Christ, B. J. 2006. The host range of *Spongospora subterranea* f. sp. *subterranea* in the United States. American Journal of Potato Research 83:343-348.

Qu, X., Kavanagh, J. A., and Egan, D. 2000. *Spongospora subterranea* f. sp. *subterranea*: molecular variation, PCR detection and isolation in vitro. Pages 75-77 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees, eds. Aberdeen, Scotland.

Qu, X. S., Kavanagh, J. A., Egan, D., and Christ, B. J. 2006. Detection and quantification of *Spongospora subterranea* f. sp. *subterranea* by PCR in host tissue and naturally infested soil. American Journal of Potato Research 83:21-30.

Ríos, D., Ghislain, M., Rodríguez, F., and Spooner, D. M. 2007. What is the origin of the European potato? evidence from Canary Island landraces. Crop Science 47:127-128.

Rodriguez, D. A., Ojeda, M., Camacaro, M. P., Gallardo, M., Valera, R., and Bittara, F. 2009. Production, powdery scab incidence and quality of potato advanced clones. Revista de la Facultad de Agronomia de la Universidad del Zulia 26:508-531.

Shah, F. A., Butler, R. C., Marshall, J. W., and Keenan, S. 2004. Relationships between *Spongospora subterranea* inoculum, powdery scab severity and potato tuber yield. Pages 172-173 in: Proceedings of the 3rd Australasian soilborne disease symposium. K. M. Keller and B. H. Hall, eds. South Australian Research and Development Institute, Adelaide.

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

Shah, F. A., Falloon, R. E., Butler, R. C., and Lister, R. A. 2012. Low amounts of *Spongospora subterranea* sporosorus inoculum cause severe powdery scab, root galling and reduced water use in potato (*Solanum tuberosum*). Australasian Plant Pathology 41:219-228.

Shah, F. A., Falloon, R. E., Butler, R. C., Lister, R. A., Thomas, S. M., and Curtin, D. 2014. Agronomic factors affect powdery scab of potato and amounts of *Spongospora subterranea* DNA in soil. Australasian Plant Pathology 43:679-689.

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

Taylor, P., Flett, S. P., de Boer, R. F., and Marshall, D. 1986. Effects of irrigation regimes on powdery scab disease and yield of potatoes. Australian Journal of Experimental Agriculture 26:745-750.

Tegg, R. S., Corkrey, R., Herdina, H., McKay, A. C., Crump, N. S., de Boer, R. F., Wiechel, T. J., and Wilson, C. R. 2015. Modeling pathogen DNA content and visual disease assessment in seed tubers to inform disease in potato progeny root, stolon, and tubers. Plant Disease 99:50-57.

Torres, H., Pacheco, M. A., and French, E. R. 1995. Resistance of potato to powdery scab (*Spongospora subterranea*) under Andean field conditions. American Potato Journal 72:355-363.

Tsror, L., Erlich, O., Hazanovsky, M., and Zig, U. I. 2009. Powdery scab occurrence and control in Israel. Page 44 in: Abstracts of the 7th international symposium on chemical and non-chemical soil and substrate disinfestation. Katholieke Universiteit Leuven, Belgium.

Tuncer, G. 2002. The effect of irrigation and nitrogen on powdery scab and yield of potatoes. Potato Research 45:153-161.

USDA. 2014. United States standards for grades of potatoes. Online: www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5091274.

USDA. 2013. Crop production report. Online: www.usda.gov/nass/PUBS/TODAYRPT/crop1113.pdf.

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

van de Graaf, P., Lees, A. K., and Duncan, J. M. 2000. Epidemiology and control of *Spongospora subterranea* f.sp. *subterranea*, with special reference to zoospore release and bait plant infection. Pages 57-60 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees, eds. Aberdeen, Scotland.

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

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

van de Haar, J. 2000. The powdery scab situation in the Netherlands. Pages 21-22 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees, eds. Aberdeen, Scotland.

Wale, S. J. 2000. Summary of the session on national potato production and the powdery scab situation. Pages 3-9 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees, eds. Aberdeen, Scotland.

Wastie, R. L. 1991. Resistance to powdery scab of seedling progenies of *Solanum tuberosum*. Potato Research 34:249-252.

Wastie, R. L., Caligari, P. D. S., and Wale, S. J. 1988. Assessing the resistance of potatoes to powdery scab (*Spongospora subterranea* (Wallr.) Lagerh.). Potato Research 31:167-171.

White, N. H. 1954. The use of decoy crops in the eradication of certain soilborne plant diseases. Australian Journal of Science 17:18-19.

Xu, X., Pan, S., Cheng, S., Zhang, B., Mu, D., Ni, P., Zhang, G., Yang, S., Li, R., Wang, J. et al. 2011. Genome sequence and analysis of the tuber crop potato. Nature 475:189–195.

Zink, R. T., Davidson, R. D., and Houser, A. 2004. Control strategies for powdery scab of potato. American Journal of Potato Research 81:95-96.

Zuckerman, L. 1998. The potato: how the humble spud rescued the western world. North Point Press, New York.

CHAPTER II. FIELD EVALUATION OF POTATO GENOTYPES FOR RESISTANCE TO POWDERY SCAB ON TUBERS AND ROOT GALL FORMATION CAUSED BY *SPONGOSPORA SUBTERRANEA*

Abstract

Spongospora subterranea causes root galls and powdery scab on potato tubers (*Solanum tuberosum* L.). Host resistance represents an economically suitable and long term approach for the management of the disease; however, the relationship between root and tuber symptoms may vary across potato genotypes. To assess susceptibility differences among genotypes, 30 potato cultivars and 83 advanced clones with varying skin type (market class) were evaluated for powdery scab and root gall formation. Five field experiments were conducted during 2011 and 2012 on naturally infested soils in Minnesota and North Dakota. Differences among genotypes in the degree of susceptibility to tuber and root symptoms were observed ($P < 0.001$). Higher powdery scab pressure ($P < 0.001$) was observed in North Dakota locations across years. Environmental conditions influenced the formation of root galls and the expression of powdery scab, with greatest variability among white- and red-skinned genotypes. Under high disease pressure, the estimates of broad-sense heritability for powdery scab incidence and severity were 0.76 and 0.63, respectively. Across environments, russet-skinned genotypes resulted in less disease on tubers, but yielded similar levels of root galls as red-skinned genotypes. Tuber scab and root gall formation indices were significantly correlated ($r = 0.47$, $P < 0.001$, $n = 80$); however, this predictor explained only 22% of the variability. Cultivars Dakota Trailblazer, Dakota Russet and Karu ranked highly resistant, whereas Shepody, Kennebec and Red LaSoda

were highly susceptible to both phases of the disease. Cultivar selection is highly recommended for disease management.

Introduction

Spongospora subterranea (Wallr.) Lagerh. is a biotrophic protozoan pathogen of solanaceous crops. On potato (*Solanum tuberosum* L.), *S. subterranea* causes root galls and powdery scab on tubers (Cavalier-Smith 1998; Harrison et al. 1997; Neuhauser et al. 2010). Members belonging to the Plasmodiophorida group develop multiple somatic structures including multinucleated plasmodia, zoosporangia, biflagellate zoospores and resting spores (Braselton 1995, Hernandez Maldonado et al. 2013). The pathogen life cycle consists of a sporangial and a sporogenic phase. Initial infections of the epidermal root cells, stolons and young tubers develop sporangial zoosporangia from which short-lived zoospores emerge. Repeated infection cycles result in a polycyclic nature of the disease (van de Graaf 2000). During the sporogenic phase, *S. subterranea* undergoes cruciform division and meiosis resulting in the development of resting spores arranged in aggregates (sporosorus). This phase is accompanied by the hyperplasia and hypertrophy of the host cortical cell; promoting the formation of root galls and pustules on tubers. When mature, hypertrophied cells burst, causing lesions and releasing masses of sporosori (Merz and Falloon 2009).

Resting spores of *S. subterranea* are highly resistant to environmental stresses and can remain viable for over 10 years (Merz and Falloon 2009). Prediction models estimate a high risk of developing powdery scab in fields in which potato is grown once at least every five years (Sparrow et al. 2015). Lesions on tubers are responsible for the spread of the pathogen to other fields (Merz and Falloon 2009). Additionally, resting spores borne on asymptomatic tubers can

induce the formation of root galls in the absence of soil-borne inoculum (Tegg et al. 2012). The severity of powdery scab, as well as the amount of inoculum borne on tubers of the cultivars Innovator and Russet Burbank, has been reported strongly associated ($P < 0.01$) with formation of root galls (Tegg et al. 2012).

Powdery scab is a quality limiting disease (Wale 2000). In the US, a lot can be downgraded when tubers have >5% surface coverage, and lots with severity >25% is considered non-marketable (Houser and Davidson 2010; USDA 2014a). However, the effect of powdery scab and root gall formation on tuber yield appears to be small, or at least not negative (Johnson and Cumming 2015; Wale 2000). Root infection by the pathogen is generally accompanied by cell necrosis, reduction of nutrient uptake and water absorption (Kole 1954; Lister et al. 2004). Under controlled conditions, the infection by *S. subterranea* was shown to reduce tuber yield; the decrease in yield and tuber weight was more pronounced on healthy tubers (Shah et al. 2005, 2012). The detrimental effect of infection by the pathogen is also likely to interact with the potato cultivar and compounded with the damage caused by other soil-borne pathogens (e.g. *Colletotrichum coccodes*) (Johnson and Cumming 2015; Merz and Falloon 2009). Furthermore, *S. subterranea* is the vector of the *Potato mop-top virus* (PMTV) (Jones and Harrison 1969), which causes internal necrotic lesions rendering tubers undesirable for consumption or processing (Davey et al. 2014). Recent reports demonstrated a significant association between the incidence of PMTV-induced tuber necrosis and the number of galls per plant ($r = 0.52$, $P = 0.05$), as well as the incidence of powdery scab on tubers ($r = 0.62$, $P = 0.02$; Domfeh et al. 2015).

In the field, the occurrence and degree of disease development is drastically limited by environmental conditions. Cool soil temperatures (10 - 20°C) are conducive for symptom

development (de Boer et al. 1985). Likewise, constant levels of high soil moisture (> -0.01 bars) are more favorable than fluctuating regimes (van de Graaf et al. 2005; 2007). Conducive levels of soil moisture can be provided by irrigation and rainfall (Johnson and Cummings 2015). Soil characteristics (i.e. texture, structure) accounting for water movement and gas interchange are likely to influence disease expression. Powdery scab is reported on a variety of soil textures, however, the disease seems to be more prevalent in organic and sandy soils (Brierley et al. 2009; Tucner 2002; van de Haar 2000).

Host resistance highly influences disease development, yet most of the commercially available grown cultivars are susceptible to some degree (de Boer 1991; Merz et al. 2012; Kirkham 1986). In field experiments conducted in Peru to assess susceptibility to powdery scab, 50 out of 467 potato genotypes evaluated were reported to have some level of resistance to the disease (Torres et al. 1995). In New Zealand, 99 cultivars and 13 breeding lines were evaluated for their susceptibility to powdery scab, and it was observed that resistance follows a continuum from very resistant to very susceptible, suggesting a trait controlled by multiple genes (Falloon et al. 2003). Elsewhere, tuber resistance has been described to be inherited in an additive manner (Wastie 1991). Susceptibility to root gall formation is likely to be governed by a number of genes similar to the tuber phase of the disease; however, the degree of association between resistance to powdery scab and root gall formation varies among genotypes (Merz et al. 2012). Tuber and root phases of the disease were reported significantly correlated, but the usefulness of this predictor was considered not suitable since cultivars showing tuber resistance can develop high rates of root galls (Hughes 1980). Two examples are the cultivars Umatilla Russet and Russet Burbank, which develop low levels of powdery scab on tubers, but are considered

susceptible to root gall formation (de Boer 1991; Houser and Davidson 2010; Manzer et al. 1964; Merz et al. 2012).

Under the described scenario, the development of management approaches aimed to control the field expression of *S. subterranea* in the short and long-term are likely to be influenced by the degree of susceptibility on roots and tubers. However, the genetic component of host resistance to the formation of root galls was reported moderately high on potato genotypes evaluated in the Columbia Basin (Nitzan et al. 2010). In that study, 18 of 24 tested potato genotypes were genetically stable before and/or after removing the environmental heterogeneity. Therefore, host resistance represents the most advantageous and long-term approach for disease management; nevertheless, most reports are focused on powdery scab on tubers and to a lesser extent root gall formation (Baldwin et al. 2008; de Boer 1991; Falloon et al. 2003; Houser and Davidson 2010; Kirkham 1986; Nitzan et al. 2008; Torres et al. 1995; Wastie et al. 1988). The objective of this research was to test the hypothesis that differences in the susceptibility levels to powdery scab and root gall formation exist among 113 potato genotypes with varying skin types.

Materials and Methods

Field experiments

Experiments were conducted on irrigated commercial fields naturally infested with *Spongospora subterranea* and with a known history of disease. A total of five trials were performed during two consecutive years at four locations: Larimore, North Dakota (47°54′N, 97°37′W, 341 m.a.s.l; Larimore-11); McCanna, ND (48°0'N, 97°42'W; 348 m.a.s.l; McCanna-11; 12); Perham, Minnesota (46°35′N, 95°34′W; 417 m.a.s.l; Perham-11) and Osage, MN

(46°55′N, 95°15′W; 455 m.a.s.l; Osage-12; Table 2.1). Plant material consisted of 113 potato cultivars and advanced clones which included red-, russet-, white- and yellow-skinned genotypes from the North Dakota State University potato breeding program and the potato breeding program of the Instituto de Investigaciones Agropecuarias (INIA) of Chile.

Table 2.1. Environments, soil texture and pH, and number of potato genotypes by skin type evaluated under field condition for susceptibility to powdery scab on tubers and root gall formation.

Environment					Skin type				Total genotypes per location
Year	**Location**	**Soil texture**	**Soil pH**	**Planting / harvest date**	**Red**	**Russet**	**White**	**Yellow**	
2011	Larimore, ND	Sandy loam	6.7	May 17 / September 27	n.t.	31	2	n.t.	33
	McCanna, ND	Sandy loam	5.6	June 18 / October 3	29	20	26	13	88
	Perham, MN	Sand	7.4	May 9 / September 14	28	11	25	13	77
2012	McCanna, ND	Sandy loam	5.6	April 27 / September 7	25	31	21	7	84
	Osage, MN	Sandy loam	6.1	May 3 / September 4	23	31	21	5	80
Total genotypes per skin type					34	35	30	14	

n.t.: not tested.

Field trials were conducted using a randomized complete block design with three replicates. Treatment plots consisted of 5-plant rows in year one and 10-plant rows for year two. Seed pieces (60 - 100 g) were planted 0.3 m apart and 0.1 m depth; the distance between rows was 0.9 m. Weather conditions during the course of field trials (June 1 - October 31) were estimated from records of the two nearest weather stations to the site of the trials (Table 2.2). At McCanna trials, irrigation was 95.3 and 518.2 mm during 2011 and 2012, respectively. At Perham in 2011, the amount of irrigation totaled 175.3 mm, and 271.78 mm at Osage during 2012.

Table 2.2. Average air temperature and total precipitation at two weather stations in Minnesota and North Dakota at experimental locations in 2011 and 2012.

State	Station	2011		2012	
		Air temperature (ºC)	Precipitation (mm)	Air temperature (ºC)	Precipitation (mm)
Minnesota	Alexandria	17.5	481.6	17.4	229.1
	Fergus Falls	18.4	327.9	17.8	199.1
North Dakota	Grand Forks	17.3	316.2	16.4	256.0
	Inkster	16.6	338.8	16.2	273.2

The amount of nitrogen applied at McCanna was 110 kg · ha^{-1} in 2011 and 319 kg · ha^{-1} in 2012. Applied nitrogen at Minnesota trials was 164.5 kg · ha^{-1} at Perham-11 and 339.3 kg · ha^{-1} in Osage-12. At McCanna, herbicide applications were conducted each year using pendimethalin (2.8 l · ha^{-1}) and rimsulfuron (105g · ha^{-1}). Pest management was performed using the insecticides thiamethoxam, imidacloprid, abamectin and esfenvalerate at manufacturer recommended rates. The control of foliar disease (early and late blight) was done using chlorothalonil, fluopyram/pyrimethanil, boscalid and azoxystrobin at recommended rates for irrigated commercial potato crops in the Upper Great Plains of the USA.

Sampling and disease assessment

Powdery scab

At harvest, all tubers over 40 g were collected, and stored for approximately 6 weeks at 12°C, and evaluated for disease. Prior to disease evaluations, tubers were washed and dried overnight at room temperature. The presence of sporosori under microscope was used to distinguish *Spongospora subterranea* from lesions caused by other pathogens such as *Streptomyces* spp. (Waterer 2010). Disease incidence was expressed as the percentage of infected tubers in a sample, and disease severity as the percentage of tuber surface covered by powdery scab lesions. Severity was estimated on each side of the tuber using an increasing percentage

graphic scale and then averaged (Falloon et al. 1995). The mean disease severity was calculated as $\left[\sum \frac{(nN_n)}{5N_0}\right] * 100$, where *n* is the disease index (0 = no disease, 1 = one pustule to 2.0%, 2 = 2.1 - 5.0%, 3 = 5.1 - 10.0%, 4 = 10.1 – 25%, 5 = > 25%) (Houser and Davidson 2010; Nakayama et al. 2007), N_n is the number of tubers with disease symptoms at level ‘‘n’’ and N_0 is the total number of tubers evaluated.

Root gall formation

Ninety days after planting, root galls were evaluated by carefully removing an entire plant from the soil with the aid of a potato fork. Root gall formation was only evaluated during the second year of research (McCanna-12 and Osage-12). Five plants per plot were sampled and the number of galls per plant was counted with the aid of a magnifying glass (1.75X). The mean number of galls per plant was calculated for each plot. The mean maximum number of galls per plant and powdery scab severity was also calculated for each location (Baldwin et al. 2008).

Statistical analysis

Statistical analysis was conducted using SAS 9.3 (SAS Institute, Cary, NC). Homogeneity of variances was assessed during data analysis using Bartlett’s test ($\alpha = 0.05$). Normal distribution of the data was evaluated using the Shapiro-Wilk test ($\alpha = 0.05$). Among genotypes, differences in the intensity of root gall formation and powdery scab parameters were estimated using Friedman’s test on ranked data. The effect of location and year on the development of powdery scab incidence and severity was assessed on a set of 15 potato cultivars across four trials. Data on powdery scab parameters were transformed ($\sqrt{y}$) and analyzed with ANOVA using a randomized complete block design with sub-sampling (potato cultivar).

Treatment means were compared using Fisher's protected least significant difference (LSD; α: 0.05).

Pearson's correlations were performed between powdery scab parameters and variables of the disease on roots and tubers for a sub-set of 50 potato genotypes. On this data set, the broad-sense heritability (H) for powdery scab incidence and severity on tubers were calculated for trials conducted in North Dakota and Minnesota. H as the proportion of the phenotypic variation given by the genotypic variance was defined as $H = \sigma^2_G / [(\sigma^2_{error} / re) + (\sigma^2_{GxE} / e) + \sigma^2_G)]$, where r = number of replicates and e = number of environments (Holland et al. 2003). The 95% confidence intervals for H were determined according to Knapp et al. (1985). The upper confidence interval was $1-[(MS1/MS2) F_{(1-\alpha/2:df2, df1)}]^{-1}$, and the lower confidence interval $1-[(MS1/MS2) F_{(\alpha/2:df2, df1)}]^{-1}$, where MS1 = mean squares for genotype and MS2 = mean squares for genotype x environment. The mean squares associated with the random effects were obtained from ANOVA analysis.

Data for powdery scab severity and root gall formation of 80 potato genotypes evaluated in at least two environments were standardized by dividing the averaged disease severity and mean number of galls by the mean maximum value of each variable at a specific environment (Y/Y_{max}). This procedure was chosen in order to adjust for major environmental differences in disease expression across trials (Falloon et al. 2003). The powdery scab and root gall formation indices of the genotypes included in this sub-set were averaged across environments, plotted and subjected to cluster analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Potato genotypes were categorized according to arbitrary cut-off points as very resistant (VR: 0 - 0.05), moderately resistant (MR: > 0.05 - 0.15), moderately susceptible (MS: > 0.15 - 0.25) and very susceptible (VS: > 0.25). Cut-off points for susceptibility ranks were

adjusted according to cluster groupings. The significance among genotype rankings for each disease phase was verified using multiple t-tests. Differences in powdery scab and root gall formation indices among skin types were tested on rank transformed data using the Median Test. The relationship between powdery scab and root gall formation indices was evaluated using Pearson's correlation and regression analysis.

Results

Susceptibility of potato genotypes to powdery scab and root gall formation

Powdery scab and root gall formation developed at all trial locations. Differences in disease expression among genotypes were significant in most of the trials; no differences were observed for powdery scab on genotypes planted at Larimore-11 and root gall formation at Osage-12 (Table 2.3). During the first year of evaluation, mean powdery scab incidence ranged from 0 to 11.4% at Perham-11. In this trial, the highest incidences were detected on the cultivars Ivory Crisp (11.4%) and Shepody (11%) and the advanced clone ND7519-1 (11.2%). In McCanna-11, disease incidence ranged from zero to 73.9%. Advanced clones ND6956b-13 (73.9%) and ND8307C-3 (48.2%) and cultivar Kennebec (50.8%) had the highest mean incidences. At Perham-11, the highest mean severities were observed on the cultivars Red LaSoda (2.8%) and Kennebec (2.7%) and the advanced clone ND7519-1 (3.0%) whereas at McCanna-11, the highest mean severities were observed on clones ND6956b-13 (23.3%), ND8307C-3 (20.7%) and T10-12 (16.6%). The cultivar Red LaSoda (2.9%) and the clone AND00272-1R (11.2%) had the highest mean maximum severities at Perham-11 and McCanna-11, respectively. Genotypes showing no powdery scab symptoms included the clones T10-12, R65A-70, R90213-6 at Perham-11, and RA 82-4, SPA 161, R65A-70 at McCanna-11.

Table 2.3. Powdery scab and root gall formation parameters evaluated for potato genotypes of four skin types (market classes) at five environments in Minnesota and North Dakota.

Disease phase	Variable		Environment				
			Larimore-11	**McCanna-11**	**Perham-11**	**McCanna-12**	**Osage-12**
Powdery scab on tuber	Incidence (%)	P-value*	0.212	<0.001	<0.001	<0.001	0.004
		Mean	1.9	11.5	1.6	9.9	1.2
		Max	13.9	73.9	11.4	52.8	18.1
	Severity (%)	P-value*	0.212	<0.001	<0.001	<0.001	0.004
		Mean	0.4	3.3	0.4	3.6	0.3
		Max	3.5	23.3	3.0	29.0	5.9
	Max severity	P-value*	0.170	<0.001	<0.001	<0.001	0.004
		Mean	0.2	1.4	0.2	3.8	0.2
		Max	1.5	11.2	2.9	40.0	2.4
Root gall formation	Galls · plant^{-1}	P-value*	n/a	n/a	n/a	<0.001	0.320
		Mean	n/a	n/a	n/a	19.3	0.1
		Max	n/a	n/a	n/a	149.6	1.7

* Friedman test on ranked data. n/a: not applicable.

In 2012, mean powdery scab incidence ranged from 0 to 18.1% at Osage-12 and from 0 to 52.8% in McCanna-12. The advanced clones ND6956b-13 (18.1%), ND7519-1 (10.2%), ND8331Cb-2 (5.6%) showed the highest incidences at Osage-12, while at McCanna-12 the highest incidences were observed on the cultivars Kennebec (52.8%), Red LaSoda (52.4%) and Dakota Pearl (50.7%). These cultivars also had the highest mean severities with 29%, 26.2% and 19.4%, respectively. At Osage-12, the highest mean severities were observed on the cultivar Shepody (1.4%) and the advanced clones ND6956b-13 (5.9%) and ND7519-1 (2.4%). The clone ND6956b-13 (2.4%) at Osage-12, and the cultivar Kennebec (40%) at McCanna-12, had the highest mean maximum severities.

At Osage-12 and McCanna-12, no powdery scab symptoms were observed on genotypes AND01804-3Russ, R91129-11, RC89-25, ‘Karu’, SPA161 and RA151-24. However, root galls developed on all genotypes tested at McCanna-12. In this trial, the highest number of galls per plant were observed on the cultivars Lamoka (149.6 galls), Kennebec (115.3 galls) and Red Pontiac (77.8 galls). Conversely, the cultivar Dakota Russet (0.6 galls) and the advanced clones

T10-12 (0.5 galls) and ND049289-1Russ (‘MonDak Gold’ x ‘Dakota Trailblazer’; > 0.1 galls) had the lowest number of galls per plant.

Across field trials, no powdery scab symptoms were observed on tubers of seven potato genotypes tested in at least two environments (Table 2.4). Most asymptomatic genotypes were russet-skinned (e.g. AND97279-5Russ, AND99362B-1Russ, ‘Dakota Russet’ and ND039194AB-1Russ) but also included a yellow-skinned clone (RA 82-4). Although the absence of root galls under conditions of high disease pressure was not observed, the advanced clone ND049289-1Russ had the lowest amount of root galls per plant across trials. This genotype yielded only one root gall in 30 plants tested at two environments.

Table 2.4. Susceptibility of potato cultivars and clones evaluated under field conditions for powdery scab and root gall formation caused by *Spongospora subterranea.*

Advanced clone / Potato cultivar	Skin type	Environments tested	Powdery scab index	Root gall formation index	Advanced clone / Potato cultivar	Skin type	Environments tested	Powdery scab index	Root gall formation index
AND00272-1R	Red	4	0.240	0.018	RC 72-35*	Red	4	0.048	0.036
ATND98459-1RY	Red	4	0.451	0.221	RC 89-25*	Red	2	0.071	0.078
Colorado Rose	Red	2	0.101	0.041	Red LaSoda	Red	4	0.613	0.153
Dakota Jewel	Red	4	0.039	0.051	Red Norland	Red	4	0.137	0.071
Dakota Rose	Red	1	0.019	n/a	Red Pontiac	Red	4	0.171	0.328
Dakota Ruby	Red	4	0.201	0.115	RG 47-3*	Red	2	0.137	0.029
Dark Red Norland	Red	4	0.033	0.033	SPA 161*	Red	4	0.002	0.083
Karu*	Red	4	0.012	0.012	T10-12*	Red	3	0.301	n/a
ND028842b-1RY	Red	2	0.114	n/a	Viking	Red	4	0.236	0.140
ND050167C-3R	Red	2	0.130	n/a	Alpine Russet	Russet	5	0.003	0.070
ND060728-5R	Red	4	0.165	0.061	Alturas	Russet	2	0.004	0.031
ND060733b-4RY	Red	2	0.008	n/a	AND00618-1RussY	Russet	3	0.052	0.016
ND4659-5R	Red	4	0.108	0.060	AND01804-3Russ	Russet	4	0.000	0.151
ND8058-11R	Red	1	0.011	n/a	AND95279-5Russ	Russet	1	0.000	n/a
ND8314-1R	Red	4	0.132	0.144	AND97279-5Russ	Russet	2	0.000	0.040
Patagonia*	Red	2	0.004	n/a	AND99362-1Russ	Russet	1	0.000	n/a
R 90070-8*	Red	4	0.018	0.086	AND99362B-1Russ	Russet	2	0.000	0.007
R 90096-5*	Red	1	0.075	0.010	AOND95292-3Russ	Russet	3	0.003	0.072
R 90160-5*	Red	2	0.533	n/a	Bannock Russet	Russet	5	0.014	0.033
R 91129-11*	Red	4	0.023	0.055	Dakota Russet	Russet	5	0.000	0.002
R90134-6*	Red	2	0.040	n/a	Dakota Trailblazer	Russet	5	0.002	0.014
R90213-6*	Red	2	0.009	n/a	ND039194-1Russ	Russet	1	0.215	n/a
RA 20-6*	Red	4	0.013	0.062	ND039194AB-1Russ	Russet	2	0.000	0.195
RA 89044-45*	Red	4	0.236	0.258	ND049289-1Russ	Russet	4	0.004	0.000
RA 90213-60*	Red	3	0.128	n/a	ND049381C-2Russ	Russet	3	0.102	0.035

Table 2.4. Susceptibility of potato cultivars and clones evaluated under field conditions for powdery scab and root gall formation caused by *Spongospora subterranea* (continued).

Advanced clone / Potato cultivar	Skin type	Environments tested	Powdery scab index	Root gall formation index	Advanced clone / Potato cultivar	Skin type	Environments tested	Powdery scab index	Root gall formation index
ND049423b-1Russ	Russet	4	0.014	0.010	Kennebec	White	4	0.644	0.567
ND049517B-1Russ	Russet	3	0.027	0.024	Lamoka	White	4	0.153	0.438
ND049546b-10Russ	Russet	5	0.017	0.013	MSL-292A	White	2	0.445	n/a
ND050082Cb-2Russ	Russet	4	0.015	0.005	ND060601CAB-2	White	2	0.020	n/a
ND050105C-1Russ	Russet	4	0.036	0.012	ND060715B-15	White	2	0.032	n/a
ND059769Ab-1Russ	Russet	2	0.029	n/a	ND060835C-4	White	4	0.122	0.025
ND060735-3Russ	Russet	3	0.017	0.019	ND060847CB-1	White	2	0.107	n/a
ND060742C-1Russ	Russet	4	0.084	0.035	ND6956b-13	White	4	0.536	0.255
ND060761B-3Russ	Russet	3	0.013	0.046	ND7519-1	White	4	0.421	0.048
ND060766b-4Russ	Russet	4	0.000	0.039	ND7550C-1	White	4	0.083	0.011
ND060770B-5Russ	Russet	3	0.044	0.089	ND8304-2	White	4	0.058	n/a
ND060796AB-1Russ	Russet	4	0.024	0.085	ND8305-1	White	2	0.076	0.202
ND070927-2Russ	Russet	3	0.041	0.036	ND8307C-3	White	4	0.338	0.080
ND6400C-1Russ	Russet	4	0.301	0.169	ND8331Cb-2	White	4	0.251	0.091
ND8068-5Russ	Russet	5	0.024	0.081	ND8331Cb-3	White	4	0.076	0.017
ND8413-7Russ	Russet	5	0.026	0.059	ND8559-20	White	4	0.013	0.084
Ranger Russet	Russet	5	0.012	0.022	Nicolet	White	4	0.136	0.457
Russet Burbank	Russet	5	0.001	0.169	NY-138	White	2	0.004	n/a
Russet Norkotah	Russet	5	0.002	0.008	NY-139	White	2	0.157	n/a
Umatilla Russet	Russet	5	0.009	0.233	R65A-70*	White	2	0.204	n/a
Atlantic	White	2	0.023	0.031	RA 151-24*	White	4	0.006	0.155
CO 95051-7W	White	2	0.010	n/a	Shepody	White	5	0.468	0.289
Dakota Crisp	White	2	0.124	0.059	Snowden	White	4	0.021	0.254
Dakota Pearl	White	2	0.367	0.174	W2717-5	White	2	0.024	n/a
Ivory Crisp	White	4	0.366	0.257	Puren*	Yellow	2	0.040	n/a

Table 2.4. Susceptibility of potato cultivars and clones evaluated under field conditions for powdery scab and root gall formation caused by *Spongospora subterranea* (continued).

Advanced clone / Potato cultivar	Skin type	Environments tested	Powdery scab index	Root gall formation index	Advanced clone / Potato cultivar	Skin type	environments tested	Powdery scab index	Root gall formation index
R 87009-28*	Yellow	3	0.081	n/a	RA 519-50*	Yellow	2	0.072	n/a
R 89045-35*	Yellow	2	0.131	n/a	RA 82-4*	Yellow	2	0.000	n/a
R 91007-5*	Yellow	4	0.101	0.065	RC 06-109*	Yellow	4	0.019	0.112
RA 148-48*	Yellow	2	0.074	n/a	RK 24-48*	Yellow	2	0.121	0.690
RA 16-5*	Yellow	2	0.059	n/a	Yagana*	Yellow	3	0.049	0.168
RA 362-54*	Yellow	2	0.032	n/a	Yukon Gold	Yellow	4	0.059	0.042
RA 517-123*	Yellow	4	0.040	0.041					

n/a: not applicable; *: genotype developed by the potato breeding program of the Chilean National Agricultural Research Institute.

Powdery scab across environments

Across locations, significant differences were observed between mean powdery scab incidence and severity developed in trials conducted in North Dakota and Minnesota ($P < 0.001$) in 2011 and 2012. However, no differences were observed for mean powdery scab incidence ($P = 0.394$) and severity ($P = 0.671$) observed in trials conducted in North Dakota or Minnesota during two consecutive years (Figure 2.1). At McCanna-11 the mean powdery scab incidence and severity on selected genotypes were 30.5% and 9.5% respectively. At McCanna-12, a similar mean disease incidence (26.9%) and severity (10.9%) was observed. The mean disease incidence and severity found at Perham-11 was 3.7% and 1% respectively, whereas at Osage during 2012, powdery scab incidence was 3.7% and disease severity was 0.9%. Overall disease incidence and severity were about seven and 10 times higher in North Dakota than the Minnesota environments.

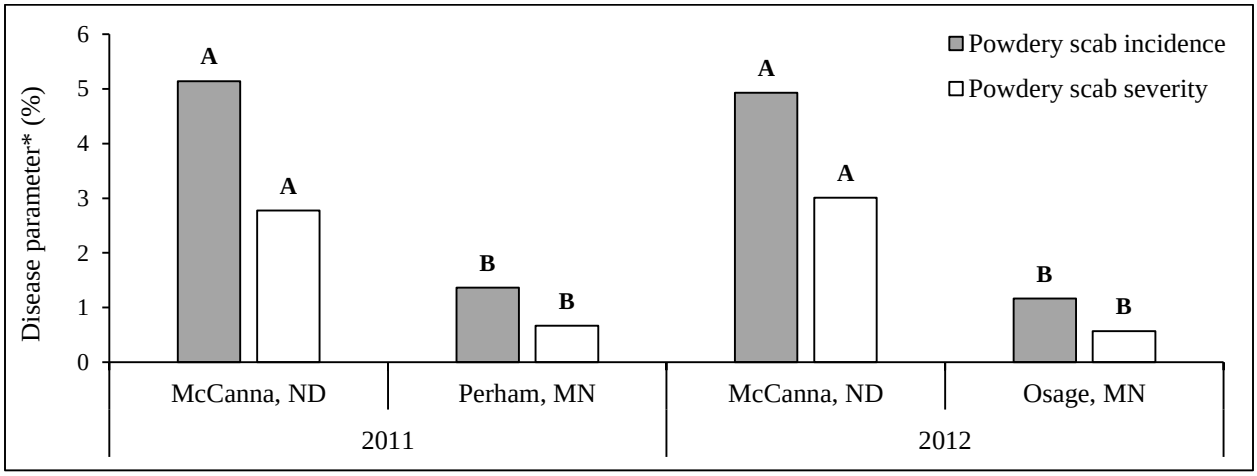

Figure 2.1. Mean powdery scab incidence (%) and severity (%) across evaluated environments in Minnesota and North Dakota during 2011 and 2012. Means of 45 five-plant plots. *: $y^* = \sqrt{y}$. Means across years with different letter are different ($LDS_{\alpha = 0.05}$).

Broad-sense heritability and the relationship of powdery scab incidence and severity

The broad-sense heritability (H) for powdery scab severity was calculated on a set of 50 potato genotypes from four environments in Minnesota and North Dakota (McCanna-11 and 12, Perham-11 and Osage-12); estimates were obtained separately for environments and combined of environments. For trials preformed in ND, H for powdery scab incidence was 0.76, and 0.63 for disease severity. The 95% confidence intervals (C. I.) for powdery scab incidence and severity were 0.57 - 0.86 and 0.35 - 0.79, respectively. In Minnesota environments, estimates of H were 0.45 for disease incidence (C. I.: 0.03 - 0.69) and 0.40 for disease severity (C. I.: 0 - 0.66). Across all environments, the broad-sense heritability for disease incidence was 0.66 (C. I.: 0.47 - 0.78) and 0.55 for disease severity (C. I.: 0.31 - 0.71).

The powdery scab mean severity was strongly correlated to the disease mean incidence ($P < 0.001$) at each tested environment. In North Dakota, Pearson's correlation coefficient (r) was 0.97 during 2011 and 2012, and in Minnesota, r was 0.98 for both Perham in 2011 and Osage during 2012 (Figure 2.3).

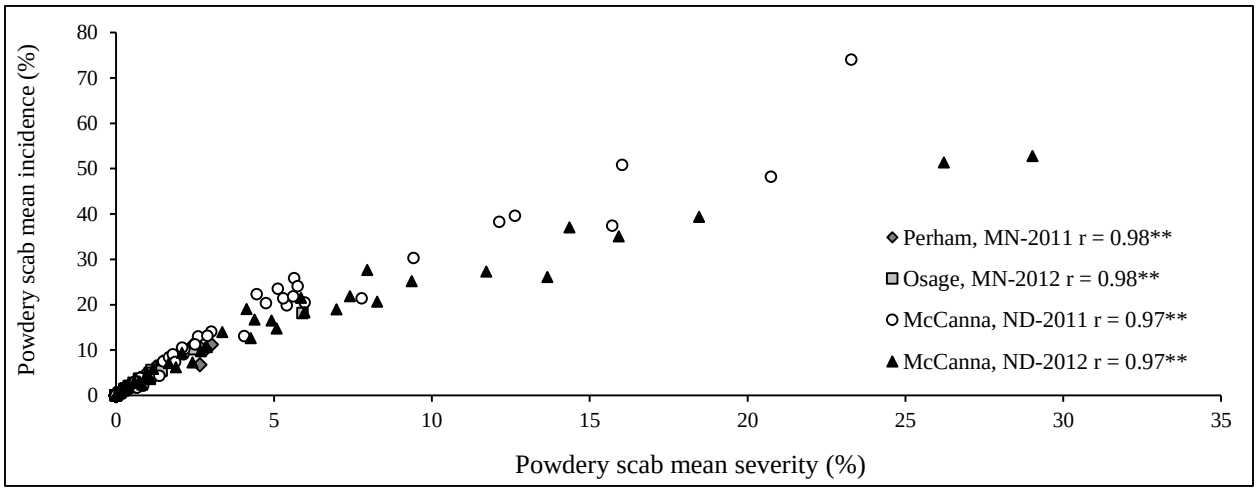

Figure 2.2. Relationship between the mean powdery scab incidence and severity for 50 potato genotypes planted at four environments in Minnesota and North Dakota during 2011 and 2012. Mean of three five-plant plots. **: $P < 0.001$.

Susceptibility ranking and association of disease phases

Powdery scab and root gall formation indices were calculated for 80 potato genotypes tested in at least two trials. Among genotypes, the indices for the tuber phase of the disease ranged from zero to a high of 0.644 for the cultivar 'Kennebec'. At the same time, low disease indices were observed on smooth-skinned cultivars such as 'Dark Red Norland' (0.033) and 'Snowden' (0.021) (Table 2.4). Root gall formation indices ranged from <0.001 for the clones ND049289-1Russ, to 0.69 on RK 24-48. Low indices for the root phase of the disease were also observed on cultivars such as 'Dakota Russet' (0.002), 'Russet Norkotah' (0.008) and 'Karu' (0.012). Overall, root and tuber indices were more widespread on red and white-skinned genotypes compared to russet and yellow-skinned genotypes (Figure 2.3A).

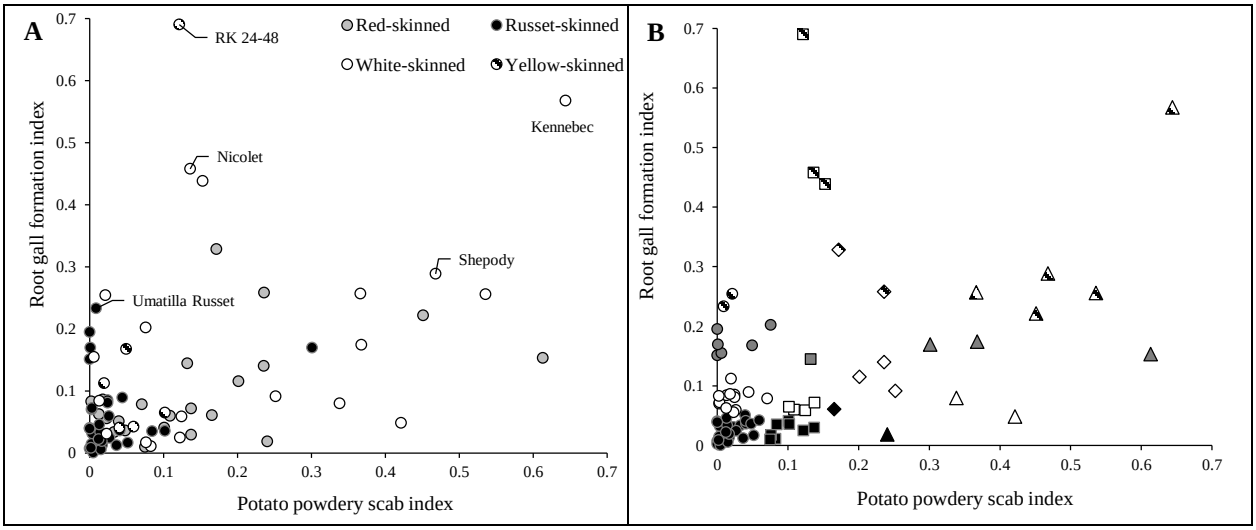

Figure 2.3. Spatial distribution based on mean disease index (Y/Ymax) of 80 potato genotypes evaluated for their susceptibility to powdery scab and root gall formation under field conditions of Minnesota and North Dakota. A: distribution by skin type; B: distribution by susceptibility rank (Powdery scab: very resistant (VR): circles, moderately resistant (MR): squares, moderately susceptible (MS): diamonds, very susceptible (VS): triangles; Root gall formation: VR: black symbols, MR: white symbols, MS: gray symbols, VS: diagonal lines symbols).

Among tuber skin types, most genotypes showing low disease indices (< 0.05) for the root and tuber phase of the disease were russet- and yellow-skinned; however, low disease indices were also observed among some red- and white-skinned genotypes. Overall, the highest powdery scab indices were observed on red- (0.613) and white-skinned genotypes (0.644) (Figure 2.4), whereas the highest root gall formation indices were observed on yellow- (0.69) and white-skinned (0.567) genotypes. Calculated indices for the root and tuber phase of the disease on russet-skinned genotypes differed significantly ($P < 0.05$) from smooth-skinned genotypes (Figure 2.4).

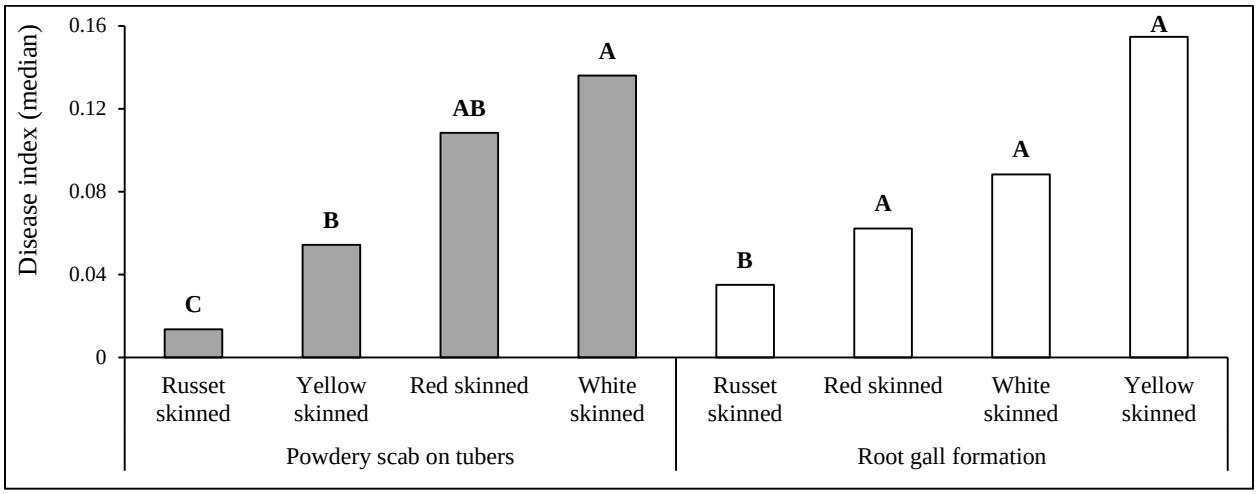

Figure 2.4. Powdery scab and root gall formation index medians of potato genotypes of four skin types (market class) planted in field trials conducted in Minnesota and North Dakota. Treatment medians with different letter are significantly different according to median test ($\alpha = 0.05$).

The degree of correlation between powdery scab on tubers and root gall formation indices of 80 potato genotypes was evaluated. A significant positive correlation (r = 0.47; $P < 0.001$) was observed among genotypes including all evaluated skin types. Similar results were observed for the relationship between disease on roots and tubers of red- (r = 0.51; $P = 0.013$; n = 23) and white-skinned genotypes (r = 0.48; $P = 0.033$; n = 20). In contrast, this association was absent on russet- (r = 0.22; $P = 0.24$; n = 31) and yellow-skinned genotypes (r = 0.64; $P = 0.173$;

n = 6). However, a significant association was observed for russet-skinned genotypes when data was combined with either red- (r = 0.47; *P* = 0.003; n = 55), white- (r = 0.58; *P* < 0.001; n = 51) or yellow-skinned genotypes (r = 0.34; *P* = 0.037; n = 38). There was no significant correlation when data of yellow-skinned genotypes was combined with red- or white-skinned genotypes. Although significant correlation was observed between powdery scab and root gall formation for all genotypes overall, some skin-types and most skin-types combinations, the percentage of variability of powdery scab on tuber explained by the formation of root galls ranged from 12 - 34%.

Potato genotypes were ranked according to their susceptibility to powdery scab and root gall formation using arbitrary cut-offs points (Table 2.5). Genotypes ranked as very susceptible to Spongospora root gall formation comprised red-, white-, and yellow-skinned cultivars and advanced clones (Table 2.5). Root gall formation indices among these genotypes ranged from 0.254 to 0.690. Conversely, among russet-skinned genotypes the highest root gall formation index was observed on cultivar Umatilla Russet (0.233). Genotypes ranked as moderately susceptible to root gall formation included red-, white-, russet-, and yellow-skinned cultivars and advanced clones (Table 2.6).

Table 2.5. Comparison among susceptibility rankings for powdery scab and root gall formation.

	Disease phase									
	Powdery scab on tubers					**Root gall formation**				
Ranking	Mean	C. I.	VR	MR	MS	Mean	C. I.	VR	M R	MS
VR (0-0.05)	0.021	0.015-0.026				0.025	0.020-0.030			
MR (>0.05-0.15)	0.112	0.099-0.125	0.019			0.079	0.070-0.089	<0.001		
MS (>0.15-0.25)	0.210	0.172-0.248	0.019	0.001		0.168	0.155-0.181	<0.001	<0.001	
VS (>0.25)	0.431	0.346-0.517	<0.001	<0.001	<0.001	0.354	0.258-0.450	<0.001	<0.001	<0.001

C. I.: 95% confidence intervals. VR: very resistant, MR: moderately resistant, MS: moderately susceptible, VS: very susceptible.

Table 2.6. Potato genotype rankings according to their susceptibility level to powdery scab on tubers and root gall formation caused by *S. subterranea*.

Tuber phase	Root phase: Very resistant		Moderately resistant	Moderately susceptible	Very susceptible
Very resistant	Alturas	ND049517B-1Russ	Alpine Russet	AND01804-3Russ	Snowden
	AND00618-1RussY	ND049546b-10Russ	AOND95292-3Russ	ND039194AB-1Russ	
	AND97279-5Russ	ND050082Cb-2Russ	ND060770B-5Russ	ND8305-1	
	AND99362B-1Russ	ND050105C-1Russ	ND060796AB-1Russ	RA 151-24	
	Atlantic	ND060735-3Russ	ND8068-5Russ	Russet Burbank	
	Bannock Russet	ND060761B-3Russ	ND8413-7Russ	Umatilla Russet	
	Dakota Jewel	ND060766b-4Russ	ND8559-20	Yagana	
	Dakota Russet	ND070927-2Russ	R 90070-8		
	Dakota Trailblazer	RA 517-123	R 91129-11		
	Dark Red Norland	Ranger Russet	RA 20-6		
	Karu	RC 72-35	RC 06-109		
	ND049289-1Russ	Russet Norkotah	RC 89-25		
	ND049423b-1Russ	Yukon Gold	SPA 161		
Moderately resistant	Colorado Rose	ND7550C-1	Dakota Crisp	ND8314-1R	Lamoka
	ND049381C-2Russ	ND8331Cb-3	ND4659-5R		Nicolet
	ND060742C-1Russ	R 90096-5	R 91007-5		RK 24-48
	ND060835C-4	RG 47-3	Red Norland		
Moderately susceptible	ND060728-5R		Dakota Ruby		RA 89044-45
			ND8331Cb-2		Red Pontiac
			Viking		
Very susceptible	AND00272-1R		ND7519-1	ATND98459-1RY	Ivory Crisp
			ND8307C-3	Dakota Pearl	Kennebec
				ND6400C-1Russ	ND6956b-13
				Red LaSoda	Shepody

Potato genotypes (n = 80) evaluated in at least two environments were ranked into four categories according to their root and tuber phase indices using arbitrary cut-off points: very resistant (0 - 0.05), moderately resistant (> 0.05 - 0.15), moderately susceptible (> 0.15 - 0.25) and very susceptible (> 0.25).

Potato genotypes ranked as very resistant to powdery scab and root gall formation accounted for 32.5% of those evaluated. Within this rank, most of the genotypes were russet-skinned (73.1%), but some white- (‘Atlantic’), red- (‘Dakota Jewel’, ‘Dark Red Norland’, ‘Karu’

and RC 72-35) and yellow-skinned (RA 517-123 and 'Yukon Gold') genotypes were also very resistant to both phases of the disease. On the other hand, only 6.25% of the evaluated genotypes ranked as very susceptible to powdery scab and root gall formation. Potato cultivars Snowden, Russet Burbank and Umatilla Russet were ranked as resistant to tuber powdery scab but were susceptible to root gall formation.

The proportion of genotypes based on skin type with resistance to powdery scab was significantly higher than the proportion of genotypes susceptible to the disease ($P = 0.002$). Similar results were observed for the development of root galls ($P = 0.033$) (Figure 2.5). In contrast, no differences were observed between the proportion of potato cultivars and advanced clones with resistance to powdery scab on tubers. Potato cultivars with resistance to powdery scab were 75%, compared to 80.8%, observed for advanced clones. These proportions differed significantly for root gall formation ($P = 0.046$). Advanced clones with resistance to root gall formation accounted for 80.8% while resistant cultivars were 57.1%.

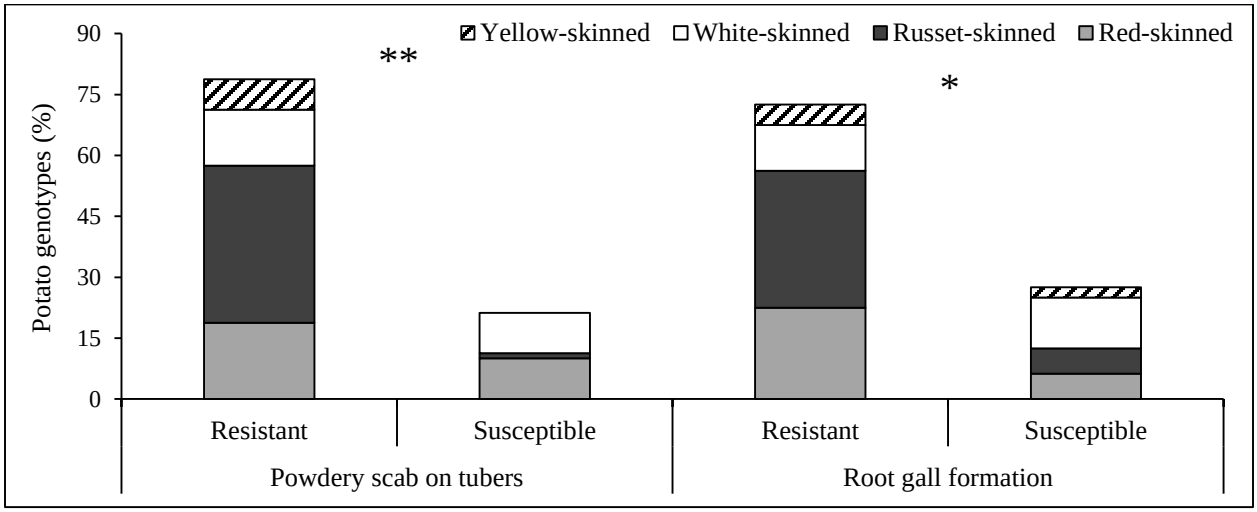

Figure 2.5. Proportion of potato genotypes of four skin types (market class) ranked as resistant or susceptible to powdery scab on tubers and root gall formation. n = 80. *: $P < 0.05$. **: $P < 0.01$.

Discussion

The main detrimental effects of powdery scab is the reduction of the quality and value of the crop. Under conducive conditions the potential effect of the infection of *S. subterranea* on tuber yield might be exacerbated (Merz and Falloon 2009; Nitzan et al. 2010; Shah et al. 2012). *Spongospora subterranea* is also the vector of the *Potato mop-top virus*, a pathogen of growing concern, as reports show an increase in distribution (David et al. 2010; Crosslin 2011; Lambert et al. 2003; Mallik and Gudmestad 2015; Whitworth and Crosslin 2013). Therefore, production intended for fresh, seed and processing markets may be affected by powdery scab. Potato production in Minnesota and North Dakota accounts for 10% of the US market (USDA 2014b). North Dakota is ranked as the second largest producing certified seed in the US, and about 33% of the potato production in North Dakota is represented by smooth-skinned cultivars (USDA 2014b). Results presented in our study focused on the evaluation of symptoms on tubers and roots as an effort to increase knowledge for disease management through host selection.

The degree of susceptibility among genotypes was tested and differences were observed. Although most of the resistant genotypes were russet-skinned, some of the smooth-skinned genotypes (e. g. ‘Atlantic’, ‘Karu’, ‘Snowden’, ‘Yagana’ and ‘Yukon Gold’) showed good levels of resistance against powdery scab. Potato cultivars such as Kennebec and Shepody were very susceptible to powdery scab in agreement with previous reports (Falloon et al. 2003, Christ and Weidner 1988; Kirkham 1986). However, cultivars resistant to powdery scab with susceptibility to root gall formation were also observed (e. g. ‘Umatilla Russet’, ‘Russet Burbank’ and ‘Snowden’). Overall, the degree of association between the two phases of the disease was significant, but the usefulness of the formation of galls as a predictor of powdery scab remains unsuitable, as it only explained about 20% of the total variation. Additionally, results described

here suggests that susceptibility to root gall formation follows a continuum from very susceptible to very resistant.

Disease pressure across environments differed between Minnesota and North Dakota locations. On average, severity of powdery scab at McCanna, ND, was 10.3 times higher than in Minnesota. Powdery scab and root gall formation are favored by cool soil temperatures (12-17°C) and abundant soil moisture (de Boer et al. 1985; van de Graaf et al. 2005, 2007); these conducive conditions appear to be more important around mid-season, coinciding with early tuber formation (Diriwätcher and Parbery 1991; Taylor et al. 1986). During the first year of this research, average air temperatures at Minnesota locations were 17.5°C in Alexandria and 18.4°C in Fergus Falls, and in North Dakota, 17.3°C and 16.7°C at Grand Forks and Inkster, respectively. During 2012, average air temperatures in Minnesota were 17.4°C at Alexandria and 17.8°C at Fergus Falls, whereas in North Dakota, temperatures were 16.4°C in Grand Forks and 16.2°C at Inkster. In field experiments in which moisture was kept at field capacity, differences in disease incidence and severity among cultivars planted on two dates in June were observed (Christ and Weidner 1988). Soil temperature was higher at late planting (mid-June) resulting in lower disease expression.

Despite environmental conditions, powdery scab and root gall formation developed in all trials at all locations. However, the severity of powdery scab varied substantially across experiments on selected susceptible cultivars such as ‘Kennebec’ (0.6 - 29%). On resistant cultivars, severity on tubers ranged from 0 to 4.1%, and on russet-skinned cultivars from 0 - 0.3%. In potato, resistance to powdery scab is based on multiple genes which have been suggested to act as buffers against environmental stresses (Falloon et al. 2003; Merz et al. 2012). Under European conditions, no evidence of a genotype x environment interaction due to

pathogen genetic diversity was observed (Merz et al. 2012). At the same time, the variability of European *S. subterranea* isolates was found lower compared to South American populations included in a global study of the diversity of the pathogen (Gau et al. 2013). In that research, the lowest variability among countries was observed in the North American populations. Furthermore, within the US, differences based on genetic variability of the pathogen were reported among, but not within, locations (Qu and Christ 2006). Although in their research the overall degree of diversity was relatively low, *S. subterranea* isolates from California, Idaho, North Dakota and Washington grouped differently than those from Colorado, Maine and Pennsylvania (Qu and Christ 2006).

Under conditions of low pathogen diversity, host resistance represents a viable alternative for disease management, which at the same time may be benefited by the presence of multiple genes of resistance on the host (Falloon et al. 2003; Gau et al. 2013). However, the genetic mechanism controlling *S. subterranea* infection appears to be different for expression of the disease in roots and on tubers (Hernandez Maldonado et al. 2013). Estimates of broad-sense heritability (H) suggest that resistance to root gall formation as a trait can be easily deployed into genotypes under development (Nitzan et al. 2010). Our estimations of H at locations under high disease pressure (i.e. North Dakota) suggest a moderately high genetic component for powdery scab incidence and severity, as values for H were calculated as 0.76 and 0.63, respectively. However, this aspect of disease resistance requires additional research to further substantiate this H value.

Spongospora subterranea is able to infect and cause disease on the cultivated potato (*S. tuberosum* ssp. *tuberosum*, spp. *andigena* and *S. phureja*); however, the amount of research focused on identifying sources of resistance among *Solanum* species is scarce. Resistance to root

infection was reported on *S. acaule* among other tested species including *S. sucrense* and *S. brevides* (Mäkäräinen et al. 1994). Other sources of resistance include clones of the species *S. phureja* (Lahuf et al. 2014; Lees et al. 2000), which has the peculiarity of developing only root galls, suggesting specific resistance to tuber infection (Gau et al. 2013). Among potato selections showing resistance to root gall formation in the Columbia Basin (WA), two common characteristic were observed: resistant genotypes derived from the introgression of *S. bulbocastanum*, and at the same time, the cultivar Summit Russet appearing more than once in their background (Nitzan et al. 2008). In our study, some of the potato genotypes ranking as very resistant to both phases of the disease were found to have the cultivar Summit Russet appearing in their pedigree (ND049289-1Russ and 'Dakota Trailblazer'). The cultivar Dakota Russet which also ranked as very resistant is an example of the introgression of *S. raphanifolium* along with other resistant cultivars such as Atlantic. Other examples of the introgression of wild species into cultivars with resistance to powdery scab and root gall formation are 'Yukon Gold' which has *S. phureja* and *S. acaule* in its background, and 'Atlantic' with *S. chacoense* (Plaisted and Hoopes 1989). The management of powdery scab on tubers and root gall formation through cultivar selection is recommended. Further research will be required in order assist breeding and selection through molecular approaches that enable resistance detection of both phases of the disease across potato market classes.

Literature Cited

Baldwin, S. J., Genet, R. A., Butler, R. C., and Jacobs, J. M. E. 2008. A greenhouse assay for powdery scab (*Spongospora subterranea* f. sp. *subterranea*) resistance in potato. Potato Research 51:163-173.

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

Brierley, J. L., Stewart, J. A., and Lees, A. K. 2009. Quantifying potato pathogen DNA in soil. Applied Soil Ecology 41:234-238.

Cavalier-Smith, T. 1998. A revised six-kingdom system of life. Biological Reviews of the Cambridge Philosophical Society 73:203–266.

Christ, B. J., and Weidner, R. J. 1988. Incidence and severity of powdery scab of potatoes in Pennsylvania. American Potato Journal 65:584-588.

Crosslin, J. M. 2011. First report of *Potato mop-top virus* on potatoes in Washington State. Plant Disease 95:1483.

Davey, T., Carnegie, S. F., Saddler, G. S., and Mitchell, W. J. 2014. The importance of the infected seed tuber and soil inoculum in transmitting *Potato mop-top virus* to potato plants. Plant Pathology 63:88-97.

David, N., Mallik, I., Crosslin, J. M., and Gudmestad, N. C. 2010. First report of *Potato mop-top virus* on potatoes in North Dakota. Plant Disease 94:1506.

de Boer, R. F. 1991. Evaluation of potato cultivars in the greenhouse and field for resistance to powdery scab. Australian Journal of Experimental Agriculture 31:699-703.

de Boer, R. F., Taylor, P. A., Flett, S. P., and Merriman, P. R. 1985. Effects of soil temperature, moisture and timing of irrigation on powdery scab of potatoes. Pages 197-198 in: Ecology and management of soilborne plant diseases. C. A. Parker, K. J. Moore, P. T. W. Wong, A. D. Rovira and J. F. Kollmorgen, eds. American Phytopatological Society, St. Paul, MN, US.

Diriwächter, G., and Parbery, D. G.1991. Infection of potato by *Spongospora subterranea*. Mycological Research 95:762-764.

Domfeh, O., Bittara, F. G., and Gudmestad, N. C. 2015. Sensitivity of potato to *Potato mop-top virus*-induced tuber necrosis. Plant Disease 99:788-796.

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

Falloon, R. E., Viljanen-Rollinson, S. L. H., Coles, G. D., and Poff, J. D. 1995. Disease severity keys for powdery and downy mildews of pea, and powdery scab of potato. New Zealand Journal of Crop and Horticultural Science 23:31-37.

Gau, R. D., Merz, U., Falloon, R. E., and Brunner, P. C. 2013. Global genetics and invasion history of the potato powdery scab pathogen, *Spongospora subterranea* f. sp. *subterranea*. PLoS ONE 8(6): e67944. doi:10.1371/journal.pone.0067944

Harrison, J. G., Searle, R. J., and Williams, N. A. 1997. Powdery scab disease of potato - a review. Plant Pathology 46:1-25.

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

Holland, J. B., Nyquist, W. E., and Cervantes-Martinez, C. T. 2003. Estimating and interpreting heritability for plant breeding: an update. Plant Breeding Reviews 22:9-112.

Houser, A. J., and Davidson, R. 2010. Development of a greenhouse assay to evaluate potato germplasm for susceptibility to powdery scab. American Journal of Potato Research 87:285-289.

Hughes, I. K. 1980. Powdery scab (*Spongospora subterranea*) of potatoes in Queensland: occurrence, cultivar susceptibility, time of infection, effect of soil pH, chemical control and temperature relations. Australian Journal of experimental Agriculture and Animal Husbandry 20:625-632.

Mallik, I., and Gudmestad, N. C. 205. First Report of *Potato mop-top virus* causing potato tuber necrosis in Colorado and New Mexico. Plant Disease 99:164.

Johnson, D. A., and Cummings, T. F. 2015. Effect of powdery scab root galls on yield of potato. Plant Disease 99:1396-1403.

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

Kirkham, R. P. 1986. Screening for resistance to powdery scab disease of potatoes. Australian Journal of Experimental Agriculture 26:245-247.

Knapp, S. J., Stroup, W. W., and Ross, W. M. 1985. Exact confidence intervals for heritability on a progeny mean basis. Crop Science 25:192-194.

Lahuf, A. A., Lees, A. K., Torrance, L., and Roberts, A. G. 2014. Identification sources of resistance to potato mop-top virus and its vector *Spongospora subterranea*. Pages 277-283 in: The Dundee Conference. Crop Protection in Northern Britain 2014. Dundee, UK.

Lambert, D. H., Levy, L., Mavrodieva, V. A. Johnson, S. B., Babcock, M. J., and Vayda, M. E. 2003. First report of *Potato mop-top virus* on Potato from the United States. Plant Disease 87: 872.

Lees, A. K. 2000. Resistance to powdery scab in potato. Pages 35-38 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees, eds. Aberdeen, Scotland.

Lister, R., Falloon, R., Curtin, D., and Butler, R. 2004. *Spongospora subterranea* reduces host (*Solanum tuberosum*) growth. Pages 135-136 in: Proceedings of the 3rd Australasian soilborne diseases symposium. K. M. Ophel Keller and B. H. Hall, eds. South Australian Research and Development Institute, Adelaide.

Manzer, F. E., Akaley, R. V., and Donald, M. 1964. Resistance to powdery scab in *Solanum tuberosum*. American Potato Journal 41:374-376.

Merz, U., and Falloon, R. E. 2009. Review: Powdery scab of potato - Increased knowledge of pathogen biology and disease epidemiology for effective disease management. Potato Research 52:17-37.

Merz, U., Lees, A. K., Sullivan, L., Schwärzel, R., Hebeisen, T., Kirk, H. G., Bouchek-Mechiche, K., and Hofferbert, H. R. 2012. Powdery scab resistance in *Solanum tuberosum*: an assessment of cultivar x environment effect. Plant Pathology 61:29-36.

Nakayama, T., Horita, M., and Shimanuki, T. 2007. *Spongospora subterranea* soil contamination and its relationship to severity of powdery scab on potatoes. Journal of General Plant Pathology 73:229-234.

Neuhauser, S., Bulman, S., and Kirchmair, M. 2010. Plasmodiophorids: the challenge to understand soil-borne, obligate biotrophs with a multiphasic life cycle. Pages 51-78 in: Molecular Identification of Fungi. Y. Gherbawy and K. Voight, eds. Springer-Verlag, Berlin-Heidelberg.

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

Nitzan, N., Haynes, K. G., Miller, J. S., Johnson, D. A., Cummings, T. F., Batchelor, D. L., Olsen, C., and Brown, C. R. 2010. Genetic stability in potato germplasm for resistance to root galling caused by the pathogen *Spongospora subterranea*. American Journal of Potato Research 87:497-501.

Plaisted, R. L., and Hoopes, R. W. 1989. The past record and future prospects for the use of exotic potato germplasm. American Potato Journal 66:603-627.

Qu, X. S., and Christ, B. J. 2006. Single cystosorus isolate production and restriction fragment length polymorphism characterization of the obligate biotroph *Spongospora subterranea* f. sp. *subterranea*. Phytopathology 98:1157-1163.

Shah, F. A., Falloon, R. E., Butler, R. C., and Lister, R. A. 2012. Low amounts of *Spongospora subterranea* sporosorus inoculum cause severe powdery scab, root galling and reduced water use in potato (*Solanum tuberosum*). Australasian Plant Pathology 41:219-228.

Shah, F. A., Keenan, R. C., Butler, R. C., and Marshall, J. W. 2005. The response of powdery scab severity and potato tuber yield to increasing levels of *Spongospora subterranea* inoculum. Page 271 in: Proceedings of the 15th Biennial Australasian Plant Pathology Society Conference Handbook 'Innovations for sustainable plant health'. Victoria, Australia.

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

Taylor, P., Flett, S. P., de Boer, R. F., and Marshall, D. 1986. Effects of irrigation regimes on powdery scab disease and yield of potatoes. Australian Journal of Experimental Agriculture 26:745-750.

Tegg, R. S., Corkrey, R., Herdina, H., McKay, A. C., Crump, N. S., de Boer, R. F., Wiechel, T. J., and Wilson, C. R. 2015. Modeling pathogen DNA content and visual disease assessment in seed tubers to inform disease in potato progeny root, stolon, and tubers. Plant Disease 99:50-57.

Torres, H., Pacheco, M. A. and French, E. R. 1995. Resistance of potato to powdery scab (*Spongospora subterranea*) under Andean field conditions. American Potato Journal 72:355-363.

Tuncer, G. 2002. The effect of irrigation and nitrogen on powdery scab and yield of potatoes. Potato Research 45:153-161.

USDA. 2014a. United States standards for grades of potatoes. Online: www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5091274.

USDA. 2014b. Vegetables and pulses yearbook data. Online: usda.mannlib.cornell.edu/usda/ers/VEGANDPULSESYEARBOOK/2014/89011.pdf.

van de Graaf, P., Lees, A. K., and Duncan, J. M. 2000. Epidemiology and control of *Spongospora subterranea* f. sp. *subterranea*, with special reference to zoospore release and bait plant infection. Pages 57-60 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees, eds. Aberdeen, Scotland.

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

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

van de Haar, J. 2000. The powdery scab situation in the Netherlands. Pages: 21-22 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees, eds. Aberdeen, Scotland.

Wale, S. 2000. Summary of the session on national potato production and the powdery scab situation. Pages 3-10 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees, eds. Aberdeen, Scotland.

Wastie, R. L. 1991. Resistance to powdery scab of seedling progenies of *Solanum tuberosum*. Potato Research 34:249-252.

Wastie, R. L., Caligari, P. D. S., and Wale, S. J. 1988. Assessing the resistance of potatoes to powdery scab (*Spongospora subterranea* (Wallr.) Lagerh.). Potato Research 31:167-171.

Waterer, D. 2010. Influence of growth regulators on skin colour and scab diseases of red-skinned potatoes. Canadian Journal of Plant Science 90:745-753.

Whitworth, J. L., and Crosslin, J. M. 2013. Detection of *Potato mop-top virus* (Furovirus) on potato in southeast Idaho. Plant Disease 97:149.

CHAPTER III. INFLUENCE OF *SPONGOSPORA SUBTERRANEA* INOCULUM LEVELS ON THE DEVELOPMENT OF POWDERY SCAB AND ROOT GALL FORMATION AND THE POTENTIAL FOR REDUCTION OF SOIL INOCULUM USING CHLOROPICRIN

Abstract

The effect of chloropicrin fumigation on the soil populations of *Spongospora subterranea* and the resulting development of powdery scab on tubers, root gall formation, and tuber yield was investigated in seven field trials conducted in Minnesota and North Dakota. Sixteen potato cultivars, with different levels of susceptibility to disease on roots and tubers, were planted in plots treated with chloropicrin at rates ranging from 29.2 to 201.8 kg a.i. · ha^{-1}. The amount of *S. subterranea* DNA in soil samples collected at pre- and post- fumigation was measured using quantitative PCR. Bioassays were conducted to further assess the effect of chloropicrin fumigation rates on root colonization by *S. subterranea* in two potato cultivars differing in susceptibility to powdery scab and root gall formation. Chloropicrin applied at rates between 70.1 to 201.8 kg a.i. · ha^{-1} significantly decreased *S. subterranea* initial inoculum in the field but, increased the amount of disease observed on roots and tubers of susceptible cultivars. Field results were confirmed in bioassays, in which the observed amount of *S. subterranea* DNA increased with the fumigant rate and remained similar between cultivars. Chloropicrin fumigation significantly increased tuber yield in cultivars such as Shepody and Umatilla Russet; the increment in yield was associated with an increase of root galls ($r = 0.30$; $P < 0.03$). This study demonstrates the unsuitability of chloropicrin fumigation for the control of powdery scab

and root gall formation, and suggests that factors other than weather conditions and host susceptibility contribute to the development of powdery scab and root gall formation epidemics.

Introduction

The obligate biotroph *Spongospora subterranea* (Wallr.) Lagerh. (Cercozoa, Phytomyxea) is the causal agent of powdery scab and root gall formation on potato (*Solanum tuberosum* L.) (Harrison et al. 1997; Neuhauser et al. 2010). The pathogen is found causing disease in most temperate potato-producing areas of the world and also in hot weather climates where production is conducted at high altitudes or under irrigation (Harrison et al. 1997; Merz and Falloon 2009; Wale 2000). Furthermore, *S. subterranea* is the vector of the *Potato mop-top virus* (Jones and Harrison 1969), which causes necrotic lesions rendering tubers undesirable for consumption or processing (Davey et al. 2014). The primary detrimental effect of the pathogen is due to tuber symptoms (powdery scab) which can greatly reduce the quality and marketability of the harvested crop (Braithwaite et al. 1994). Tubers intended for seed and fresh consumption are more likely to be subject to rejection and quality downgrade; for an example, losses due to unmarketable tubers have ranged from 50% in Australia (Hughes 1980) to 100% in Venezuela (García et al. 2004). Although the tuber phase of the disease has been more extensively studied, a number of reports have suggested the deleterious effect of the *S. subterranea* root infection on host growth and tuber yield (Gilchrist et al. 2011; Shah et al. 2004; 2012). Additionally, in a series of pot experiments in which *S. subterranea* was added to the plant growth medium, evidence of root disruption was found, as water and nutrient uptake was reduced in the susceptible host (Falloon et al. 2005; Hernandez Maldonado et al. 2013; Lister et al. 2004). Tuber yield losses ranging from 5 - 12 $t \cdot ha^{-1}$ were associated with root infection by *S.*

subterranea in potato fields planted with russet-skinned cultivars in the Columbia Basin (WA) (Brown et al. 2007; Nitzan et al. 2008). However, in a four-year field study in which the cultivars Shepody and Umatilla Russet were included, no evidence of a negative effect on tuber yield was observed due to the development of symptoms on roots and tubers (Johnson and Cummings 2015). Moreover, no tuber yield reduction was observed when plants were grown in naturally infested and pathogen-free soils (Johnson and Cummings 2015).

Root galls and lesions on tubers are filled with masses of resting spore aggregates (sporosori) contributing to an increase in inoculum infestation levels and the spread of the pathogen to other locations (Brierley et al. 2009; Merz 2008). In a series of shadehouse experiments, a curvilinear relationship was observed between the severity of powdery scab and the inoculum amount which ranged from zero to 10^5 sporosori · ml soil^{-1} (Shah et al. 2012). However, this relationship was not clear for inoculum concentrations greater than 1000 sporosori · ml soil^{-1}. Inoculated pot experiments, in which the pathogen concentration ranged from 5 - 50 sporosori · ml soil^{-1}, resulted in no differences in observed level of disease on tubers and roots (van de Graaf et al. 2005; 2007). Similar results were reported in field studies in which a weak or non-existent relationship between the initial inoculum amount and the resulting degree of powdery scab were noted (Merz et al. 2012; Nakayama et al. 2007; Shah et al. 2014). In contrast, a significant positive association between the initial inoculum level (0 – 14400 sporosori g · soil^{-1}) and powdery scab incidence was observed for 17 potato fields in the US (Qu et al. 2006). Additionally, when soil samples of 27 potato fields in Japan were assessed for the pathogen, a significant association was observed between the level of soil infection potential (i.e. infecting zoospores) and powdery scab severity (Nakayama et al. 2007). These results suggest that conditions other than the initial inoculum level influence the resulting degree of disease

development (Burnett 1991); however, a larger amount of initial inoculum might increase the chances for the pathogen to infect and disease to occur in the field (Brierley et al. 2013; Tegg et al. 2015).

The control of Spongospora root gall formation and tuber scab is difficult. A number of chemical compounds applied to the soil and seed have shown some efficacy in disease reduction (Braithwaite et al. 1994; Falloon et al. 1996; Hughes 1980); however, no chemical treatment eliminates the disease (Falloon 2008; Merz and Falloon 2009). The chemical compounds fluazinam and flusulfamide are among the few chemicals registered elsewhere for powdery scab management (Falloon 2008). In the United States, the use of fluazinam applied in-furrow to the soil have shown disease reduction (40%) and increased of the number of healthy tubers (27%); nevertheless, in some instances these results were not reproducible (Davidson and Houser 2009; Zink et al. 2004).

In 2013, the potato crop in the US constituted the most valuable vegetable grown, with a production value around $4.3 billion (USDA 2014). In order to satisfy the demand for quality, the potato industry commonly relies on soil fumigation for the management of soil-borne pathogens affecting the crop (Hirnyck and Downey 2007; Gudmestad et al. 2007; Pasche et al. 2014). In major potato producing states such as Idaho, Oregon and Washington, the use of soil fumigation ranges from 50 to 90% of the planted area (Hirnyck and Downey 2007). However, although a number of chemical compounds are available as result of the methyl bromide phase-out (Ajwa et al. 2002), metam sodium remains as the most widely fumigant used (Cox 2006). Metam sodium is regularly applied pre-plant for the management of major disease problems such as Verticillium wilt (*Verticillium dahliae* Kleb.) and black dot (*Colletotichum coccodes* Wallr.; Gudmestad et al. 2007; Taylor et al. 2005). However, its use for the management of powdery

scab is mixed, with some studies demonstrating positive results (Nachmias and Krikun 1988; Tsror 2009; 2014), as well as limited efficacy, or no disease control (Christ 2001; de Boer and Theodore 1997).

Recent studies have shown promising results with the application of chloropicrin for the control of powdery scab (Tsror et al. 2009; Tsror 2014), in which disease reduction was in most cases accompanied by an increase in tuber yield. Nevertheless, the efficacy of soil fumigation can be affected by several factors, including the pathogen soil inoculum level, the motility of the chemical within the soil and soil properties (e.g. texture and structure) (de Boer and Theodore 1997; Lembright 1990); therefore, the evaluation of chloropicrin soil fumigation under cropping condition of the North Dakota and Minnesota is necessary. The objective of this study was to investigate the effect of chloropicrin soil fumigation on the soil populations of *S. subterranea* and to assess their effect on the resulting development of disease on tubers and roots and total tuber yield.

Materials and Methods

Soil sampling

In order to assess the effect of chloropicrin fumigation rates on the soil populations of *S. subterranea*, soil samples were collected from pre-established plots pre- (October - November) and post-fumigation (June – July) for experiments conducted during 2012 and 2013. In experiments conducted in 2011, only post-fumigation soil samples were collected. In 2012 and 2013, post-fumigation sampling was performed 50 days after planting (DAP) and 9 DAP during 2011. In the 2012 and 2013 experiments, a total of 48 sampling points (3 sites per whole plot) were flagged pre-fumigation, to form a 12 x 4 or a 24 x 2 (sampling points x row) grid, in which

each row was parallel to the fumigation line. During 2011, soil samples were collected at arbitrary sampling points (>10 sites). For all conducted experiments, sampling points were located between the two middle rows of each main plot, from which a compound sample (approx. 0.5 l) was obtained by bulking ten soil subsamples obtained at arbitrarily points within one meter diameter area around each marked site. Sampling was performed using a soil auger at two depths (0 – 0.1 m and 0.1 – 0.2 m). Each compound sample was air-dried at 25ºC for 7 days in the dark and kept at 4ºC until use. The aggregation pattern of *S. subterranea* at pre-planting was investigated using Lloyd's Index of Patchiness (LIP) and calculated as $LIP = \frac{(\bar{X}+S^2 / \bar{X}-1)}{\bar{X}}$ (Lloyd 1967). LIP values >1 indicate aggregation, <1 uniform distribution and = 1 indicates random distribution.

Bioassay

During 2013, two bioassays were conducted using chloropicrin fumigated soil at four rates (0, 70.1, 140.1 and 201.8 kg a.i. · ha^{-1}). Large soil samples (10 kg) were collected from two fields located at Perham and Park Rapids, MN. A single sampling site was positioned at the center of each main plot replicate (fumigation rate). In total, four soil samples (0 – 0.2 m depth) were collected for each fumigation treatment and bulked. Large debris clumps and crop residuals were removed using a sieve (0.25 cm mesh); then, 300 g of soil were added to black plastic cups (350 ml capacity) with drain holes in the bottom. The cups were placed into a growth chamber in a randomized complete block design (4 blocks) and maintained at 15ºC in dark for 7 days. After the incubation period, one sprouted disease-free mini-tuber was planted in each cup. The cultivars Dakota Trailblazer and Shepody were selected for the experiment based on their susceptibility reaction to powdery scab and root gall formation caused by *S. subterranea*

observed in the field during the course of this research. Plants were grown at 17/15ºC (day/night) with 16 hours of cool white fluorescent lighting. The soil water content was inspected on a daily basis and high moisture conditions were kept by watering at periodic intervals. At each sampling date, one plant per block of each treatment combination (2 potato cultivars x 2 locations x 4 fumigant rates x 6 sampling dates) was carefully removed. The plant root system was washed free of soil and inspected for the presence of root galls. Root samples were freeze-dried, ground to a fine powder and kept at 4ºC until use. The experiment was performed twice and the observed root galls were expressed as number of galls · g^{-1} dry weight.

Pathogen molecular quantification from soil and host tissue

DNA extraction from soil and root tissue

Soil samples were sieved to a fine grain (<5 mm) and DNA was extracted from 0.25 g (in triplicate) using the PowerSoil® DNA isolation kit according to manufacturer's instruction with few modifications. Sample homogenization and cell lysis was achieved using a FastPrep® instrument (MP Biomedicals, Santa Ana, California) at speed 5.5 for 40s and an additional 15 min on a horizontal vortex platform at medium speed. An additional 2 min of incubation was added to step 20. The extracted DNA was eluted in 100 µl of solution C6 (10 mM Tris, pH 8.0). Root samples were washed free of soil particles using a soft brush under tap water. The plant root system was freeze-dried and ground to a fine powder from which 10 mg were used for DNA extractions (Hernandez Maldonado et al. 2013). The PowerPlant Pro® extraction kit (MoBio, Carlsbad, CA) was used following manufacturer's instruction with few modifications. Sample homogenization and cell lysis was achieved using a FastPrep® instrument (MP Biomedicals) at speed 5.0 for 30s, and an additional 10 min of horizontal vortex agitation. The DNA extracts

were incubated for 2 min in the Spin Filter prior to elution in 100 µl of solution PD7 (10 mM Tris, pH 8.0).

Artificially infested soils

Spongospora subterranea sporosori were obtained from heavily infected tubers of the cv. Shepody (Merz 1989). A sample of the sporosori retained between the 45 - 150 µm mesh sieves was used to estimate the mean number of resting spores per sporosori and the average sporosori volume (Falloon et al. 2011). The sample size (± 5%) was determined as $= \frac{(t^2)(S^2)}{(d^2)(\bar{x}^2)}$, with $t_{n-1;\frac{\alpha}{2}}$ (n = sampled population; α = 0.05), and where *d* is half of the total length of the confidence interval of the mean ($\bar{x}$) expressed as a proportion (Neher and Campbell 1997).

A total of three soil samples [A: organic matter (OM) 4.2%, clay 40%, pH 7.9; B: OM 4.2%, clay 30.9%, pH 7.6; C: OM 2.5%, clay 34.9%, pH 7.3] previously tested as pathogen-free (below), were inoculated with a sporosori suspension to a final concentration of 10^6 resting spores · g^{-1} soil (d. w.). DNA was extracted from the infested soil samples and tenfold serial dilutions were prepared in order to obtain concentrations equivalents to 10^5, 10^4, 10^3, 10^2 and 10^1 resting spores · g^{-1} soil.

Real-time PCR and pathogen quantification from soil and host tissue

The *S. subterranea* specific primer/probe set SsTQF1 (5´- CCG GCA GACCCA AAA CC -3´), SsTQR1 (5´- CGG GCG TCACCCTTC A-3´) and TaqMan® probe SsTQP1 (5´FAM - CAGACA ATC GCA CCC AGG TTC TCA TG-3´) (van de Graaf et al. 2003) were used in the course of this research. The real-time quantitative PCR (qPCR) reactions were performed using a Stratagene Mx3005P® qPCR System (Agilent Technologies, Santa Clara, CA) in which each

reaction consisted of 2 µl of sample DNA (fivefold diluted for soil samples) and 23 µl of reaction mix [1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM each primer, 0.1 µM of the probe and one unit GoTaq® DNA polymerase (Promega, Madison, WI)]. The qPCR thermal conditions used consisted of an initial cycle at 98°C for two min, followed by 40 cycles of 98°C for 10s and 58°C for 45s.

The absolute quantification of the pathogen was performed using a standard curve containing different amounts of a plasmid carrying the *S. subterranea* ITS gene. The target DNA (63 bp) was amplified using the SsTQF1/SsTQR1 primer set, and cloned using the pGEM®-T Easy Vector System (Promega) according manufacturer's instructions. The correct insert was verified by PCR and DNA sequencing, followed by plasmid purification using the Plasmid Mini Kit (QIAGEN) and spectrophotometric quantification. A series of tenfold dilutions of the plasmid-DNA, with concentrations ranging from 1×10^{-2} to 1×10^{-8} ng plasmid-DNA μl^{-1}, was analyzed in triplicates. The resulting Ct values were plotted against the amount of plasmid-DNA to construct a standard curve. This procedure was also used to create a standard curve between the amounts of plasmid-DNA and *S. subterranea* resting spores $\cdot$ g^{-1} soil, using the artificially infested soils A, B and C and taking in account the initial soil sample weight and the amount of soil DNA in each qPCR reaction. The efficiency of each assay was calculated as $E = [(10^{-1/slope}) - 1] \times 100$; across qPCRs E was kept between 90 - 110%. The regression coefficient (r^2) of each assay was kept at $r^2 > 0.99$. Among assays, the standard curves were compared using the heterogeneity of regression coefficients test ($\alpha = 0.05$) (Burns et al. 2005; Bustin et al. 2009). Samples were tested in three individual assays in which triplicate reactions were included for each sample and standard concentration; additionally, a non-template reaction was included in each plate. Results are expressed as femtograms (fg) of *S. subterranea* DNA $\cdot$ μl^{-1} (root samples)

or fg *S. subterranea* DNA · g^{-1} soil. Prior to analysis, data on fg *S. subterranea* DNA · μl^{-1} were log-transformed; data on *S. subterranea* DNA · g^{-1} soil were square-transformed.

Field experiments

A total of seven large-scale field experiments were conducted from 2011 to 2013 in five irrigated commercial fields in North Dakota and Minnesota (Table 3.1). Fields were selected based on history of previous powdery scab occurrence. The trials were aimed at assessing the effect of chloropicrin soil fumigation rate on the development of powdery scab, root gall formation, and tuber-yield using a randomized split-plot design with four replicates with exception of the trial conducted at Larimore, ND, during 2011, which was a randomized strip-plot design with four replicates. The whole plot (or horizontal factor) was represented by the fumigant rate which ranged from zero (non-treated) to 201.8 kg a.i. · ha^{-1} (Table 3.1).

Chloropicrin soil fumigations [Pic Plus Fumigant ™ = Chloropicrin (CCl_3NO_2) – 85.5% active ingredient, Triest AG Group Inc., Greenville, NC] was applied in-row to a depth of 0.3 m using a standard, commercial shank injector system. Alternatively, broadcast injections were performed using a commercial coulter rig. Soil fumigations, with exception of the trial conducted at McCanna, ND, during 2011, were carried out during the fall (Table 3.1) preceding each cropping season (May to September). Spring soil fumigation at McCanna (2011) was conducted on May 26, followed by late planting on June 20. During fumigation procedures, soil temperatures to a depth of 0.1 m ranged between 3.2 – 10.6°C. A buffer area (approx. 15 m) was left between whole plots in order to reach the fumigation rate required for each soil treatment.

Table 3.1 Overview of potato cultivars and chloropicrin rates evaluated in field trials conducted in Minnesota and North Dakota between 2011 and 2013.

Year	Location	Soil texture / pH	Fumigation Date	Planting / harvest date	In-row fumigant rate (kg a.i. · ha^{-1})	Split-plot	Cultivars
2011	Perham (Huebsch), MN	Sand / 7.4	October, 2010	May 10 / September 15	0 (non-treated) 74.7* 149.4*	3 rows of 9 m long each	Alpine Russet, Bannock Russet, Dakota Trailblazer, Ivory Crisp, Kennebec, Lamoka, Nicolet, Ranger Russet, Red LaSoda, Red Norland, Red Pontiac, Russet Burbank, Russet Norkotah, Shepody, Umatilla Russet, Yukon Gold
	Larimore, ND	Sandy loam / 6.7	October, 2010	May 17 / September 27	0 74.7* 149.4*	3 rows of 9 m long each	Alpine Russet, Bannock Russet, Dakota Trailblazer, Ranger Russet, Russet Burbank, Russet Norkotah, Shepody, Umatilla Russet
	McCanna (Elm Grove), ND	Sandy loam / 5.6	May 26, 2011	June 20 / October 3	0 29.2* 58.3* 112.1 154.1 196.1	3 rows of 4.5 m long each	Alpine Russet, Bannock Russet, Dakota Trailblazer, Ivory Crisp, Kennebec, Ranger Russet, Red LaSoda, Red Norland, Red Pontiac, Russet Burbank, Russet Norkotah, Shepody, Umatilla Russet, Yukon Gold
2012	Osage, MN	Sandy loam / 6.1	October 8, 2011	May 2 / September 3	0 112.1 154.1 196.1	4 rows of 4.5 m long each	Dakota Trailblazer, Ivory Crisp, Kennebec, Ranger Russet, Red LaSoda, Red Pontiac, Russet Burbank, Shepody, Umatilla Russet, Yukon Gold
	McCanna (Elm Grove), MN	Sandy loam /5.6	October 11, 2011	April 25 / September 6			
2013	Perham (Zimatic), MN	Loamy sandy /5.8	October 24, 2012	May 24 / September 25	0 70.1 140.1 201.8	6 row of 9 m long each	Red Norland, Russet Burbank, Shepody
	Park Rapids, MN	Sand / 7.2	November 1, 2012	May 27 / September 27			

* broadcast rather than applied in-row, in-row equivalent presented.

A set of potato cultivars ranging in susceptibility to powdery scab and root gall formation (Houser and Davidson 2010) were randomly assigned to each split-plot (or vertical factors). Each sub-plots was separated by a 0.9 m buffer area. Whole tubers or tuber sections (60 – 100 g) were obtained from certified seed-tubers. In the field, each seed-tuber was planted 0.3 m apart and 0.1 m depth; the distances between rows was 0.9 m. Soil texture among experimental field ranged from sand to sandy loam and loamy sand. Crop management and pest and diseases control was conducted by the growers according to recommendations developed for the area.

Field sampling, disease and tuber-yield assessment

Powdery scab

At harvest, all tubers over 40 g were collected and stored for approximately six weeks at 12°C. Prior to disease evaluations, tubers were washed and dried overnight at room temperature. A total of 100 tubers were evaluated per plot; when this amount was not available, all tubers were evaluated. Severity was estimated on each side of the tuber using an increasing percentage graphic scale, and then averaged (Falloon et al. 1995). The mean disease severity was calculated as $\left[\sum \frac{(nN_n)}{5N_0}\right] * 100$, where *n* is the disease index (0 = no disease, 1 = one pustule to 2.0%, 2 = 2.1 - 5.0%, 3 = 5.1 - 10.0%, 4 = 10.1 – 25%, 5 = > 25%) (Houser and Davidson 2010; Nakayama et al. 2007), N_n is the number of tubers with disease symptoms at level ‘‘n’’ and N_0 is the total number of tubers evaluated. A powdery scab index was obtained from multiplying the mean disease severity by the proportion of symptomatic tubers in each plot (Houser and Davidson 2010). Data were log-transformed prior analysis.

Root gall formation

Three plants were carefully removed from the soil at 60, 75 and 90 DAP. Plants were arbitrarily sampled from the outermost row to be selected for tuber yield. Large soil particles were removed by gently shaking the plant root system. Galls on roots were counted using a magnifying glass (1.75X) and the number of galls per plant averaged for each plot. The area under disease progress curve (AUDPC) for each plot was calculated as $AUDPC = \sum_{j=1}^{n_j-1} \left(\frac{Y_j+Y_{j+1}}{2}\right)\left(t_{j+1} - t_j\right)$ (Madden et al. 2007). Data were log-transformed prior analysis.

Tuber yield

Total tuber yield was obtained at harvest (approximately 115-120 DAP) by weighing the harvested tubers obtained in the middle row of each plot. Tuber yield from each row was then converted to tonnes (t) per hectare.

Statistical analysis

Statistical analysis was carried out using SAS 9.3 (SAS Institute, Cary, NC). The Bartlett's test was used to assess the homogeneity of variances and the Wilk-Shapiro test was used to assess the normality of the residuals. Data on amount of *S. subterranea* DNA in soil at pre- and post-fumigation was analyzed with ANCOVA. Data from bioassays were analyzed with ANOVA in which plants presenting no galls during weekly evaluations were excluded from the analysis to avoid underestimation of the variation (Hernandez Maldonado et al. 2013). Data from field experiments conducted during 2011 were analyzed individually with ANOVA; treatments from 2012 and 2013 were combined before analysis. Treatment means were compared using a protected LSD and contrasts ($\alpha = 5\%$). Regression analysis was conducted between the amounts of *S. subterranea* plasmid-DNA and number of resting spores in artificially infested soils. Spearman's rank correlation was used to assess the degree of linear association among evaluated parameters.

Results

Spongospora subterranea quantification in soil and spatial distribution

Regression analysis of the quantity of *S. subterranea* plasmid-DNA and the number of spiked resting spores resulted in a linear function (Figure 3.1A). The detection limit of the qPCR

assay varied among artificially infested soils and pathogen levels equivalent to 500 resting spores · g^{-1} soil (equivalent to 0.25 sporosori · g^{-1} soil) were detected in three of six spiked soils. Pathogen levels equivalent to 5000 resting spores · g^{-1} soil (2.5 sporosori · g^{-1} soil) were detected for all spiked soils. The amount of *S. subterranea* in non-treated soil samples at pre-fumigation was assessed among location trials, for which significant differences were observed (P <0.001; Figure 3.1B). The highest amount of the pathogen were detected at Larimore, ND, and Perham (Huebsch), MN, where the average levels of pathogen in the soil were 2.0 x 10^5 and 1.8 x 10^5 resting spores · g^{-1} soil, respectively (in the same order 97.7 and 90.6 sporosori · g^{-1} soil). At pre-fumigation, the average amount of pathogen across sampled fields was 1.5 x 10^5 resting spores · g^{-1} (74.3 sporosori · g^{-1} soil), while the highest level observed was 1.1 x 10^6 resting spores · g^{-1} (563.6 sporosori · g^{-1} soil) at Park Rapids, MN, during 2013. *Spongospora subterranea* was detected in all collected soil samples; however, pathogen populations at Park Rapid, MN, had an aggregated distribution as LIP was calculated as 1.03. Pathogen distribution at McCanna, ND, and Osage and Perham, MN, was uniform with LIP values of 0.67, 0.57 and 0.32, respectively.

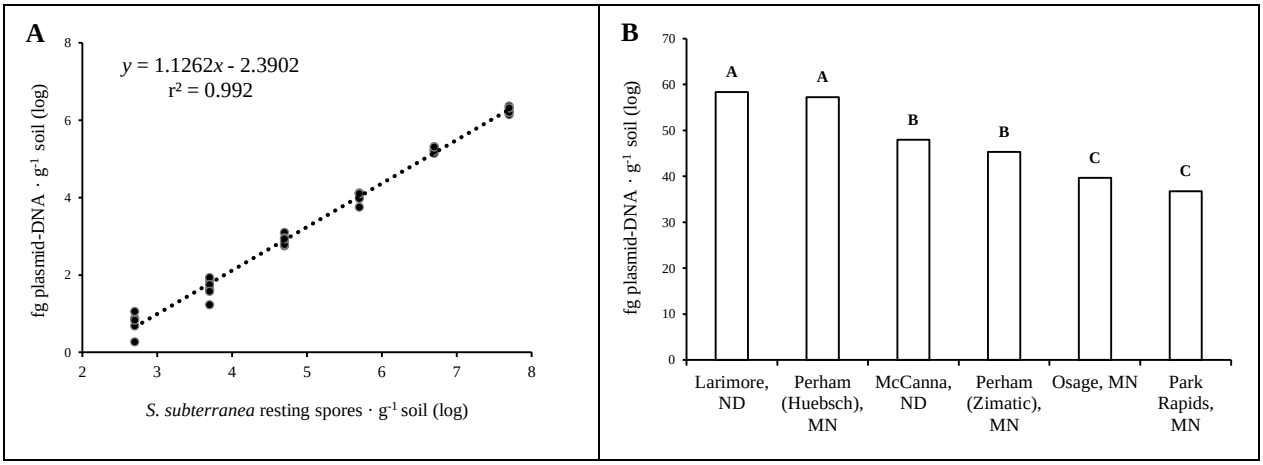

Figure 3.1. A: linear regression between femtogram (fg) of *S. subterranea* plasmid-DNA and number of resting spores in artificially infested soils (n = 6); B: Mean fg of *S. subterranea* plasmid-DNA in pre-fumigation soil samples from six trial locations in Minnesota and North Dakota.

Effect of chloropicrin fumigation rates on *S. subterranea* soil levels

Results from *S. subterranea* quantification in fumigated soil were statistically analyzed to determined differences between the amount of pathogen detected in the 0 – 10 and 10 – 20 cm strata, and then combined. Analysis of variance was then conducted to assess the effect of fumigant rates on pathogen populations at pre- and post-fumigation.

During 2011, soil samples were collected at post-fumigation only. At Larimore, ND chloropicrin fumigation significantly decreased *S. subterranea* populations compared to the non-treated control ($P = 0.014$). At this location, significant reduction of the pathogen level in soil was achieved at the high fumigant rate evaluated (149.4 kg a.i. · ha^{-1}; 18.6%). At Perham (Huebsch), MN, soil fumigation with chloropicrin resulted in a reduction of pathogen level in soil compared to the non-treated control ($P = 0.042$); however, at this location soil pathogen levels were reduced at the low fumigant rate of 74.7 kg a.i. · ha^{-1} (10.8%; Figure 3.2A).

In 2012, a significant reduction of *S. subterranea* at post-fumigation was observed at McCanna, ND ($P < 0.001$), and Osage, MN ($P < 0.001$; Figure 3.2B). In McCanna, ND, soil populations of the pathogen were significantly reduced when chloropicrin was applied at rates of 154.1 and 196.1 kg a.i. · ha^{-1}. At these two rates, inoculum was reduced in 31.5% and 32.6% respectively compared to the non-treated control (Figure 3.2B). In addition, the analysis of main effects resulted in a significant reduction of *S. subterranea* soil populations at post-fumigation compared to the amount of pathogen DNA detected at pre-fumigation ($P < 0.001$; 21.2%). At Osage, MN, a significant reduction of *S. subterranea* soil populations was achieved with all chloropicrin rates (Figure 3.2B). The percentage of inoculum reduction in comparison to the non-treated control was 11.5% at 112.1 kg a.i. · ha^{-1}, 12.9% at 154.1 kg a.i. · ha^{-1} and 14.2% at 196.1 kg a.i. · ha^{-1} (Figure 3.2B). At post-fumigation, the analysis of main effects revealed a

significant reduction of *S. subterranea* DNA compared to the pathogen soil populations at pre-fumigation ($P < 0.001$; 13.5%).

In 2013, a significant reduction of *S. subterranea* soil populations was found at Park Rapids ($P < 0.001$) and Perham, MN ($P < 0.001$; Figure 3.2C). At these two locations, all evaluated chloropicrin rates (70.1, 140.1 and 208.8 kg a.i. · ha^{-1}) resulted in a significant reduction of pathogen populations when compared to the non-treated control. At Park Rapids, reduction of *S. subterranea* soil populations ranged from 17.7% at 70.1 kg a.i. · ha^{-1} to 34.7% at 208.8 kg a.i. · ha^{-1} (33.7% at 140.1 kg a.i. · ha^{-1}; Figure 3.2C). Additionally, the analysis of main effects resulted in significant differences for the amount of *S. subterranea* DNA ($P < 0.001$) detected at post-fumigation compared to the amount of pathogen DNA at pre-fumigation, such reduction was of 38.6%. At Perham, MN, the percentage of inoculum reduction in comparison to the non-treated control was 15.9% at 70.1 kg a.i. · ha^{-1}, 30.9% at 140.1 kg a.i. · ha^{-1} and 24.9% at 208.8 kg a.i. · ha^{-1} (Figure 3.2CB). Similar to previous results, the analysis of main effects revealed a significant reduction of the amount of pathogen DNA at post-fumigation compared to the amount of pathogen DNA observed at pre-fumigation ($P < 0.001$; 28.4%).

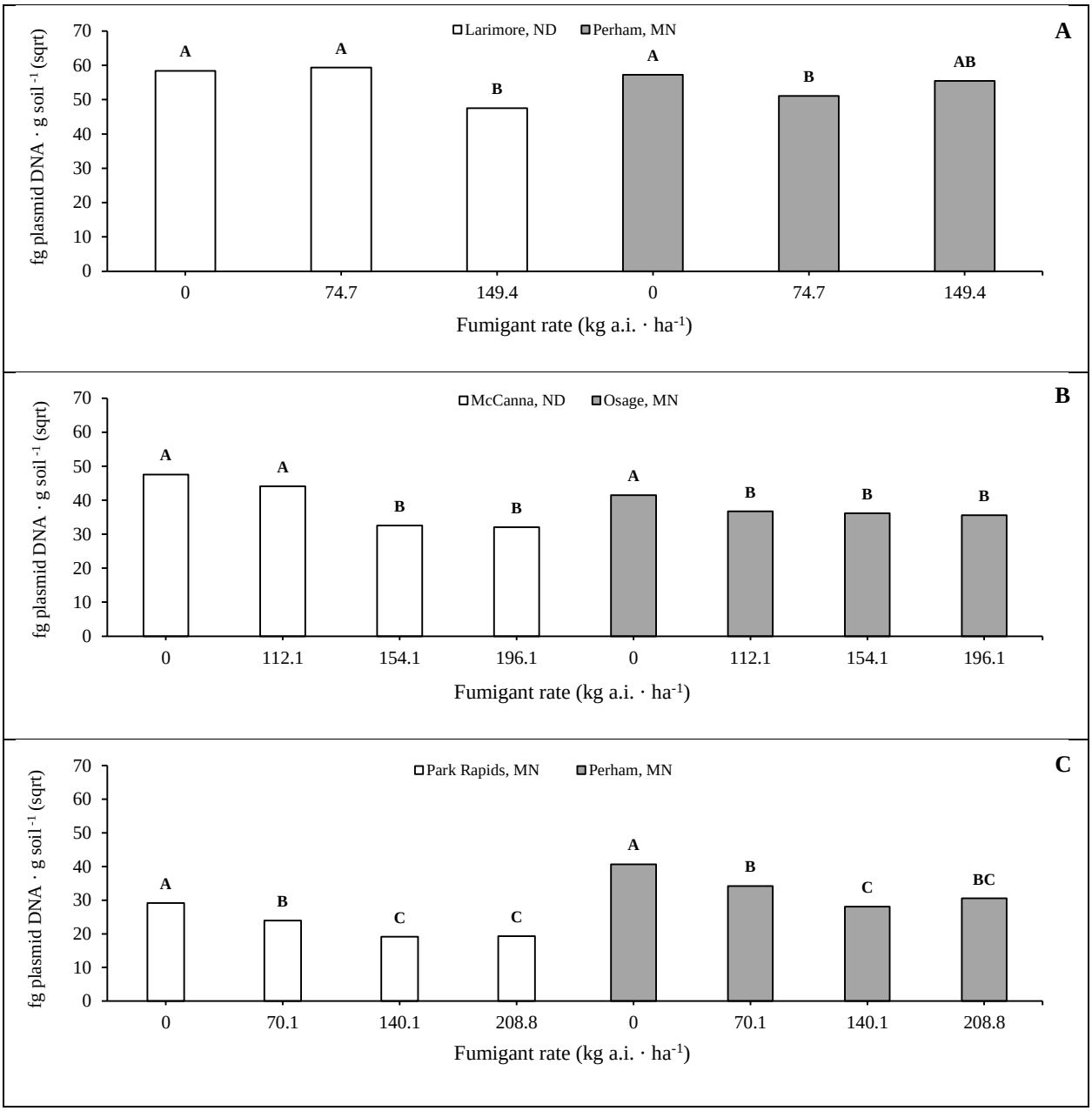

Figure 3.2. Effect of chloropicrin rates on *Spongospora subterranea* soil populations at post-fumigation in A: Larimore, ND and Perham, MN in 2011; B: Osage, MN and McCanna, ND in 2012, and C: Perham and Park Rapids, MN in 2013.

Bioassays

Quantification of *S. subterranea* in roots

The quantification of *S. subterranea* DNA in roots revealed significant variations in the amount of pathogen across sampling dates ($P_{\text{main effect}} < 0.001$). The highest amount of pathogen was detected at Perham, MN, three weeks after planting, while at Park Rapids, MN, maximum population levels were recorded five weeks after planting. Conversely, the amount of *S. subterranea* DNA detected in 'Dakota Trailblazer' and 'Shepody' did not differ significantly throughout sampling dates at each location (Figure 3.3A). Chloropicrin fumigation increased the amount of pathogen DNA from 1.4 to 4.3 times, compared to the non-treated control ($P_{\text{main effect}} < 0.001$). However, for each fumigant rate tested, similar amounts of pathogen DNA were observed for each cultivar ($P = 0.430$; Figure 3.3B).

Root gall formation

Root galls on cv. Shepody and Dakota Trailblazer were observed five and six weeks after planting, respectively. In addition, a significant location effect was observed, resulting in remarked differences in the number of galls observed throughout the experiment ($P = 0.015$). The highest amount of galls $\cdot$ g^{-1} were recorded in 'Shepody' at Perham, MN, six weeks after planting (Figure 3.3C). A significant chloropicrin rate x potato cultivar interaction was observed ($P < 0.001$). Chloropicrin fumigation increased the number of galls observed in the susceptible cultivar Shepody compared to the non-control treatment; however, similar numbers of galls were observed at rates of 140.1 and 201.8 kg a.i.$\cdot$ ha^{-1}. Conversely, the number of galls observed in the resistant cultivar Dakota Trailblazer were similar across all chloropicrin rates evaluated compared to the non-control treatment (Figure 3.3D).

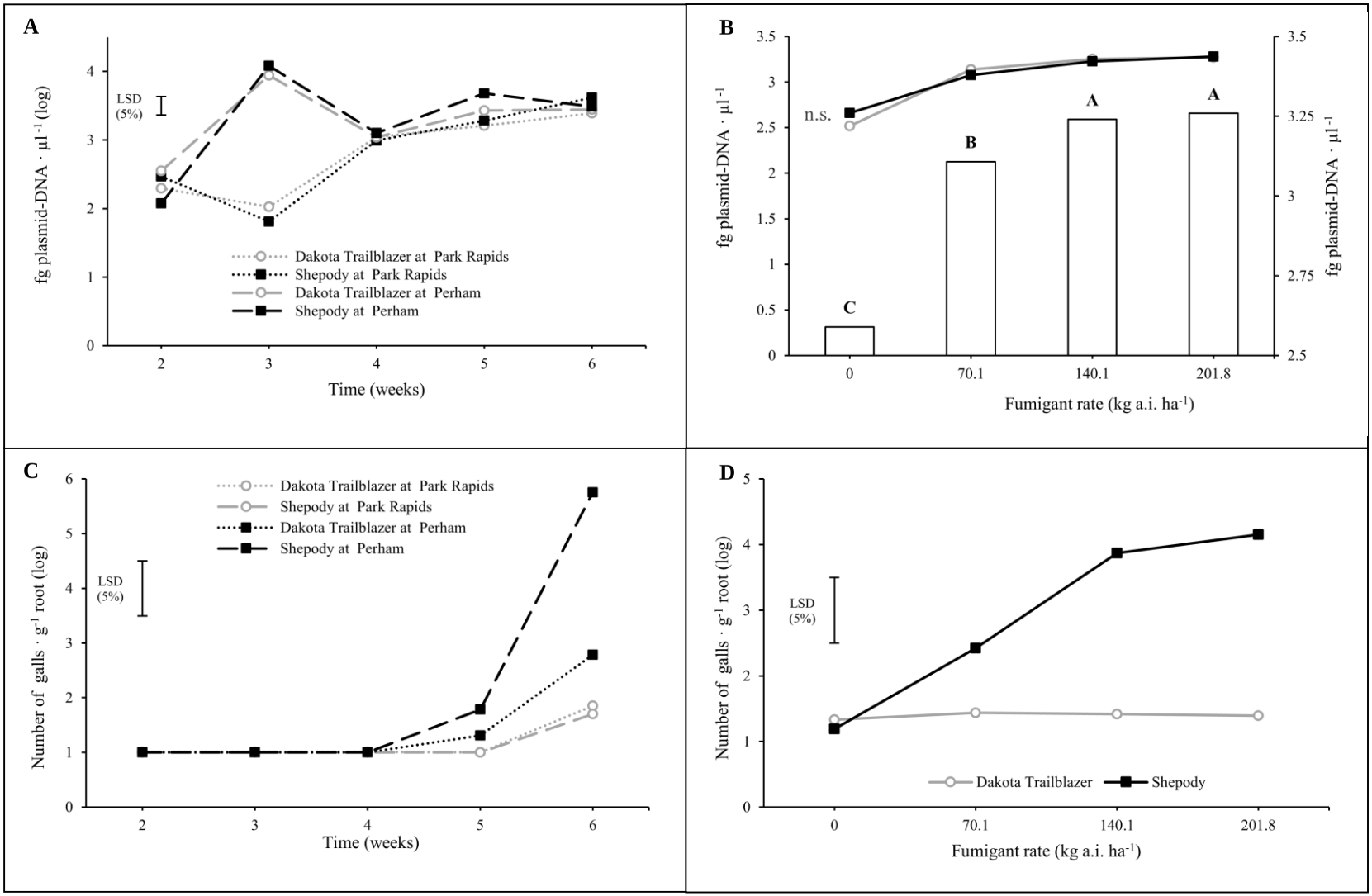

Figure 3.3. Mean amounts (fg plasmid-DNA · μ^{-1}) of *S. subterranea* DNA (A) and number of galls per gram in roots (C) of cvs. Shepody and Dakota Trailblazer planted in chloropicrin treated soil from Perham and Park Rapid, MN and mean amounts of *S. subterranea* DNA (B) and number of galls per gram in roots (D) of cvs. Shepody and Dakota Trailblazer across chloropicrin fumigation rates evaluated in 2013.

The number of galls per plant observed across chloropicrin rates for 'Shepody' were significantly associated with the amounts of pathogen DNA detected in roots collected from week 2 to week 5 ($r = 0.426 – 0.633$; $P = 0.001$ - 0.016). The highest degree of association was observed five weeks after inoculation ($r = 0.636$; $P < 0.001$). Although root gall formation was observed in the cultivar Dakota Trailblazer, no significant association was observed between the number of galls and the amount of pathogen DNA ($P > 0.05$).

Interaction of chloropicrin fumigation rates and potato cultivar on the formation of root galls

In 2011, no significant chloropicrin rate x potato cultivar interaction was observed at Perham (Huebsch), MN ($P = 0.518$). At this trial location, significant differences in AUDPC were found among cultivars ($P < 0.001$), but no effect of fumigant rate was observed ($P = 0.415$). Cultivars such as Bannock Russet, Russet Norkotah and Ranger Russet had the lowest AUDPC, whereas 'Ivory Crisp', 'Russet Burbank' and 'Nicolet' had the highest. Although no significant differences were detected among evaluated fumigant rates, mean AUDPC at the 74.7 and 149.4 kg a.i. $\cdot$ ha^{-1} rates were higher than for the non-treated control (Figure 3.4A). Conversely, a significant chloropicrin rate x potato cultivar interaction was observed at Larimore, ND ($P < 0.001$), where similar fumigant rates were applied (Figure 3.4B). The AUDPC in cultivars such as Shepody and Russet Burbank in comparison to the non-treated control increased at chloropicrin rates of 74.7 and 149.4 kg a.i. $\cdot$ ha^{-1}; however, the amount of galls on roots did not differ significantly between these two fumigant rates (Figure 3.4B).

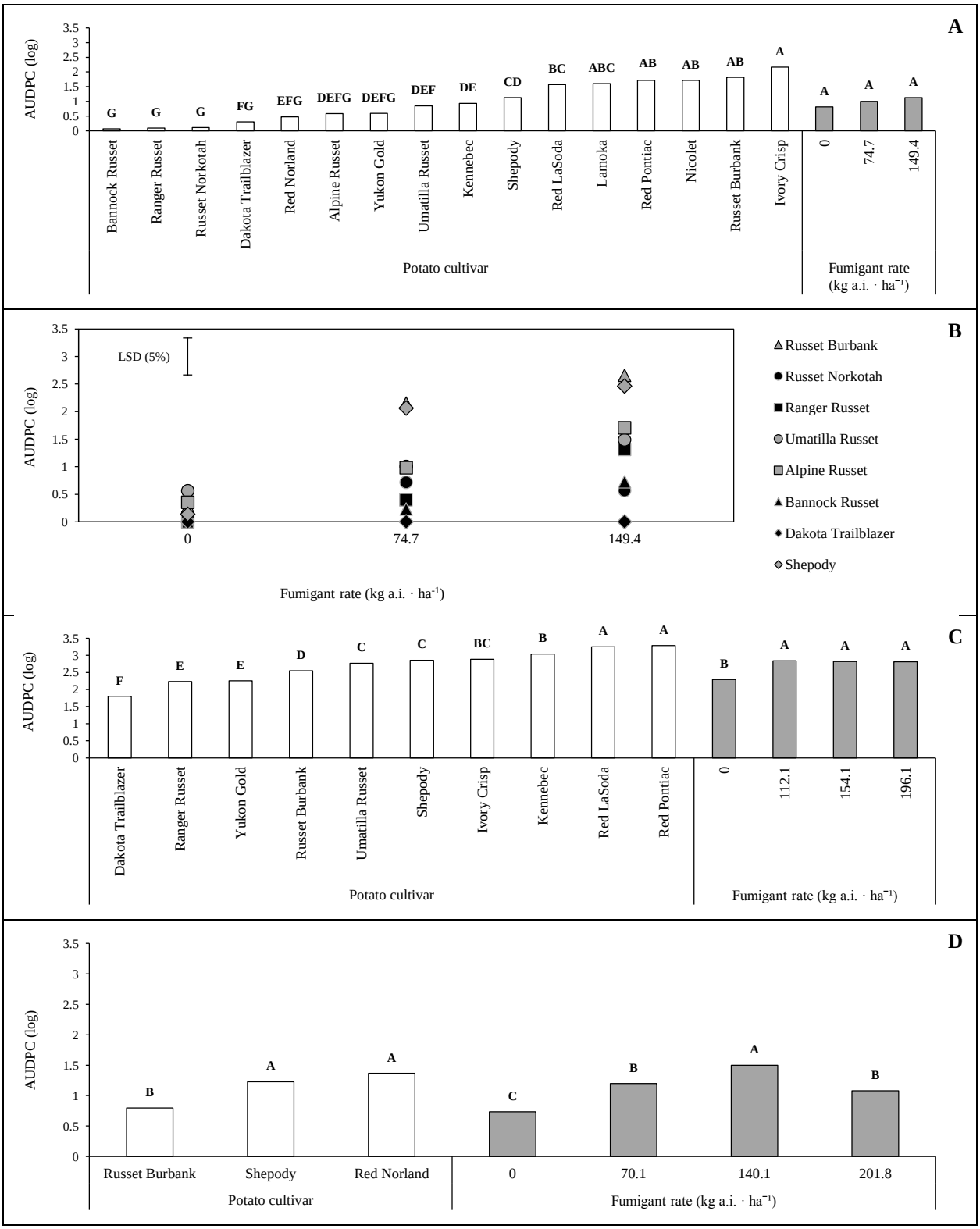

Figure 3.4. Effect of chloropicrin fumigation on root gall AUDPC in potato cultivars evaluated at Perham, MN (A) and Larimore, ND (B) in 2011; Osage, MN and McCanna, ND (C) in 2012, and Perham and Park Rapids, MN in 2013.

The AUDPC for cultivars such as Ranger Russert and Umatilla Russet was significantly higher at the highest fumigant rate tested in this trial (149.4 kg a.i. · ha^{-1}), compared to the non-treated control. Additionally, no galls where observed on roots of cv. Dakota Trailblazer across evaluated fumigant rates.

In 2012, no significant chloropicrin rate x potato cultivar interaction was found (P = 0.613); however, significant differences in AUDPC values were detected among potato cultivars ($P < 0.001$) and among fumigant rates ($P < 0.001$). The cv. Dakota Trailblazer had the lowest AUDPC, followed by cv. Ranger Russet and Yukon Gold; whereas, Red LaSoda and Red Pontiac developed the highest number of root galls during the course of the experiment (Figure 3.4C). During 2012, AUDPC increased with chloropicrin fumigation compared to the non-treated control; however, the number of root galls did not differ significantly among fumigant rates. The overall increase in AUDPC when chloropicrin was applied to the soil ranged from 104 to 179%.

In 2013, no significant chloropicrin rate x potato cultivar interaction was observed (P = 0.981). Unlike powdery scab on tubers, numerous root galls were found on each evaluated cultivar; however, AUDPC in ‘Shepody’ (65.8) and ‘Red Norland’ (81.8) were significantly higher than ‘Russet Burbank’ (14.9; $P < 0.001$). Similar to previous years, soil fumigation with chloropicrin increased AUDPC values compared to the non-treated control ($P < 0.001$); however, an increase in chloropicrin rate did significantly affect root gall formation and resultant AUDPC values (Figure 3.4D), despite an overall increase of AUDPC among chloropicrin rates ranging from 99 to 356%.

Interaction of chloropicrin fumigation rates and potato cultivar on the expression of powdery scab

In 2011, no significant fumigant rate x potato cultivar interactions were observed for the experiments conducted at McCanna, ND ($P = 0.269$) and Perham, MN ($P = 0.343$). The analysis of the main effects at McCanna, ND, revealed significant differences among potato cultivars ($P < 0.001$), but no effect of the chloropicrin fumigation ($P = 0.109$; Figure 3.5A). Potato cultivars such as Bannock Russet, Yukon Gold and Red Noland had the lowest powdery scab indices, whereas Shepody, Red LaSoda and Ivory Crisp had the highest. Although no effect of the chloropicrin fumigation was observed, the mean powdery scab index at fumigant rates of 74.7 and 149.4 kg a.i. · ha^{-1} resulted in higher disease indices compared to the non-treated control. At Perham, MN, a similar scenario was observed. Significant differences in powdery scab indices were found among evaluated cultivars ($P < 0.001$; Figure 3.5B). At this trial location, cultivars such as Bannock Russet, Yukon Gold and Red Noland had the lowest disease indices, whereas Shepody, Red LaSoda and Ivory Crisp had the highest. Additionally, chloropicrin soil fumigation had no effect ($P = 0.240$) on the expression of powdery scab compared to the non-treated control; however, higher disease indices were observed at rates of 29.2, 58.3 and 112.1 kg a.i. · ha^{-1}.

In 2012, a significant chloropicrin rate x potato cultivar interaction was observed ($P = 0.035$). The powdery scab index in cultivars such as Shepody, Red Pontiac and Kennebec increased significantly at each fumigant rates evaluated compared to the non-treated control; whereas, disease index on cv. Red LaSoda increased significantly at chloropicrin rates of 154.1 and 196.1 kg a.i. · ha^{-1} compared to the non-treated control (Figure 3.5C). Conversely, for cultivars such as Dakota Trailblazer, Ranger Russet, Russet Burbank and Yukon Gold no

increase in powdery scab indices were observed across fumigant rates, compared to non-treated control (Figure 3.5C). During this year, the overall increase in powdery scab indices in regard to the non-treated control ranged from 43 to 90%.

In 2013, no significant chloropicrin rate x potato cultivar was observed ($P = 0.263$); however, the analysis of main effects resulted in significant differences for powdery scab indices among potato cultivars ($P < 0.001$) and fumigant rates ($P = 0.011$; Figure 3.5D). Among evaluated cultivars, the highest disease index was found in 'Shepody' (703.35) whereas in 'Russet Burbank' few symptomatic tubers were recorded, resulting in a low disease index (0.03). Chloropicrin soil fumigation significantly increased powdery scab indices compared to the non-treated control (Figure 3.5D), but disease indices evaluated among fumigant rates did not differ significantly. Overall, powdery scab indices increased from 133 to 315% when chloropicrin soil fumigation was applied.

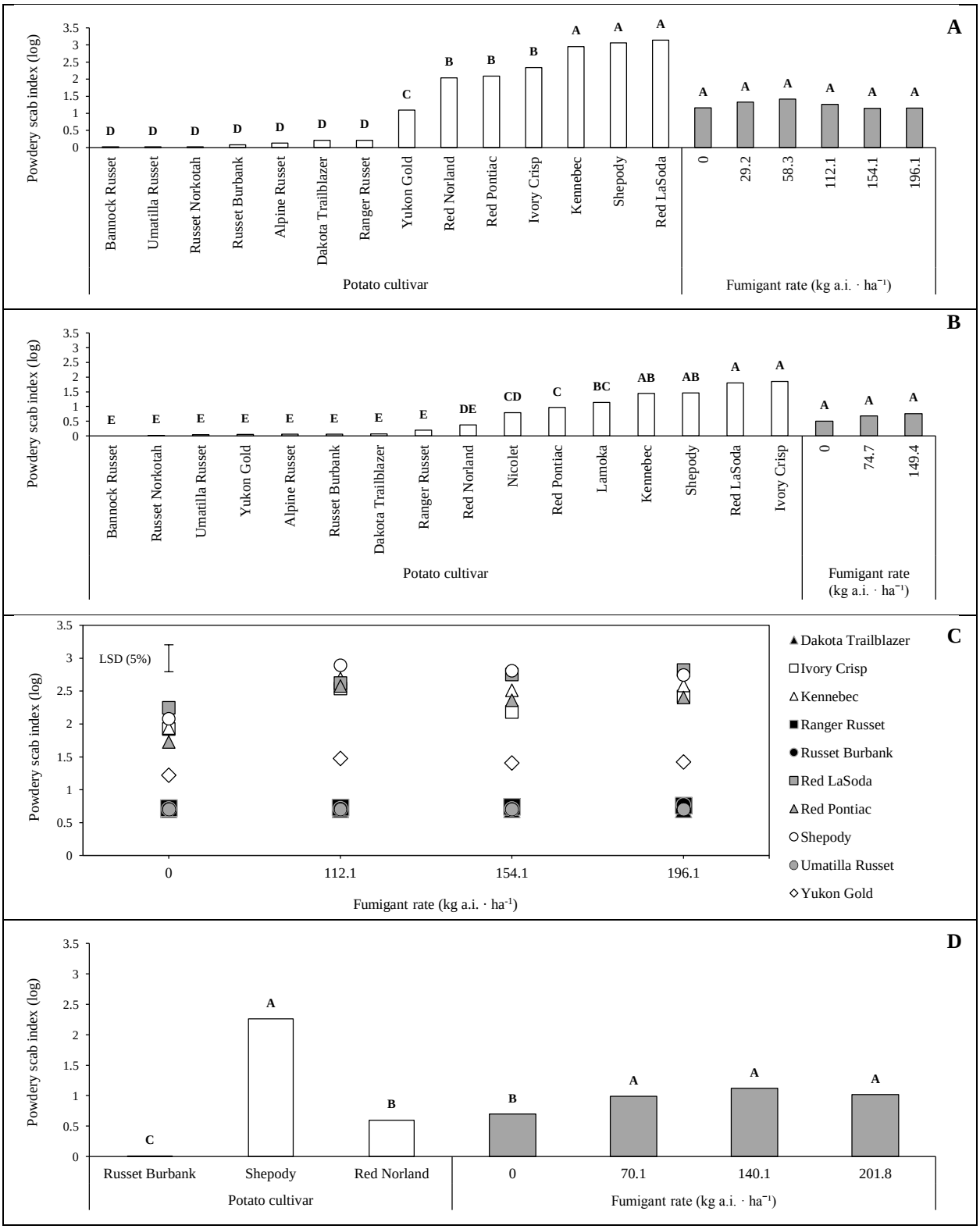

Figure 3.5. Effect of chloropicrin fumigation on powdery scab indices in potato cultivars evaluated at McCanna, ND (A) and Perham, MN (B) in 2011; Osage, MN and McCanna, ND (C) in 2012, and Perham and Park Rapids, MN in 2013.

Effect of chloropicrin fumigation rates on tuber yield

In 2011, no significant chloropicrin rate x potato cultivar interaction was observed for tuber yield at Perham, MN ($P = 0.457$). The analysis of main effects revealed significant differences in tuber yield among cultivars ($P < 0.001$), but no effect of the chloropicrin fumigation ($P = 0.308$; Figure 3.6A). Among cultivars, Red Pontiac had the highest yield, whereas yields were lowest for cv. Yukon Gold. At Larimore, ND, no significant chloropicrin rate x potato cultivar interaction was observed ($P = 0.444$); however, significant differences for tuber yield were observed among cultivars ($P < 0.001$) and fumigant rates ($P = 0.004$; Figure 3.6B). Cultivars such Alpine Russet and Russet Norkotah had the highest tuber yield, whereas Bannock Russet had the lowest. On average, tuber yield increased 18% when chloropicrin fumigation was applied; however, among fumigant rates no differences in yield were observed.

In 2012, no significant chloropicrin rate x potato cultivar interaction was observed ($P = 0.406$); however, tuber yield among cultivars varied significantly ($P < 0.001$). Net tuber yield for potato cultivars such as Umatilla Russet and Kennebec were significantly higher than 'Yukon Gold', but did not differ from 'Dakota Trailblazer' and 'Red Pontiac' (Figure 3.6C). Chloropicrin did not significantly increase tuber yield ($P = 0.443$) compared to the non-treated control. Similar to previous years, no significant chloropicrin x potato cultivar interaction was observed in 2013 ($P = 0.612$); however, the analysis of main effects revealed significant differences in tuber yield among potato cultivars ($P = 0.004$). During this year, tuber yield for 'Red Norland' was significantly higher than 'Shepody', but did not differ from 'Russet Burbank'. Chloropicrin soil fumigation significantly increased net tuber yield ($P = 0.004$) compared to the non-treated control; however, no significant variation among chloropicrin rates was observed. Overall, chloropicrin increased net tuber yield from eight to 14% (Figure 3.6D).

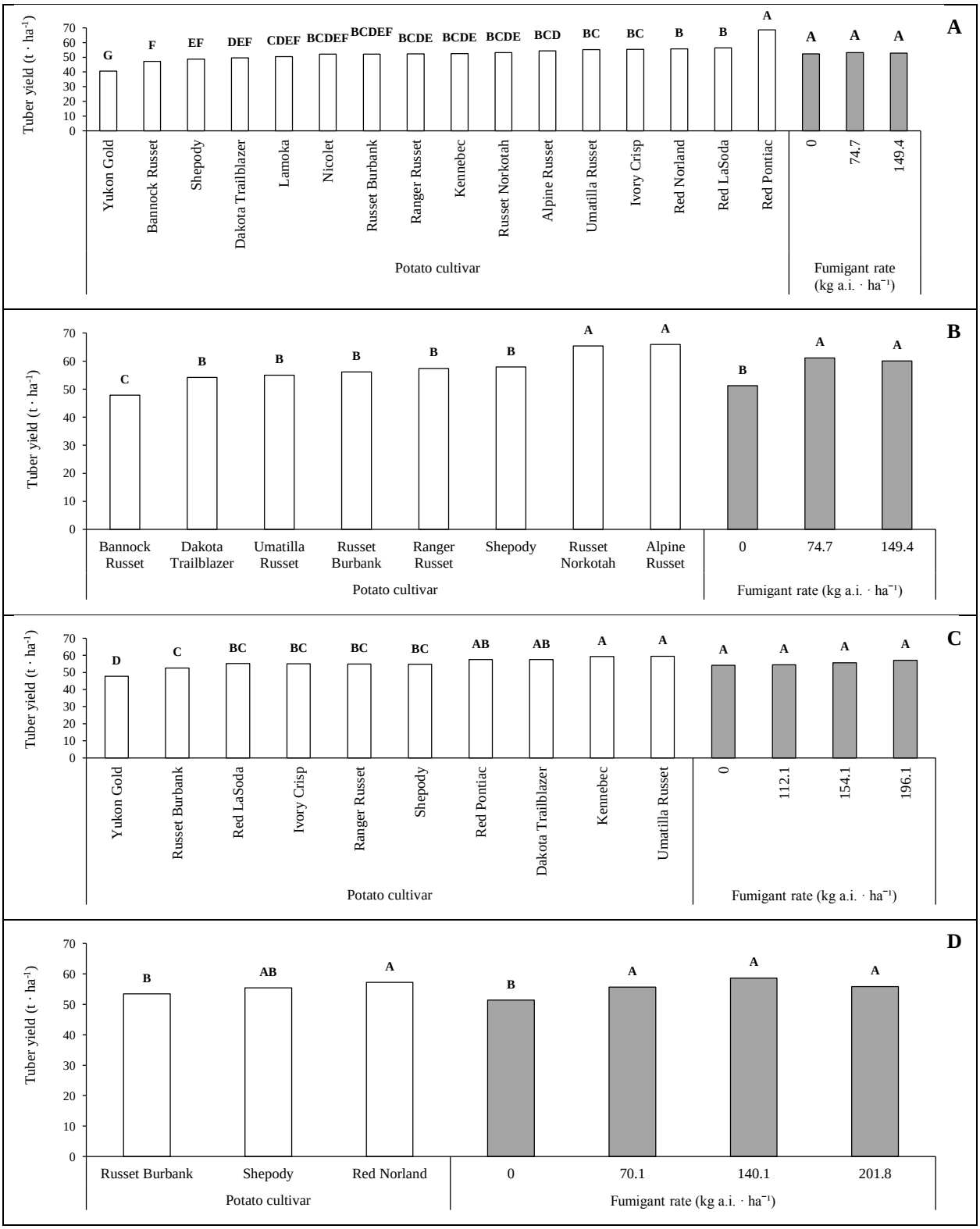

Figure 3.6. Effect of chloropicrin fumigation on tuber yields of potato cultivars evaluated at Perham, MN (A) and Larimore, ND, MN (B) in 2011; Osage, MN and McCanna, ND (C) in 2012, and Perham and Park Rapids, MN in 2013.

Association of *S. subterranea* initial inoculum, chloropicrin rate, disease and yield

A significant negative correlation was observed across experiments between chloropicrin rates and the levels of *S. subterranea* detected at post-fumigation (Sspf; Table 3.2). For these two variables, the degree of association (r) for selected sub-plots ranged from -0.27 to -0.33. In addition, a significant negative association was observed between Sspf and the resulting root gall formation AUDPC for cultivars such as Dakota Trailblazer (r = -0.28; $P = 0.027$) and Red LaSoda (r = -0.32; $P = 0.021$). However, no relationship was observed between Sspf and powdery scab index and tuber yield. No significant relationship ($P > 0.05$) was observed between *S. subterranea* populations in non-treated plots and the expression of the disease on tubers and roots. Chloropicrin fumigation was found associated with increasing AUDPC levels in all selected cultivars (r: 0.29 – 0.45; $P < 0.05$), while in cultivars such as Shepody and Red LaSoda a significant association between chloropicrin fumigation and powdery scab index ($P < 0.05$) was observed. This association was not significant among russet-skinned cultivars Dakota Trailblazer (r = -0.1; $P = 0.495$) and Umatilla Russet (r = 0.12; $P = 0.354$). In addition, chloropicrin fumigation was found to be significantly associated with tuber yield in four of six selected cultivars (r: 0.29 – 0.45; $P < 0.05$; Table 3.2). In cultivars such as Umatilla Russet and Dakota Trailblazer a significant positive association between tuber yield and AUDPC was observed ($P < 0.05$); whereas, for ‘Shepody’ and ‘Yukon Gold’, tuber yield was found to be significantly associated with AUDPC and the powdery scab index. The degree of association of tuber yield and AUDPC ranged from 0.23 to 0.50. Furthermore, a significant relationship between AUDPC and powdery scab index was observed among selected cultivars (r: 0.29 – 0.75; $P < 0.005$), but this relationship was not significant in ‘Dakota Trailblazer’ ($P = 0.289$) and ‘Umatilla Russet’ ($P = 0.771$).

Table 3.2. Spearman rank correlation among chloropicrin rates, *S. subterranea* inoculum at post-fumigation, disease on tubers and roots and tuber yield in field experiment conducted in Minnesota and North Dakota from 2011 to 2013.

Cultivar			Chloropicrin rate	Tuber Yield	Sspf	AUDPC
Shepody (n = 96)	Tuber Yield	r	0.302			
		P	0.003			
	Sspf	r	-0.17	-0.182		
		P	0.093	0.076		
	AUDPC	r	0.328	0.229	0.193	
		P	0.001	0.025	0.061	
	Powdery scab index	r	0.362	0.404	-0.111	0.684
		P	0.001	0.001	0.281	<0.001
Yukon Gold (n = 52)	Tuber Yield	r	0.212			
		P	0.131			
	Sspf	r	-0.325	0.027		
		P	0.019	0.851		
	AUDPC	r	0.354	0.501	-0.377	
		P	0.011	0.001	0.018	
	Powdery scab index	r	0.279	0.711	-0.199	0.749
		P	0.045	<0.001	0.155	<0.001
Red LaSoda (n = 52)	Tuber Yield	r	-0.166			
		P	0.238			
	Sspf	r	-0.325	0.303		
		P	0.019	0.029		
	AUDPC	r	0.447	-0.176	-0.32	
		P	0.01	0.211	0.021	
	Powdery scab index	r	0.373	0.132	-0.02	0.594
		P	0.007	0.349	0.9	<0.001
Dakota Trailblazer (n = 64)	Tuber Yield	r	0.335			
		P	0.007			
	Sspf	r	-0.27	-0.223		
		P	0.033	0.077		
	AUDPC	r	0.409	0.437	-0.278	
		P	0.001	0.001	0.027	
	Powdery scab index	r	-0.09	-0.142	0.053	-0.134
		P	0.495	0.262	0.675	0.289
Russet Burbank (n = 96)	Tuber Yield	r	0.248			
		P	0.015			
	Sspf	r	-0.201	-0.109		
		P	0.052	0.291		
	AUDPC	r	0.292	-0.034	0.4208	
		P	0.004	0.742	<0.001	
	Powdery scab index	r	0.183	0.021	0.103	0.289
		P	0.076	0.846	0.322	0.005
Umatilla Russet (n = 64)	Tuber Yield	r	0.305			
		P	0.015			
	Sspf	r	-0.268	-0.057		
		P	0.033	0.653		
	AUDPC	r	0.345	0.4261	-0.2	
		P	0.005	0.001	0.122	
	Powdery scab index	r	0.118	0.098	-0.1	0.037
		P	0.354	0.442	0.451	0.771

Sspf: *S. subterranea* inoculum at post-fumigation; AUDPC: area under disease progress curve for root gall formation.

Discussion

This study represents the first report of large-scale experiments aimed to evaluate the effect chloropicrin soil fumigation on the soil populations of *S. subterranea* and its concomitant effect on disease expression in the United States. To date, alternatives of control for disease caused by *S. subterranea* are limited, which increases the difficulty in establishing cost-effective management approaches, and at the same time highlights the necessity of integration of control methods that can mitigate disease expression (Falloon 2008). *Spongospora subterranea* is an obligate parasite that survives long-term in the soil and on tubers via resting spores (Braselton 2001). Once in the plant, the pathogen undergoes multiple infection cycles increasing the amount of short-lived inoculum (sporangial stage). This biphasic nature of the pathogen allows significant amount of disease to develop from relatively low levels of sporosori when conducive conditions are present (Brierley et al. 2013). However, a number of reports suggest that under field conditions, a higher amount of initial inoculum will likely increase the chances for the pathogen to infect and cause disease (Brierley et al. 2013; Qu et al. 2006; Shah et al. 2014; Sparrow et al. 2015).

In this study, chloropicrin rates ranging from 74.7 to 196.1 kg a.i. · ha^{-1} were effective at reducing *S. subterranea* inoculum in a series of sandy soils. Overall, pathogen reduction due to chloropicrin fumigation ranged from 10.8% to 34.7%, but the fumigation efficiency varied largely among trial locations and fumigant rates. For example, in 2012 chloropicrin applied at 196.1 kg a.i. · ha^{-1} resulted in a 32.6% soil inoculum reduction at McCanna, ND; whereas at Osage, MN, inoculum decreased 14.2%. Furthermore, in 2012, soil fumigation at rate of 112.1 kg a.i. · ha^{-1} resulted in a reduction of the pathogen populations of 11.5% at Osage, MN but no significant reduction at McCanna, ND; whereas, in 2013 chloropicrin applied at rate of 70.1 kg

a.i. · ha^{-1} resulted in reductions of 15.9% at Perham, MN and 17.7% at Park Rapids, MN. Several factors, including inoculum level, soil texture and temperature, are involved in fumigant efficacy once applied to the soil (Lembright 1990). Fumigation described in this research was performed at soil temperatures between 3 and 11°C, which falls into the temperature range routinely used in the area of study and EPA recommendations (Gudmestad et al. 2007; Pasche et al. 2014; Taylor et al. 2005). Across fields, the average amount of inoculum in soil was 1.5 x 10^5 resting spores · g^{-1} (74.3 sporosori · g^{-1}); however, at pre-fumigation the amount of inoculum varied significantly among and within fields. These differences are likely to have contributed to observed results.

The global potato industry is continuously challenged by quality parameters necessary to satisfy a very dynamic market (Bonnel 2008; Pasche et al. 2014). The use of fumigation in some instances constitutes a requirement to meet quality standards, as well as, the sustainability of the crop (Ajwa et al. 2002; Duniway 202; Pasche et al. 2014). In the US, metam sodium is by far the most commonly used fumigant in the potato industry (Cox 2006); however, fumigant alternatives like chloropicrin alone and in combination with 1,3-dichloropropene (1,3-D) have shown increased profitability in crops such as strawberries and tomatoes (Sydorovych et al. 2006; 2008), constituting a comparative advantage for chloropicrin. In this study, it was hypothesized that chloropicrin soil fumigation would result in disease control as a consequence of the reduction of *S. subterranea* initial populations in soil. However, our results, although null, revealed valuable pieces of information regarding the epidemiology of *S. subterranea*.

Powdery scab and root gall formation were significantly increased when chloropicrin was applied to the soil at rates ranging from 70.1 to 201.8 kg a.i. · ha^{-1}. This increasing effect was more frequently observed on the formation of root galls than powdery scab on tubers. A similar increase of powdery scab was observed when chloropicrin was applied in combination with 1,3-

D. In general, disease on tubers and roots was higher in smooth-skinned cultivars such as Red LaSoda and Shepody, while on russet-skinned cultivars such as Umatilla Russet and Russet Burbank disease on tubers was limited from one to few lesions, though they developed several root galls. The lowest levels of disease on tubers and roots were observed for 'Bannock Russet', 'Dakota Trailblazer' and 'Ranger Russet'. Disease expression for smooth-skinned cultivars was higher at increasing chloropicrin rates, but remained relatively constant in those cultivars with resistance to powdery scab and/or root gall formation.

Field results were further confirmed in controlled condition experiments, where chloropicrin fumigation significantly increased the amount of *S. subterranea* DNA in roots of 'Dakota Trailblazer' and 'Shepody'. Differences were especially pronounced at the 201.8 kg a.i. $\cdot$ ha^{-1} rate, where levels of pathogen DNA detected in roots were four times higher than for the non-treated control. This effect was observed at both locations; however, at Perham, MN, the amount of pathogen DNA quantified was two times more than levels observed at Park Rapids. The amount of *S. subterranea* DNA detected in cv. Shepody was two times higher than observed in 'Dakota Trailblazer'; however, these differences were not significant between cultivars, or between cultivars across soil fumigation rates. In contrast, the number of root galls observed in each cultivar differed strikingly, especially for 'Shepody', which unlike 'Dakota Trailblazer', had a higher number of root galls as chloropicrin rate increased.

These results suggest that higher levels of *S. subterranea* infection are likely to increase the degree of disease development on roots and tubers in susceptible cultivars, and at the same time, propose that defense factors acting against the sporangial phase of the pathogen might differ from those controlling the formation of symptoms (Hernandez Maldonado et al. 2013). *Spongospora subterranea*, as well as other plasmodiophorids, is able to infect a number of plant

species across a wide range of families (Iftikhar and Ahmad 2005; Qu and Christ 2006; Shah et al. 2010), but the ability of the pathogen to stimulate symptoms and form resting spores has been found limited to a number of host species (Qu and Christ 2006). It remains a matter of speculation regarding the factors inherent to the pathogen and host responsible for disease induction. In addition, the results described in this research raise new questions regarding the role played by soil microbiota in the development of powdery scab and root galls. Previous reports described an increase of powdery scab when mancozeb (2.3 kg· ha^{-1}) was applied to the soil at pre-planting (Falloon et al. 1996). In that study, the authors suggested a plausible effect of the fungicide rate in prolonging the susceptibility of the host or offering a sub-lethal effect on the pathogen population in soil. As the number of reports showing evidence of disease control with the use of antagonistic biological agents (Nakayama et al. 2013; Nielsen and Larsen 2004) increases, the chances that chloropicrin fumigation effect on *S. subterranea* root colonization is due to reduction of non-target organisms in the soil.

A singular aspect of this research was that tuber yield was generally increased along the amount of disease observed in roots but as well as on tubers. Chloropicrin fumigations are known to deplete populations of soil microorganisms, resulting in changes in the amounts of nitrogen available to the plant (Stromberger et al. 2005). During the course of this research the analysis of multiple soil samples collected at pre- and post-fumigation indicated similar levels of nutrients in soil (data not shown). Evidence of the adverse effects of root infection by *S. subterranea* on tuber yield and size has been reported in a number of studies (Falloon et al. 2015; Shah et al. 2012). Our results indicated a significant increase in *S. subterranea* DNA amounts with the increase of fumigant rate. It is therefore likely that a greater amount of root cell disruption has taken place in treatments with increased pathogen DNA, resulting in yield

reductions. Future research will be required in order to elucidate the effect of root infection under field conditions and at inoculum levels commonly found in fields.

In summary, the results described in this research show that chloropicrin fumigation, although effectively reduced the amount *S. subterranea* in soil, remains unsuitable for control of disease on tubers and roots. Further research will be required in order to determine whether control strategies aimed at the sporangial stage of the pathogen, rather than initial inoculum, are efficacious in controlling the disease. Additionally, the importance of biological interactions between *S. subterranea* and soil microbiota in the development of powdery scab and root galls, remains relatively unexplored (Kole 1959).

Literature Cited

Ajwa, H. A., Trout, T., Mueller, J., Wilhelm, S., Nelson, S. D., Soppe, R., and Shatley, D. 2002. Application of alternative fumigants through drip irrigation systems. Phytopathology 92:1349-1355.

Bonnel, E. 2008. Potato breeding: a challenge, as ever!. Potato Research 51:327-332.

Braithwaite, M., Falloon, R., Genet, R., Wallace, A., Fletcher, J., and Braam, W. 1994. Control of powdery scab of potatoes with chemical seed tuber treatments. New Zealand Journal of Crop and Horticultural Science 22:121-128.

Braselton, J. P. 2001. Plasmodiophoromycota. Pages 81-91 in: The Mycota VII, Part A, Systematics and Evolution. D. J. McLaughlin, E. G. McLaughlin and P. A. Lemke, eds. Springer-Verlag. Berlin-Heidelberg.

Brierley, J. L., Stewart, J. A., and Lees, A. K. 2009. Quantifying potato pathogen DNA in soil. Applied Soil Ecology 41:234-238.

Brierley, J. L., Sullivan, L., Wale, S. J., Hilton, A. J., Kiezebrink, D. T., and Lees, A. K. 2013. Relationship between *Spongospora subterranea* f. sp. *subterranea* soil inoculum level, host resistance and powdery scab on potato tubers in the field. Plant Pathology 62:413-420.

Brown, C. R., Vandemark, G. J., Cummings, T., Batchelor, D., Miller, J., Olsen, C., and Johnson, D. 2007. Resistance to powdery scab in potato. Potato Progress 7:1-4.

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

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., and Wittwer, C. T. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry 55:611-622.

Christ, B. J. 2001. Powdery scab workshop – summary notes. Online: http://www.uidaho.edu/ag/plantdisease/scabnote.

Cox, C. 2006. Metam sodium: fumigant factsheet. Journal of Pesticide Reform 26: 12–16.

Davey, T., Carnegie, S. F., Saddler, G. S., and Mitchell, W. J. 2014. The importance of the infected seed tuber and soil inoculum in transmitting *Potato mop-top virus* to potato plants. Plant Pathology 63:88-97.

Davidson, R. D., and Houser, A. 2009. Evaluation of fluazinam application methods in-furrow for control of powdery scab on potato. American Journal of Potato Research 86:140.

de Boer, R., and Theodore, M. 1997. The epidemiology and control of powdery scab of potatoes. Horticultural Research and Development Corporation. PT303. 45 p.

Duniway, J. M. 2002. Status of chemical alternatives to methyl bromide for pre-plant fumigation of soil. Phytopathology 92:1337-1343.

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

Falloon, R. E., Curtin, D., Lister, R. A., and Butler, R. C. 2005. Root function and growth of potato plants reduced by *Spongospora subterranea* infection. American Journal of Potato Research 82:68.

Falloon, R. E., Merz, U., Butler, R. C., Curtin, D., Lister, R. A., and Thomas, S. M. 2015. Root infection of potato by *Spongospora subterranea*: knowledge review and evidence for decreased plant productivity. Plant Pathology. DOI: 10.1111 /ppa.12419.

Falloon, R., Merz, U., Lister, R. A., Wallace, A. R., and Hayes, S. P. 2011. Morphological enumeration of resting spores in sporosori of the plant pathogen *Spongospora subterranea*. Acta Protozoologica 50:121-132.

Falloon, R. E., Viljanen-Rollinson, S. L. H., Coles, G. D., and Poff, J. D. 1995. Disease severity keys for powdery and downy mildews of pea, and powdery scab of potato. New Zealand Journal of Crop and Horticultural Science 23:31-37.

Falloon, R., Wallace, A., Braithwaite, M., Genet, R., Nott, H., Fletcher, J., and Braam, W. 1996. Assessment of seed tuber, in-furrow, and foliar chemical treatments for control of powdery scab (*Spongospora subterranea* f. sp. *subterranea*) of potato. New Zealand Journal of Crop and Horticultural Science 24:341-353.

García, R., Garnica, J., and Espinosa, Y. 2004. Estudio de epifitias en la roña de la papa (*Spongospora subterranea*) en el Estado Mérida, Venezuela. Revista Latinoamericana de la Papa. Suplemento especial, Marzo, Oral 40.

Gilchrist, E., Soler, J., Merz, U., and Reynaldi, S. 2011. Powdery scab effect on the potato *Solanum tuberosum* ssp. *andigena* growth and yield. Tropical Plant Pathology 36:350-355.

Gudmestad, N. C., Taylor, R. J., and Pasche, J. S. 2007. Management of soilborne diseases of potato. Australasian Plant Pathology 366:109-115.

Harrison, J. G., Searle, R. J., and Williams, N. A. 1997. Powdery scab disease of potato - a review. Plant Pathology 46:1-25.

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

Hirnyck, R., and Downey, L. 2007. Pest management strategic plan for Pacific Northwest potato production-REVISION. Pages 16-20 in: Summary of PMSP workshop. S. O'Neal Coates, ed. Pocatello, ID, US.

Houser, A. J., and Davidson, R. 2010. Development of a greenhouse assay to evaluate potato germplasm for susceptibility to powdery scab. American Journal of Potato Research 87:285-289.

Hughes, I. K. 1980. Powdery scab (*Spongospora subterranea*) of potatoes in Queensland: occurence, cultivar susceptibility, time of infection, effect of soil pH, chemical control. Australian Journal of Experimental Agriculture and Animal Husbandry 20:625-632.

Iftikhar, S., and Ahmad, I. 2005. Alternate hosts of *Spongospora subterranea* f. sp. *subterranea*, the causal organism of powdery scab of potato. American Journal of Potato Research 82:74-75.

Johnson, D. A., and Cummings, T. F. 2015. Effect of powdery scab root galls on yield of potato. Plant Disease 99:1396-1403.

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

Kole, A. P. 1959. *Plasmodiophora brassicae* en *Spongospora subterranea*, punten van overeenkomst en verschil. Tijdschrift over Plantenziekten 65: 47-55.

Lembright, H. W. 1990. Soil fumigation: principles and application technology. Journal of Nematology (Supplement) 22:632-644.

Lister, R. A., Falloon, R. E., Curtin, D., and Butler, R. C. 2004. *Spongospora subterranea* reduces host (*Solanum tuberosum*) growth. Pages 135-136 in: Proceedings of the 3rd Australasian soilborne diseases symposium. K. M. Ophel Keller and B. H. Hall, eds. South Australian Research and Development Institute, Adelaide.

Lloyd, M. 1967. Mean crowding. Journal of Animal Ecology 36:1-30.

Madden, L. V., Hughes, G. and van den Bosch, F. 2007. Temporal analysis I: quantifying and comparing epidemics. Pages 63-116 in: The study of plant disease epidemics. American Phytopathological Society, St. Paul, MN, US.

Merz, U. 1989. Infectivity, inoculum density and germination of *Spongospora subterranea* resting spores: a solution-culture test system. Bulletin OEPP 19:585-592.

Merz, U. 2008. Powdery scab of potatoes - occurrence, life cycle and epidemiology. American Journal of Potato Research 85:239-246.

Merz, U., and Falloon, R. E. 2009. Review: Powdery scab of potato - Increased knowledge of pathogen biology and disease epidemiology for effective disease management. Potato Research 52:17-37.

Merz, U., Lees, A. K., Sullivan, L., Schwärzel, R., Hebeisen, T., Kirk, H. G., Bouchek-Mechiche, K., and Hofferbert, H. R. 2012. Powdery scab resistance in *Solanum tuberosum*: an assessment of cultivar x environment effect. Plant Pathology 61:29-36.

Nachmias, A., and Krikun, J. 1988. Etiology and control of powdery scab of potato in a semi-arid region of Israel. Phytoparasitica 16:33-38.

Nakayama, T., Horita, M., and Shimanuki, T. 2007. *Spongospora subterranea* soil contamination and its relationship to severity of powdery scab on potatoes. Journal of General Plant Pathology 73:229-234.

Nakayama, T., Sayama, M., and Merz, U. 2013. Suppression of potato powdery scab caused by *Spongospora subterranea* using an antagonistic fungus *Aspergillus versicolor* isolated from potato roots. Pages 53-54 in: Proceedings of the 9th symposium of the international working group on plant viruses with fungal vectors. Obihiro, Hokkaido, Japan.

Neher, D. A., and Campbell, C. L. 1997. Determining sample size. Pages 12-15 in: Exercises in plant disease epidemiology. L. J. Francl and D. A. Neher, eds. American Phytopathological Society, St. Paul, MN, US.

Neuhauser, S., Bulman, S., and Kirchmair, M. 2010. Plasmodiophorids: the challenge to understand soil-borne, obligate biotrophs with a multiphasic life cycle. Pages 51-78 in: Molecular Identification of Fungi. Y. Gherbawy and K. Voigt, eds. Springer-Verlag.

Nielsen, S. L., and Larsen, J. 2004. Two Trichoderma harzinum-based bio-control agents reduce tomato root infection with *Spongospora subterranea* (Wallr.) Lagerh. f. sp. *subterranea*, the vector of *Potato moptop virus*. Zeitschr Pflanzenkr Pflanzensch 111:145-150.

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

Pasche, J. S., Taylor, R. J., David, N. L., and Gudmestad, N. C. 2014. Effect of soil temperature, injection depth, and metam sodium rate on the management of Verticillium wilt of potato. American Journal of Potato Research 91:227-290.

Qu, X. S., and Christ, B. J. 2006. The host range of *Spongospora subterranea* f. sp. *subterranea* in the United States. American Journal of Potato Research 83:343-348.

Qu, X. S., Kavanagh, J. A., Egan, D., and Christ, B. J. 2006. Detection and quantification of *Spongospora subterranea* f. sp. *subterranea* by PCR in host tissue and naturally infested soil. American Journal of Potato Research 83:21-30.

Shah, F. A., Butler, R. C., Marshall, J. W., and Keenan, S. 2004. Relationships between *Spongospora subterranea* inoculum, powdery scab severity and potato tuber yield. Pages 172-173 in: Proceedings of the 3rd Australasian soilborne disease symposium. K. M. Keller and B. H. Hal, eds. South Australian Research and Development Institute, Adelaide.

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

Shah, F. A., Falloon, R. E., Butler, R. C., and Lister, R. A. 2012. Low amounts of *Spongospora subterranea* sporosorus inoculum cause severe powdery scab, root galling and reduced water use in potato (*Solanum tuberosum*). Australasian Plant Pathology 41:219-228.

Shah, F. A., Falloon, R. E., Butler, R. C., Lister, R. A., Thomas, S. M., and Curtin, D. 2014. Agronomic factors affect powdery scab of potato and amounts of *Spongospora subterranea* DNA in soil. Australasian Plant Pathology 43:679-689.

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

Stromberger, M. E., Klose, S., Ajwa, H., Trout, T., and Fennimore, S. 2005. Microbial populations and enzyme activities in soils fumigated with methyl bromide alternatives. Soil Science Society of America Journal 69:1987-1999.

Sydorovych, O., Safley, C. D., Ferguson, L. M., Poling, E. B., Fernandez, G. E., Brannen, P. M., Monks, D. M., and Louws, F. J. 2006. Economic evaluation of methyl bromide alternatives for the production of strawberries in the Southern United States. HortTechnology 16:11705-713.

Sydorovych, O., Safley, C. D., Welker, R. M., Ferguson, L. M., Monks, D. W., Jennings, K., Driver, J., and Louws, F. J. 2008. Economic evaluation of methyl bromide alternatives for the production of tomatoes in North Carolina. HortTechnology 18: 118-128.

Taylor, R. J., Pasche, J. S., and Gudmestad, N. C. 2005. Influence of tillage and method of metam sodium application on distribution and survival of *Verticillium dahliae* in the soil and the development of potato early dying disease. American Journal of Potato Research 82:451-461.

Tegg, R. S., Corkrey, R., Herdina, H., McKay, A. C., Crump, N. S., de Boer, R. F., Wiechel, T. J., and Wilson, C. R. 2015. Modeling pathogen DNA content and visual disease assessment in seed tubers to inform disease in potato progeny root, stolon, and tubers. Plant Disease 99:50-57.

Tsror, L. 2014. Epidemiology and management of powdery scab in Israel. Second international powdery scab workshop. Pretoria, South Africa. July 29th - August 1st. Online: ww.spongospora.ethz.ch/SA_2014/docus/day3/PS_WS_Tsror_F.pdf.

Tsror, L., Erlich, O., Hazanovsky, M., and Zig, U. I. 2009. Powdery scab occurrence and control in Israel. Page 44 in: Abstracts of the 7th international symposium on chemical and non-chemical soil and substrate disinfestation. Katholieke Universiteit Leuven, Belgium.

USDA. 2014. Vegetables and pulses yearbook data. Online: usda.mannlib.cornell.edu/usda/ers/VEGANDPULSESYEARBOOK/2014/89011.pdf.

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

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

Wale, S. J. 2000. Summary of the session on national potato production and the powdery scab situation. Pages 3-9 in: Proceedings of the first European powdery scab workshop. U. Merz and A. K. Lees, eds. Aberdeen, Scotland.

Zink, R. T., Davidson, R. D., and Houser, A. 2004. Control strategies for powdery scab of potato. American Journal of Potato Research 81:95-96.

CHAPTER IV. EVALUATION OF THE EFFECT OF RECIPROCAL GRAFTING BETWEEN TWO POTATO CULTIVARS ON THE INFECTION AND DEVELOPMENT OF ROOT GALLS CAUSED BY *SPONGOSPORA SUBTERRANEA*

Abstract

The biotroph protozoan *Spongospora subterranea* causes powdery scab and root gall formation on potato. The intrinsic nature of the pathogen has hampered the efforts focused on disease control and has further emphasized the need to integrate strategies for control, among which host resistance represents the most suitable alternative. However, the mechanisms of resistance involved in disease expression in tubers and roots remain to be elucidated. The cultivars Shepody (S) and Dakota Trailblazer (D), susceptible and resistant to gall formation respectively, were employed as either scion or rootstock to prepared grafted plants in order to investigate the effect of reciprocal grafting on the infection and development of root galls by *S. subterranea*. At 24 hours post-inoculation, the amount of *S. subterranea* DNA detected in graft combinations involving 'Shepody' as rootstock (S/S and D/S) and inoculated in combination with all graft treatments was significantly higher than grafted plants with 'Dakota Trailblazer' as scion and rootstock (D/D). Overall, the amount of pathogen DNA detected at 24 hours post-inoculation was positively associated with the number of root galls developed at six and eight weeks ($r = 0.3$; $P < 0.008$). The number of root galls that developed in graft combinations S/S, D/S and S/D were significantly higher than D/D across all trials. Root gall formation in S/D was five to 50 times higher than the number of galls observed in D/D. These results suggest the

translocation of host factors present in ‘Shepody’ that are involved in the host response to the development of root galls by the pathogen.

Introduction

Spongospora subterranea (Wallr.) Lagerh. is an obligate biotrophic parasite that causes powdery scab and root gall formation in potato (*Solanum tuberosum* L.; Harrison et al. 1997; Merz and Falloon 2009). Additionally, *S. subterranea* is the vector of the *Potato mop-top virus* (Jones and Harrison 1969). The pathogen is currently classified as a member of Phytomyxea within the protist super-group Rhizaria (Cercozoa) (Adl et al. 2005; Cavalier-Smith and Chao 2003). Phytomyxea is a monophyletic group comprising two orders: Phagomyxida (phagomyxids) and Plasmodiophorida (plasmodiophorids); in which *S. subterranea* is classified along with other members of agricultural importance such as *Plasmidiophora brassicae* Woronin and *Polymyxa betae* Keskin (Bulman and Braselton 2014; Neuhauser et al. 2011). Members of the Phytomyxea are characterized by having cruciform nuclear division, obligate intracellular parasitism, biflagellated zoospores, and environmentally resistant resting spores (Bulman et al. 2001; Braselton 1995; Qu and Christ 2004).

The life history of *S. subterranea* begins with germination of resting spores, from which a single haploid zoospore is released (Harrison et al. 1997). Once in contact with the host, zoospores infect the epidermal cells of roots, stolons and tubers, and develop a multinucleate primary plasmodium (sporangial) (Braselton 1995). Multiple infection and re-infection cycles are produced by the pathogen, giving the production of new flushes of secondary zoospores every three to four days (Falloon et al. 2015). This inoculum build-up is followed by the development of secondary plasmodia (sporogenic) as result of the infection by either primary or

secondary zoospores into cortical cells of infected organs (Braselton 1995; Harrison et al. 1997). The development of sporogenic zoosporangia is accompanied by the promotion of hypertrophied tissue, resulting in the formation of galls on roots, and pustules on tubers leading to powdery scab lesions.

Susceptibility to powdery scab and presumably to root gall formation, has been described as the consequence of the interaction of multiple host genes acting at different moments throughout disease development, including the release of primary zoospores (Falloon et al. 2003; Harrison et al. 1997). Although the germination of *S. subterranea* resting spores is reported to occur spontaneously across a wide range of temperatures (3 – 25°C) (Fornier 1997; Harrison et al. 1997), an increase in the intensity of root infection was reported when plants were inoculated with a sporosori suspension previously confronted by root exudates of solanaceous and non-solanaceous crops (Fornier 1997; Merz 1993). In a series of greenhouse experiments, differences in the amount of zoosporangial root infection were observed among 15 potato cultivars inoculated with a suspension of *S. subterranea* sporosori. The intensity of the infection observed at 21 days after inoculation was generally associated with the expression of powdery scab, however, exceptions were noted (Falloon et al. 2003). Despite these findings, the intensity of zoosporangium root infection observed in roots of two potato cultivars with contrasting susceptibility to powdery scab and root gall formation was temporally and quantitatively similar (Falloon et al. 2015; Hernandez Maldonado et al. 2013). Previous research of other members of the plasmodiophorids have demonstrated that the development of zoosporangium infection in host roots contrasts with the formation of symptoms in roots, suggesting the onset of resistance mechanisms to this specific stage of the pathogen during symptom induction (Hernandez Maldonado et al. 2013; Ludwig-Müller and Schuller 2008).

Research in host/pathogen interaction during clubroot of crucifer disease development caused by *P. brassicae* demonstrate the ability of the pathogen to break down the host basal resistance mechanisms and to modify the host hormone homeostasis (Chu et al. 2014; Devos et al. 2006; Feng et al. 2013; Ludwig-Müller et al. 2009; Schwelm et al. 2015). Although changes in host primary and secondary metabolism are triggered by primary infection of the pathogen (Devos et al. 2006; Jameson 2000; Ludwig-Müller et al. 2009), the highest concentration of hormones such as auxins and cytokinins are reported during the development of root galls later in the disease process (Ludwig-Müller and Schuller 2008; Schwelm et al. 2015). As a consequence, a *de novo* meristematic area is established which acts as a sink for host nutrients such as carbohydrates, amino acids and lipids (Jameson 2000; Ludwig-Müller et al. 2009). Nevertheless, the extent of the symptom development is dependent on the translocation and interaction among host factors (e. g. salicylic acid and flavonoids) involved in resistance against the pathogen (Jameson 2000; Ludwig-Müller et al. 2009).

The involvement of the pathogen in the host hormonal balance is also reported among other biotroph plant pathogens (Ishibashi and Shimizu 1970) and is suggested to occur in *S. subterranea* hypertrophied tissues as culturability of root galls in absence of plant regulators has been demonstrated (Bulman et al. 2011). At the molecular level, evidence of a close host/pathogen interaction is provided by the absence of genes encoding for proteins involved in the uptake of nutrients and biosynthesis of amino acids (Ludwig-Müller 2009; Schwelm et al. 2015). This is a characteristic recently reported for plasmodiophorids such as *P. brassicae* and *S. subterranea* (Schwelm et al. 2015).

The management of powdery scab and root gall formation is particularly difficult due to the ability of the pathogen to persist for long periods of time in the soil (> 10 years), as well as

the limited effect of current options for control (Falloon 2008). Thus, management of this disease requires the integration of control measures, among which host resistance represents the most sustainable and long-term approach for disease control (Merz and Falloon 2009). Nevertheless, development of cultivars in breeding programs requires information on the physiology and mechanisms involved in the development of powdery scab and root gall formation. The goal of the research reported here was to investigate the potential of unknown host factors in the development of root galls caused by *S. subterranea*. This was accomplished by utilizing rootstock and scion combinations in grafts between two potato cultivars with contrasting disease susceptibility and resistance to *S. subterranea*.

Materials and Methods

Preparation of grafts

Reciprocal grafts were made between two potato cultivars with marked differences in susceptibility to root gall formation and powdery scab in tubers (Domfeh et al. 2015). Cultivars Shepody (very susceptible) and Dakota Trailblazer (very resistant) were selected and four scion/rootstock combinations (i.e. graft treatment) were prepared: Shepody/Shepody (S/S), Shepody/Dakota Trailblazer (S/D), Dakota Trailblazer/Dakota Trailblazer (D/D) and Dakota Trailblazer/Shepody (D/S). Sprouts from disease-free mini-tubers or tuber buds (2 - 4 g) were surface sterilized by immersion in 80% ethanol for 30 s followed by 0.5% sodium chloride for 15 min and rinsed three times with sterile reverse osmosis water (Curry and Cassells 1999). The mini-tubers or tuber sections were placed into black plastic cups (350 ml) with drain holes in the bottom containing 400 g of sterilized coarse grain sand. Cups were placed into growth chambers and kept at 18/16°C (day/night) and 75% relative humidity. Soft-white fluorescent lights were

used to provide 16 hours of lighting. The tubers in the cups were irrigated by capillarity with three-fold Merz nutritive solution (Merz 1989).

Plants were grown for three weeks to a height of approximately 10 cm before grafting. Prior to grafting, large leaves and secondary shoots were removed by trimming. The apical portion of the plant was removed and the lower part was used as rootstock. Grafting was performed using the wedge (cleft) grafting technique where the scion cambium tissue was exposed by slicing off two opposite cortical stem sections. A vertical incision (approximately 2 cm) was made in the stem of the rootstock in which the "V" shaped scion was inserted. The scion/rootstock combination was held together with the aid of a grafting clip and misted periodically with reverse osmosis water. After grafting, plants were grown for an additional 10 days at 25/20°C (day/night) and watered with 2-fold nutrient solution by capillarity. During this period, all new shoots growing under the scion/rootstock scar were removed.

Inoculum source and preparation

Spongospora subterranea sporosori were obtained from lesions of heavily infected tubers of the cultivar Shepody. Tubers were washed under tap water and air dried overnight. Periderm scrapings containing sporosori from tubers were homogenized in 750 ml of reverse osmosis water in a conventional blender for five min and the slurry passed through a series of sieves (Merz 1989). Sporosori retained between 150 - 45 µm was dried overnight and kept in the dark at 4°C until use. A sporosori suspension was prepared by soaking the inoculum in 200 ml of full strength nutrient solution for 20 min.

Bioassays

Three trials were conducted in plastic boxes (30 x 20 x 10 cm) painted black on the outside and filled with 2 l of a sporosori suspension in full strength nutrient solution (250 sporosori · ml^{-1}). Sporosori were quantified by counting the number of propagules in 2 µl of nutrient solution using a hemocytometer. The prepared sporosori concentration was equivalent to 5 x 10^5 resting spores · ml^{-1} (Falloon et al. 2011). The boxes were transferred to a growth chamber in which the sporosori suspension was incubated for nine days at 15ºC in the dark (Merz et al. 2004). At the end of the incubation period, four grafted plants were added to each box and grown at 17/15°C (day/night) and 16 h of light. In the first trial (T1), one grafted plant of each scion/rootstock combination was included in each box, while in trials 2 (T2) and 3 (T3) four grafted plants of the same scion/rootstock combination were added (Table 4.1).

Table 4.1. Overview of graft treatments and treatment combinations evaluated.

Trial	Root sampling *S. subterranea* root infection	Root gall formation	Post-inoculation treatment combinations
T1	24 hours	6 weeks	All graft treatments in box
T2	24 hours	6 weeks	Same graft treatments in box
T3	24 hours	8 weeks	Same graft treatments in box

Sampling and disease evaluation

Root samples were collected at two intervals, 24 h post-inoculation (PI) for all trials, six weeks PI in trials 1 and 2 and eight weeks PI in trial 3 (Table 4.1). The number of root galls in each grafted plant were counted under a magnifying glass at 6 and 8 weeks after inoculation and expressed as number galls · g^{-1} root (d. w.). At each sampling date, the entire root system of each grafted plant was washed with tap water, freeze-dried and ground to a fine powder to obtain a homogeneous sample (Hernandez Maldonado et al. 2013).

Pathogen molecular quantification in roots

DNA extraction

Total DNA extraction from roots samples were performed using the PowerPlant Pro® extraction kit (MoBio, Carlsbad, CA). Ten milligrams (mg) of root tissue were homogenized in a FastPrep® instrument (MP Biomedicals, Santa Ana, California) at speed 5 for 30s, and then following instructions of the manufacturer. The quality and quality of DNA was assessed using a NanoDrop® 2000 spectrophotometer (NanoDrop Tech, Wilmington, DE).

Real time PCR and pathogen quantification in host tissue

The quantification of the pathogen was performed using the *S. subterranea* ITS specific primer/probe set SsTQF1 (5´- CCG GCA GACCCA AAA CC -3´) and SsTQR1 (5´- CGG GCG TCACCCTTC A-3´) and TaqMan® probe SsTQP1 (5´FAM - CAGACA ATC GCA CCC AGG TTC TCA TG-3´) (van de Graaf et al. 2003). Real-time quantitative PCR (qPCR) was performed using a Stratagene Mx3005P® qPCR System (Agilent Technologies, Santa Clara, CA). Each qPCR reaction included two µl of sample DNA and 23 µl of reaction mix containing 1x PCR buffer (Promega, Madison, WI), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM each primer, 0.1 µM of the probe and 1 unit GoTaq® DNA polymerase (Promega). Thermal conditions used for each qPCR consisted of an initial cycle at 98°C for 2 min, followed by 40 cycles of 98°C for 10s and 58°C for 45s.

The *S. subterranea* ITS gene was amplified using the SsTQF1/SsTQR1 and the resulting amplicon (63 bp) was cloned using the pGEM®-T Easy Vector System (Promega) according to manufacturer's instructions. The identity of the insert was verified by qPCR and sequencing followed by plasmid purification using the Plasmid Mini Kit (QIAGEN) according to

manufacturer's instructions and quantified spectrophotometrically. A tenfold dilution series ranging from 1 x 10^{-2} to 1 x 10^{-8} ng $\cdot \mu l^{-1}$ of plasmid standard was analyzed in triplicate and plotted against Ct values observed to construct the standard curve used to quantify the amounts of pathogen in samples. From these regressions, the assay efficiency was calculated as $E = [(10^{-1/slope}) - 1] \times 100$. The regression coefficient (r^2) and *E* for each assay was kept at $r^2 > 0.99$ and between 90 - 110% respectively. Standard curves used in each assay were compared using the heterogeneity of regression coefficients test ($\alpha = 0.05$) (Burns et al. 2005, Bustin et al. 2009). Root samples were tested in three individual assays in which triplicate reactions were included for each sample and standard concentration; a non-template reaction was included in each plate. Results were expressed as picogram (pg) *S. subterranea* DNA $\cdot g^{-1}$ root (d. w.).

Statistical analysis

Experimental design and statistical analysis

Data from trials were analyzed using SAS 9.3 (SAS Institute, Cary, NC). Trial 1 was conducted using a complete randomized block design. Trials 2 and 3 were performed using a complete randomized design with sampling. At each sampling date, a total 12 replicates (blocks) per graft treatment were evaluated in T1 and T3, and eight replicates in T2. Data on the amount of *S. subterranea* DNA at 24h, root gall formation at six and eight weeks or *S. subterranea* DNA at six and eight weeks post-inoculation, were analyzed separately for each trial. In order to normalize treatment variances, data on the number of number galls $\cdot g^{-1}$ root and pg *S. subterranea* DNA $\cdot g^{-1}$ root at 24h were log-transformed. Picograms *S. subterranea* DNA $\cdot g^{-1}$ root at six and eight weeks post-inoculation were square root-transformed. Normality of the residuals and homogeneity of variances were assessed using Wilk-Shapiro ($\alpha = 0.05$), and

Bartlett's test ($\alpha = 0.05$) respectively. ANOVA was used to analyze the data and treatment means were compared using a protected LSD or multiple t-test ($\alpha = 0.05$). Spearman's rank correlation coefficient was used to assess the degree of association among trials and amount of pathogen DNA at 24 h post-inoculation and the number of root galls · g^{-1} root.

Results

Spongospora subterranea infection of grafted potato plants

The amount of *S. subterranea* DNA per gram of root observed at 24 h post-inoculation in Trial 1 varied significantly among graft treatments ($P < 0.001$; Figure 4.1A). As a consequence, the amount of pathogen DNA detected in graft treatments involving cv. Shepody as rootstock (i.e. S/S or D/S) was significantly higher than the amount of *S. subterranea* DNA found in graft combination D/D. In addition, the quantity of pathogen DNA measured in graft treatment S/D was similar to that observed in D/D or D/S, but significantly lower than S/S. The increased amount of *S. subterranea* DNA observed in graft treatment S/S was 1.3 and 1.2 times higher than the quantity of pathogen DNA detected in D/D and S/D respectively. In contrast, no differences among graft treatments were observed for the amount of *S. subterranea* DNA per gram of root detected at 24h post-inoculation in Trial 2 ($P = 0.605$; Figure 4.1B) or Trial 3 ($P = 0.153$; Figure 4.1C).

For the graft treatments involving 'Shepody' as scion and/or rootstock, significantly higher amounts of *S. subterranea* DNA were observed in two (S/S and D/S) of nine graft combinations evaluated across all three trials (Figure 4.1A-C), suggesting no effect of 'Shepody' as scion ($P = 0.998$) on the amount of pathogen DNA detected at 24h post-inoculation. Nevertheless, the amount of pathogen DNA detected at 24h post-inoculation in Trial 1 was

significantly higher (P <0.001) than the amount of pathogen DNA found in Trials 2 and Trial 3. As a result, the amount of *S. subterranea* DNA observed in T1 was 3.9 times higher than T2 and T3.

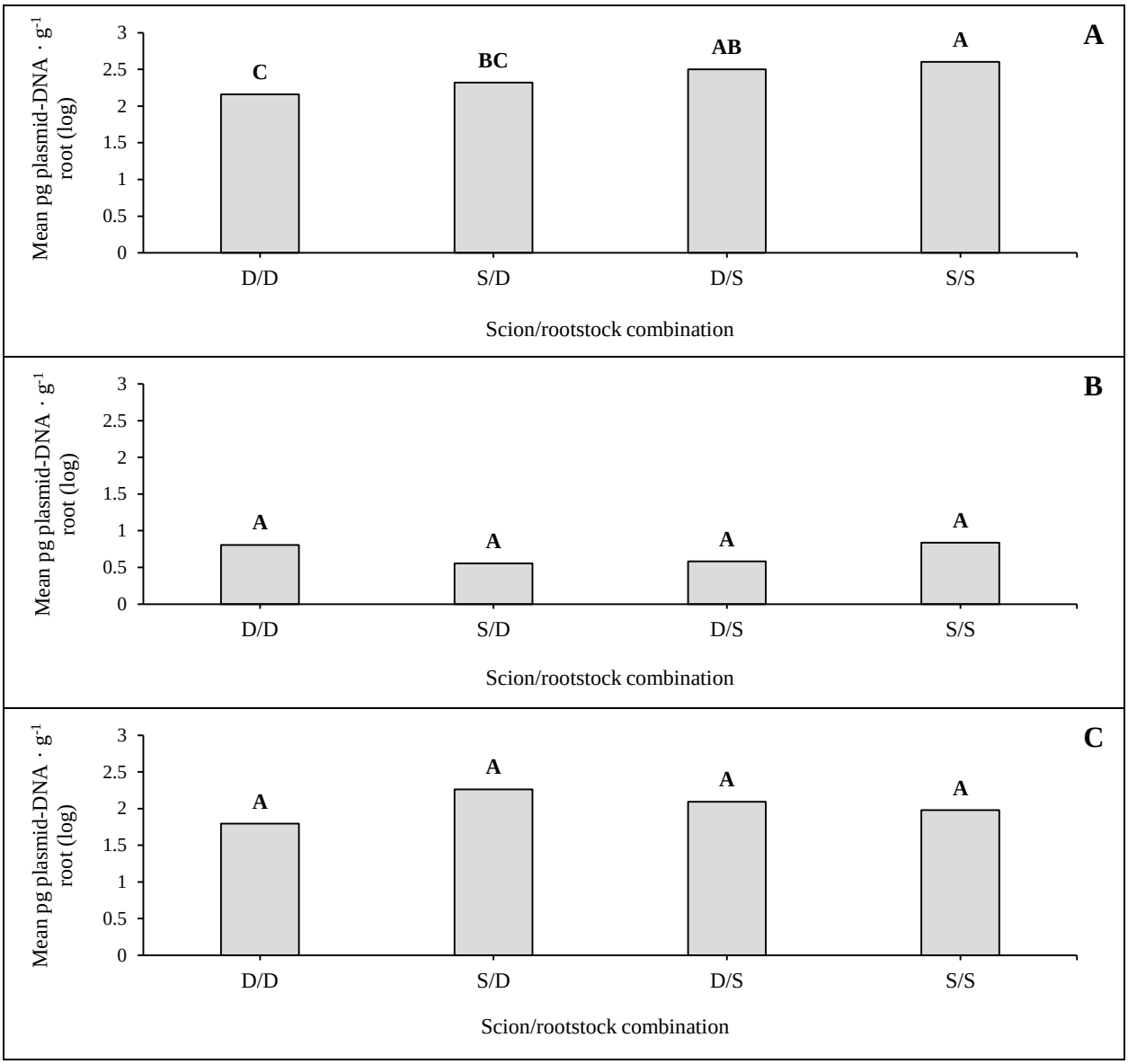

Figure 4.1. Amount of *S. subterranea* DNA at 24 hours post-inoculation in roots of grafted potato plants using 'Dakota Trailblazer' (D; resistant) and 'Shepody' (S; susceptible) as either scion and/or rootstock in Trial 1 (A), Trial 2 (B) or Trial 3 (C). Treatment means with different letters are significantly different ($LSD_{\alpha = 0.05}$).

Root gall formation in grafted potato plants

Root galls were observed in plants of each scion/rootstock combination across all three trials. In Trial 1, significant differences were observed for the number of galls · g^{-1} root developed at six weeks post-inoculation (P <0.001). Overall, the mean number of galls · g^{-1} root observed in the graft treatment D/D was significantly less the than the number found in all other graft combinations (Figure 4.2A). The highest number of galls · g^{-1} root galls was observed in graft treatment S/S followed by D/S; the latter yielded 0.6 times less number of root galls than S/S. In contrast, the number of galls observed in roots of graft treatment S/D was five times larger than D/D (Figure 4.2A). In Trial 2, the number of root galls developed at six weeks post-inoculation in graft combination D/D was significantly less than the number of root galls observed in all other graft treatments (P <0.001). However, no differences were observed among graft treatments involving 'Shepody' as rootstock and/or scion. In graft combinations including 'Shepody' as rootstock, graft treatment D/S developed 0.4 times less root galls than S/S, while in treatments with 'Dakota Trailblazer' as rootstock, S/D developed 54 times more root galls than D/D (Figure 4.2A).

Similar results were observed at 8 weeks after inoculation in trial 3. The number of galls · g^{-1} root observed in the graft treatment D/D was significantly less than disease developed in all other graft treatments (P < 0.001); however, the amount of galls observed on roots of S/S and D/S were significantly higher than the number of galls found on S/D (Figure 4.2C). For graft treatments involving 'Dakota Trailblazer' as the rootstock, the number of galls · g^{-1} that developed on roots of S/D was two times higher than the number of galls observed on D/D. Between graft treatments with 'Shepody' as rootstock, no significant differences were observed; however, the mean number of root galls developed on D/S was 0.2 times less than the number of

root galls observed on S/S (Figure 4.2C). In addition, a significantly positive correlation was observed between the number of galls · g^{-1} developed at six weeks post-inoculation in T1 and the number of root galls observed at eight week post-inoculation in T3 (r = 0.70; $P < 0.001$).

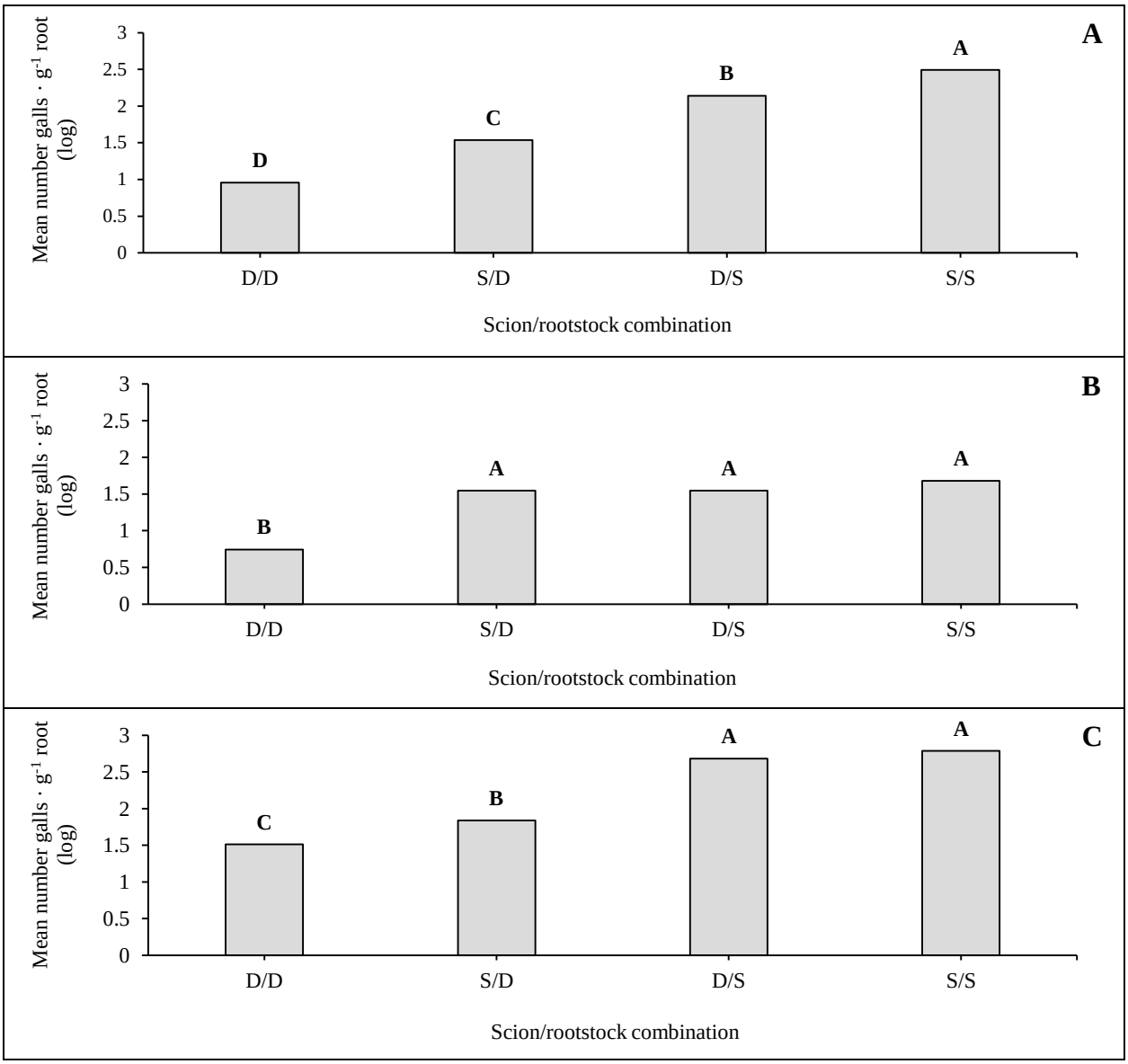

Figure 4.2. Root gall formation in grafted potato plants using 'Dakota Trailblazer' (D; resistant) and 'Shepody' (S; susceptible) as either scion and/or rootstock at six weeks post-inoculation in Trial 1 (A) and Trial 2 (B). C: root gall formation in grafted potato plants at eight weeks post-inoculation (Trial 3). Treatment means with different letters are significantly different ($LSD_{\alpha = 0.05}$).

No graft combination involving Shepody as either rootstock or scion developed higher number of galls · g^{-1} root than graft treatment S/S. However, all graft combinations involving 'Shepody' (S/S, D/S and S/D) evaluated across all three trials, consistently developed significantly higher numbers of root galls · g^{-1} root than graft treatment D/D ($P = 0.004$).

The amount of *S. subterranea* DNA detected at 24h post-inoculation was observed to be positively associated with the number of root galls observed in graft combinations across all conducted trials ($r = 0.30$; $P < 0.001$). However, among trials, this association was found to be significant only in T1 ($r = 0.38$; $P = 0.008$). Furthermore, the number of galls developed in Trial 1 was significantly higher than the number of galls observed in plants of Trials 2 or Trial 3 ($P < 0.001$).

The amount of *S. subterranea* DNA detected at six and eight weeks post-inoculation varied significantly among graft treatments in Trial 1 ($P < 0.001$), Trial 2 ($P = 0.003$) and Trial 3 ($P < 0.001$). For Trials 1 and 3, the amount of pathogen DNA observed in graft combinations involving 'Shepody' as rootstock was significantly higher than the amount of *S. subterranea* DNA detected in graft combinations S/D and D/D (Table 4.2). In Trial 3, the amount of *S. subterranea* DNA detected in graft treatment D/D was significantly lower than the amount of pathogen DNA observed in S/S and D/S (Table 4.2). The amount of pathogen DNA observed in graft combination S/D was significantly lower than S/S, but similar to that detected in D/S and D/D.

Table 4.2. Mean amount of *S. subterranea* DNA in roots of grafted potato plants at six (Trials 1 and 2) and eight weeks post-inoculation (Trial 3).

Graft (scion / rootstock) combination	**Trial 1** pg plasmid-DNA·g^{-1} root (sqrt)	**Trial 2** pg plasmid-DNA·g^{-1} root (sqrt)	**Trial 3** pg plasmid-DNA·g^{-1} root (sqrt)
Dakota Trailblazer / Dakota Trailblazer	189.30 B	165.09 C	232.88 B
Shepody / Dakota Trailblazer	203.08 B	225.92 BC	226.40 B
Dakota Trailblazer / Shepody	334.54 A	269.56 AB	413.78 A
Shepody / Shepody	330.98 A	303.93 A	374.86 A

Dakota Trailblazer (resistant), Shepody (susceptible). Treatment means in each column with different letters are significantly different ($LSD_{\alpha = 0.05}$).

Discussion

The management of powdery scab and root gall formation caused by to *S. subterranea* is difficult due in part to the ability of the pathogen to survive harsh environmental conditions in the absence of a host. Factors controlling the emergence of zoospores are poorly understood, although evidence suggests that zoospores release occurs in a staggered fashion, but spore dormancy is absent (Falloon et al. 2011; Harrison et al. 1997; Merz 1993). Germination of *S. subetarranea* resting spores has been documented occurring across a variety of conditions, but increased in the presence of root exudates of either host or non-host plants (Fornier 1997; Merz 1993). Root exudates are composed of an array of compounds such as amino acids, organic acids, polysaccharides and proteins acting as chemical attractants and repellants to microorganisms of the rhizosphere (Flores et al. 1999; Walker et al. 2003) promoting germination in a direct or indirect manner. For example, germination of *P. brassicae* resting spores is stimulated by root exudate compounds such as caffeic and coumalic acid which may be universally present in roots of host and non-host, but also by hydrolytes obtained from the enzymatic activity of *P. brassicae* on root exudate compounds which are involved in the process of host recognition by the pathogen (Feng et al. 2010; Ohi et al. 2003). Our results suggest a

selective infection by the pathogen toward roots of the susceptible cultivar Shepody, which may represent an advantageous feature for pathogen survival in the field as infection of susceptible hosts results in larger amounts of pathogen inoculum (Bouchek-Méchiche et al. 2005) required for further infection cycles and facilitating the establishment of the pathogen in the field. Furthermore, no evidence of an effect of 'Shepody' as scion was observed, as similar amounts of pathogen DNA were observed in graft treatment S/D and D/D across all performed trials.

Among plasmodiophorids, root infections are reported occurring in a broad range of plant families (Feng et al. 2010; 2013; Ludwig-Müller et al. 1999; Neuhauser et al. 2014), but the development of symptoms is restricted to a narrower number of hosts. For plasmodiophorids such as *P. brassicae*, this phenomenon has been attributed to the inability of the pathogen to overcome the basal mechanisms of resistance of non-hosts; however, in susceptible hosts, the extent of symptom development is dependent on the interaction of a number of factors required for the establishment of a *de novo* meristem and the reproduction (Feng et al. 2010; 2013; Ludwig-Müller et al. 1999). An analogous scenario has been described for *S. subterranea* for which root infections are reported in members of monocot and dicot plant families (Qu and Christ 2006; Neuhauser et al. 2014), but gall formation and production of resting spores is limited to members of the Solanaceae (Nitzan et al. 2009; Shah et al. 2010; Qu and Christ 2006). The results obtained in this research demonstrate the effect of the selected scion on the development of galls on the resistant cultivar Dakota Trailblazer, determined by an increase in the formation of root galls. These results suggest the presence of susceptibility factors in 'Shepody' contributing to the development of symptoms in 'Dakota Trailblazer'. Although a decrease in the number of galls in graft treatment D/S was observed, these differences were not significant. However, the number of root galls recorded on D/S was consistently lower than S/S,

suggesting a contribution of ‘Dakota Trailblazer’ when used as scion on the development of galls, which might be attributed to a masking effect of the susceptible rootstock ‘Shepody’. To our knowledge, no reports of research focused in determining the changes in host metabolism and defense mechanisms upon *S. subterranea* infection are available; however, it is highly likely that the mechanism involved in root gall formation in potato shares similarities to those observed in *P. brassicae* during clubroot development. Based on our results we hypothesize those pathogenicity factors involved in the formation of galls in ‘Shepody’ are transferable, resulting in a stimulus for *S. subterranea* to manipulate the host hormonal balance to its favor and cause disease. However, this hypothesis may not hold true for tubers, as additional factors may be involved (Perla et al. 2014). Future research will be required in order to elucidate the underlying mechanisms of the pathogen-root interactions.

Literature Cited

Adl, S. M., Simpson, A. G., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., et al. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of Protists. Journal of Eukaryotic Microbiology 52:399-451.

Bouchek-Méchiche, K., Montfort, F., and Andrivon, D. 2005. Assessing seed borne inoculum as a source for soil contamination by *S. subterranea* using nested PCR and real-time PCR. American Journal of Potato Research 82:59.

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

Bulman, S. R. and Braselton, J. P. 2014. Rhizaria: Phytomyxea. Pages 99-112 in: The Mycota VII, Systematics and Evolution, Part A. 2nd ed. D. J. McLaughlin and J. W. Spatafora, eds. Springer-Verlag, Berlin-Heidelberg.

Bulman, S., Candy, J. M., Fiers, M., Lister, R., Conner, A. J., and Eady, C. C. 2011. Genomics of biotrophic, plant-infecting plasmodiophorids using in vitro dual cultures. Protist 162: 449-461.

Bulman, S., Kühn, S., Marshal, J., and Schnepf, E. 2001. A phylogenetic analysis of the SSU rRNA from members of the Plasmodiophorida and Phagomyxida. Protist 152:43-51.

Burns, M. J., Nixon, G. J., Carole, A. F., and Harris, N. 2005. Standardisation of data from real-time quantitative PCR methods – evaluation of outliers and comparison of calibration curves. BMC Biotechnology 5:31.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., and Wittwer, C. T. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry 55:611-622.

Cavalier-Smith, T., and Chao, E. E. Y. 2003. Phylogeny and classification of phylum Cercozoa (Protozoa). Protist 154:341-358.

Chu, M., Song, T., Falk, K. C., Zhang, X., Liu, X., Chang, A., Lahlali, R., McGregor, L., Gossen, B. D., Yu, F., and Peng, G. 2014. Fine mapping of Rcr1 and analyses of its effect on transcriptome patterns during infection by *Plasmodiophora brassicae*. BMC Genomics 15:1166.

Curry, R. F., and Cassells, A. C. 1999. Callus initiation, maintenance, and shoot induction in potato – Monitoring of spontaneous genetic variability in vitro and in vivo. Pages 31-42 in: Plant Cell Culture Protocols. Humana Press Inc., Totowa, NJ, US.

Devos, S., Laukens, K., Deckers, P., Van Der Straeten, D., Beeckman, T., Inzé, D., Van Onckelen, H., Witters, E., and Prinsen, E. 2006. A hormone and proteome approach to picturing the initial metabolic events during *Plasmodiophora brassicae* infection on Arabidopsis. Molecular Plant-Microbe Interactions 19:1431-1443.

Falloon, R. E. 2008. Control of powdery scab of potato: towards integrated disease management. American Journal of Potato Research 85:253-260.

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

Falloon, R. E., Merz, U., Butler, R. C., Curtin, D., Lister, R. A., and Thomas, S. M. 2015. Root infection of potato by *Spongospora subterranea*: knowledge review and evidence for decreased plant productivity. Plant Pathology. DOI: 10.1111/ppa.12419.

Feng, J., Hwang, S. -F., and Strelkov, S. E. 2013. Studies into primary and secondary infection processes by *Plasmodiophora brassicae* on canola. Plant Pathology 62:177-183.

Feng, J., Hwang, R., Hwang, S. -F., Strelkov, S. E., Gossen, B. D., Zhou, Q. -X., and Peng, G. 2010. Molecular characterization of a serine protease Pro1 from *Plasmodiophora brassicae* that stimulates resting spore germination. Molecular Plant Pathology 11:503-512.

Feng, J., Xiao, Q., Hwang, S. -F., Strelkov, S. E., and Gossen, B. D. 2012. Infection of canola by secondary zoospores of *Plasmodiophora brassicae* produced on a nonhost. European Journal of Plant Pathology 132:309-315.

Flores, H. E., Vivanco, J. M., and Loyola-Vargas, V. M. 1999. 'Radicle' biochemistry: the biology of root-specific metabolism. Trends in Plant Science 4:220-226.

Fornier, N. 1997. Epidemiology of *Spongospora subterranea*, the cause of powdery scab of potatoes. University of Aberdeen. Aberdeen, UK.

Harrison, J. G., Searle, R. J., and Williams, N. A. 1997. Powdery scab disease of potato—a review. Plant Pathology 46:1-25.

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

Iftikhar, S., and Ahmad, I. 2005. Alternate hosts of *Spongospora subterranea* f. sp. *subterranea*, the causal organism of powdery scab of potato. American Journal of Potato Research 82: 74-75.

Ishibashi, N., and Zhimisu, K. 1970. Gall formation by root-knot nematode, *Meloidogyne incognita* (KOFOID et. WHITE, 1919) CHITWOOD, 1949, in the grafted tomato plants, and accumulation of phosphates on gall tissues (Nematoda: Tylenchida). Applied Entomology and Zoology 5:105-111.

Jameson, P. E. 2000. Cytokinins and auxins in plant-pathogen interactions - An overview. Plant Growth Regulation 32:369-380.

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

Ludwig-Müller, J. 2009. Plant defence - what can we learn from clubroots?. Australasian Plant Pathology 38:318-324.

Ludwig-Müller, J., Bennett, R. N., Kiddle, G., Ihmig, S., Ruppel, M., and Hilgenberg, W. 1999. The host range of *Plasmodiophora brassicae* and its relationship to endogenous glucosinolate content. New Phytologist 141:443-458.

Ludwig-Müller, J., and Schuller, A. 2008. What can we learn from clubroots: alterations in host roots and hormone homeostasis caused by *Plasmodiophora brassicae*. European Journal of Plant Pathology 121:291-302.

Ludwig-Müller, J., Prinsen, E., Rolfe, S. A., and Scholes, J. D. 2009. Metabolism and plant hormone action during clubroot disease. Journal of Plant Growth Regulation 28:229-244.

Merz, U. 1993. Epidemiological aspects of powdery scab of potatoes caused by *Spongospora subterranea*. Pages 104-106 in: Proceedings of the 2nd symposium of the international working group on plant viruses with fungal vectors. C. Hiruki, ed. Montreal, Canada.

Merz, U., and Falloon, R. E. 2009. Review: powdery scab of potato—increased knowledge of pathogen biology and disease epidemiology for effective disease management. Potato Research 52:17-37.

Merz, U., Martinez, V., and Schwaerzel, R. 2004. The potential for the rapid screening of potato cultivars (*Solanum tuberosum*) for resistance to powdery scab (*Spongospora subterranea*) using a laboratory bioassay. European Journal of Plant Pathology 110:71-77.

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

Neuhauser, S., Kirchmair, M., and Gleason, F. H. 2011. The ecological potentials of Phytomyxea ("plasmodiophorids") in aquatic food webs. Hydrobiologia 659:23-35.

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

Ohi, M., Kitamura, T., and Hata, S. 2003. Stimulation by caffeic acid, coumalic acid and corilagin of germination of resting spores of the clubroot pathogen *Plasmodiophora brassicae*. Bioscience, Biotechnology, and Biochemistry 67:170-173.

Perla, V., Jayanty, S. S., Holm, D. G., and Davidson, R. D. 2014. Relationship between tuber storage proteins and tuber powdery scab resistance in potato. American Journal of Potato Research 91:233-245.

Qu, X. S., and Christ, B. J. 2004. Genetic variation and phylogeny of *Spongospora subterranea* f. sp. *subterranea* based on ribosomal DNA sequence analysis. American Journal of Potato Research 81:385-394.

Qu, X. S., and Christ, B. J. 2006. The host range of *Spongospora subterranea* f. sp. *subterranea* in the United States. American Journal of Potato Research 83:343-8.

Schwelm, A., Fogelqvist, J., Knaust, A., Jülke, S., Lilja, T., Bonilla-Rosso, G., Karlsson, M., Shevchenko, A., Dhandapani, V., Choi, S. R., Kim, H. G., Park, J. Y., Lim, Y. P., Ludwig-Müller, J., and Dixelius, C. 2015. The *Plasmodiophora brassicae* genome reveals insights in its life cycle and ancestry of chitin synthases. Scientific Reports, 5. Art. 11153.

Shah, F. A., Falloon, R. E., Bulman, S. R. 2010. Nightshade weeds (*Solanum* spp.) confirmed as hosts of the potato pathogens *Meloidogyne fallax* and *Spongospora subterranea* f. sp. *subterranea*. Australasian Plant Pathology 39:492-8.

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

Walker, T. S., Bais, H. P., Grotewold, E., and Vivanco, J. M. 2003. Root exudation and rhizosphere biology. Plant Physiology 132:44-51.

CHAPTER V. GENERAL CONCLUSIONS

Spongospora subterranea (Wall.) Lagerh. is an obligate soil- and tuber-borne pathogen that causes powdery scab on tubers and root gall formation in potato (*Solanum tuberosum* L.). The disease affects directly the quality of tubers intended for fresh, processing and seed markets, and disease on roots have been associated with yield reduction (Harrison et al. 1997; Nitzan et al. 2008). Lesions on tubers and roots bear masses of pathogen resting spores arranged in aggregates (sporosori; syn. cystosori, sporeball) which contribute to inoculum built-up and pathogen spread to new locations (Brierley et al. 2013; Merz and Falloon 2009). Furthermore, *S. subterranea* is the vector of *Potato mop-top virus*, which causes economic losses by inducing necrotic symptoms in the flesh of tubers (Davey et al. 2014; Domfeh et al. 2015), representing an emerging disease in the US. *Spongospora subterranea* causes disease in cool weather regions as well as in warm climate areas where potato is grown under irrigation or at high altitudes (de Boer et al. 1985; Harrison et al. 1997). In the US, *S. subterranea* was first recorded in 1913 on potato shipments imported from Europe and Canada (Güssow 1913), and is now reported in many potato growing regions of the US including Colorado, Idaho, Maine, North Dakota and Washington (Draper et al. 1997; Melhus 1914).

The control of powdery scab and root gall formation is difficult partly due to the biphasic nature of the pathogen that produces long-lived resting spores (>10 years) resistant to a number of environmental stresses (Brierley et al. 2013; Merz and Falloon 2009). In the host, *S. subterranea* goes through multiple infection and re-infection cycles that increases the amount of short-lived inoculum available to cause infection; however, larger amounts of pathogen inoculum in soil are likely to increase the chances for the pathogen to infect and cause disease (Brierley et al. 2013; Harrison et al. 1997). Available approaches for disease management are limited, so one

approach is to reduce the expression of symptoms in tubers and roots. However, most commercial cultivars are susceptible in some degree to disease on tubers or roots. Therefore, the management of the disease requires of the integration of multiple management methods and evaluation of measures of control that can be easily adopted into cropping systems.

The main objective of this dissertation was to evaluate approaches for the control of powdery scab and root gall formation, using as main strategies the identification of potato genotypes with resistance to disease on tubers and roots, and reduction of pathogen initial inoculum. Chapter One focuses on disease management through cultivar resistance, and Chapter Two focuses on elimination of initial inoculum by soil fumigation with chloropicrin. Chapter Three investigates the involvement of unknown host factors on root infection and development of root galls caused by *S. subterranea* by reciprocal grafting of susceptible and resistant potato cultivars.

In the first research chapter, a total of 30 potato cultivars and 83 advanced clones with varying skin-types (market class) were evaluated for susceptibility to powdery scab on tubers and root galls under field conditions in Minnesota and North Dakota. Variability in the degree of susceptibility to powdery scab and root gall formation was observed among potato genotypes. Resistance to powdery scab and root gall formation followed a continuum from very resistant to very susceptible. About 32% of the evaluated genotypes were ranked as very resistant to disease on tubers and roots. Most resistant genotypes were russet-skinned, but high levels of resistance were observed among some red-, white- and yellow-skinned genotypes. The expression of powdery scab and root gall formation was influenced by the environment; nevertheless, the genotypes ranked as resistant had the lowest variability in disease expression across environments.

In the second chapter, the effect of chloropicrin soil fumigations on the soil populations of *S. subterranea* and the subsequent development of powdery scab, root gall formation and tuber yield were investigated in a series of field trials conducted in Minnesota and North Dakota. Chloropicrin soil fumigation at rates ranging from 70.1 to 201.8 kg a.i. · ha^{-1} significantly reduced the amount of *S. subterranea* inoculum at post-fumigation compared to the non-treated control. However, the amount of powdery scab and root gall formation observed on susceptible cultivars increased with fumigant rate. These results were confirmed in controlled condition experiments, in which the amount of *S. subterranea* DNA detected in roots of cultivars 'Shepody' (susceptible to powdery scab and root gall formation) and 'Dakota Trailblazer' (resistant) increased significantly with chloropicrin rate. In contrast, similar amounts of pathogen DNA were observed between both potato cultivars. Chloropicrin fumigation significantly increase tuber yield in most environments, and in cultivars such as Umatilla Russet and Shepody, a significant positive association between the amount of root galls and tuber yield was observed. Factors other than environmental conditions and host resistance are suggested to be involved in powdery scab and root gall formation epidemics.

In the third chapter, the potential effect of unknown host factors on the infection and development of root galls by *S. subterranea* was investigated using reciprocal grafting between the powdery scab susceptible cultivar Shepody and powdery scab resistant cultivar Dakota Trailblazer. Scion/rootstock grafting combinations were prepared between the potato cultivars and evaluated over three experiments. Higher number of root galls per plant were observed in graft combination involving 'Shepody' as scion and/or rootstock compared to graft combinations using 'Dakota Trailblazer' as scion and rootstock. In addition, the amount of *S. subterranea* DNA detected at 24 hours post-inoculation was found to be significantly associated to the

number of root galls observed at six and eight weeks post-inoculation. Results suggest the translocation of unknown factors present in ‘Shepody’ involved in the host response to the development of root symptoms by *S. subterranea*. Further work is necessary to identify putative factors.

Host selection is highly recommended for disease management, and further research will be required in order to assist breeding programs in the selection and development of genotypes conferring resistance to powdery scab and root gall formation.

Literature Cited

Brierley, J. L., Sullivan, L., Wale, S. J., Hilton, A. J., Kiezebrink, D. T., and Lees, A. K. 2013. Relationship between Spongospora subterranea f. sp. subterranea soil inoculum level, host resistance and powdery scab on potato tubers in the field. Plant Pathology 62:413-420.

Davey, T., Carnegie, S. F., Saddler, G. S., and Mitchell, W. J. 2014. The importance of the infected seed tuber and soil inoculum in transmitting Potato mop-top virus to potato plants. Plant Pathology 63:88-97.

de Boer, R. F., Taylor, P. A., Flett, S. P., and Merriman, P. R. 1985. Effects of soil temperature, moisture and timing of irrigation on powdery scab of potatoes. Pages 197-198 in: Ecology and management of soilborne plant pathogens. Proceedings of section five of the fourth International Congress of Plant Pathology. C. A. Parker, A. D. Rovira, K. J. Moore, and P. T. W. Wong, eds. Melbourne, Australia.

Domfeh, O., Bittara, F. G., and Gudmestad, N. C. 2015. Sensitivity of potato to Potato mop-top virus-induced tuber necrosis. Plant Disease 99:788-796.

Draper, M. A., Secor, G. A., and Gudmestad, N. C. 1997. First report of potato powdery scab, caused by Spongospora subterranea f. sp. subterranea, in North Dakota. Plant Disease 81:693.

Güssow, H. T. 1913. Powdery scab of potatoes, Spongospora subterranea (Wallr.) Johns. Phytopathology 3:18-19.

Harrison, J. G., Searle, R. J., and Williams, N. A. 1997. Powdery scab disease of potato - a review. Plant Pathology 46:1-25.

Melhus, I. E. 1914. Powdery scab (Spongospora subterranea) of potatoes. Bulletin of the U. S. Department of Agriculture, No. 82. Pp. 1-16.

Merz, U., and Falloon, R. E. 2009. Review: Powdery scab of potato - Increased knowledge of pathogen biology and disease epidemiology for effective disease management. Potato Research 52:17-37.

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

APPENDIX

Table A1. Mean (rank) powdery scab incidence, severity and maximum severity on potato genotypes evaluated at Larimore, ND during 2011.

Potato cultivar or advanced clone	Incidence	Group	Potato cultivar or advanced clone	Severity	Group	Potato cultivar or advanced clone	Maximum severity	Group
ND049381C-2Russ	26.50	A	ND060742C-1Russ	27.50	A	ND049381C-2Russ	26.50	A
Ranger Russet	25.50	A	ND049381C-2Russ	26.50	A	Ranger Russet	25.50	A
ND6400C-1Russ	25.33	A	Ranger Russet	25.33	A	ND6400C-1Russ	25.33	A
ND060742C-1Russ	24.33	A	ND060770B-5Russ	23.83	AB	ND060742C-1Russ	24.33	A
ND039194-1Russ	24.33	A	ND039194-1Russ	23.83	AB	ND039194-1Russ	24.33	A
Dakota Trailblazer	22.83	AB	ND060796AB-1Russ	22.00	AB	Dakota Trailblazer	22.83	AB
ND060770B-5Russ	22.67	AB	ND6400C-1Russ	22.00	AB	ND060770B-5Russ	22.67	AB
Russet Norkotah	21.67	AB	Russet Norkotah	22.00	AB	Russet Norkotah	21.67	AB
ND060796AB-1Russ	20.67	AB	Dakota Trailblazer	20.83	AB	ND060796AB-1Russ	20.67	AB
Shepody	18.50	AB	ND049517B-1Russ	18.33	AB	Shepody	18.50	AB
AND01804-3Russ	18.17	AB	AOND95292-3Russ	18.17	AB	AND01804-3Russ	18.17	AB
ND049546b-10Russ	17.83	AB	ND070927-2Russ	18.17	AB	ND049546b-10Russ	17.83	AB
AOND95292-3Russ	17.83	AB	AND01804-3Russ	18.17	AB	AOND95292-3Russ	17.83	AB
ND049517B-1Russ	17.50	AB	ND050105C-1Russ	17.33	AB	ND049517B-1Russ	17.50	AB
ND050105C-1Russ	17.17	AB	ND049423b-1Russ	17.33	AB	ND050105C-1Russ	17.17	AB
ND049423b-1Russ	17.00	AB	ND049546b-10Russ	16.50	AB	ND049423b-1Russ	17.00	AB
ND070927-2Russ	16.83	AB	ND060735-3Russ	16.50	AB	ND070927-2Russ	16.83	AB
ND8413-7Russ	16.50	AB	Umatilla Russet	16.17	AB	ND8413-7Russ	16.50	AB
ND060735-3Russ	16.50	AB	Shepody	16.17	AB	ND060735-3Russ	16.50	AB
Umatilla Russet	15.83	AB	AND00618-1RussY	16.17	AB	Umatilla Russet	15.83	AB
AND00618-1RussY	15.50	AB	ND8413-7Russ	16.17	AB	AND00618-1RussY	15.50	AB
Russet Burbank	11.83	B	Russet Burbank	11.83	B	Russet Burbank	11.83	B
Alpine Russet	11.83	B	Alpine Russet	11.83	B	Alpine Russet	11.83	B
Bannock Russet	11.83	B	Bannock Russet	11.83	B	Bannock Russet	11.83	B
ND8068-5Russ	11.83	B	ND8068-5Russ	11.83	B	ND8068-5Russ	11.83	B
ND8229-3	11.83	B	ND8229-3	11.83	B	ND8229-3	11.83	B
AND99362-1Russ	11.83	B	AND99362-1Russ	11.83	B	AND99362-1Russ	11.83	B
AND95279-5Russ	11.83	B	AND95279-5Russ	11.83	B	AND95279-5Russ	11.83	B
ND060761B-3Russ	11.83	B	ND060761B-3Russ	11.83	B	ND060761B-3Russ	11.83	B
ND060766b-4Russ	11.83	B	ND060766b-4Russ	11.83	B	ND060766b-4Russ	11.83	B
ND050082Cb-2Russ	11.83	B	ND050082Cb-2Russ	11.83	B	ND050082Cb-2Russ	11.83	B
ND059769Ab-1Russ	11.83	B	ND059769Ab-1Russ	11.83	B	ND059769Ab-1Russ	11.83	B
ND049289-1Russ	11.83	B	ND049289-1Russ	11.83	B	ND049289-1Russ	11.83	B
P-value	0.212		*P*-value	0.212		*P*-value	0.170	
$LSD_{(0.05)}$	12.338		$LSD_{(0.05)}$	12.338		$LSD_{(0.05)}$	12.151	

Table A2. Mean (rank) powdery scab incidence, severity and maximum severity on potato genotypes evaluated at McCanna, ND during 2011.

Potato cultivar or advanced clone	Inc.	Group	Potato cultivar or advanced clone	Sev.	Group	Potato cultivar or advanced clone	Max. Sev.	Group
ND6956b-13	87.33	A	AND00272-1R	85.83	A	ND6956b-13	86.67	A
ND6400C-1Russ	84.17	AB	ATND98459-1RY	83.50	AB	ND6400C-1Russ	85.00	AB
Kennebec	83.33	AB	ND8307C-3	82.33	ABC	AND00272-1R	84.67	AB
ND8307C-3	82.00	ABC	ND6400C-1Russ	80.67	ABCD	T10-12	83.83	AB
AND00272-1R	82.00	ABC	ND6956b-13	78.67	ABCDE	Kennebec	83.00	ABC
T10-12	81.83	ABC	Kennebec	78.33	ABCDEF	ND8307C-3	82.83	ABC
ATND98459-1RY	81.33	ABC	T10-12	77.17	ABCDEFG	ATND98459-1RY	82.00	ABC
ND060728-5R	76.33	ABCD	ND060728-5R	76.17	ABCDEFGH	ND060728-5R	76.67	ABCD
R 91007-5	76.33	ABCD	Red LaSoda	75.33	ABCDEFGH	ND8331Cb-2	74.50	ABCDE
ND8331Cb-2	75.17	ABCDE	Viking	72.83	ABCDEFGHI	Viking	74.00	ABCDE
R 89045-35	75.00	ABCDE	ND8331Cb-3	71.83	ABCDEFGHI	R 89045-35	73.33	ABCDEF
RA 89044-45	72.83	ABCDEF	ND8555-8R	71.33	ABCDEFGHI	R 91007-5	72.67	ABCDEFG
ND8331Cb-3	72.67	ABCDEF	ND8314-1R	71.17	ABCDEFGHIJ	ND8331Cb-3	72.00	ABCDEFG
NY-139	71.00	ABCDEFG	ND8331Cb-2	70.83	ABCDEFGHIJ	ND028842b-1RY	71.33	ABCDEFG
Shepody	69.83	ABCDEFGH	ND028842b-1RY	70.50	ABCDEFGHIJ	Red LaSoda	70.00	ABCDEFG
ND028842b-1RY	69.33	ABCDEFGH	RA 89044-45	68.67	ABCDEFGHIJK	NY-139	69.67	ABCDEFGH
Viking	68.67	ABCDEFGHI	R 89045-35	68.67	ABCDEFGHIJK	Shepody	69.50	ABCDEFGH
ND7519-1	68.50	ABCDEFGHI	Shepody	68.50	ABCDEFGHIJK	RA 89044-45	69.17	ABCDEFGH
Red LaSoda	67.83	ABCDEFGHI	RA 90213-60	67.17	ABCDEFGHIJK	ND8314-1R	69.00	ABCDEFGH
ND8314-1R	67.67	ABCDEFGHIJ	R 91007-5	66.33	ABCDEFGHIJKL	ND8555-8R	68.67	ABCDEFGH
ND8555-8R	65.67	ABCDEFGHIJK	NY-139	65.50	ABCDEFGHIJKLM	ND7519-1	64.50	BCDEFGHI
RA 519-50	63.83	BCDEFGHIJKL	RA 519-50	65.17	ABCDEFGHIJKLM	RA 519-50	63.83	BCDEFGHIJ
Ivory Crisp	63.00	BCDEFGHIJKL	ND060847CB-1	61.67	BCDEFGHIJKLMN	Lamoka	61.00	CDEFGHIJK

Table A2. Mean (rank) powdery scab incidence, severity and maximum severity on potato genotypes evaluated at McCanna, ND during 2011 (continued).

Potato cultivar or advanced clone	Inc.	Group	Potato cultivar or advanced clone	Sev.	Group	Potato cultivar or advanced clone	Max. Sev.	Group
Lamoka	61.00	CDEFGHIJKLM	ND4659-5R	60.00	BCDEFGHIJKLMNO	Ivory Crisp	59.17	DEFGHIJKL
ND4659-5R	58.33	DEFGHIJKLMN	ND060742C-1Russ	59.67	CDEFGHIJKLMNO	ND4659-5R	57.33	DEFGHIJKL
ND060847CB-1	57.67	DEFGHIJKLMN	Dakota Jewel	59.50	CDEFGHIJKLMNO	ND060847CB-1	57.00	DEFGHIJKL
RA 16-5	56.33	DEFGHIJKLMN	Lamoka	59.17	CDEFGHIJKLMNO	ND050167C-3R	56.67	DEFGHIJKL
R 87009-28	56.33	DEFGHIJKLMN	R90134-6	57.83	DEFGHIJKLMNO	R 87009-28	55.67	DEFGHIJKL
ND060742C-1Russ	55.33	DEFGHIJKLMN	RA 517-123	57.50	DEFGHIJKLMNOP	RA 517-123	54.33	EFGHIJKLM
RA 517-123	54.67	DEFGHIJKLMNO	R 91129-11	57.17	DEFGHIJKLMNOPQ	RA 16-5	53.83	EFGHIJKLM
ND050167C-3R	54.50	DEFGHIJKLMNO	ND050167C-3R	56.50	EFGHIJKLMNOPQ	ND8304-2	53.67	EFGHIJKLM
ND060835C-4	54.00	EFGHIJKLMNO	ND060835C-4	56.33	EFGHIJKLMNOPQ	Dakota Jewel	53.67	EFGHIJKLM
ND8304-2	54.00	EFGHIJKLMNO	Yukon Gold	54.83	FGHIJKLMNOPQ	RA 90213-60	53.67	EFGHIJKLM
Dakota Jewel	53.50	EFGHIJKLMNO	Ivory Crisp	54.17	GHIJKLMNOPQR	ND060835C-4	53.00	EFGHIJKLM
ND7550C-1	53.33	EFGHIJKLMNO	ND7519-1	54.00	GHIJKLMNOPQR	ND060742C-1Russ	52.83	EFGHIJKLM
R 91129-11	52.50	FGHIJKLMNO	R 87009-28	53.83	GHIJKLMNOPQR	ND7550C-1	52.67	EFGHIJKLM
Yukon Gold	51.00	FGHIJKLMNOP	ND8304-2	52.83	HIJKLMNOPQRS	Yukon Gold	51.83	FGHIJKLMN
RA 90213-60	49.33	GHIJKLMNOPQ	RA 16-5	50.00	IJKLMNOPQRST	R90134-6	51.33	FGHIJKLMN
R90134-6	48.67	HIJKLMNOPQR	ND7550C-1	47.67	JKLMNOPQRSTU	R 91129-11	50.83	GHIJKLMNO
R 90070-8	48.50	HIJKLMNOPQRS	ND059769Ab-1Russ	46.50	KLMNOPQRSTUV	R 90070-8	47.83	HIJKLMNOP
RC 06-109	47.17	IJKLMNOPQRST	R 90070-8	46.33	KLMNOPQRSTUV	RC 06-109	46.00	IJKLMNOPQ
Puren	46.83	IJKLMNOPQRST	Dark Red Norland	45.83	KLMNOPQRSTUVW	Yagana	45.17	IJKLMNOPQR
Yagana	45.83	JKLMNOPQRSTU	ND060601CAB-2	45.50	KLMNOPQRSTUVW	ND059769Ab-1Russ	44.83	IJKLMNOPQRS
ND059769Ab-1Russ	45.17	KLMNOPQRSTUV	Red Pontiac	43.33	LMNOPQRSTUVW	Puren	44.67	IJKLMNOPQRS
RA 362-54	44.83	KLMNOPQRSTUVW	RC 06-109	42.33	MNOPQRSTUVW	Red Pontiac	44.33	IJKLMNOPQRS
R 90160-5	43.83	KLMNOPQRSTUVWX	Puren	40.17	NOPQRSTUVW	R 90160-5	43.17	IJKLMNOPQRST
Red Pontiac	43.00	LMNOPQRSTUVWXY	RA 148-48	39.17	NOPQRSTUVWX	RA 362-54	42.50	IJKLMNOPQRSTU
ND060715B-15	40.67	MNOPQRSTUVWXYZ	R 90160-5	39.00	NOPQRSTUVWX	Dark Red Norland	41.83	JKLMNOPQRSTUV
ND060601CAB-2	40.00	MNOPQRSTUVWXYZ	RA 362-54	38.17	NOPQRSTUVWX	ND060601CAB-2	40.00	KLMNOPQRSTUV
RA 148-48	38.67	NOPQRSTUVWXYZ	ND060796AB-1Russ	38.00	OPQRSTUVWX	ND060715B-15	39.33	KLMNOPQRSTUV
W2717-5	38.33	NOPQRSTUVWXYZ	Yagana	38.0	OPQRSTUVWX	RA 148-48	39.33	KLMNOPQRSTUV
Dark Red Norland	38.17	NOPQRSTUVWXYZ	ND060715B-15	37.50	OPQRSTUVWX	W2717-5	37.33	LMNOPQRSTUVW
Nicolet	33.17	OPQRSTUVWXYZa	RA 20-6	37.50	OPQRSTUVWX	ND060796AB-1Russ	32.33	MNOPQRSTUVW
ND060796AB-1Russ	32.83	OPQRSTUVWXYZa	Red Norland	34.00	PQRSTUVWX	Nicolet	32.50	MNOPQRSTUVW
CO 95051-7W	30.17	PQRSTUVWXYZa	ND8068-5Russ	34.00	PQRSTUVWX	RA 20-6	30.50	NOPQRSTUVW
Red Norland	29.83	PQRSTUVWXYZa	W2717-5	33.83	QRSTUVWX	CO 95051-7W	30.17	NOPQRSTUVW
ND8413-7Russ	29.33	PQRSTUVWXYZa	CO 95051-7W	30.67	RSTUVWX	Red Norland	29.83	NOPQRSTUVW
ND060733b-4RY	27.50	QRSTUVWXYZa	R90213-6	29.83	STUVWX	ND8413-7Russ	29.17	OPQRSTUVW
RA 151-24	27.00	RSTUVWXYZa	Nicolet	29.67	STUVWX	ND8068-5Russ	28.83	OPQRSTUVW
ND050105C-1Russ	26.67	STUVWXYZa	ND060733b-4RY	29.67	STUVWX	ND060733b-4RY	27.50	PQRSTUVW
RC 72-35	26.67	STUVWXYZa	ND8413-7Russ	27.67	TUVWX	ND050105C-1Russ	26.67	PQRSTUVW
RA 20-6	26.17	TUVWXYZa	Snowden	26.50	TUVWX	RC 72-35	26.67	PQRSTUVW
R90213-6	26.17	TUVWXYZa	Patagonia	26.50	TUVWX	RA 151-24	26.33	PQRSTUVW
ND8068-5Russ	25.67	TUVWXYZa	RA 151-24	26.50	TUVWX	R90213-6	25.67	QRSTUVW
ND049289-1Russ	24.83	UVWXYZa	Karu	24.33	UVWX	ND049289-1Russ	24.83	QRSTUVW
Karu	24.67	UVWXYZa	ND050105C-1Russ	23.33	VWX	Karu	24.67	QRSTUVW
MSL-292A	23.50	VWXYZa	ND049289-1Russ	23.33	VWX	MSL-292A	23.50	RSTUVW
ND8559-20	23.17	WXYZa	ND8058-11R	23.33	VWX	ND8559-20	23.17	RSTUVW
ND8058-11R	22.83	XYZa	RC 72-35	23.33	VWX	ND8058-11R	22.83	STUVW
Patagonia	21.50	YZa	MSL-292A	22.33	WX	Patagonia	21.50	TUVW
NY-138	21.00	Za	NY-138	22.33	WX	NY-138	21.00	UVW
Snowden	20.17	Za	ND8559-20	22.33	WX	Snowden	20.17	VW
Russet Burbank	16.00	a	Russet Burbank	16.00	X	Russet Burbank	16.00	W
Russet Norkotah	16.00	a	Russet Norkotah	16.00	X	Russet Norkotah	16.00	W
Ranger Russet	16.00	a	Ranger Russet	16.00	X	Ranger Russet	16.00	W
Umatilla Russet	16.00	a	Umatilla Russet	16.00	X	Umatilla Russet	16.00	W
Alpine Russet	16.00	a	Alpine Russet	16.00	X	Alpine Russet	16.00	W
Bannock Russet	16.00	a	Bannock Russet	16.00	X	Bannock Russet	16.00	W
Dakota Trailblazer	16.00	a	Dakota Trailblazer	16.00	X	Dakota Trailblazer	16.00	W
ND060766b-4Russ	16.00	a	ND060766b-4Russ	16.00	X	ND060766b-4Russ	16.00	W
ND050082Cb-2Russ	16.00	a	ND050082Cb-2Russ	16.00	X	ND050082Cb-2Russ	16.00	W
AND01804-3Russ	16.00	a	AND01804-3Russ	16.00	X	AND01804-3Russ	16.00	W
ND049546b-10Russ	16.00	a	ND049546b-10Russ	16.00	X	ND049546b-10Russ	16.00	W
ND049423b-1Russ	16.00	a	ND049423b-1Russ	16.00	X	ND049423b-1Russ	16.00	W
ND8229-3	16.00	a	ND8229-3	16.00	X	ND8229-3	16.00	W
RA 82-4	16.00	a	RA 82-4	16.00	X	RA 82-4	16.00	W
SPA 161	16.00	a	SPA 161	16.00	X	SPA 161	16.00	W
R65A-70	16.00	a	R65A-70	16.00	X	R65A-70	16.00	W
P-value		<0.001	*P*-value		<0.001	*P*-value		<0.001
$LSD_{(0.05)}$		21.956	$LSD_{(0.05)}$		22.052	$LSD_{(0.05)}$		23.578

Inc.: incidence; Sev.: severity; Max. Sev.: maximum severity.

Table A3. Mean (rank) powdery scab incidence, severity and maximum severity on potato genotypes evaluated at Perham, MN during 2011.

Potato cultivar or advanced clone	Incidence	Group	Potato cultivar or advanced clone	Severity	Group	Potato cultivar or advanced clone	Maximum severity	Group
Shepody	75.00	A	Shepody	74.67	A	ND7519-1	74.83	A
Viking	72.33	AB	Red LaSoda	72.67	AB	Red LaSoda	72.83	AB
Red LaSoda	71.33	ABC	ND7519-1	72.00	AB	ATND98459-1RY	71.67	ABC
Kennebec	69.17	ABCD	Viking	71.33	AB	Kennebec	71.00	ABCD
ND7519-1	69.00	ABCD	Kennebec	70.67	ABC	Shepody	69.83	ABCD
ND028842b-1RY	68.00	ABCD	ND028842b-1RY	67.67	ABCD	ND028842b-1RY	69.00	ABCD
Nicolet	66.00	ABCD	ATND98459-1RY	66.33	ABCD	Nicolet	65.83	ABCD
ATND98459-1RY	65.67	ABCD	Nicolet	65.67	ABCD	Viking	65.50	ABCD
Ivory Crisp	60.33	ABCDE	Ivory Crisp	60.00	ABCDE	Ivory Crisp	58.17	ABCDE
Lamoka	56.33	ABCDEF	Lamoka	56.33	ABCDEF	Lamoka	57.50	ABCDE
ND060835C-4	56.33	ABCDEF	ND060835C-4	56.00	ABCDEF	NY-139	54.17	ABCDE
ND050167C-3R	56.33	ABCDEF	ND050167C-3R	55.50	ABCDEF	R 87009-28	54.00	ABCDE
R90134-6	55.50	ABCDEF	R90134-6	55.50	ABCDEF	ND050167C-3R	53.67	BCDE
RA 89044-45	54.83	ABCDEF	RA 89044-45	54.50	ABCDEF	R90134-6	52.83	BCDE
NY-139	51.83	BCDEF	NY-139	51.83	BCDEF	ND060835C-4	52.50	BCDE
R 87009-28	50.67	CDEF	R 87009-28	50.33	CDEF	AND00272-1R	52.50	BCDE
ND4659-5R	49.50	DEFG	AND00272-1R	49.17	DEFG	RA 16-5	51.50	CDE
AND00272-1R	49.17	DEFG	ND4659-5R	49.17	DEFG	RA 89044-45	50.67	DE
RA 16-5	48.83	DEFG	RA 16-5	48.83	DEFG	ND4659-5R	50.17	DE
Puren	43.00	EFG	Puren	43.00	EFG	Puren	43.50	EF
Bannock Russet	42.33	EFG	Bannock Russet	42.33	EFG	NY-138	42.67	EF
NY-138	42.17	EFG	NY-138	42.17	EFG	MSL-292A	42.33	EF
ND060728-5R	41.83	EFG	ND060601CAB-2	41.83	EFG	Red Pontiac	41.17	EF
ND060601CAB-2	41.83	EFG	ND060728-5R	41.83	EFG	ND060601CAB-2	40.17	EF
ND060847CB-1	41.50	EFG	ND060847CB-1	41.50	EFG	ND060715B-15	40.17	EF
ND8307C-3	40.83	EFG	ND8307C-3	40.83	EFG	ND8331Cb-3	40.17	EF
ND060715B-15	40.67	EFG	ND060715B-15	40.67	EFG	Bannock Russet	39.67	EF
R 91007-5	40.50	EFG	R 91007-5	40.17	EFG	Snowden	39.67	EF
Red Pontiac	40.17	EFG	RA 82-4	40.00	EFG	ND060728-5R	39.67	EF
RA 82-4	40.00	EFG	Red Pontiac	39.83	EFG	R 91129-11	39.67	EF
RA 519-50	39.67	EFG	RA 519-50	39.33	EFG	RA 82-4	39.67	EF
ND8331Cb-3	38.50	FG	ND8331Cb-3	38.50	FG	ND060847CB-1	38.83	EF
Snowden	38.33	FG	Snowden	38.33	FG	ND8307C-3	38.83	EF
MSL-292A	38.33	FG	MSL-292A	38.00	FG	R 91007-5	38.83	EF
Yagana	38.33	FG	Yagana	38.00	FG	RA 519-50	38.83	EF
R 89045-35	37.83	FG	RC 72-35	37.67	FG	Yagana	38.83	EF
RC 72-35	37.67	FG	R 89045-35	37.50	FG	R 89045-35	38.83	EF
Russet Burbank	28.33	G	Russet Burbank	28.33	G	Russet Burbank	28.33	F
Russet Norkotah	28.33	G	Russet Norkotah	28.33	G	Russet Norkotah	28.33	F
Ranger Russet	28.33	G	Ranger Russet	28.33	G	Ranger Russet	28.33	F
Umatilla Russet	28.33	G	Umatilla Russet	28.33	G	Umatilla Russet	28.33	F
Alpine Russet	28.33	G	Alpine Russet	28.33	G	Alpine Russet	28.33	F
Dakota Trailblazer	28.33	G	Dakota Trailblazer	28.33	G	Dakota Trailblazer	28.33	F
Yukon Gold	28.33	G	Yukon Gold	28.33	G	Yukon Gold	28.33	F
Red Norland	28.33	G	Red Norland	28.33	G	Red Norland	28.33	F
Dark Red Norland	28.33	G	Dark Red Norland	28.33	G	Dark Red Norland	28.33	F
CO 95051-7W	28.33	G	CO 95051-7W	28.33	G	CO 95051-7W	28.33	F
W2717-5	28.33	G	W2717-5	28.33	G	W2717-5	28.33	F
ND7550C-1	28.33	G	ND7550C-1	28.33	G	ND7550C-1	28.33	F
ND060733b-4RY	28.33	G	ND060733b-4RY	28.33	G	ND060733b-4RY	28.33	F
ND049546b-10Russ	28.33	G	ND049546b-10Russ	28.33	G	ND049546b-10Russ	28.33	F
ND8555-8R	28.33	G	ND8555-8R	28.33	G	ND8555-8R	28.33	F
ND8559-20	28.33	G	ND8559-20	28.33	G	ND8559-20	28.33	F
ND8304-2	28.33	G	ND8304-2	28.33	G	ND8304-2	28.33	F
ND8314-1R	28.33	G	ND8314-1R	28.33	G	ND8314-1R	28.33	F
ND8331Cb-2	28.33	G	ND8331Cb-2	28.33	G	ND8331Cb-2	28.33	F
ND8068-5Russ	28.33	G	ND8068-5Russ	28.33	G	ND8068-5Russ	28.33	F
ND8229-3	28.33	G	ND8229-3	28.33	G	ND8229-3	28.33	F
ND6956b-13	28.33	G	ND6956b-13	28.33	G	ND6956b-13	28.33	F
ND8413-7Russ	28.33	G	ND8413-7Russ	28.33	G	ND8413-7Russ	28.33	F
Dakota Jewel	28.33	G	Dakota Jewel	28.33	G	Dakota Jewel	28.33	F
R 90070-8	28.33	G	R 90070-8	28.33	G	RC 72-35	28.33	F
R 91129-11	28.33	G	R 91129-11	28.33	G	R 90070-8	28.33	F
R 90160-5	28.33	G	R 90160-5	28.33	G	R 90160-5	28.33	F
RC 06-109	28.33	G	RC 06-109	28.33	G	RC 06-109	28.33	F
RA 517-123	28.33	G	RA 517-123	28.33	G	RA 517-123	28.33	F
Patagonia	28.33	G	Patagonia	28.33	G	Patagonia	28.33	F
RA 362-54	28.33	G	RA 362-54	28.33	G	RA 362-54	28.33	F
RA 20-6	28.33	G	RA 20-6	28.33	G	RA 20-6	28.33	F
RA 90213-60	28.33	G	RA 90213-60	28.33	G	RA 90213-60	28.33	F
Karu	28.33	G	Karu	28.33	G	Karu	28.33	F

Table A3. Mean (rank) powdery scab incidence, severity and maximum severity on potato genotypes evaluated at Perham, MN during 2011 (continued).

Potato cultivar or advanced clone	Incidence	Group	Potato cultivar or advanced clone	Severity	Group	Potato cultivar or advanced clone	Maximum severity	Group
SPA 161	28.33	G	SPA 161	28.33	G	SPA 161	28.33	F
RA 148-48	28.33	G	RA 148-48	28.33	G	RA 148-48	28.33	F
RA 151-24	28.33	G	RA 151-24	28.33	G	RA 151-24	28.33	F
T10-12	28.33	G	T10-12	28.33	G	T10-12	28.33	F
R65A-70	28.33	G	R65A-70	28.33	G	R65A-70	28.33	F
R90213-6	28.33	G	R90213-6	28.33	G	R90213-6	28.33	F
P-value	<0.001		*P*-value	<0.001		*P*-value	<0.001	
$LSD_{(0.05)}$	21.183		$LSD_{(0.05)}$	20.942		$LSD_{(0.05)}$	20.904	

Table A4. Mean (rank) powdery scab incidence, severity and maximum severity on potato genotypes evaluated at McCanna, ND during 2012.

Potato cultivar or advanced clone	Inc.	Group	Potato cultivar or advanced clone	Sev.	Group	Potato cultivar or advanced clone	Max. Sev.	Group
Dakota Pearl	81.67	A	Kennebec	82.33	A	Kennebec	81.83	A
Kennebec	81.33	AB	Red LaSoda	81.67	AB	Red LaSoda	80.33	AB
Red LaSoda	81.17	AB	ATND98459-1RY	79.67	ABC	ATND98459-1RY	79.83	AB
Red Pontiac	78.67	ABC	Dakota Pearl	79.33	ABC	Shepody	78.67	ABC
ATND98459-1RY	78.33	ABC	Shepody	78.00	ABC	ND8555-8R	78.00	ABCD
Shepody	77.67	ABCD	Red Pontiac	78.00	ABC	Dakota Pearl	74.83	ABCDE
ND8555-8R	73.50	ABCDE	ND8555-8R	76.67	ABCD	ND8307C-3	74.67	ABCDE
ND8331Cb-2	72.67	ABCDEF	Ivory Crisp	73.00	ABCDE	Red Pontiac	71.83	ABCDEF
Ivory Crisp	71.83	ABCDEFG	ND8314-1R	70.33	ABCDEF	Ivory Crisp	71.50	ABCDEF
ND8314-1R	70.67	ABCDEFG	ND8331Cb-2	70.00	ABCDEFG	ND8314-1R	69.50	ABCDEFG
RA 89044-45	68.33	ABCDEFGH	Red Norland	69.17	ABCDEFG	Colorado Rose	69.17	ABCDEFGH
Viking	68.00	ABCDEFGH	RA 89044-45	68.67	ABCDEFG	Red Norland	68.00	ABCDEFGHI
ND6956b-13	67.83	ABCDEFGH	ND8307C-3	68.33	ABCDEFG	ND8331Cb-2	67.83	ABCDEFGHI
Dakota Crisp	67.67	ABCDEFGHI	Dakota Crisp	68.00	ABCDEFG	RA 89044-45	67.83	ABCDEFGHI
ND8307C-3	66.67	ABCDEFGHI	ND049381C-2Russ	67.33	ABCDEFGH	Dakota Crisp	67.50	ABCDEFGHI
Red Norland	65.67	ABCDEFGHIJ	ND7550C-1	66.33	ABCDEFGHI	ND049381C-2Russ	66.67	ABCDEFGHI
ND7550C-1	65.67	ABCDEFGHIJ	Viking	65.00	ABCDEFGHIJ	Viking	64.17	ABCDEFGHIJ
ND049381C-2Russ	64.83	ABCDEFGHIJK	Colorado Rose	64.00	ABCDEFGHIJK	Lamoka	62.50	ABCDEFGHIJK
Lamoka	61.33	BCDEFGHIJKL	Lamoka	62.67	ABCDEFGHIJKL	ND7550C-1	62.33	ABCDEFGHIJK
Colorado Rose	60.67	CDEFGHIJKL	ND6956b-13	62.17	BCDEFGHIJKLM	ND060835C-4	61.17	ABCDEFGHIJKL
RK 24-48	58.67	CDEFGHIJKLM	ND060835C-4	61.00	CDEFGHIJKLM	ND4659-5R	59.50	BCDEFGHIJKLM
AND00272-1R	58.50	CDEFGHIJKLM	ND060742C-1Russ	57.67	DEFGHIJKLMN	RK 24-48	57.33	CDEFGHIJKLMN
ND060835C-4	58.00	DEFGHIJKLMN	RG 47-3	55.67	EFGHIJKLMNO	ND060742C-1Russ	57.17	DEFGHIJKLMNO
ND060742C-1Russ	56.50	EFGHIJKLMNO	RK 24-48	55.67	EFGHIJKLMNO	RG 47-3	56.33	EFGHIJKLMNOP
RG 47-3	55.67	EFGHIJKLMNO	AND00272-1R	55.33	EFGHIJKLMNO	ND8305-1	55.83	EFGHIJKLMNOP
ND4659-5R	55.50	EFGHIJKLMNO	ND4659-5R	55.00	EFGHIJKLMNOP	RA 90213-60	55.00	EFGHIJKLMNOP
Nicolet	54.00	EFGHIJKLMNOP	ND8305-1	53.00	FGHIJKLMNOPQ	ND8068-5Russ	53.67	EFGHIJKLMNOPQ
ND8305-1	53.67	EFGHIJKLMNOP	ND8068-5Russ	52.17	FGHIJKLMNOPQ	Dark Red Norland	50.67	FGHIJKLMNOPQR
Dark Red Norland	52.67	FGHIJKLMNOPQ	T10-12	52.17	FGHIJKLMNOPQ	Dakota Jewel	50.67	FGHIJKLMNOPQR
ND060728-5R	52.33	GHIJKLMNOPQ	Nicolet	50.67	FGHIJKLMNOPQR	Yukon Gold	50.00	GHIJKLMNOPQR
ND8068-5Russ	52.33	GHIJKLMNOPQ	Dark Red Norland	50.33	GHIJKLMNOPQRS	AND00272-1R	49.67	GHIJKLMNOPQR
T10-12	49.67	HIJKLMNOPQR	ND060728-5R	50.33	GHIJKLMNOPQRS	ND6956b-13	49.00	GHIJKLMNOPQR
Yukon Gold	48.67	HIJKLMNOPQR	RA 90213-60	50.33	GHIJKLMNOPQRS	R 90096-5	48.83	GHIJKLMNOPQR
ND6400C-1Russ	47.50	IJKLMNOPQRS	Yukon Gold	47.67	HIJKLMNOPQRST	ND7519-1	47.83	HIJKLMNOPQRS
Dakota Jewel	46.33	JKLMNOPQRST	Dakota Jewel	47.00	IJKLMNOPQRSTU	ND060728-5R	46.83	IJKLMNOPQRS
RA 90213-60	46.00	JKLMNOPQRST	ND6400C-1Russ	45.33	JKLMNOPQRSTU	ND6400C-1Russ	44.50	JKLMNOPQRST
Atlantic	45.33	KLMNOPQRST	Atlantic	44.33	KLMNOPQRSTUV	ND8413-7Russ	44.33	JKLMNOPQRSTU
R 91007-5	43.33	LMNOPQRSTU	ND7519-1	44.33	KLMNOPQRSTUV	Nicolet	43.83	JKLMNOPQRSTUV
Yagana	42.17	LMNOPQRSTUV	R 90096-5	43.17	LMNOPQRSTUVW	Atlantic	41.83	KLMNOPQRSTUV
ND7519-1	41.67	LMNOPQRSTUV	R 91007-5	42.33	MNOPQRSTUVWX	ND8331Cb-3	40.17	LMNOPQRSTUV
ND8559-20	40.00	MNOPQRSTUV	Yagana	39.50	NOPQRSTUVWXY	T10-12	39.17	MNOPQRSTUV
R 90096-5	38.50	MNOPQRSTUV	ND8559-20	37.67	OPQRSTUVWXY	R 91007-5	38.00	NOPQRSTUV
ND8413-7Russ	38.00	NOPQRSTUV	ND8413-7Russ	37.67	OPQRSTUVWXY	Yagana	37.67	NOPQRSTUVW
ND070927-2Russ	36.33	OPQRSTUVW	AND00618-1RussY	35.33	PQRSTUVWXYZ	ND050105C-1Russ	37.17	NOPQRSTUVW
AND00618-1RussY	35.00	PQRSTUVW	RA 517-123	35.00	QRSTUVWXYZ	AND00618-1RussY	37.00	NOPQRSTUVW
R 91129-11	34.83	PQRSTUVW	ND8331Cb-3	34.67	QRSTUVWXYZ	ND060761B-3Russ	36.83	NOPQRSTUVW
R 87009-28	34.50	PQRSTUVW	ND070927-2Russ	34.67	QRSTUVWXYZ	R 87009-28	35.83	OPQRSTUVW
RA 517-123	34.33	PQRSTUVW	ND050105C-1Russ	33.67	QRSTUVWXYZ	RA 517-123	35.00	PQRSTUVW
ND050105C-1Russ	33.83	PQRSTUVW	R 87009-28	33.50	QRSTUVWXYZ	R 90070-8	33.33	QRSTUVW
ND8331Cb-3	33.17	QRSTUVW	R 91129-11	33.50	QRSTUVWXYZ	ND8559-20	33.17	QRSTUVW
RA 20-6	32.00	RSTUVW	RA 20-6	31.33	RSTUVWXYZ	RA 20-6	32.33	QRSTUVW
RC 72-35	30.67	RSTUVW	ND060761B-3Russ	31.17	RSTUVWXYZ	Karu	32.17	RSTUVW
Snowden	30.50	RSTUVW	Karu	30.50	STUVWXYZ	Alturas	32.00	RSTUVW
Karu	29.83	RSTUVW	RC 72-35	30.00	TUVWXYZ	ND070927-2Russ	31.83	RSTUVW
Alturas	27.83	STUVW	Snowden	29.83	TUVWXYZ	RC 72-35	31.67	RSTUVW
R 90070-8	27.67	STUVW	Alturas	27.83	TUVWXYZ	Snowden	30.67	RSTUVW
ND060761B-3Russ	27.00	TUVW	R 90070-8	27.67	UVWXYZ	R 91129-11	29.33	RSTUVW
AOND95292-3Russ	25.00	UVW	SPA 161	24.83	VWXYZ	SPA 161	27.00	STUVW
SPA 161	24.83	UVW	Russet Norkotah	24.33	WXYZ	AOND95292-3Russ	25.33	TUVW
Russet Norkotah	24.33	UVW	AOND95292-3Russ	24.33	WXYZ	ND049423b-1Russ	23.50	TUVW
Alpine Russet	23.50	UVW	ND049423b-1Russ	23.50	WXYZ	RA 151-24	23.50	TUVW
ND049423b-1Russ	23.50	UVW	Alpine Russet	23.17	XYZ	Ranger Russet	23.00	UVW

Table A4. Mean (rank) powdery scab incidence, severity and maximum severity on potato genotypes evaluated at McCanna, ND during 2012 (continued).

Potato cultivar or advanced clone	Inc.	Group	Potato cultivar or advanced clone	Sev.	Group	Potato cultivar or advanced clone	Max. Sev.	Group
Ranger Russet	22.50	VW	Ranger Russet	22.50	XYZ	Alpine Russet	23.00	UVW
RA 151-24	22.17	VW	RA 151-24	22.17	YZ	Russet Norkotah	22.83	VW
Russet Burbank	16.50	W	Russet Burbank	16.50	Z	Russet Burbank	16.50	W
Umatilla Russet	16.50	W	Umatilla Russet	16.50	Z	Umatilla Russet	16.50	W
Bannock Russet	16.50	W	Bannock Russet	16.50	Z	Bannock Russet	16.50	W
Dakota Trailblazer	16.50	W	Dakota Trailblazer	16.50	Z	Dakota Trailblazer	16.50	W
ND049546b-10Russ	16.50	W	ND049546b-10Russ	16.50	Z	ND049546b-10Russ	16.50	W
ND8229-3	16.50	W	ND8229-3	16.50	Z	ND8229-3	16.50	W
AND99362B-1Russ	16.50	W	AND99362B-1Russ	16.50	Z	AND99362B-1Russ	16.50	W
AND97279-5Russ	16.50	W	AND97279-5Russ	16.50	Z	AND97279-5Russ	16.50	W
ND060735-3Russ	16.50	W	ND060735-3Russ	16.50	Z	ND060735-3Russ	16.50	W
ND060766b-4Russ	16.50	W	ND060766b-4Russ	16.50	Z	ND060766b-4Russ	16.50	W
ND060770B-5Russ	16.50	W	ND060770B-5Russ	16.50	Z	ND060770B-5Russ	16.50	W
ND060796AB-1Russ	16.50	W	ND060796AB-1Russ	16.50	Z	ND060796AB-1Russ	16.50	W
ND050082Cb-2Russ	16.50	W	ND050082Cb-2Russ	16.50	Z	ND050082Cb-2Russ	16.50	W
ND049517B-1Russ	16.50	W	ND049517B-1Russ	16.50	Z	ND049517B-1Russ	16.50	W
ND039194AB-1Russ	16.50	W	ND039194AB-1Russ	16.50	Z	ND039194AB-1Russ	16.50	W
ND049289-1Russ	16.50	W	ND049289-1Russ	16.50	Z	ND049289-1Russ	16.50	W
AND01804-3Russ	16.50	W	AND01804-3Russ	16.50	Z	AND01804-3Russ	16.50	W
RC 06-109	16.50	W	RC 06-109	16.50	Z	RC 06-109	16.50	W
RC 89-25	16.50	W	RC 89-25	16.50	Z	RC 89-25	16.50	W
P-value	<0.001		*P*-value	<0.001		*P*-value	<0.001	
$LSD_{(0.05)}$	20.168		$LSD_{(0.05)}$	19.951		$LSD_{(0.05)}$	21.385	

Inc.: incidence; Sev.: severity; Max. Sev.: maximum severity.

Table A5. Mean (rank) powdery scab incidence, severity and maximum severity on potato genotypes evaluated at Osage, MN during 2012.

Potato cultivar or advanced clone	Incidence	Group	Potato cultivar or advanced clone	Severity	Group	Potato cultivar or advanced clone	Maximum severity	Group
ND6956b-13	65.33	A	ND6956b-13	65.33	A	ND6956b-13	65.00	A
ND7519-1	63.83	A	ND7519-1	63.17	A	ND7519-1	62.00	A
ND8331Cb-2	62.17	A	ATND98459-1RY	63.00	A	RA 89044-45	62.00	A
ATND98459-1RY	62.00	A	ND8331Cb-2	61.83	A	ATND98459-1RY	61.33	A
RG 47-3	61.33	A	RG 47-3	61.33	A	Ivory Crisp	60.83	A
RA 89044-45	60.00	A	RA 89044-45	60.00	A	R 91007-5	60.67	A
ND8305-1	59.50	A	Ivory Crisp	59.67	A	RG 47-3	60.00	A
Ivory Crisp	59.00	A	ND8305-1	58.83	A	ND8331Cb-2	59.83	A
Dakota Pearl	59.00	A	RC 72-35	58.83	A	Dakota Pearl	59.17	A
RC 72-35	58.83	A	Dakota Pearl	58.67	A	ND8305-1	59.00	A
R 91007-5	58.50	A	R 91007-5	58.50	A	RC 72-35	59.00	A
Red LaSoda	58.00	A	Red LaSoda	57.67	A	Red LaSoda	58.17	A
RC 89-25	49.17	AB	RC 89-25	49.17	AB	ND6400C-1Russ	49.50	AB
Kennebec	48.83	AB	ND6400C-1Russ	48.83	AB	RK 24-48	49.33	AB
AND00272-1R	48.50	AB	Kennebec	48.50	AB	Colorado Rose	48.67	AB
Shepody	48.33	AB	Shepody	48.33	AB	AND00272-1R	48.67	AB
ND8314-1R	48.17	AB	AND00272-1R	48.17	AB	RC 89-25	48.67	AB
ND8307C-3	48.00	AB	ND8307C-3	48.00	AB	Shepody	48.33	AB
RK 24-48	47.83	AB	RK 24-48	47.83	AB	ND8331Cb-3	47.33	AB
ND6400C-1Russ	47.83	AB	ND8314-1R	47.83	AB	Kennebec	46.67	AB
ND050082Cb-2Russ	47.50	AB	Red Pontiac	47.33	AB	ND8314-1R	46.67	AB
Red Pontiac	47.33	AB	ND050082Cb-2Russ	47.17	AB	RA 517-123	46.67	AB
RA 517-123	46.83	AB	Colorado Rose	46.83	AB	Red Pontiac	46.50	AB
Dakota Rose	46.00	AB	RA 517-123	46.83	AB	ND8307C-3	46.50	AB
Colorado Rose	45.83	AB	Dakota Rose	46.00	AB	Dakota Rose	46.50	AB
ND8304-2	45.50	AB	ND8304-2	45.50	AB	ND8304-2	45.50	AB
ND8331Cb-3	44.83	AB	ND8331Cb-3	44.83	AB	ND050082Cb-2Russ	45.50	AB
Russet Burbank	34.00	B	Russet Burbank	34.00	B	Russet Burbank	34.00	B
Russet Norkotah	34.00	B	Russet Norkotah	34.00	B	Russet Norkotah	34.00	B
Ranger Russet	34.00	B	Ranger Russet	34.00	B	Ranger Russet	34.00	B
Umatilla Russet	34.00	B	Umatilla Russet	34.00	B	Umatilla Russet	34.00	B
Alpine Russet	34.00	B	Alpine Russet	34.00	B	Alpine Russet	34.00	B
Bannock Russet	34.00	B	Bannock Russet	34.00	B	Bannock Russet	34.00	B
Dakota Trailblazer	34.00	B	Dakota Trailblazer	34.00	B	Dakota Trailblazer	34.00	B
Lamoka	34.00	B	Lamoka	34.00	B	Lamoka	34.00	B
Nicolet	34.00	B	Nicolet	34.00	B	Nicolet	34.00	B
Yukon Gold	34.00	B	Yukon Gold	34.00	B	Yukon Gold	34.00	B

Table A5. Mean (rank) powdery scab incidence, severity and maximum severity on potato genotypes evaluated at Osage, MN during 2012 (continued).

Red Norland	34.00	B	Red Norland	34.00	B	Red Norland	34.00	B
Dark Red Norland	34.00	B	Dark Red Norland	34.00	B	Dark Red Norland	34.00	B
Viking	34.00	B	Viking	34.00	B	Viking	34.00	B
Alturas	34.00	B	Alturas	34.00	B	Alturas	34.00	B
Snowden	34.00	B	Snowden	34.00	B	Snowden	34.00	B
Dakota Crisp	34.00	B	Dakota Crisp	34.00	B	Dakota Crisp	34.00	B
Atlantic	34.00	B	Atlantic	34.00	B	Atlantic	34.00	B
ND7550C-1	34.00	B	ND7550C-1	34.00	B	ND7550C-1	34.00	B
ND060728-5R	34.00	B	ND060728-5R	34.00	B	ND060728-5R	34.00	B
ND060835C-4	34.00	B	ND060835C-4	34.00	B	ND060835C-4	34.00	B
ND8555-8R	34.00	B	ND8555-8R	34.00	B	ND8555-8R	34.00	B
ND8559-20	34.00	B	ND8559-20	34.00	B	ND8559-20	34.00	B
ND4659-5R	34.00	B	ND4659-5R	34.00	B	ND4659-5R	34.00	B
Dakota Jewel	34.00	B	Dakota Jewel	34.00	B	Dakota Jewel	34.00	B
ND049546b-10Russ	34.00	B	ND049546b-10Russ	34.00	B	ND049546b-10Russ	34.00	B
AND00618-1RussY	34.00	B	AND00618-1RussY	34.00	B	AND00618-1RussY	34.00	B
ND8068-5Russ	34.00	B	ND8068-5Russ	34.00	B	ND8068-5Russ	34.00	B
ND8229-3	34.00	B	ND8229-3	34.00	B	ND8229-3	34.00	B
AND99362B-1Russ	34.00	B	AND99362B-1Russ	34.00	B	AND99362B-1Russ	34.00	B
AND97279-5Russ	34.00	B	AND97279-5Russ	34.00	B	AND97279-5Russ	34.00	B
AOND95292-3Russ	34.00	B	AOND95292-3Russ	34.00	B	AOND95292-3Russ	34.00	B
ND060735-3Russ	34.00	B	ND060735-3Russ	34.00	B	ND060735-3Russ	34.00	B
ND060742C-1Russ	34.00	B	ND060742C-1Russ	34.00	B	ND060742C-1Russ	34.00	B
ND060761B-3Russ	34.00	B	ND060761B-3Russ	34.00	B	ND060761B-3Russ	34.00	B
ND060766b-4Russ	34.00	B	ND060766b-4Russ	34.00	B	ND060766b-4Russ	34.00	B
ND060770B-5Russ	34.00	B	ND060770B-5Russ	34.00	B	ND060770B-5Russ	34.00	B
ND060796AB-1Russ	34.00	B	ND060796AB-1Russ	34.00	B	ND060796AB-1Russ	34.00	B
ND070927-2Russ	34.00	B	ND070927-2Russ	34.00	B	ND070927-2Russ	34.00	B
ND050105C-1Russ	34.00	B	ND050105C-1Russ	34.00	B	ND050105C-1Russ	34.00	B
ND049517B-1Russ	34.00	B	ND049517B-1Russ	34.00	B	ND049517B-1Russ	34.00	B
ND039194AB-1Russ	34.00	B	ND039194AB-1Russ	34.00	B	ND039194AB-1Russ	34.00	B
ND049289-1Russ	34.00	B	ND049289-1Russ	34.00	B	ND049289-1Russ	34.00	B
ND049381C-2Russ	34.00	B	ND049381C-2Russ	34.00	B	ND049381C-2Russ	34.00	B
ND049423b-1Russ	34.00	B	ND049423b-1Russ	34.00	B	ND049423b-1Russ	34.00	B
ND8413-7Russ	34.00	B	ND8413-7Russ	34.00	B	ND8413-7Russ	34.00	B
AND01804-3Russ	34.00	B	AND01804-3Russ	34.00	B	AND01804-3Russ	34.00	B
R 90070-8	34.00	B	R 90070-8	34.00	B	R 90070-8	34.00	B
R 91129-11	34.00	B	R 91129-11	34.00	B	R 91129-11	34.00	B
RC 06-109	34.00	B	RC 06-109	34.00	B	RC 06-109	34.00	B
RA 20-6	34.00	B	RA 20-6	34.00	B	RA 20-6	34.00	B
Karu	34.00	B	Karu	34.00	B	Karu	34.00	B
SPA 161	34.00	B	SPA 161	34.00	B	SPA 161	34.00	B
RA 151-24	34.00	B	RA 151-24	34.00	B	RA 151-24	34.00	B
P-value	0.004		*P*-value	0.004		*P*-value	0.004	
$LSD_{(0.05)}$	21.735		$LSD_{(0.05)}$	21.755		$LSD_{(0.05)}$	21.732	

Table A6. Mean (rank) Spongospora root gall formation on potato genotypes evaluated at McCanna, ND and Osage, MN during 2012.

McCanna, ND			Osage, MN		
Potato cultivar or advanced clone	**Galls · plant^{-1}**	**Group**	**Potato cultivar or advanced clone**	**Galls · plant^{-1}**	**Group**
Red Pontiac	79.33	A	ND6956b-13	64.17	A
Kennebec	78.33	A	Dakota Rose	62.00	A
Snowden	77.67	AB	RA 151-24	61.83	A
Shepody	77.33	AB	Ivory Crisp	60.33	A
RK 24-48	74.33	ABC	Kennebec	59.83	A
ATND98459-1RY	72.67	ABCD	RA 89044-45	59.83	A
RC 06-109	71.00	ABCDE	ND8555-8R	57.50	A
Nicolet	70.00	ABCDEF	ND8559-20	56.83	A
Lamoka	68.33	ABCDEFG	ND039194AB-1Russ	49.83	A
ND060770B-5Russ	67.67	ABCDEFGH	Dakota Pearl	49.17	A
ND8305-1	66.33	ABCDEFGHI	RK 24-48	48.83	A
RA 89044-45	65.33	ABCDEFGHIJ	Viking	48.17	A
Umatilla Russet	65.17	ABCDEFGHIJ	ND8305-1	48.17	A
AND01804-3Russ	65.00	ABCDEFGHIJ	RC 89-25	48.17	A
ND8555-8R	64.67	ABCDEFGHIJK	Red LaSoda	48.00	A
SPA 161	64.17	ABCDEFGHIJKL	Red Pontiac	47.50	A
Red LaSoda	64.00	ABCDEFGHIJKL	Dakota Jewel	47.50	A
ND8331Cb-2	62.50	ABCDEFGHIJKLM	Russet Burbank	47.17	A

Table A6. Mean (rank) Spongospora root gall formation on potato genotypes evaluated at McCanna, ND and Osage, MN during 2012 (continued).

McCanna, ND			Osage, MN		
Potato cultivar or advanced clone	**Galls · plant^{-1}**	**Group**	**Potato cultivar or advanced clone**	**Galls · plant^{-1}**	**Group**
ND6400C-1Russ	62.33	ABCDEFGHIJKLMN	ND8314-1R	47.17	A
Yagana	62.00	ABCDEFGHIJKLMN	ATND98459-1RY	47.17	A
Alpine Russet	61.00	ABCDEFGHIJKLMN	ND060796AB-1Russ	47.17	A
R 90070-8	61.00	ABCDEFGHIJKLMN	ND6400C-1Russ	47.17	A
ND8307C-3	60.00	ABCDEFGHIJKLMNO	Bannock Russet	46.17	A
RA 20-6	55.50	ABCDEFGHIJKLMNOP	Nicolet	46.17	A
Dakota Pearl	55.00	ABCDEFGHIJKLMNOPQ	Dakota Crisp	46.17	A
AOND95292-3Russ	54.50	ABCDEFGHIJKLMNOPQR	ND060728-5R	46.17	A
ND8068-5Russ	52.17	BCDEFGHIJKLMNOPQRS	ND8331Cb-2	46.17	A
R 91129-11	52.00	BCDEFGHIJKLMNOPQRS	ND4659-5R	45.67	A
ND8314-1R	50.50	CDEFGHIJKLMNOPQRST	RC 72-35	45.67	A
Russet Burbank	48.17	DEFGHIJKLMNOPQRSTU	RG 47-3	44.67	A
Colorado Rose	47.00	DEFGHIJKLMNOPQRSTUV	ND7519-1	44.67	A
Viking	46.50	EFGHIJKLMNOPQRSTUVW	AOND95292-3Russ	44.67	A
Ivory Crisp	45.83	EFGHIJKLMNOPQRSTUVWX	ND060742C-1Russ	44.67	A
RA 517-123	45.83	EFGHIJKLMNOPQRSTUVWX	R 91007-5	44.67	A
ND060796AB-1Russ	45.33	EFGHIJKLMNOPQRSTUVWX	Russet Norkotah	33.50	A
R 91007-5	44.67	FGHIJKLMNOPQRSTUVWX	Ranger Russet	33.50	A
R 87009-28	44.17	FGHIJKLMNOPQRSTUVWXY	Umatilla Russet	33.50	A
RA 90213-60	44.17	FGHIJKLMNOPQRSTUVWXY	Alpine Russet	33.50	A
ND060728-5R	44.00	GHIJKLMNOPQRSTUVWXYZ	Dakota Trailblazer	33.50	A
ND8559-20	43.83	GHIJKLMNOPQRSTUVWXYZa	Shepody	33.50	A
ND060766b-4Russ	43.67	GHIJKLMNOPQRSTUVWXYZa	Lamoka	33.50	A
ND049381C-2Russ	42.00	HIJKLMNOPQRSTUVWXYZab	Yukon Gold	33.50	A
Dark Red Norland	41.83	HIJKLMNOPQRSTUVWXYZab	Red Norland	33.50	A
ND070927-2Russ	41.83	HIJKLMNOPQRSTUVWXYZab	Dark Red Norland	33.50	A
ND8413-7Russ	41.67	IJKLMNOPQRSTUVWXYZab	Alturas	33.50	A
ND4659-5R	40.00	JKLMNOPQRSTUVWXYZabc	Snowden	33.50	A
Atlantic	39.50	JKLMNOPQRSTUVWXYZabc	Atlantic	33.50	A
ND6956b-13	38.83	KLMNOPQRSTUVWXYZabcd	Colorado Rose	33.50	A
Alturas	38.67	LMNOPQRSTUVWXYZabcd	ND7550C-1	33.50	A
ND7519-1	36.67	MNOPQRSTUVWXYZabcde	ND060835C-4	33.50	A
Yukon Gold	36.50	NOPQRSTUVWXYZabcde	ND8304-2	33.50	A
AND97279-5Russ	34.17	OPQRSTUVWXYZabcde	ND8331Cb-3	33.50	A
ND060761B-3Russ	32.50	PQRSTUVWXYZabcdef	AND00272-1R	33.50	A
Bannock Russet	32.17	PQRSTUVWXYZabcdef	ND8307C-3	33.50	A
ND060835C-4	31.33	PQRSTUVWXYZabcdefg	ND049546b-10Russ	33.50	A
ND049517B-1Russ	29.17	QRSTUVWXYZabcdefg	AND00618-1RussY	33.50	A
Red Norland	29.00	RSTUVWXYZabcdefg	ND8068-5Russ	33.50	A
Dakota Crisp	27.00	STUVWXYZabcdefgh	ND8229-3	33.50	A
ND8331Cb-3	26.00	TUVWXYZabcdefgh	AND99362B-1Russ	33.50	A
Ranger Russet	25.50	TUVWXYZabcdefgh	AND97279-5Russ	33.50	A
AND00272-1R	25.33	TUVWXYZabcdefgh	ND060735-3Russ	33.50	A
RC 72-35	25.17	TUVWXYZabcdefgh	ND060761B-3Russ	33.50	A
ND060735-3Russ	24.50	UVWXYZabcdefgh	ND060766b-4Russ	33.50	A
AND00618-1RussY	24.00	UVWXYZabcdefgh	ND060770B-5Russ	33.50	A
RC 89-25	23.67	UVWXYZabcdefgh	ND070927-2Russ	33.50	A
RA 151-24	23.00	UVWXYZabcdefgh	ND050082Cb-2Russ	33.50	A
ND039194AB-1Russ	22.00	VWXYZabcdefgh	ND050105C-1Russ	33.50	A
ND060742C-1Russ	20.67	WXYZabcdefgh	ND049517B-1Russ	33.50	A
ND050105C-1Russ	20.67	WXYZabcdefgh	ND049289-1Russ	33.50	A
ND7550C-1	20.17	XYZabcdefgh	ND049381C-2Russ	33.50	A
Dakota Trailblazer	18.67	YZabcdefgh	ND049423b-1Russ	33.50	A
Dakota Jewel	18.33	YZabcdefgh	ND8413-7Russ	33.50	A
Karu	18.17	Zabcdefgh	AND01804-3Russ	33.50	A
ND049546b-10Russ	18.00	abcdefgh	R 90070-8	33.50	A
RG 47-3	17.17	bcdefgh	R 91129-11	33.50	A
Russet Norkotah	15.50	cdefgh	RC 06-109	33.50	A
AND99362B-1Russ	13.33	defgh	RA 517-123	33.50	A
R 90096-5	13.00	defgh	RA 20-6	33.50	A
ND049423b-1Russ	12.67	efgh	Karu	33.50	A
ND050082Cb-2Russ	8.17	fgh	SPA 161	33.50	A
ND8229-3	7.33	fgh			
T10-12	6.17	gh			
ND049289-1Russ	3.00	h			
P-value		<0.001	*P*-value	0.320	
$LSD_{(0.05)}$		25.863	$LSD_{(0.05)}$	n/a	

n/a: not applicable.