THESIS

EVALUATION OF COVER CROPS IN REDUCING *SPONGOSPORA SUBTERRANEA* INOCULUM THROUGH QPCR AND MICROSCOPY ASSAYS

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2021

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ABSTRACT

EVALUATION OF COVER CROPS IN REDUCING *SPONGOSPORA SUBTERRANEA* INOCULUM THROUGH QPCR AND MICROSCOPY ASSAYS

Cover crops have been used for years as an effective practice to manage soil-borne pathogens. Beside their other beneficial properties in enhancing soil quality and fertility, they can suppress soil-borne pathogens and reduce their populations in the soil. *Spongospora subterranea* is a soil-borne obligate biotrophic plasmodiophorid that causes powdery scab on potato tubers and gall formation on roots. Powdery scab tuber lesions are filled with resting spores (sporosori) and reduce potato quality and marketability. *S. subterranea* also vectors Potato mop-top virus, which causes necrotic arcs and spraing in tuber flesh. Currently, there are no effective methods to manage *S. subterranea*, which has a wide host range in addition to potato. This study was conducted to determine whether cover crops can decrease *S. subterranea* population levels in the soil. Eighteen cover crops, including legumes and mustards were assessed, and for each plant line, five plants were grown in individual pot that was inoculated with 10 sporosori/g of potting mix by comparison with five plants that were grown in non-inoculated potting mix. After harvest, plant roots were stained using trypan blue and examined under the light microscope, and qPCR was performed to determine *S. subterranea* inoculum level in the potting mix. The results suggested that *S. subterranea* invaded all the cover crop roots; however, the pathogen was unable to complete its life cycle on eleven out of eighteen cover crops based on post-harvest qPCR results. To confirm our results, a follow up experiment was conducted by inoculating the potting mix with 40 sporosori/g to better detect the pathogen, in addition to autoclaving the peat moss prior to planting.

The roots were stained with DAPI after harvest and examined under the fluorescence microscope and *S. subterranea* DNA levels was quantified in the eleven cover crops by qPCR. The results showed that buckwheat, barley, and legumes have the potential to stop the pathogen from increasing in the soil where there was no significant increase in the inoculated samples in most of the tested cover crops. The results from this experiment will be used to guide in-field cover crop experiments and to advise farmers on cover crops that may not increase the inoculum levels of *S. subterranea* in the soil.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my esteemed advisor, Dr. Amy Charkowski for giving me the opportunity to undertake my studies at the department of Agricultural biology, for her continuous support, and all her invaluable advice during my master's study. I would also like to thank Dr. Ana Cristina Fulladolsa, and Dr. Yuan Zeng for their mentorship, immense knowledge, and plentiful experience that have guided me to this success. I also thank Dr. Chakradhar Mattupalli for his great work and help in the San Luis Valley. My gratitude extends to my graduate committee members Dr. Jane Stewart and Dr. Kelly Wrighton, and to all our lab members for their kind help and support. I also thank USDA-ARS and USDA-APHIS for funding my research project.

DEDICATION

I am dedicating this thesis to my loving parents who always been there for me and taught me to work hard and fight for what I want, to my siblings who mean so much to me and have been supporting and encouraging me throughout this journey. To my beloved husband and my wonderful daughters, for their understanding, constant help, and support during the challenges of graduate school and life. I also dedicate this work to my academic advisor who guided me in this process, kept me on track, and made all this possible.

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CHAPTER ONE – BACKGROUND

Potato is one of the most important crop productions globally. Potato plants may be infected by many different soilborne diseases that negatively affect plant development, tuber quality, and reduce potato production and marketability. Soilborne diseases can be caused by bacteria, fungi, oomycetes, plasmodiophords, or nematodes or by viruses carried by plasmodiophorids or nematodes. Some of the most important and common soilborne diseases include: common scab, caused by the bacterium *Streptomyces scabies*; fusarium dry rot, caused by several fungal species belong to *Fusarium* genus; early blight of potato, caused by the fungus *Alternaria solani;* black scurf and rhizoctonia canker, caused by the fungus *Rhizoctonia solani;* silver scurf, caused by the fungus *Helminthosporium solani*; black dot, caused by the fungus *Colletotrichum coccodes*; pink rot, caused by the oomycete *Phytophthora erythroseptica;* late blight, caused by the oomycete *Phytophthora infestans*; and powdery scab of potatoes, caused by the biotrophic plasmodiophorid pathogen *Spongospora subterranea* f.sp. *subterranea* (Larkin & Lynch, 2018, and Scheufele, 2016).

Spongospora subterranea is responsible for significant financial losses in the potato industry globally (Balendres, Clark, Tegg, and Wilson, 2018). It belongs to the family Plasmodiophoridae in the Protozoa kingdom. It is technically difficult to study this pathogen due to its biological characteristics. It is an obligate biotroph and it is unculturable (Thangavel, Tegg, and Wilson, 2015, and Balendres, Clark, Tegg, and Wilson, 2018). It can invade host roots, stolons, and tubers, where it develops a plasmodium that can cause galls that eventually become filled with powdery masses of resting spores (sporosori) (Fig. 1). These sporosori will be released into the soil causing increase in soil inoculum. On tubers, these lesions caused by *S. subterranea* negatively

affect potatoes cosmetically, leading to low potato quality and marketability. These scab-like lesions that are filled with sporosori form another source of inoculum when the infected tubers are used as seed tubers (Merz, 2008). *S. subterranea* does not form lesions on all varieties of potato tubers and russet skin potatoes tend to be tolerant to tuber lesions. However, even these tuber-tolerant varieties may have root galls, resulting in soil inoculum increase each year that potato is grown (Van de Graaf, Wale, and Lees, 2007). *S. subterranea* also vectors an economically important pathogen, the potato-mop-top virus (PMTV), which causes necrotic arcs in potato tubers (Merz, 2008, and Zeng et al., 2020) (Fig. 2 and 3)

Figure 1: Powdery scab on potato tubers.

Figure 2: PMTV symptoms in tuber.

Figure 3: The rare foliar symptoms of PMTV.

Powdery scab was first recorded in Europe in Germany in 1841, and it was found in South America in 1891, and in 1913 it was first recognized in North America. Now, the disease is threatening most potato growing regions, where it can spread easily (Johnson, 2002, and Merz, 2008). There are several factors contributing in the occurrence of powdery scab and increase its incidence, such as: using susceptible potato cultivars, banning of mercury, which was an efficient seed tuber treatment previously used, overdose irrigation, and the increase of potato production (Merz, 2008). Previous studies on tomato show that temperature play an important role for root hair infection by *S. subterranea,* in which the optimum temperature to initiate the infection is between 16-17 °C with a minimum of 11 °C and a maximum of 22-25 °C. However, not much is known about the optimum and favoring conditions for root infection and galling by *S. subterranea* on potato (Van de Graaf, Wale, and Lees, 2007). Recently, *S. subterranea* was reported in peat-based potting mix and since early generation seed potato tubers are often grown in potting mix, this may explain the swift spread of this pathogen in global potato production (Mallik, I, et al., 2019).

S. subterranea has a complicated life cycle with two major phases (Fig. 4), each initiated by single plasmodia that infect the host cell. The sporangial, asexual phase (the inner circle of Fig. 4): Many secondary zoospores, which are motile asexual spores that uses a flagellum for locomotion, are formed in compartments within thin-walled zoosporangia (the asexual structure in which the zoospores develop), which develop from multinucleate sporangiogenous plasmodia in the host root epidermal cells or root hairs. These secondary biflagellate zoospores exit the host and initiate further infection cycles. The sporogenic, sexual phase (the outer circle of Fig. 4): The pathogen produces spore balls (sporosori), after nuclear divisions and cleavage within the

sporogenic plasmodia, each sporosori consists of thick-walled resting spores that are highly persistent. Each resting spore releases a single biflagellate primary zoospore (Merz, 2008).

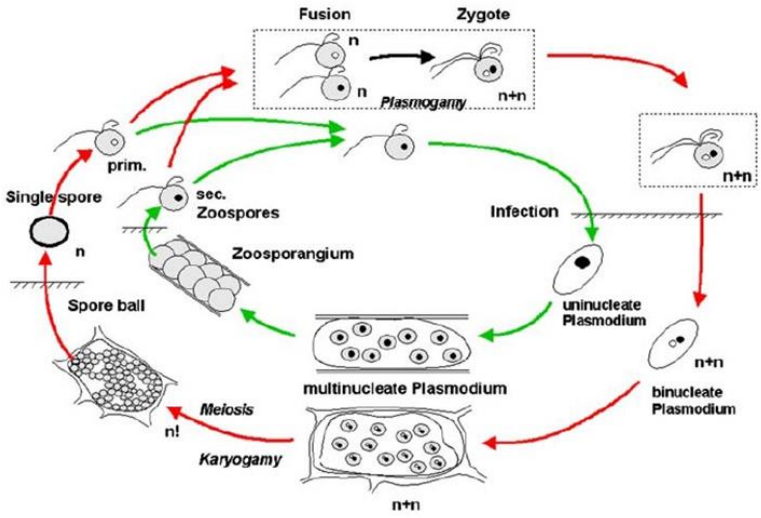

Figure 4: Tentative life cycle of Spongospora subterranea f.sp. subterranea. The outer path represents sexual reproduction, and the inner path represents asexual reproduction. (Reproduced from Merz (2008) with permission of Springer).

During the sexual phase, a dikaryotic zoospore (with two separate haploid nuclei, n+n) is formed by the fusion of two zoospores, and then it infects the roots, which initiate the infection. Once it inside the host, the dikaryotic zoospore develops into a binucleate plasmodium (one pair of nuclei, n+n). This plasmodium will undergo mitotic division and replicate its nucleus to create a multinucleate plasmodium (many pairs of nuclei, n+n). These pairs will fuse together by karyogamy, which is the fusion of two haploid cell nuclei, and the plasmodium will divide quickly into many sporosori each contain numerous resting spores. The walls of these resting spores are very thick, consisting of three layers, which makes them extremely resistant to different environmental conditions. Sporosori can persist in the soil for more than 10 years and can survive dry heat temperatures of up to 121°C. In the asexual phase, the infection starts by infecting the

root tissue by a zoospore, which forms a uninucleate plasmodium. This plasmodium will develop into multinucleate plasmodium through mitotic nuclear division, which creates many nuclei within a single cell. Then, zoosporangium will develop from the multinucleate plasmodium, which eventually release more zoospores. These zoospores are secondary zoospores which represent an important source of secondary inoculum in the field (Merz, 2008).

Zoospores initiate the infection by attaching to the outer surface of a host root hairs, encysting, and then penetrating the epidermis through stomata and lenticels. Once inside, more zoospores are produced by the division of the multinucleate plasmodium. The plasmodium will force the infected host cells to multiply rapidly and enlarge into a gall (hypertrophy). Uninucleate cells will develop from this rapid multiplication and aggregates to form sporosori that look like a powdery mass within the gall, hence the name is powdery scab. The gall will eventually swell and bursts out of the epidermis, releasing the spores back into the soil. The severity of the infection depends on inoculum level, environment, and potato cultivar (Merz, 2008).

The favorable growth temperature for *Spongospora subterranea* is between 12°C and 15°C, and it requires free water for spread (Merz, 2008, and Van de Graaf, Wale, and Lees, 2007). According to Van de Graaf (2007), temperature has a great effect on the severity of the disease incidence on both potato root infection and the formation of galls. They found that the percentage of plants with infected roots increased with the increase of temperature, in which 86% of the plant roots were infected at 17°C, 53% of the plant roots were infected at 12°C, and 38% of the plant roots were infected at 9°C. In addition, root galls were formed on all the plants grown at 17°C, where there was no gall formation on the plants grown at 9°C, and only 33% of the plants grown at 12°C had galls. The severity of the infection and root galling was significantly greater at 17°C compared to 12°C and 9°C. *S. subterranea* has the ability to infect plants at early stages of plant

development. The pathogen was found in 76% of the potato roots harvested at tuber initiation, and gall formation was also found at early stages; however, the severity of galls was greater at plant maturity. The formation of powdery scab symptoms on potato tubers had no relationship with root infection, where some plants had tuber symptoms without root infection, and other plants had infected roots with gall formation with symptomless tubers. However, most of the potatoes with severe tuber symptoms, had root gall formation (Van de Graaf et al., 2007).

Spongospora subterranea causes obvious damage on potato skin as it produces lesions filled with powdery scab on the tubers. For consumers, this damage is only cosmetic and can be easily removed by peeling off the skin, but for potato producers it causes a serious problem as it reduces potato quality. In addition, infected tubers cannot be used as seed, which will cause seed producers big financial losses. Powdery scab can also reduce stored tubers yield since the lesions formed on the tubers cause tuber shrinkage as they weaken the skin and allow it for gas exchange, and it also serve as an entrance for other pathogens. *S. subterranea* also causes root galls which weakens the root function resulting in lower yield and quality (Merz, 2008).

S. subterranea management options are very limited due to its multiplex life cycle and the longevity of its sporosori (Merz, 2008; Clarke et al., 2020; Zeng et al., 2020; Tsror et al., 2020). Using resistant cultivar is the best management method for disease, however; for powdery scab, most available potato cultivars are susceptible, and developing resistant cultivars has not been achieved yet (Merz, 2008; Clarke et al., 2020; Zeng et al., 2020). Since the emergence of this pathogen, researchers have been trying to find an effective management control that can be easily applied by all farmers. There are some factors that may reduce the incidence of powdery scab, such as improving soil drainage capacity, controlling planting date, and not watering late in the season. However, there are very few studies to support these proposed methods. Chemical

management strategies have been applied to reduce powdery scab inoculum level in the soil, treat the seeds, and reduce the pathogen incidence, but with little efficacy. In Colorado, farmers have tried Omega® 500F and FOLI-R-PLUS RIDEZ™, but they are not effective in reducing disease severity or inoculum (Zeng et al., 2020). Chloropicrin, which is a soil fumigant, has been tested for powdery scab management since it worked effectively with common scab, however; it was not effective with powdery scab. In some cases, both chloropicrin and RIDEZ showed increased disease incidence or inoculum (Clarke et al., 2020, and Zeng et al., 2020). In addition, fumigants such as chloropicrin harmful effects on the environment, are not affordable by everyone, and are not registered in many countries (Tsror et al., 2020). According to Clarke (2020), a low-dose treatment of the herbicide 2,4-D, which mimics the actions of the plant growth hormone auxin, had significantly reduced the severity of powdery scab symptoms on tubers, however; it did not show any reduction on root galling. Therefore, it cannot be considered as an effective treatment for *S. subterranea* because root galls represent a source of inoculum since it contains sporosori and can increase soil inoculum level. Due to the economic importance and impact of this pathogen on the potato industry and the lack of management practices to control powdery scab, our goal is to find an effective solution that can be easily utilized by farmers and all of whom in interest. This can be achieved by choosing the right cover crops that can help reduce the disease incidence, decrease soil inoculum build-up, or at least stop the pathogen from increasing in the soil and thus enhancing potato yield.

According to Dupont (2015), cover crops have been successfully used to suppress Verticillium wilt in tomato. Their study was conducted from 2010 till 2013 in tomato field with a history of Verticillium wilt. Cover crops that had biofumigant properties was the focus of this study, where they used mustards cv, caliente 119, and Sudangrass. These crops have

chemical substances and enzymes that when chopped and incorporated with the soil, they come in contact to each other and become toxic to soil-borne pathogens. Tomato yield was increased after using Sudangrass and mustards as rotational crops in 2011. However, they did not notice any increase in yield in 2013 after using the same cover crops. They assume that this is because 2013 was a wet year, and tomatoes were not that stressed by Verticillium wilt as in a dry year since this disease works by disabling the plant from taking water from its roots to the rest of the plant by clogging up the vascular system of the plant. They concluded that getting variable result is common when managing soil-borne diseases with cover crops because many factors could affect the results.

Another example of soil-borne disease suppression by cover crops is presented in Everts (2016) study, where they discuss the different mechanisms behind this suppression. Cover crops can increase the competition with plant pathogens for nutrients by increasing the diversity and overall activity of the soil microbial community. In addition, they produce unfavorable compounds to the pathogens that interfere with their ability to develop and grow. An example of these compounds is glucosinulates, which are sulfur compounds, produced by mustard green that can successfully suppress *Rhizoctonia* occurrence on potatoes by releasing isothiocyanates into the soil. This mechanism in known as general suppression. Another mechanism induced by cover crops is specific suppression, where they enhance individual beneficial organisms. Beans soil-borne pathogens such as *Pythium* and *Fusarium* spp. can be suppressed by the fungus *Trichoderma harzianum.* This fungus can colonize a wide variety of cover crops including annual ryegrass, red clover, hairy vetch, and winter wheat. Its suppression mechanism based on nutrients competition with the plant pathogens. Cover crops can also increase the abundance of mycorrhizae in soils, which are fungi that benefit the plants and live-in association with the roots and can suppress

individual pathogens. The increased population is also observed in the subsequent cash crop, where it was observed in peach, tomato, and watermelon after rotating with crimson clover and hairy vetch cover crop. Mycorrhizal colonization of the watermelon roots was improved after these cover crops, and it also reduced Fusarium wilt. Cover crops can successfully manage soil-borne diseases under the right conditions and the right choice of crops.

CHAPTER TWO – INTRODUCTION

Potato (*Solanum tuberosum*) is the third most important food crop globally (CIP, International Potato Center). Among a number of diseases that threaten potato production, powdery scab (PS) of potatoes caused by the plasmodiophorid, *Spongospora subterranea* (Ss), is one of the major diseases that impacts potato production worldwide. The pathogen *S. subterranea* is common in the United States and has been reported in the major potato producing states in the U.S. (Bittara, Robinson, and Gudmestad, 2018). The disease causes dramatic losses in both ware and seed potato production, where the potato market worth approximately $4 billion annually in the US. Powdery scab affects 4% of this production and it reduces tuber quality and marketability because it causes small lesions on the tuber skin that develop later into wart-like masses filled with sporosori (Lahkim et al., 2020, and Balendres, Tegg, and Wilson, 2017). The pathogen also causes gall formation on the roots (Thangavel, Tegg, and Wilson, 2015, and Merz, 2008) that reduces potato ability to grow and thus reduces yields. In addition, *S. subterranea* transmits Potato Mop-Top Virus (PMTV), which causes necrotic arcs in potato tubers (Thangavel, Tegg, and Wilson, 2015, and Ciaghi, Neuhauser, and Schwelm, 2018).

S. subterranea is an obligate biotroph and therefore requires a living host and cannot be cultured in vitro. *S. subterranea* has a biphasic life cycle, allowing it to persist in soil with the production of resting spore aggregates (sporosori) and also spreading through the formation of short-lived zoospores in the host when environmental conditions are favorable. Detection in the soil and on tubers is based on the presence of sporosori, the resting spores of the pathogen, which are formed in the lesions found on the tuber surfaces and root galls. Sporosori can survive extreme environmental conditions and can persist in the soil for up to 10 years (Merz, 2008). These

sporosori release motile primary zoospores that swim toward its host's roots or tubers, encyst, and penetrate the root cells to initiate infection (Balendres, Clark, Tegg, and Wilson, 2018, and Thangavel, Tegg, and Wilson, 2015). After a successful infection, the pathogen forms plasmodium that develop to sporangium, which produce secondary zoospores to initiate further infection when released to the soil (Thangavel, Tegg, and Wilson, 2015, and Merz, 2008). Unlike the resting spores, the zoospores cannot tolerate harsh environmental conditions. Zoospores need free water to swim and once emerged, they survive approximately 2-5 h to find a host (Balendres, Clark, Tegg, and Wilson, 2018, and Merz, 2008). One of the significant challenges to manage *S. subterranea* is the high numbers of long-lived resting spores produced by the host plant during the infection cycles, which are released into the soil and provide a source of inoculum. These spores are highly persistence, with three layered walls that allow them to survive extreme environmental conditions (Merz, 2008). Therefore, we are aiming to find cover crops that can decrease the presence of these spores in the soil by preventing the pathogen from completing its life cycle in the host.

Disease management is primarily based on preventative measures that include using noncontaminated fields, disease-free seed tubers, and resistant cultivars. These strategies rely on reducing the soil inoculum levels of the pathogens, however none of them is fully effective (Balendres, Clark, Tegg, and Wilson, 2018). The lack of management strategies and methods of control is due to the pathogen's complicated life cycle, and its nature as a soil-borne unculturable pathogen, which make it technically difficult to study (Merz, 2008, and Thangavel, Tegg, and Wilson, 2015). In addition, *S. subterranea* can be found in un-inoculated potting mix (Mallik, I, et al., 2019).

Currently, there are no cost-effective chemical treatment for powdery scab and there are very few tolerant potato varieties (Van de Graaf, Wale, and Lees, 2007, and Balendres, Tegg, and Wilson, 2017). Furthermore, the treatments that are currently used, including Omega® 500F (fluazinam) and FOLI-R-PLUS RIDEZ™ (fertilizer) are ineffective in reducing the disease incidence (Zeng et al., 2020). Therefore, there is an urgent need to develop effective management practice(s) in order to reduce soil inoculum levels of *S. subterranea* to control powdery scab. Cover crops are one attractive possibility for management of *S. subterranean*, especially if cover crops that promote spore germination, but that do not allow the pathogen to complete its life cycle, can be identified.

Cover cropping is an effective and important practice that can manage soil-borne diseases by reducing inoculum build-up resulting from consecutive cultivation of susceptible plant species. *S. subterranea* has a wide range of hosts in the zoosporangium phase, but not all the hosts allow it to complete its life cycle and produce sporosori that can increase the soil inoculum (Lahkim et al., 2020). It is postulated that improving soil drainage capacity, controlling planting date, soil temperature, soil fumigation, and chemical treatments may provide an effective tool to manage PS, however, most of the earlier studies show that these tools are not fully effective. Therefore, cultural practice such as cover cropping should be used to reduce inoculum, where an ideal cover crop would cause the spores to germinate but disable them from completing their life cycle.

In Colorado, the San Luis Valley is the primary region where potato is grown. The San Luis Valley is a 5,120,000-acre high altitude valley located in south-central Colorado and it is characterized by dry, cold weather, and sandy soil. It is ideal for potato production, but it is limited by temperature, water availability, and altitude for production of many other crops, including some common cover crops. The goal of this study is to use greenhouse assays to identify cover crops

suitable for use in the San Luis Valley that also may reduce soil inoculum levels of the pathogen. The most promising cover crops may then be tested in future field studies to determine if they provide effective management of powdery scab. In addition, 8 diploid potato lines were tested to determine if any show tolerance or resistance to powdery scab. The specific objectives for this study were a) qPCR-based detection and quantification the pathogen in 18 cover crops and 8 diploid potato species; b) light microscopy-based visualization of the different stages of the pathogen's life cycle under plasmodium, and zoosporangium stages; c) fluorescence microscopy-based visualization of the presence of *S. subterranea* in the cover crops that reduced the inoculum levels of the pathogen as determined in Objective 1 and 2.

CHAPTER THREE - MATERIALS AND METHODS

Experimental design

Experiments with *S. subterranea* tend to have high variability and to take at least three months to complete due to the nature of the pathogen life cycle. Therefore, our overall experimental design was to conduct two separate greenhouse assays in order to obtain data needed to design future field experiments. In our first greenhouse experiment, we evaluated 18 cover crops and 8 diploid potato lines. In the second greenhouse experiment, a subset of the cover crops that grew well under greenhouse conditions and that appeared to be either the most suppressive or the most susceptible were evaluated.

Both experiments were conducted in a greenhouse of San Luis Valley in south-central Colorado (37.7250° N, 105.8506° W). The first was conducted from February 5 to May 7, 2019, and the second from December 18, 2020, to March 18, 2021. The greenhouse temperature was set to 10-15.5 °C with 70% relative humidity and a 12-hour daylight setting. The plants were grown in prepared potting mix (PM= 1:1:2 – vermiculite: peat moss: sand). According to the method of Zeng et al., (2020), two treatments were evaluated for each plant species, including a control treatment (uninoculated) and an inoculated treatment. In the first experiment, 10 sporosori/g of potting mix was used in the inoculated treatment and in the second, 40 sporosori/g of potting mix was used. Inoculum level was limited by the amount of inoculum available. Five plants of each species were used for each treatment (control or inoculated), with 18 cover crops and 8 diploid potato lines tested in the first experiment and 11 cover crops tested in the second experiment.

During the course of the first experiment, we discovered that *S. subterranea* is common in commercial peat used in potting mix (Mallik, I, et al., 2019). To eliminate *S. subterranea* inoculum from potting mix, it must be moistened with water and autoclaved at least twice (Fulladolsa-Palma, pers. comm.). Therefore, for the second experiment, the peat moss used to prepare the potting mix was autoclaved three times for 60 min per cycle prior to use.

Prior to planting, the required amount of inoculum was mixed with the potting mix and all the pots were filled with the appropriate amount of potting mix. After planting the seeds, each pot was watered to saturation using approximately 500 mL of water. From the planting date the pots were irrigated by hand, and after 24 days they were irrigated automatically by a drip system connected with ½ gallons per hour (GPH) pressure pinch drip emitters (DripWorks, Willits, CA, USA) that was installed in each pot to apply water 2 min every 8 hours. After 75 days of planting, the drip system was adjusted to apply water 5 min every 8 hours until harvest (Zeng, Y, et al., 2020).

Inoculum preparation

The inoculum of *S. subterranea* was obtained from lesions on infected potato tubers with powdery scab. Infected tubers were washed, and air dried overnight, and the lesions were scraped using a razor blade. The collected powder from the lesions was washed and sieved through three different mesh sizes (850 µm, 150 µm, and 74 µm). After that, the inoculum was left in the hood to air dry, and it was stored at 4°C. The number of sporosori per gram (sp/g) of inoculum was determined with a hemocytometer (Qu and Christ, 2006).

Sampling and disease assessment

The plants were harvested approximately 120 days after planting, and the potting mix, tubers, and roots were collected from each pot and transferred to the laboratory at Fort Collins, CO, for further analysis. The collected potting mix samples were air dried, ground, and sieved to perform DNA extraction. DNA was extracted from 0.25 g per potting mix sample using DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) as per manufacturers' instructions. Quantitative PCR (qPCR) was used to determine and quantify the amount of *S. subterranea* present in the collected samples using the primers and conditions described in detail previously (Mallik, I, et al., 2019; Zeng, Y, et al., 2020). All qPCR assays were performed in a 96-well plate using an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA). The SsTQF1-SsTQP1-SsTQR1 primer-probe set was used to target the amount of the sporosori DNA of *S. subterranea.* Each individual sample was replicated twice on a 96-well plate, and the results were analyzed by t-test. The qPCR results were compared to a standard curve obtained from potting mix DNA extraction with sporosori prepared in water from infected tubers (5, 52, 516, 5,160, 51,600 sporosori per gram of soil for experiment one and 38.125, 381.25, 3,812, 38,125 sporosori per gram of soil for experiment two). The collected tubers were examined to determine whether they are tuber resistant (no powdery scab symptoms) or tuber susceptible (show powdery scab symptoms). The roots were stained with trypan blue based on the method of Hernandez Maldonado, et al., (2013). For each plant group we had 10 replicates (5 inoculated and 5 control), and 3 slides were prepared and examined under the microscope for each replicate to detect the presence of the different stages of *S. subterranea* life cycle in each sample (plasmodium and zoosporangium). In experiment one, we used trypan blue staining and in experiment two, the roots were stained with DAPI to better visualize the sporangial stage of the pathogen and obtain high

quality images. For each plant group we had 10 replicates (5 inoculated with 40 sp/g of potting mix, and 5 un-inoculated), and for each plant sample one slide was prepared and examined under the microscope to detect the presence of *S. subterranea* in the plant roots.

Statistical Analysis

The two experiments were analyzed separately. Since there was a high level of variability among the results, the data were transformed by Box Cox transformation to better fit the normal distribution and obtain normality. T-Test was performed on all the qPCR results using R software (R version 3.6.2 / 2019-12-12) to compare the means of the control and the inoculated samples and determine if there was a significant difference between the two groups. The t-test was performed at a significant level of $\alpha = 0.05$ to determine the significance of the obtained p-values. An ANOVA test was performed to test the treatment effect on each cover crop and to compare between the amount of sporosori pre-harvest and post-harvest (sporosori change) on the eleven cover crops tested. Three variables were tested to check their effect on the amount of sporosori/g of potting soil, treatment (control vs inoculated), plant species, and the interaction between the treatment and plant species.

CHAPTER FOUR – RESULTS

Experiment 1

Based on both qPCR and microscopy results, all the diploid potatoes became infected with *S. subterranea*, although some were more tolerant than the others (Table 1, Fig. 5 & 6). In the inoculated treatments, the number of sporosori detected with the qPCR assay was higher for all of the diploid potatoes than any of the cover crops, except for diploid potato line Dp8 (Table 1). However, even in the diploid potato, the sporosori levels were relatively low in lines Dp2, Dp3, and Dp5 compared to cultivated potato, where we typically see 500 to 250,000 sporosori/g of potting mix in inoculated treatments (Zeng et al., 2020).

The qPCR results for the cover crops showed that the majority of legumes, barley, and buckwheat did not increase soil inoculum levels. For these cover crops, there was not a significant difference between the inoculated and uninoculated treatments and the number of sporosori detected in the inoculated treatment was approximately10 sporosori/g or less (Table 1). The variability was high and as a result, even cover crops that had more than 10 sporosori/g of potting mix at the end of the experiment did not show significant difference between the inoculated and uninoculated treatments. However, there was statistically significant difference between the inoculated treatment compared to each other, and mostly it was between the mustards compared to the other cover crops (Table 2).

Of the cover crops, those in the mustard family may have potential to increase soil inoculum levels, in which the amount of sporosori/g of potting soil in almost all the samples were above 10 sporosori/g (Table 1). Dwarf essex rape, terra nova radish, and caliente mustard had the

highest amount of sporosori/g in the inoculated samples, whereas Pacific gold mustards had the lowest amount of sporosori/g in this family. Among all the cover crop tested, Cahaba vetch had the lowest inoculated mean sp/g of potting soil (0.7 sporosori/g) followed by Common vetch (1.7 sporosori/g), and Anika peas (6.0 sporosori/g), which all belong to the legume family.

An ANOVA test was performed to test the treatment effect on each cover crop and to compare between the amount of sporosori pre-harvest and post-harvest (sporosori change) on the eleven cover crops tested (Table 4). All three of the variables were tested, including treatment (control vs inoculated), plant species, and the interaction between the treatment and plant species were significant. A Tukey test was also performed for a multiple comparison of the group means to determine if the cover crops are statistically different from one another. The potato group means (both control and inoculated) were significantly greater than all the cover crops group means. In addition, there was a statistically significant difference between the control and inoculated group means among all the cover crop tested. However, there was no statistically significant difference between most of the cover crops control groups mean. The only significant difference among the control groups mean was between buckwheat and dixie, and between buckwheat and green peas, where the p-value was 0.05 for both.

Table 1: qPCR assay results from Experiment One for cover crops and diploid potato lines (P-values obtained from T-test), and the presence of plasmodium and zoosporangium in the roots.

Cover Crops	Control Mean (sp/g)	Inoculated Mean (sp/g)	P-Values	Fold Change Increase	Plasmodium	Zoosporangium
Legumes (Fabaceae Family)						**Inoculated**
1. Alfalfa	1.63	11.55	0.31		+	10 % (1/20)
2. Anika Peas	4.20	5.96	0.08		+	0 %
3. Green Peas	98.13	7.68	0.55		+	0 %
4. Cahaba Vetch	0.58	0.72	0.09	1.2	+	0 %
5. Chickling Vetch	1.34	21.90	0.06	16	+	0 %
6. Common Vetch	0	1.71	0.02	2	+	0 %
7. Hairy Vetch	28.76	9.87	0.23		+	0 %
8. Dixie	8.62	7.87	0.88		+	0 %
9. YBSC	1.51	36.84	0.25		+	10 % (1/10)
Mustards (Brassicaceae Family)						**Control & inoculated**
10. Caliente	17.45	54.21	0.23		+	30 % (3/10)
11. Daikon Radish	9.26	14.78	0.46		+	0 %
12. Dwarf Essex Rape	7.50	75.95	0.20		+	44.4 % (4/9)
13. Pacific Gold Mustard	1.29	7.79	0.18		+	10 % (1/10)
14. Terra Nova Radish	15.31	70.99	0.70		+	30 % (3/10)
15. Winfred	5.41	35.24	0.67		+	0 %
Poaceae Family						
16. Scarlet Barley	2.63	8.02	0.05	3	+	0 %
Polygonaceae Family						
17. Buckwheat	0.93	9.33	0.17		+	0 %
Sorghum x Sudangrass						**Inoculated**
18. Sordan 79	5.07	27.14	0.24		+	28.5 % (2/7)
Diploid Potatoes						**Control & inoculated**
1. Dp7 (BS 297 S438) (Tuber resistant)	1601.61	229.24	0.14		+	40 % (4/10)
2. Dp8 (GS5 US-W 0457) (Tuber resistant)	25.71	32.03	0.25		+	18.2 % (2/11)
3. Dp4 (GS12 US-W 0730) (Tuber resistant)	72.13	1867.09	0.20		+	28.6 % (2/7)
4. Dp5 (BS 231 M10) (Tuber susceptible)	340.66	132.01	0.06	0.4	+	50 % (5/10)
5. Dp2 (GS22 US-W 3573) (Tuber susceptible)	141.61	231.81	0.71		+	55.5 % (5/9)
6. Dp6 (BS 298 S440) (Tuber susceptible)	19.27	2629.19	0.01	136	+	33.3 % (3/9)
7. Dp3 (GS23 US-W 4519) (Tuber susceptible)	0.95	158.18	0.04	158	+	25 % (2/8)
8. Dp9 (M6) (Tuber susceptible)	0.80	3662.50	0.04	4600	+	33.3 % (3/9)

Table 2: Multiple comparison between the cover crops inoculated treatment from Experiment One obtained from the Tukey test.

INOCULATED CHANGE	P-VALUE
Daikon Cc17-Anika Peas Cc15	0.017
Daikon Cc17-Barley Cc11	0.000
Daikon Cc17-Cahaba Vetch Cc18	0.000
Daikon Cc17- Green Peas Cc4	0.000
Daikon Cc17- Dixie Cc14	0.000
Daikon Cc17- Hairy Vetch Cc2	0.036
Daikon Cc17- PG Mustard Cc6	0.000
Daikon Cc17- Sordan Cc8	0.000
Daikon Cc17- Terra Nova Radish Cc5	0.005
Daikon Cc17- Winfred Cc7	0.002
Daikon Cc17- YBSC Cc12	0.001
Common Vetch. Cc16-Barley Cc11	0.010
Common Vetch. Cc16-Dixie Cc14	0.051
Common Vetch. Cc16-Sordan Cc8	0.001
Caliente Cc13-Sordan Cc8	0.038

The selected potato varieties showed differences in the tolerance to powdery scab in tubers stages (Table 1). The roots of all the varieties developed galls and the tubers of five of the lines developed lesions. Plasmodia and zoosporangia were observed in all potato lines with light microscopy (Fig. 5, Fig. 6). Although, zoosporangia were observed in all the groups of diploid potato varieties, there was a high variability within replicates. The presence of zoosporangia ranged from 11.1% to 40% and 25% to 55.5% in the resistant and susceptible tuber varieties, respectively (Table 1).

Root galls were not observed in the cover crops. The light microscopy results showed that all the cover crop roots had plasmodium, however, zoosporangium was only found in some cover crops. In the legume's family, zoosporangia were only found in alfalfa and YBSC (Table 1, Fig. 8), and also in sordan 79 (Fig. 12); however, it was not abundant. On the other hand, zoosporangia were found in four plant groups in the mustards family out of six plants tested, and it was abundant

in Dwarf Essex Rape, Terra Nova Radish, and Caliente mustard (44.4 %, 30 %, and 30 % respectively) (Table 1, Fig. 10).

In the diploid potatoes and the mustards family, zoosporangia were found in both the control and the inoculated samples, whereas in the legumes and sordan 79 it was only found in the inoculated samples. In addition, there was a direct relationship between the amount of sporosori/g present in each sample and the presence of zoosporangium. All the samples that had high numbers of sporosori/g of potting soil had zoosporangium in their roots, and most of the samples that had low numbers of sporosori/g of potting soil did not have zoosporangium in their roots.

Experiment 2

Among the eleven cover crops tested in experiment 2, buckwheat had the lowest amount of sp/g of potting soil in the inoculated samples, followed by anika peas, and cahaba vetch where the group mean was (43.84, 57.84, and 69.42 sp/g respectively) (Table 3). Scarlet barley, dixie, and common vetch had the highest amount of sp/g of potting soil in the inoculated samples where the group mean was (140.84, 131.02, and 124.88 sp/g respectively). All the legumes, buckwheat, and barley had more sp/g in the potting mix than the inoculated amount of 40 sp/g in the inoculated samples; however, all the control samples had a low amount of sp/g in the potting mix ranging from 0.55 sp/g for buckwheat to 28.99 sp/g for common vetch. On the other hand, caliente, which belongs to the mustard family, had a sp/g level that was above the rest of the cover crops in both control and inoculated samples (114.35 sp/g and 347.18 sp/g respectively), suggesting that the pathogen may be multiplying in this cover crop.

The potato cv. “Cherry Red” had a high population level of *S. subterranea* in both control and inoculated samples (792.96 and 25340.14 sp/g, respectively) (Table 3). We used seed tubers

for this experiment that did not have visible powdery scab symptoms, but that may have been infested. The positive control results also again clearly demonstrate the potential for potato to increase soilborne inoculum compared to cover crops.

In our second experiment, we only looked for zoosporangia since these structures are more distinctive than plasmodia. Zoosporangia were only found in three of the 11 cover crops tested and the results were consistent with the first experiment, except that zoosporangium was found in chickling vetch in the second experiment (Fig. 13). The zoosporangia were only in the inoculated samples and were not abundant. However, it was found in both control and inoculated samples in the caliente roots (Fig. 14). As expected, zoosporangia were also found in the potato Cherry red potato roots, and it was abundant in both control and inoculated samples (Fig. 15).

Table 3: The abundance of sporosori/g of potting soil in the cover crops obtained from qPCR results represented with their p-values, and plant group means, and the abundance of zoosporangium found in the roots represented in percentage based on fluorescence microscopy results. (Experiment Two).

	Cover Crop	Control Mean (sp/g)	Inoculated Mean (sp/g)	p-value	Zoosporangium Inoculated
1.	Buckwheat	0.55	43.84	0.006	0 %
2.	Anika Peas	4.58	57.84	0.001	0 %
3.	Cahaba Vetch	25.78	69.42	0.3	0 %
4.	Hairy Vetch	0.97	79.13	0.01	0 %
5.	Chickling Vetch	0.91	100.56	0.002	10 % (1/10)
6.	Green Peas	4.99	103.33	0.02	0 %
7.	Alfalfa	4.08	124.63	0.09	10 % (1/10)
8.	Common Vetch	28.99	124.88	0.2	0 %
9.	Dixie	5.51	131.02	0.009	0 %
10.	Scarlet Barley	2.82	140.84	0.04	0 %
					Control & inoculated
11.	Caliente	114.35	347.18	0.4	40 % (4/10)
	Positive Control				**Control & inoculated**
12.	Cherry Red Potato	792.96	25340.14	0.03	60 % (6/10)

Table 4: ANOVA test results, which was performed on the second experiment to see the treatment effect on each cover crop and to compare between groups mean.

ANOVA TEST

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TREATMENT	1	45577	45577	1.641e+05	< 2e-16 ***
COVER CROP	11	42	4	1.369e+01	2.24e-15 ***
TREATMENT:COVER CROP	11	15	1	4.832e+00	6.73e-06 ***
RESIDUALS	96	27	0		

CHAPTER FIVE - DISCUSSION

S. subterranea is a challenging pathogen to study because it is an obligate pathogen that only infects below-ground plant organs and because of its complex life cycle. We used qPCR and microscopy assays to evaluate diploid potato and cover crops to determine if they are susceptible to this pathogen and to determine if they can increase inoculum. We found that some cover crops routinely used in the San Luis Valley do not result in an increase in *S. subterranea* inoculum, at least in greenhouse experiments. Some Brassicaceae plants may allow a low level of pathogen multiplication. We also found that some diploid potato may be tolerant to *S. subterranea*.

Our first qPCR experiment demonstrated that all the tested diploid potatoes were infected with powdery scab, and that the inoculum level of *S. subterranea* in the potting mix used to grow these potatoes was increased. The amount of increase varied, however, and was quite low for some of the diploid lines. Some of the diploid potatoes did not show tuber symptoms while others did; however, all of them had both plasmodium and zoosporangium in their roots. These results indicate that the pathogen completed its life cycle in these plants and the spores can be released into the soil and thus increasing soil inoculum level. Since potatoes are the main host for this pathogen, these results were expected, and because we did not find any resistant in the diploid potatoes, we focused our second experiment solely on the cover crops. However, the variability among these diploid lines suggests that useful genetic experiments could be designed to identify susceptibility genes.

The selection of the cover crops used in this experiment was based on the most commonly used crops by farmers as cover crops, and these crops have been used for years as an effective practice to manage different soil-borne diseases (Marr, Janke, & Conway, 1998). A cover crop is

a crop that is often planted after harvesting a vegetable crop and removed before the next vegetable crop is planted. In addition to their ability in suppressing soil-borne diseases, they provide many benefits to the soil (Nair, Kaspar, & Nonnecke, 2015). They can improve soil structure by increasing soil organic matter, improve soil moisture and nutrient holding capacity, enhance soil biodiversity, increase nitrogen fixation, reduce the amount of bacterial and fungal disease in the soil resulting in breaking disease cycles, and suppress weed germination (Wallace, 2012, Dupont, 2015, and Nair, Kaspar, & Nonnecke, 2015).

Previous studies show that the mustards family have the ability to reduce soil-borne diseases including powdery scab (Larkin & Lynch, 2018); however, our results were contradictory, where the mustards family had the potential to increase soil inoculum level comparing to the other cover crops. This contradiction maybe due to the different ways of using the cover crops, where previously they used it as a green manure in which they incorporate the crop with the soil. Using this process with the mustards family resulted in reducing soil-borne diseases based on Larkin & Lynch, 2018 study. The mustards family produce chemical (sulfur compound) known as glucosinolates that when incorporated into the soil break down to produce isothiocyanates, which can act like a fumigant and are toxic to many soil-borne pathogens (Dupont, 2015, and Larkin & Lynch, 2018). While in our study we used these crops as cover crops without chopping and incorporating them into the soil to study and evaluate their ability to reduce the disease incidence as a host or non-host crop.

Our first experiment demonstrated that the mustards family had the highest inoculated mean sp/g of potting soil compared to the legume family and other cover crops. Among all the mustards tested Caliente mustard (54.21 sp/g of potting soil) was filled with both plasmodium and zoosporangium, which means that *S. subterranea* completed its life cycle in this crop and thus can

increase soil inoculum level. The presence of zoosporangium in four out of six mustards tested indicated that these plants could be a suitable host for this pathogen. On the other hand, the legumes family, buckwheat, barley, and sordan 79 all had plasmodium, but zoosporangium was only present in 3 out of 12 plants tested, indicating that the pathogen could not complete its life cycle in the presence of these plants and thus the inoculum level can be reduced overtime. The low numbers of sp/g obtained for these plants from the qPCR assay with the microscopy results show that these plants (legumes, barley, and buckwheat) might be the solution in reducing the incidence of powdery scab.

There are two possibilities that could explain the high level of variability in the obtained results of the qPCR, and the presence of plasmodium in all the samples; first, since *S. subterranea* share some similarities with other plasmodiophorid and fungus pathogens, which all produce plasmodium, other pathogens may be the cause of the plasmodium found in all the roots. Second, sporosori are common in uninoculated potting mix based on previous studies (Mallik, I, et al., 2019), and thus it was found in the control samples. Distinguishing *S. subterranea* from other pathogens under the light microscopy was one of the challenges we faced especially that the roots were invaded with many other pathogens. Therefore, in the second experiment we used DAPI (a blue-fluorescent DNA stain) to stain the roots to obtain a better overview of the sporangial stage of *S. subterranea* found in the roots.

The second experiment was conducted to confirm our results and to assure that the chosen cover crops have the potential to reduce the population levels of *S. subterranea* in the soil. Out of 18 cover crops tested, 11 cover crops were chosen based on their low amounts of sp/g found in the DNA that was obtained from qPCR assay. These crops included buckwheat, scarlet barley, legume family, and caliente. Among all the 11 chosen cover crops, buckwheat was the best crop where it

had the lowest amount of sp/g of potting soil in both control and inoculated samples, and zoosporangium was not detected in the roots. Scarlet barley and all the legumes had low amount of sp/g in the control samples, indicating that autoclaving the peat moss prior to use in the second experiment reduced the sporosori abundance in commercial peat moss. All the cover crops had above inoculated detectable level of 40 sp/g of potting mix in the inoculated samples; however, the increase was not significant, and they had fewer amount of sp/g of potting mix than caliente that belongs to the mustard family. Zoosporangium was found in alfalfa and chickling vetch, but it was not that abundant, and it was only found in one of the inoculated samples. The important thing here is that the pathogen does not form sporosori (the resting spores that can survive in the soil for more than 10 years), and these cover crops tend to act as trapping plants where they trigger the germination of the zoospore, allow the infection, but prevent the pathogen from producing new spores. Using these cover crops could reduce the abundance of the spores in the soil over time or at least don't increase the soil inoculum level.

Caliente, which belongs to the mustard family, was chosen for the second experiment to check if it has the potential to increase soil inoculum level and based on our results from both first and second experiment, we concluded that it may increase the sporosori level in the soil. Caliente had sp/g above the inoculated detectable level in experiment 1 and 2, and their roots were highly invaded with zoosporangia in both control and inoculated samples. The high amount of sp/g found in the cherry red potato (our positive control), and the abundance of zoosporangium was expected as potatoes are the main host for this pathogen.

These results made us wonder if there is a sub-population of *S. subterranea* or if it is a heterogenous population and the one that infects potatoes belong to a different population than what's infecting other cover crops since we only got high amount of sp/g in the potatoes. Genome

sequencing may be the answer for our questions, and it can be the focus of the future studies regarding this pathogen.

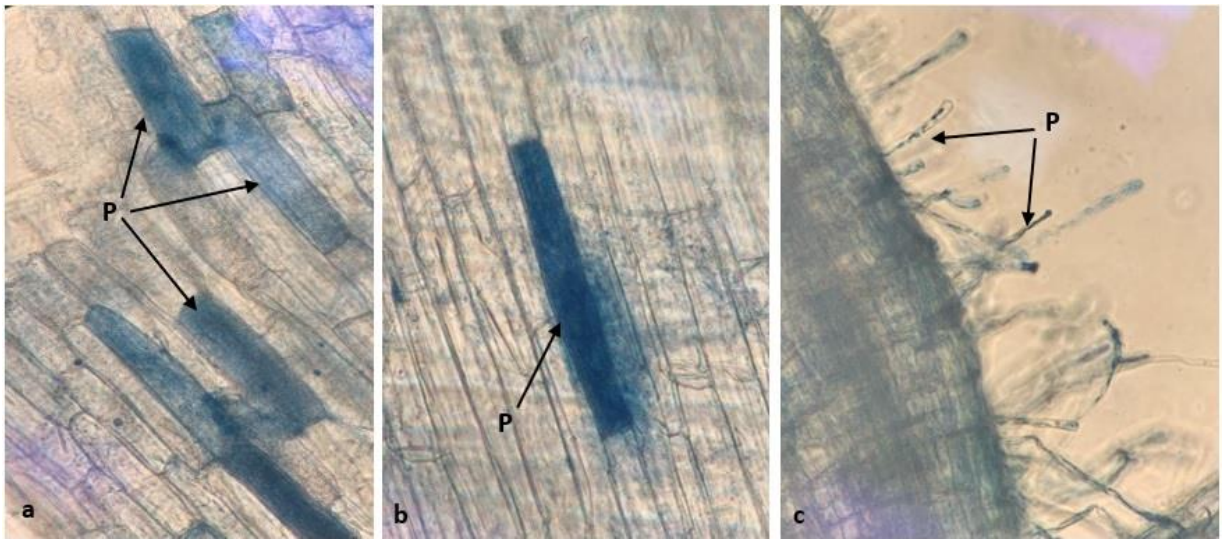

Figure 5: Plasmodium found in diploid potato 1 root cells (a), Plasmodium found in diploid potato 4 root cells (b), and plasmodium found in diploid potato 6 root hairs (c). Similar results were observed for all the varieties (data not presented).

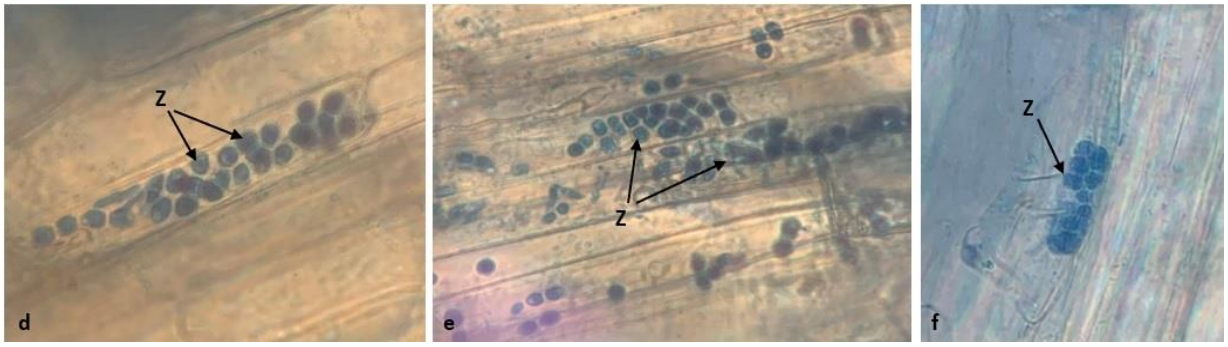

Figure 6: Zoosporangium found in diploid potato 1 root cells (d), zoosporangium found in diploid potato 5 root cells (e), and zoosporangium found in diploid potato 6 root cells (f). Similar results were observed for all the varieties (data not presented).

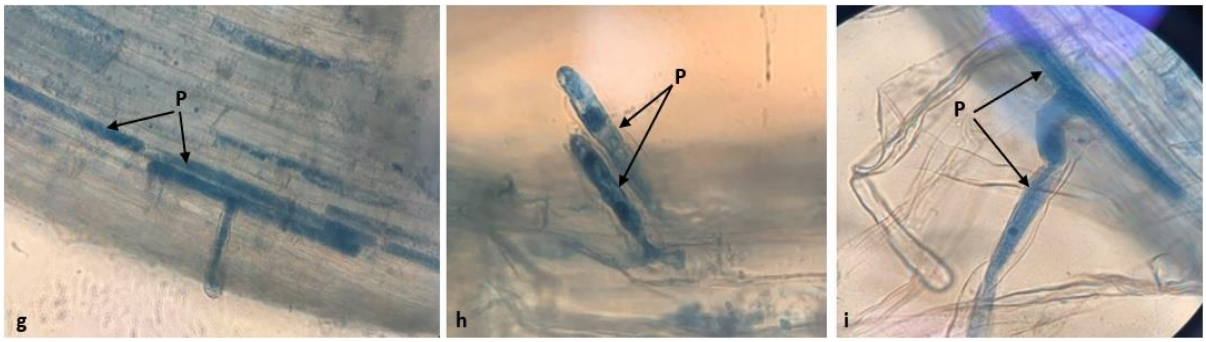

Figure 7: Plasmodium found in the roots of the legume's family. (g) Green peas, (h) Alfalfa, and (i) Dixie. Similar results were observed for all the varieties (data not presented).

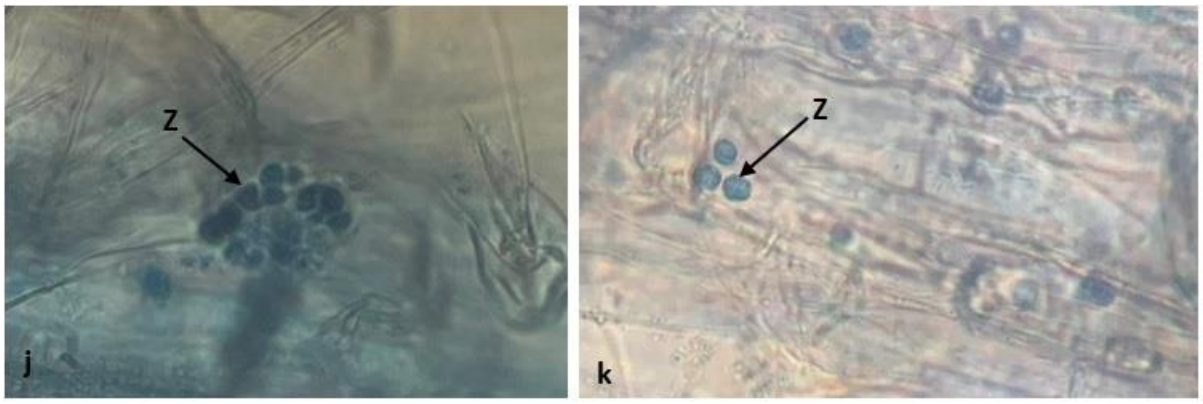

Figure 8: Zoosporangium found in the roots of the legume's family. (j) Alfalfa, and (k) YBSC. It was only found in these varieties.

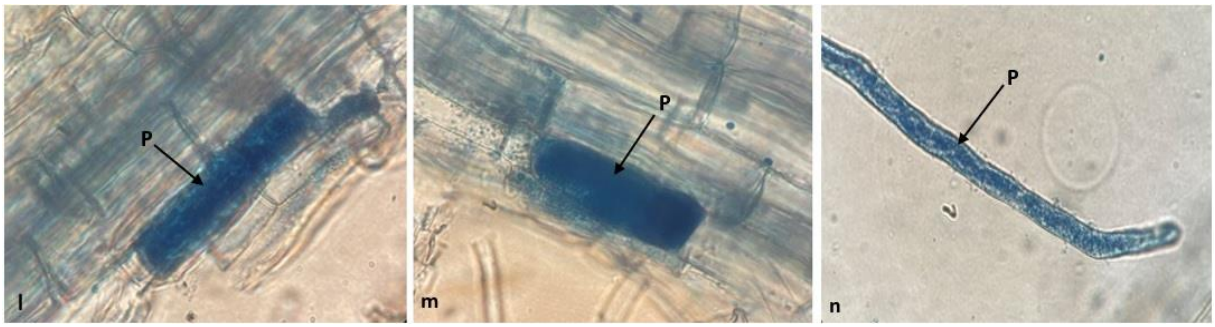

Figure 9: Plasmodium found in the roots of the mustard's family. (l) Pacific Gold Mustards, (m) Caliente, and (n) Dwarf Essex Rape. Similar results were observed for all the varieties (data not presented).

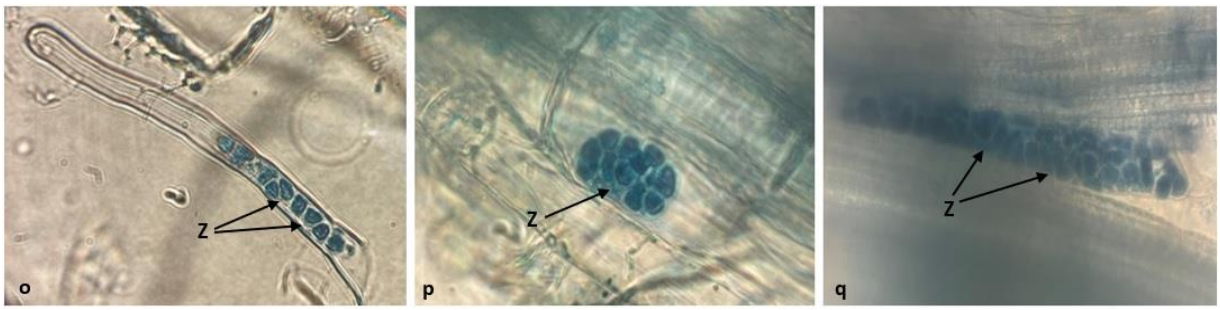

Figure 10: Zoosporangium found in the roots of the mustard's family. (o) zoosporangium in Caliente root hair, (p) zoosporangium in Caliente root cells, and (q) zoosporangium in Pacific Gold Mustards root cells. Similar results were observed in Terra Nova Radish and Dwarf Essex Rape (data not presented).

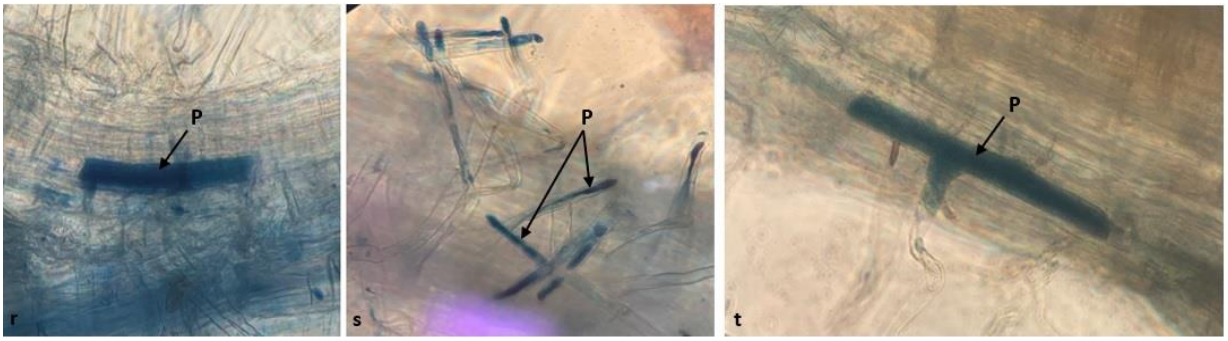

Figure 11: Plasmodium found in the roots of (r) Scarlet Barley, (s) Sordan 79, and (t) Buckwheat.

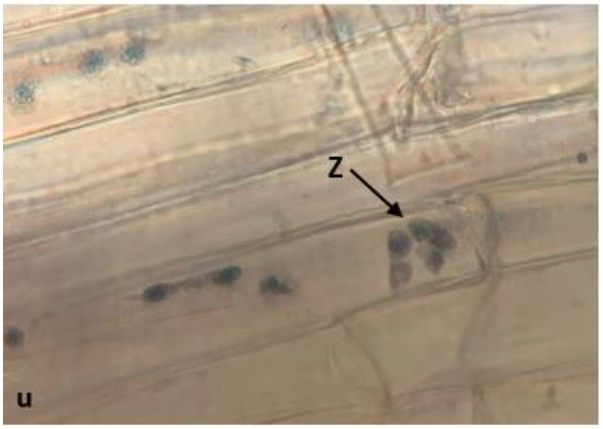

Figure 12: Zoosporangium found in Sordan 79.

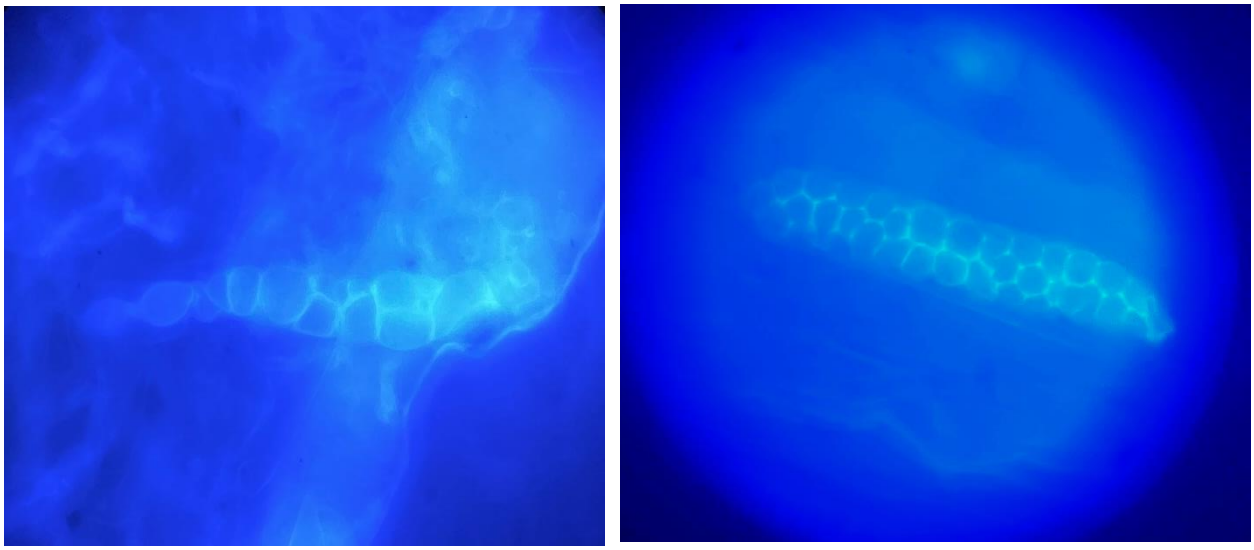

Figure 13: Zoosporangium found in the inoculated sample of alfalfa roots (on the left), and zoosporangium found in chickling vetch inoculated sample (on the right). Experiment 2.

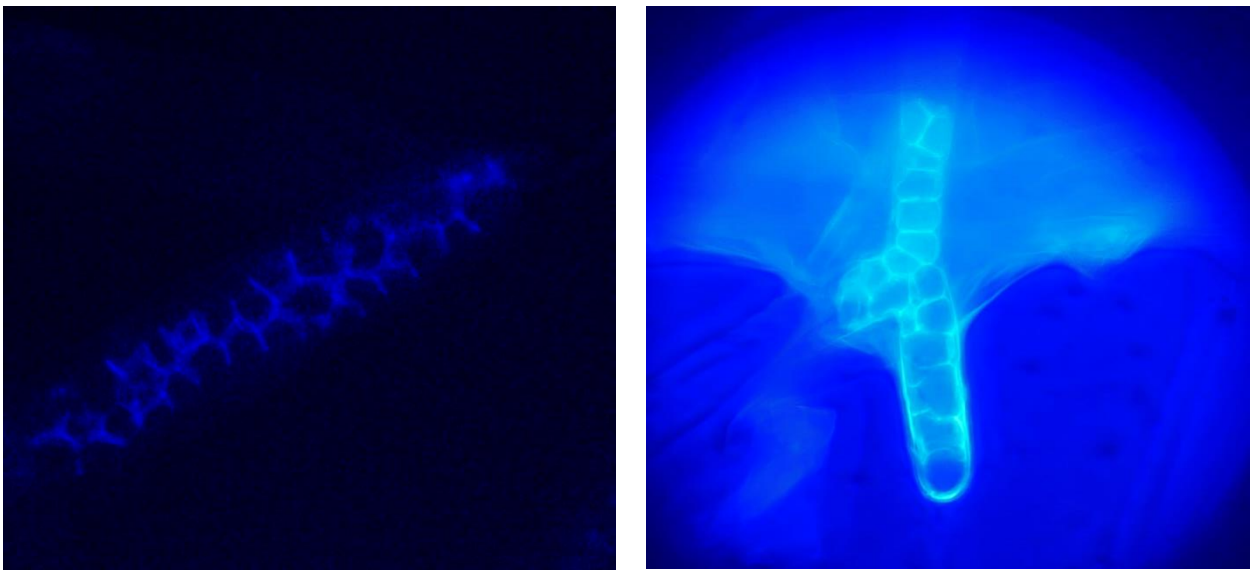

Figure 14: Zoosporangium found in both control and inoculated samples of caliente roots. Experiment 2.

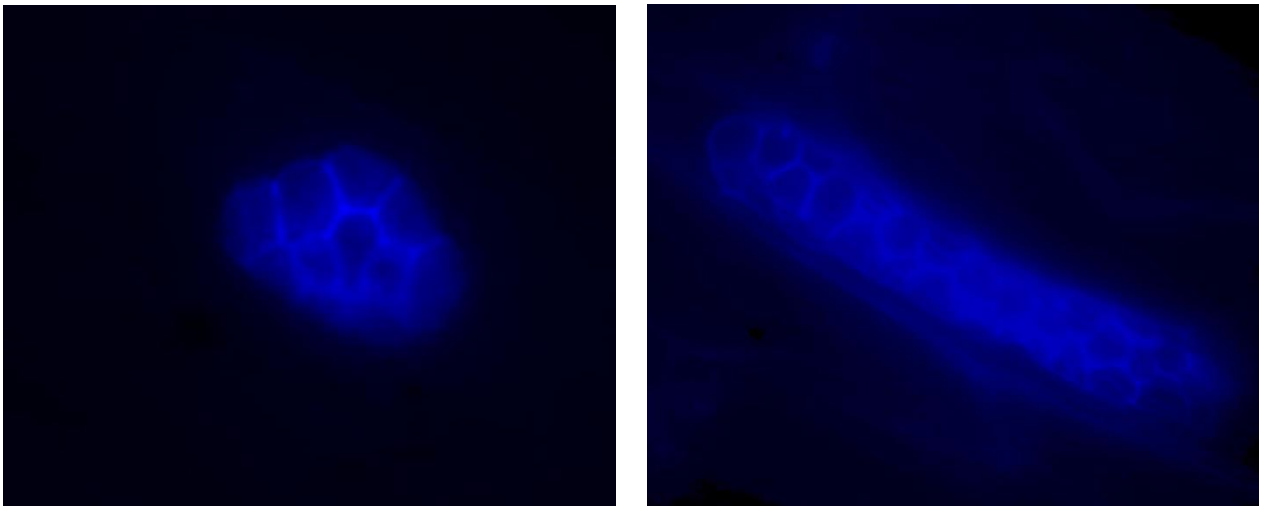

Figure 15: Zoosporangium found in both control and inoculated samples of cherry red potato roots. Experiment 2.

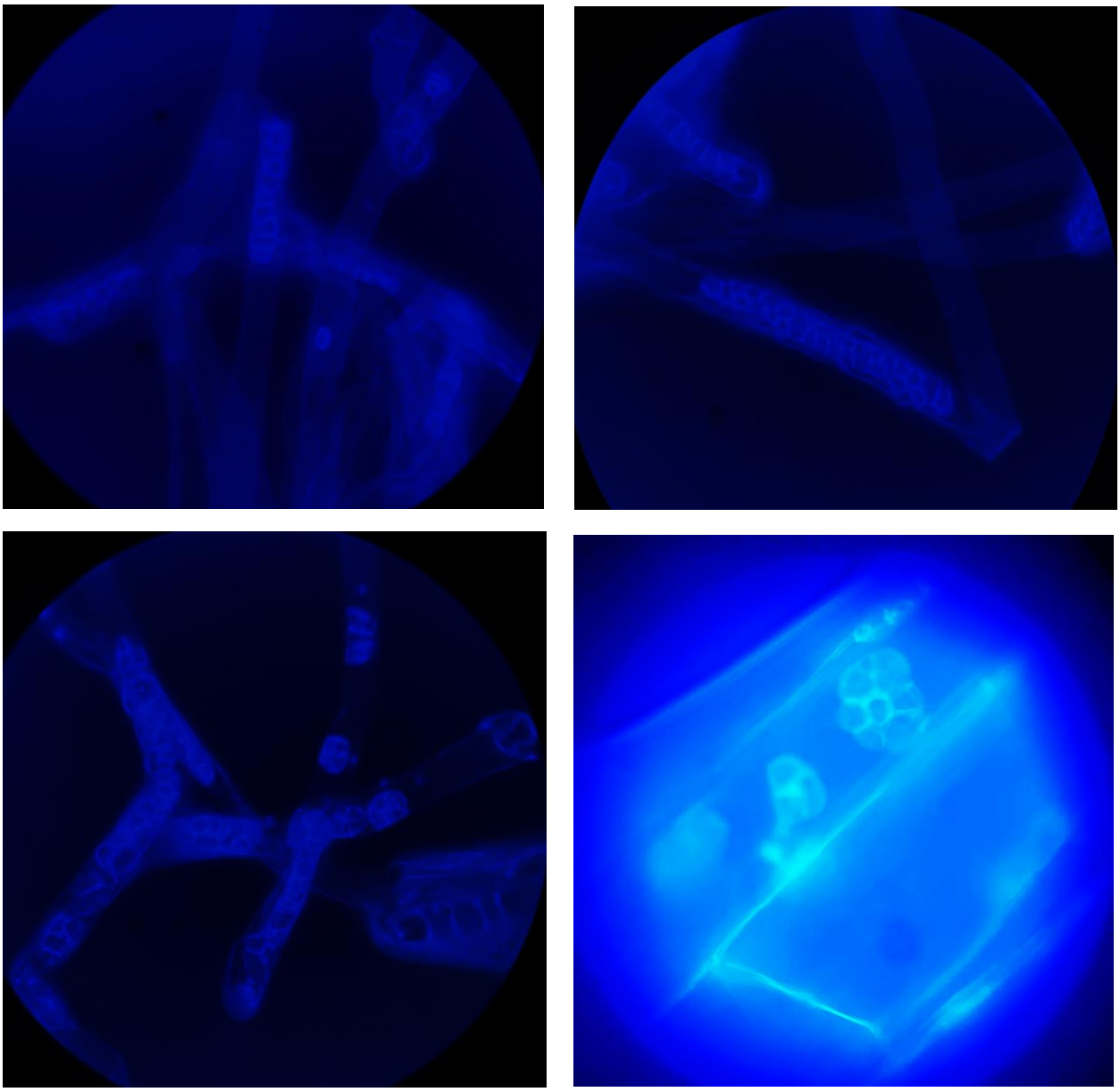

Figure 16: The abundance of zoosporangium in cherry red potato roots.

REFERENCES

Van de Graaf, P., Wale, S. J., & Lees, A. K. (2007). Factors affecting the incidence and severity of Spongospora subterranea infection and galling in potato roots. Plant Pathology, 56(6), 1005-1013.

Van de Graaf, P., Lees, A. K., Cullen, D. W., & Duncan, J. M. (2003). Detection and quantification of Spongospora subterranea in soil, water and plant tissue samples using real-time PCR. *European Journal of Plant Pathology*, *109*(6), 589-597.

Balendres, M. A., Tegg, R. S., & Wilson, C. R. (2017). Resting spore dormancy and infectivity characteristics of the potato powdery scab pathogen Spongospora subterranea. Journal of Phytopathology, 165(5), 323-330.

Tsror, L., Shapira, R., Erlich, O., Hazanovsky, M., & Lebiush, S. (2020). Characterization of weeds and rotational crops as alternative hosts of Spongospora subterranea, the causal agent of powdery scab in Israel. Plant Pathology.

Thangavel, T., Tegg, R. S., & Wilson, C. R. (2015). Monitoring Spongospora subterranea development in potato roots reveals distinct infection patterns and enables efficient assessment of disease control methods. PLoS One, 10(9).

Merz, U. (2008). Powdery scab of potato—occurrence, life cycle and epidemiology. American Journal of Potato Research, 85(4), 241.

Ciaghi, S., Neuhauser, S., & Schwelm, A. (2018). Draft genome resource for the potato powdery scab pathogen Spongospora subterranea. Molecular plant-microbe interactions, 31(12), 1227-1229.

Bittara, F. G., Robinson, A. P., & Gudmestad, N. C. (2018). North Dakota State University. Retrieved June 09, 2020, from https://www.ag.ndsu.edu/publications/crops/powdery-scab-of-potatoes

Balendres, M. A., Clark, T. J., Tegg, R. S., & Wilson, C. R. (2018). Germinate to exterminate: chemical stimulation of Spongospora subterranea resting spore germination and its potential to diminish soil inoculum. Plant Pathology, 67(4), 902-908.

Zeng, Y., Fulladolsa, A. C., Cordova, A. M., O'Neill, P., Gray, S., & Charkowski, A. O. (2020). Evaluation of effects of chemical soil treatments and potato cultivars on Spongospora subterranea soil inoculum and the incidence of powdery scab and potato mop-top virus in potato. Plant Disease, (ja).

Mallik, I., Fulladolsa, A. C., Yellareddygari, S. K. R., Bittara, F. G., Charkowski, A. O., & Gudmestad, N. C. (2019). Detection and quantification of Spongospora subterranea sporosori in soil by quantitative real-time PCR. Plant Disease, 103(12), 3189-3198.

Larkin, R. P., & Lynch, R. P. (2018). Use and effects of different Brassica and other rotation crops on soilborne diseases and yield of potato. Horticulturae, 4(4), 37.

Scheufele, S. B. (2016, November 16). Potato, Identifying Diseases. Retrieved January 03, 2021, from https://ag.umass.edu/vegetable/fact-sheets/potato-identifying-diseases

Johnson, S. B. (2002). Bulletin #2436, Potato Facts: Powdery Scab of Potatoes - Cooperative Extension Publications - University of Maine Cooperative Extension. Retrieved January 03, 2021, from https://extension.umaine.edu/publications/2436e/

Clarke, C. R., Tegg, R. S., Thompson, H. K., Frederick, C., Haynes, K. G., Kramer, M., & Wilson, C. R. (2020). Low-dose foliar treatments of the auxin analog 2, 4-D reduce potato common scab and powdery scab for multiple potato cultivars and enhance root development. *Crop Protection*, 105208.

Tsror, L., Lebiush, S., Hazanovsky, M., & Erlich, O. Control of potato powdery scab caused by Spongospora subterranea by foliage cover and soil application of chemicals under field conditions with naturally infested soil. *Plant Pathology*.

Hernandez Maldonado, M. L., Falloon, R. E., Butler, R. C., Conner, A. J., & Bulman, S. R. (2013). Spongospora subterranea root infection assessed in two potato cultivars differing in susceptibility to tuber powdery scab. *Plant Pathology*, *62*(5), 1089-1096.

Marr, C. W., Janke, R., & Conway, P. (1998, November). COVER CROPS FOR VEGETABLE GROWERS. Retrieved 2021, from https://mccc.msu.edu/wp-content/uploads/2016/10/KS_1998_covercropsfor-vegetablegrowers.pdf

Nair, A., Kaspar, T., & Nonnecke, G. (2015, June). Cover Crops in Vegetable Production Systems. Retrieved 2021, from https://mccc.msu.edu/wp-content/uploads/2016/09/IA_2015_Cover-Crops-in-Vegetable-Production-Systems.pdf

Dupont, T. (2015, January 14). Reducing soil borne diseases with cover crops. Retrieved April, 2021, from https://extension.psu.edu/reducing-soil-borne-diseases-with-cover-crops

Wallace, J. (2012, Summer). COVER CROPS FOR DISEASE SUPPRESSION. Retrieved 2021, from https://cdn.dal.ca/content/dam/dalhousie/pdf/faculty/agriculture/oacc/en/tcog/TCOG_2012_Cover_Crops.pdf

Encyclopedia Staff. (2020, March 13). San Luis Valley. *Colorado Encyclopedia*. Retrieved from https://coloradoencyclopedia.org/article/san-luis-valley

Everts, K. (2016, August). *Soil Biology: Cover Crops and Disease Suppression*. SARE Southern. https://southern.sare.org/resources/soil-biology-cover-crops-and-disease-suppression/.