Investigating Management Alternatives for Southern Blight on Vegetables in the mid-Atlantic United States.

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ABSTRACT

Incidence and severity of southern blight caused by *Sclerotium rolfsii* Sacc. is increasing in the mid-Atlantic region of the U.S. affecting both conventional and organic vegetable production systems. Traditional southern blight management relied on fumigants and fungicides with often inconsistent and uneconomical results. Moreover, with the phase-out of methyl bromide, and the high cost and toxicity of other available fumigants, it is necessary to identify effective and economical southern blight management alternatives. Therefore, the objectives of this research were to 1) evaluate the effect of planting date and potato (*Solanum tuberosum* L.) cultivar selection on southern blight incidence and tuber yield and quality; 2) compare potato cultivar tuber susceptibility to *S. rolfsii* in post-harvest settings; and 3) assess the suitability of six cool-season cover crop biomasses and three locally organic materials as carbon sources for anaerobic soil disinfestation (ASD) treatments and their impact on *S. rolfsii* viability and soil. In a three-year field study, ten commercial potato cultivars and four planting dates per year were evaluated on the Eastern Shore of Virginia. Later potato plantings generally produced greater disease incidence (85-94%) and lower tuber yield (1.8-9.4 Mg ha^{-1}) and quality (47-78%), likely driven by humid, warm weather later in the season that was conducive to disease and detrimental to the potato crop. While no potato cultivar was completely immune to *S. rolfsii* incidence, cultivar 'Accumulator' consistently had one of the lowest disease incidences (12-71%) and greatest tuber yield among cultivars (6.2-37.3 Mg ha^{-1}), and 'Adirondack Blue' and 'Red Norland' had the overall greatest disease incidence (33-100%). Following harvest of the field experiment, the susceptibility of tubers from the ten cultivars to post-harvest rot caused by *S. rolfsii* was

compared in a laboratory experiment under controlled conditions. ‘Atlantic’ followed by ‘Accumulator’ were the least susceptible (3.7-12.6 g of diseased tissue) whereas ‘Adirondack Blue’, followed by red-skinned potato cultivars had the greatest severity of post-harvest tuber rot (12-17.5 g of diseased tissue). Following three weeks of ASD treatments in growth chamber pot experiments utilizing nine carbon sources incubated at 15/25°C 12/12-h cycle, most carbon sources induced soil anaerobicity (33-90 % iron oxide paint removal), but they did not reduce sclerotial viability compared to nonamended aerobic controls. However, most amended soils undergoing ASD induced greater soil microbial activity (0.7-2.0 % CO_2 in vol.) compared to nonamended controls (0.1-0.7 CO_2 in vol.), increased soil pH, and some amendments increased soil nitrate accumulation. Overall, results of these studies indicate that southern blight can be effectively managed in potato by coupling early planting dates with cultivar selection, but though promising, additional studies are needed to determine the parameters to effectively decrease *S. rolfsii* viability with ASD treatments.

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GENERAL AUDIENCE ABSTRACT

The fungal disease southern blight, caused by the soilborne plant pathogen *Sclerotium rolfsii* Sacc., is increasing in the mid-Atlantic U.S. The increase of southern blight is likely due to a multitude of issues such as climate fluctuations, reduction in the use of fumigants, increased soil organic matter through conservation practices, and cropping systems that favor soilborne pathogens. Traditional management of *S. rolfsii* relied on pesticides and fumigants, but control was often inconsistent and expensive. Moreover, the use of some soil fumigants has been restricted due to environmental concerns, which has limited the options for the management of plant pathogens present in the soil, and management alternatives are needed. Through a series of field, laboratory, and greenhouse experiments, this study 1) evaluated the effect of planting date and potato (*Solanum tuberosum* L.) cultivar selection on southern blight incidence and tuber yield and quality; 2) compared potato cultivar tuber susceptibility to *S. rolfsii* in post-harvest settings; and 3) assessed the suitability of six cool-season cover crop biomasses and three locally-sourced organic materials as carbon sources in anaerobic soil disinfestation (ASD) treatments and their impact on *S. rolfsii* viability and soil. Results of a three-year field study demonstrated that an integrated approach to the management of southern blight that utilizes moderately resistant potato cultivars such as ‘Accumulator’ and early (March to early April) planting dates will minimize risk of disease and maximize tuber yield. Following harvest, inoculation of potato tubers under controlled conditions revealed that the cultivar ‘Atlantic’ followed by ‘Accumulator’ can avoid greater losses due to less tuber rot, while 'Adirondack Blue' followed by red-skinned potatoes have a greater risk of suffering post-harvest tuber decay

from southern blight. The anaerobic soil disinfestation (ASD) treatments examined in this study indicated that cover crops biomass and poultry litter are suitable carbon sources to induce soil anaerobic conditions, but they may not reduce the survival of *S. rolfsii* propagules if treatment temperatures are below 25°C. However, ASD showed benefits such as increased activity of soil microbial communities compared to nonamended soil, increased soil acidity, and some amendments such as mushroom compost, poultry litter, and Austrian winter pea (*Pisum sativum* subsp. *arvense*) can contribute soil nutrients such as nitrate. Overall, this research demonstrates that choosing less susceptible cultivars coupled with early planting dates can minimize the negative impact of southern blight on potato yields and quality, and consequently minimize the use of pesticides as the main option to control southern blight on potato across the mid-Atlantic region. Though promising, additional studies are needed to determine the parameters that optimize ASD as an alternative to manage *S. rolfsii* with ASD treatments.

DEDICATION

I dedicate this dissertation to my beloved parents, Jose Domingo Garcia and Herminia Gonzalez, for being models, inspiration, heroes, and their immense and unconditional support through my life and studies.

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TABLE OF CONTENTS

LIST OF TABLES

Chapter 2.

Chapter 3.

LIST OF FIGURES

CHAPTER 1: Introduction and Literature Review

Potato and tomato production

In Virginia, a variety of vegetable crops are grown annually and among them, potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) play an important role in Virginia's agriculture industry. Historically, Virginia has been recognized as a major potato producer within the U.S. averaging 48,562 ha harvested annually from 1917 to 1931, representing 3.5% of the total harvested nationally (Brittingham et al. 1963). However, over the past century, the average potato area planted has declined to 2,023 ha in 2018 ranking 21st in the nation (USDA-National Agricultural Statistics Service 2019c). In 2018, the 850 ha planted to tomato ranked Virginia 10th in the U.S. for tomato production (USDA-National Agricultural Statistics Service 2020). Of the 5,301-ha planted to vegetables in Virginia for 2018, potato and tomato accounted for 53% of the total area planted with a production of 47,000 and 19,000 metric tons, respectively. The total value for both crops was in excess of $44 million (USDA-National Agricultural Statistics Service 2019b, c, 2020). Roughly 80% of the potato and tomato production is located in the Eastern Shore of Virginia (ESVA), the southern part of the Delmarva Peninsula (USDA-National Agricultural Statistics Service 2019a).

In Virginia, it is recommended to plant potato seed pieces from March 10 to April 5, with planting dates happening later in more northern states in the mid-Atlantic region (Kuhar et al. 2019). Seed potato are purchased from certified producing states, with Maine leading the region with 62.7% of the total 3,175 metric tons imported in 2019 (Richardson 2020). Before planting, the seed is cut into pieces that contain at least one bud. During cutting or soon afterward, the seed pieces are generally treated with a fungicide such as mancozeb, metiram, captan,

thiophanate methyl, or fludioxonil, to protect against certain soilborne pathogens. It is recommended that potato seed pieces be planted on 0.90 m spaced rows and a 0.18-0.30 m within-row spacing; however, this varies depending on the potato type, seed piece size, and target market (Kuhar et al. 2019). The recommended target soil pH for cultivars susceptible to potato scab (*Streptomyces scabies*) is 5.2, and soil pH of 6.2 for resistant cultivars. Fertilizer recommendations average 168-202 kg ha^{-1} of nitrogen (N), 224 kg ha^{-1} of phosphorus-P_2O_5 (P), and 224 kg ha^{-1} of potash-K_2O (P). The total P, K, and one-third of the total N are broadcast and disked-in before planting, and the rest of N is recommended 4-5 weeks after planting (Kuhar et al. 2019; Reiter et al. 2009). Herbicides and insecticides are applied based on regular crop inspections and pest alerts. Depending on the cultivar and market, potato tubers are harvested 90-160 days after planting (Kuhar et al. 2019). Virginia produces fresh-market and processing potato tubers and harvest generally occurs from early June to late August (Richardson 2020).

For tomato, most of the production in Virginia follows standard guidelines for commercial plasticulture production systems. After land preparations and raised bed formation, plastic mulch with drip tape underneath is placed. In some conventional farming production systems, soil fumigation is performed to control soilborne diseases, insects, and weeds. Fumigants commonly include chloropicrin, 1,3-Dichloropropene, metam-sodium, and allyl isothiocyanate; and are applied simultaneously when the plastic mulch is installed (Kuhar et al. 2019). Fresh market tomato seedlings are transplanted starting from April 10 in the warmer areas of the mid-Atlantic. Seedlings are subsequently staked and strung throughout the growing season (O'Dell et al. 2009). Rows are typically spaced 1.5-1.8 m apart with transplants spaced 0.4-0.6 m apart within rows. Fertilizer recommendations depend upon soil nutrient analysis, but generally, polyethylene-mulched fresh market tomato crops require 168-235 kg ha^{-1} of N, 112-224 kg ha^{-1}

of P, and 112-336 kg ha^{-1} of K. All the P and half of the total K is recommended to be broadcast and disked-in, while roughly one-third of the N is incorporated into the bed before laying the plastic mulch. The rest of N and K is fertigated throughout the growing vegetative stage (Kuhar et al. 2019). Throughout the growing season, pesticides are used based on regular crop inspections and recommended schedules and constitute a major production expense. For organic production systems fumigants are not applied and limited pesticide use occurs, thus increasing the challenges of pest and disease management. Production and pest management costs of conventionally produced fresh market tomato on the ESVA range from $19,000-$30,000 per hectare (Fiedler 2014).

Southern blight

Southern blight can occur in both potato and tomato crops and is caused by *Sclerotium rolfsii* Sacc (teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough*)*, a necrotrophic soilborne fungal pathogen that is included among the most important pathogenic agents in the basidiomycete group (Schumann and D'Arcy 2010). This pathogen was discovered in Florida by Henry Rolfs in 1892 causing blight in tomato plantings (Aycock 1966; Rolfs 1893). The disease is present worldwide with more prevalence in tropic and subtropic regions (Aycock 1966; Mullen 2001). Domestically, southern blight is more problematic in the southern states (Aycock 1966). The occurrence of southern blight in Virginia has been reported since the beginning of the 20th century. Most research in the U.S. has focused on management in peanut (*Arachis hypogaea* L.) (Bowen 2003; Garren 1958; Garren and Bailey 1963; Hagan et al. 2001) and tomato (Keinath and DuBose 2017). However, limited reports and recommendations for southern blight in potato exist to date (Brittingham et al. 1963). Recently, southern blight has become an

emerging problem on potato and other vegetables on the ESVA and the rest of the mid-Atlantic region (Pethybridge et al. 2019; Rideout, personal comm.).

Southern blight symptoms and signs

Symptoms on crops vary due to plant age and weather conditions. Generally, on potato, early symptoms manifest as slight wilting with pale green and yellowing leaves (Aycock 1966; Weber 1923); which resemble those caused by *Fusarium*, *Verticillium*, or bacteria (*Ralstonia solanacearum*) (Edson and Shapovalov 1923). Wilting increases as the day gets warmer and slightly recovers during night periods or cold and dry weather (Xie and Vallad 2010). Other symptoms include a slight drop of some of the younger leaves and leaf tips, and within four days, the entire shoots wilt and fail to recover. On tomato, *S. rolfsii* stem lesions start as small irregular light brown water soaked lesion at the base of the plant (Aycock 1966; Xie and Vallad 2010). In tomato and potato, a closer inspection will show the outer stem tissue decaying at ground level (Carpenter 1920; Xie and Vallad 2010). During moist weather conditions, white mycelium is present on stem lesions. The teleomorph phase can appear at this stage when basidiospores are produced, but this rarely happens under field conditions (Punja 1985; Xie and Vallad 2010). Later, numerous small round white structures with a smooth surface and a hard texture known as sclerotia are observed. The sclerotia become yellow and subsequently dark-brown when they mature (Carpenter 1920; Weber 1923). The fungus can attack the stem one to two inches below the soil line or slightly above (Weber 1923). However, plants with fleshy roots or tubers are prone to underground infections (Aycock 1966; Edson and Shapovalov 1923).

Lesions on tubers commonly appear on the stem and lenticels. They start as small, round, and sunken, with a brownish margin; later, the lesion becomes deeply sunken and turns yellow or

tan. A network of mycelium often adheres to the tuber surface. As the disease progresses, the tissue becomes soft and disintegrates, producing odorless white, opaque, cheesy material; which produces sclerotia in the cavities (Aycock 1966; Weber 1943). The growth of white, thin, silky, radial-growing appressed mycelium is often diagnostic of the disease (Ramsey et al. 1949).

Biology and life cycle of *Sclerotium rolfsii*

Mycelia that survive in decaying organic matter, along with sclerotia that can survive in the soil for years, can serve as primary inoculum (Mullen 2001). *Sclerotium rolfsii* produces a thick mat of white mycelium 3-4 days after the infection starts under ideal weather conditions (Aycock 1966; Mullen 2001). Infection occurs at any stage of the host commonly at the soil line and extends a few centimeters up the stem (Mullen 2001; Roberts et al. 2014). The main hyphae are 5-9 microns in diameter, hyaline, thin-walled, with scattered septa, and clamp connections (Mullen 2001; Townsend and Willetts 1954). Smaller feeding branches measure 2-4 microns in diameter, and are responsible for penetrating the plant tissue (Mullen 2001). Initially, the mycelium is perceived as silky white to the naked eye, and as the pathogen continues developing the mycelium becomes opaque and darker (Aycock 1966). *Sclerotium rolfsii* produces oxalic acid and pectinolytic and cellulolytic enzymes that degrade host tissue and enables infection (Le 2011; Leoni et al. 2014; Punja 1985). The mass of hyphae develops infection cushions that contain crystals of calcium oxalate that disintegrate the plant tissue; subsequently, appressoria are developed at the hyphal tips to extract plant cell nutrients (Smith et al. 1986). The mycelium can grow inter- and intracellularly (Aycock 1966). During the early stage of infection, *S. rolfsii* expresses lectin genes that facilitate the initial attachment of the mycelium to the plant surface (Tarafdar et al. 2018). A mass of hyphae (3-12 strands) assembled in parallel elicits the

formation of sclerotia, which later becomes mere compact or hard masses of mycelium (Aycock 1966).

Sclerotia, which serve as an asexual reproductive structure that can overwinter in harsh climatic conditions, are essential in the continuation of the pathogen's life cycle (Townsend and Willetts 1954). Sclerotia are asexual, multicellular, rigid resting structures that are formed from a hyphae arranged in parallel that later become a round shape (Aycock 1966; Chet and Henis 1975). The small sclerotia (0.5 to 2 mm in diameter) are generally produced after seven days but can start to form as early as four days (Aycock 1966; Mullen 2001). Their color changes from white to tan or dark brown as they mature (Aycock 1966; Mullen 2001). The sclerotia are composed of a thick cuticle that encloses an outer melanized rind two to four cell-layers thick, thin-walled cell cortex, and a medulla of loosely interlacing hyphal strands (Aycock 1966; Punja 1985; Townsend and Willetts 1954). The cuticle is composed of chitin and laminarin and may be developed from a remnant of the membranous material surrounding the sclerotial exudation drops or the result from the polymerization of compounds in the sclerotial exudation (Punja 1985). The germination of sclerotia can be hyphal or eruptive. Hyphal refers to the growth of separate strands on the sclerotial surface that originate from the medulla cells. In contrast, eruptive (destructive) germination refers to a mass of mycelium breaking through the rind. The eruptive germination can lead to formation of secondary sclerotia (Punja 1985).

Athelia rolfsii, the perfect stage of *S. rolfsii* is uncommon under natural conditions (Mullen 2001; Xie and Vallad 2010). Research performed in the early 1930s reported the presence of basidiospores, and Goto concluded that *Corticium centrifugum* should be the name of the perfect stage (Aycock 1966). In addition, Mario Curzi contributed to the research of the

perfect stage in 1932; concluding that isolates in his study possessed a significant affinity to *Corticium rolfsii* (Aycock 1966). Later studies found that the basidial stages belong to the genus *Athelia*, and the new name combination *Athelia rolfsii* (Curzi) was proposed in 1978 by Tu and Kimbrough (Tu and Kimbrough 1978). The role of basidiospores as a source of inoculum is unclear (Punja 1985; Xie and Vallad 2010). While some researchers have stated that the basidiospores are not successful in producing infections (Aycock 1966; Goto 1938), others have expressed that they contribute to a secondary life cycle (Mullen 2001). *S. rolfsii* produces basidia (spore-bearing structure) that contain sterigmata, forming 2 to 4 haploid hyaline, ovoid, thin-walled basidiospores (Roberts et al. 2014; Tu and Kimbrough 1978). The basidia are produced in a white, yellow, or buff-colored unprotected hymenium (Mullen 2001).

Epidemiology

Sclerotium rolfsii is commonly found in tropical and subtropical regions where humid and warm conditions favor growth and plant infection by the fungus (Aycock 1966; Mullen 2001). In the U.S., outbreaks of plant disease caused by *S. rolfsii* have been reported in the northern region (Peltier 1916; Pethybridge et al. 2019), but it is generally more problematic in southern states (Aycock 1966). The optimal temperature for mycelium growth is 27-35°C when coupled with high relative humidity (>90%), but growth can occur over a wide range of temperatures between 8-40°C (Mullen 2001). Well-aerated and drained sandy soils are more prone to *S. rolfsii* development (Punja 1985). Mycelium growth in the soil is promoted by volatile compounds released when undecomposed organic materials are remoistened (Punja 1985).

Sclerotial formation is greatest at 27-30°C (Aycock 1966); when humidity is high; soil pH is below 7; and within well-aerated, light soils (Mullen 2001). Studies have shown that sclerotia have been produced in 4 days at 33°C, reaching maturity in 7 days. Increasing the temperature affects the formation of sclerotia. Increasing the temperature above 35°C decreases the size and number of sclerotia produced, and the mycelial growth is also hindered (Aycock 1966). Conversely, sclerotial formation has been observed at 10°C but with very slow maturation, or in some cases there was a complete absence of formation (Aycock 1966). However, sclerotia have been found to survive below freezing temperatures (Mullen 2001). Generally, the sclerotia survive best near the soil surface. Laboratory studies have found that no sclerotia survived buried 15 cm deep for 45 days in (Chowdhury 1948; Mullen 2001). Other factors such as high soil moisture (higher matric potential) harm sclerotial survival, and the suppression of sclerotial survival is even greater when combined with high temperature (Beute and Rodriguez-Kabana 1981; Punja 1985).

S. rolfsii is dispersed through any mechanism that moves infested soil, infected seed tuber or plant parts, or the use of equipment contaminated with mycelia or sclerotia (Fiers et al. 2012; Franke et al. 1998; Xie and Vallad 2010; Xu et al. 2008). In naturally infested fields, the sclerotia are generally clustered; therefore the disease is initially distributed in the same pattern (Punja 1985). Spread to nearby plants has been significant only in high-density plantings like carrots (*Daucus carota* L.) and beets (*Beta vulgaris* spp. *vulgaris*), and in low-density crops, the disease impact is correlated with the initial number of disease foci (Punja 1985). Sclerotial germination is greater near the soil surface where senesced plant tissue and organic matter serves as food source (Beute and Rodriguez-Kabana 1981; Jenkins and Averre 1986). The decomposition of organic matter releases volatile compounds (alcohols and aldehydes) that trigger sclerotial

germination (Punja et al. 1984), thus the potential for infection is higher when plan debris is available near the susceptible crops.

When a potato crop is planted in an infested field, the germination of the seed tuber is endangered if conditions are favorable for infection by *S. rolfsii*. A study has shown that many tubers did not sprout in infested fields during May planting dates in Virginia (Edson and Shapovalov 1923). Once the roots or tuber are infected, the development of the plant is stopped, and eventually it dies, either before or after emerging (Edson and Shapovalov 1923). As a result, weak or irregular emergence is observed. For emerged plants, *S. rolfsii* continues to be a threat. Infected plants produce smaller tubers, and the possibility of infection is greater when they are near the soil surface (Rolfs 1893). After the pathogen infects the plant at the crown, it can descend and reach the stolons, and eventually infect the potato tubers (Edson and Shapovalov 1923).

Economic importance

Sclerotium rolfsii can infect over 1,200 different plants in more than 500 plant species (Farr and Rossman 2017; Keinath and DuBose 2017; Mullen 2001). The host range is extensive and includes not only important plant crops, but less economically important plants as well (Aycock 1966). Due to the wide host range, it is difficult to accurately quantify the economic losses due to *S. rolfsii* (Aycock 1966). Early reports showed losses of 7-70% in fields of solanaceous crops such as tomato (Rolfs 1893), and the U.S. Department of Agriculture reported in 1928 that *S. rolfsii* and root-knot nematodes (*Meloidogyne* spp.), had caused more damage to crops than any other plant pathogens (Aycock 1966; Mullen 2001). In the U.S., pod yield losses in peanut are estimated between 10-25% but can surpass 80% in heavily infested fields (Mehan

et al. 1995). In Georgia, total losses and management costs were estimated at over $38 million from 2004 to 2007 (Le 2011).

Although losses in potato due to southern blight are not well estimated, in the U.S., the report of potato as a host of *S. rolfsii* has occurred since the first studies of the pathogen (Rolfs 1893). Subsequently, many reports worldwide have occurred, beginning with the isolation of *S. rolfsii* made in 1912 in India (Shaw and Ajrekar 1915) to recent reports of the first infection on potato and other solanaceous crops in Spain (Remesal et al. 2010; Roca et al. 2016). *Sclerotium rolfsii* is included among the most economically important fungal pathogens for potatoes worldwide, and in the U.S., it is more problematic in the southern states (Aycock 1966; Kirk and Wharton 2014). In India, in-field incidence of 1-40% has been reported in some regions, and up to 25% tuber rot in storage (Anahosur 2001). In Virginia, although the disease was occasionally a problem for potato in early reports (Brittingham et al. 1963), over the last decade, the incidence of *S. rolfsii* in potato plantings on the ESVA has increased, coinciding with the increase in the application of soil conservation practices, organic farming productions, and climatic conditions that favor the pathogen propagules survival. In fact, an entire field of over 24 hectares was lost to the disease in 2016 on the ESVA (Rideout, personal comm.), and *S. rolfsii* outbreaks have arisen across the mid-Atlantic region (Pethybridge et al. 2019).

Southern blight management strategies

Physical and cultural methods

Soil solarization significantly reduces sclerotium viability. Exposure to temperatures of 50°C and 55°C for six and three hours, respectively, can render the sclerotia nonviable (Xie and Vallad 2010). Under field conditions, solarization for one month decreased onion mortality

caused by *S. rolfsii* by 24% compared to not solarized treatments in the central Mexico region (Flores-Moctezuma et al. 2006). The effectiveness of solarization treatments depends on the availability of adequate temperatures/sunlight, duration of the treatment, geographic location, and other factors. While it is suitable for small areas, it is considered infeasible for large acreage farms (Xie and Vallad 2010).

Cultural control alters the environment to make the cropping system less favorable for pest development (Gabryś and Kordan 2013). Though simple in theory, applications of specific cultural control practices to reduce plant diseases requires extensive knowledge of the interaction between the host, causal agent, and environment to achieve economic disease suppression (Howard 1996). Reducing the amount of inoculum has been demonstrated to effectively manage *S. rolfsii* in some production systems. Mycelium of *S. rolfsii* is generally short-lived; however, growth chamber studies have found that in dry soils, the mycelia can survive up to 6 months at 15°C and 30°C with greater survival at the soil surface (Beute and Rodriguez-Kabana 1981). On the other hand, sclerotia can survive in soil for months to several years (Xu et al. 2008), and due to this, it typically serves as the primary inoculum source (Leoni et al. 2014). Studies in peanut have found that 73% of sclerotia were still viable after 10 months following peanut harvest (Beute and Rodriguez-Kabana 1981).

Deep plowing buries the sclerotia and crop residues preventing host-pathogen contact for the subsequent crop, thus limiting disease development. This has been one of the most effective management practices commonly suggested by researchers and extension personnel (Aycock 1966; Garren and Duke 1958). In fields with high levels of *S. rolfsii* inoculum, burying the organic matter at least 4 in deep prior to planting in combination with non-hilling cultivation (no

soil thrown around the base of the plants), led to less southern blight development compared to deep plowing coupled with non-hilling cultivation (Aycock 1966; Garren and Duke 1958). Studies have shown that sclerotia buried more than one inch have reduced survival (Smith et al. 1989). After a month of sclerotial burial, germination was reduced to 11% and 17% at 5 and 10 cm, respectively, compared to sclerotia at the soil surface (65% germination), and to less than 5% after 9-month burial compared to sclerotia on soil surface (26%) (Smith et al. 1989).

The benefits of deep plowing are not achieved in production systems where soil conservation practices like reduced tillage or strip-till are utilized. Furthermore, when the same practice is used in successive years, there is a potential that the inoculum can be brought back to the soil surface (Mullen 2001). The critics of this control method stated that a yield reduction can occur because the layer of soil brought to the surface is poor in nutrients and prone to erosion (Aycock 1966; Harrison 1958). Furthermore, in susceptible crops such as carrots, where low inoculum levels can result in high disease incidence, deep plowing alone does not reduce disease and should be combined with fungicides (Punja 1985). In addition, the increase of reduced tillage and cover cropping systems across Virginia (Schonbeck and Morse 2007) has created more ideal conditions for the survival of the pathogen (Langston, personal comm.). Thus, alternative cultural control methods are needed.

Planting density has proven to be an important factor impacting the incidence of southern blight in some crops. Significant spread to nearby plants occurs in high density planted crops such as sugar beets and carrots (Punja 1985). In processing carrots, for example, it was found that disease severity was higher when high plant densities were used (Smith et al. 1988). In peanut, the microclimate influenced by canopy density affects southern blight incidence (Mehan

et al. 1995; Rideout 2002). Higher canopy relative humidity was the factor most consistently related to the increase in symptomatic peanut plants infected by *S. rolfsii* (Rideout 2002).

Crop rotation with certain gramineous crops can reduce the prevalence of inoculum in the soil (Bost et al. 2014). For example, a continuous crop rotation of sweet pepper – fallow led to higher sclerotial density than when sweet pepper was followed by onion, fallow, and black oat (Leoni et al. 2014). When using an integrated approach, even just one year of rotation might help increase the efficacy of fungicides due to the reduction of inoculum (Keinath and DuBose 2017). However, in many instances, these practices are not economically feasible when land is limited for high value crops.

Soil amendment with inorganic and organic inputs have shown some suppression of southern blight. Nitrogen-based inorganic fertilizers have been shown to decrease disease incidence (Bark and Khan 1981; Punja 1985; Xie and Vallad 2010), likely due to inhibition of sclerotial germination and mycelial growth or alteration of the susceptibility of the host or soil microbiome (Punja 1985). However, some organic amendments have been shown to provide greater control of southern blight compared to synthetic fertilizers (Bulluck III and Ristaino 2002; Stapleton et al. 2010). In tilled bare-soil receiving synthetic fertilizers, disease incidence was higher (67%) compared to soil amendments of cotton gin waste, swine manure, or rye-vetch green manure (6.8-17.5%) (Bulluck III and Ristaino 2002). Fertilization with only farmyard manure (10 t ha^{-1}) resulted in the least amount of potato tuber infection (5.5-7.5%) compared with a 100% NPK fertilization program (10-11.5%) (Basu and Maiti 2006; Fiers et al. 2012). The use of organic amendments must be careful since the incorporation of some legume cover crops such as black bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata* L.), lupine (*Lupinus*

angustifolius and *Lupinus albus*), and hairy vetch (*Vicia villosa*) can increase sclerotial populations due to saprophytic growth and new sclerotial formation (Leoni et al. 2014). Increasing levels of sclerotia may be due to the germination of the sclerotia promoted by the organic components released by the decomposition of the plant residues, and the subsequent colonization of the substrates (Flores-Moctezuma et al. 2006; Leoni et al. 2014; Punja 1985). On the contrary, some grasses such as sorghum-sudangrass (*Sorghum bicolor* x S. *sudanense*) and foxtail millet (*Setaria italica*) have shown a reduction of sclerotial densities (Leoni et al. 2014; Stapleton et al. 2010). When decomposed, some grasses release toxic chemical compounds (dhurrin, isothiocyanates, saponins, flavonoids, phytoalexins, or avenanthramides) that are detrimental to sclerotia (Leoni et al. 2014). The time of incorporation of crop residues is critical, however. The incorporation of winter green manures in Uruguay significantly decreased sclerotial viability after 90 days due to the influence of warmer temperatures compared to summer green manure (Leoni et al. 2014). The inconsistent results and feasibility of applications of physical and cultural methods to manage southern blight compels the need to seek further alternatives.

Due to a lack of resistant cultivars, cultivar selection as a cultural practice has not been widely applied to manage southern blight (Mullen 2001). Studies have reported some resistance in peanut cultivars "Florida-07' and 'Tamspan-90' (Besler et al. 1997; Hagan et al. 2015) and hosta (*Hosta* spp. 'Halcyon') (Edmunds et al. 2003). Early studies on potato showed high susceptibility to *S. rolfsii* of 'Irish Cobbler' and 'Bliss Triumph' (Edson and Shapovalov 1923). Additionally, the severity of the infection depended on the strain of *S. rolfsii* that was present within the field; as one isolate caused more damage in April 8 plantings than May 6 plantings (Edson and Shapovalov 1923). In India, two potato cultivars were rated as moderately resistant

(33-47 % incidence), while the other 18 cultivars were rated as moderate to highly susceptible (>60% incidence) (Anahosur 2001). A bioassay in Tunisia found the potato cultivar 'Tango" among the most susceptible, while 'Daisy' had more tolerance to *S. rolfsii* tuber infection. In the U.S., it has also been stated that the cultivar 'Ute Russet', released in 1986, presents resistance to *S. rolfsii* (Holm et al. 1987), and Voss et al. (1984) reported that some of the screened cultivars and breeding lines were at least partially resistant to *S. rolfsii.* However, little research for *S. rolfsii* cultivar resistance on potato has been performed in the U.S on commercially available cultivars. Studies in grafting tomato ('Cherokee Purple' and 'German Johnson') with resistant rootstocks ('Big Power', 'Beaufort', 'Maxifort') have noted a reduction of southern blight incidence by over 93% (Rivard et al. 2010). However, this practice is mainly used in greenhouses or high tunnels and its high cost could limit its applicability in larger commercial open field tomato plantations.

Biological control

In 1932 Weindling described that *Trichoderma viride* (former *T. lignorum*) possesses antagonistic effects to *S. rolfsii* (Weindling 1932). Since then, many studies have evaluated and recommended *Trichoderma* species to control *S. rolfsii* and other soilborne pathogens (Aycock 1966; Elad et al. 1980a; Hino and Endo 1940; Wells et al. 1972). *Trichoderma harzianum* produced disease reduction of 97% in bean seedlings (*Phaseolus vulgaris* L. 'Brittle Wax') in a greenhouse study (Elad et al. 1980a). Moreover, greater control was achieved with higher rates of *Trichoderma* grown on wheat bran (5 g kg^{-1} of soil) compared to rates of <3g kg^{-1} of soil, and almost complete control was achieved when combined with soil heat treatments (Elad et al. 1980b). In vitro studies have shown that *T. viride, T. harzianum, and T. pseudokoningii* significantly inhibit *S. rolfsii* mycelial growth (68%, 57%, and 53%, respectively), and therefore

a reduction in the formation of sclerotia (Javaid and Ali 2016; Yaqub and Shahzad 2010). Further studies in pots under greenhouse conditions indicated that a combination of *T. harzianum* with *Glomus clarum* (arbuscular mycorrhizal fungus) provided more control than each fungus alone in certain Jerusalem artichokes (*Helianthus tuberosus* L.), evidently due to the synergistic effects (Sennoi et al. 2013). In addition, when *T. harzianum* is combined with fungicide (carboxin + thiram) and mustard oil cake, the control is higher than each element separately (Rubayet and Bhuiyan 2016). Field applications of *Trichoderma* formulated in sandy loam soil mixed with ryegrass seed (*Lolium multiflorum* Lam.) (10:1 ratio) at a rate of 1.5 g cm^{-1} of row, noted significant control (<10% incidence) of *S. rolfsii* in tomato compared to the nontreated controls (78% incidence) (Wells et al. 1972).

The efficacy of *Trichoderma* depends on formulation type used and inoculation timing. Experiments in greenhouses have shown that *T. harzianum* growth on wheat bran is more efficient than the liquid conidial suspension (Chet and Henis 1975; Elad et al. 1980a). However, one of the advantages of the liquid conidial formulations is its ease of large-scale production through fermentation tanks (Cumagun 2014). Wells et al. (1972) found that more healthy tomato plants were achieved in *S. rolfsii* naturally infested fields when three applications of *T. harzianum* were made compared to a single application utilizing 1.3 g cm^{-1} per row of *T. harzianum* inoculum prepared in ryegrass seed, soil, and sieved in 2-mm mesh (Wells et al. 1972).

Other soil microorganisms have been studied for management of southern blight with partial success. Studies in the 1950s in Louisiana showed that two strains of *Bacillus subtilis* prevented mycelium growth (Aycock 1966). Laboratory and glasshouse trials to evaluate the

effect of *Bacillus subtilis* on *S. rolfsii* showed that this organism effectively decreased in vitro *S. rolfsii* growth on potato dextrose agar; however, when examined in soil under controlled environments, no significant reduction of grapevine infections was observed. The authors stated that the *Bacillus* likely does not produce the fungistatic compounds in soil (Keyser and Ferreira 1988). In another in vitro experiment, it was reported that *B. subtilis* reduced mycelial growth by 11% (Naik 2012). Researchers have found that *Serratia marcescens* can exert a biocontrol effect of *S. rolfsii* (Ordentlich et al. 1987). This antagonistic bacterium produces chitinolytic enzymes that degrade the hyphae of *S. rolfsii.* In a greenhouse study, the disease incidence in beans (*Phaseolus vulgaris* L. 'Brittle Wax') sown in *S. rolfsii* infested soil treated with *S. marcescens* was significantly lower (<20%) compared to non-treated soil (around 50%) (Ordentlich et al. 1988). In addition, some actinomycetes and mycorrhizal organisms have been shown to provide some suppression of *S. rolfsii* (Errakhi et al. 2009; Mullen 2001). Most of the biological control studies have shown promising results in laboratory and greenhouse studies but results in field settings are inconsistent (Elad et al. 1980a).

Anaerobic soil disinfestation

With the phase-out of methyl bromide, it is necessary to explore alternative methods to control soilborne plant diseases. Methyl bromide was, for decades, the most reliable fumigant for managing soilborne pests and diseases, but due to its implication as an ozone layer depleter, its use was globally restricted as part of the Montreal Protocol in 1987 (Butler et al. 2012b; Rosskopf et al. 2005). Anaerobic soil disinfestation (ASD), also known as biological soil disinfestation, is a technique used to manage soilborne diseases that was developed in Japan and the Netherlands in the early 2000s (Blok et al. 2000; Butler et al. 2012b; Momma et al. 2006). In the last decade, ASD studies have shown promise for managing soilborne diseases in vegetables

(Butler et al. 2012a; Shrestha et al. 2018; Testen and Miller 2018). The ASD process consists of creating anaerobic conditions by applying carbon (C) source amendments, covering with polyethylene film, and then irrigating to field capacity for 2 to 8 weeks (Blok et al. 2000; Butler et al. 2014; Strauss and Kluepfel 2015). Anaerobic conditions are created when oxygen is depleted by soil microflora (Blok et al. 2000) shifting soil microbial populations to facultative and obligate anaerobic organisms (Butler et al. 2012b).

In the U.S., ASD research has been performed primarily in Florida, California, Tennessee, and Ohio (Butler et al. 2012a; Shrestha et al. 2018; Strauss and Kluepfel 2015; Testen and Miller 2018), with promising results in managing *Verticillium dahliae*, *Fusarium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pyrenochaeta lycopersici*, *Colletotrichum coccodes*, some oomycetes, nematodes, plant pathogenic bacteria, and certain weeds (Butler et al. 2012b; Butler et al. 2012a; Strauss and Kluepfel 2015; Testen and Miller 2018). For example, Butler et al. (2012a) reported that *Fusarium oxysporum* survival was reduced by more than 97% in all C sources compared to nonamended control. Choosing the proper C source is very important for optimization of ASD (Strauss and Kluepfel 2015). Studies have evaluated the use of dry and blackstrap molasses (10-20 Mg ha^{-1}), wheat bran (2-10 Mg ha^{-1}), rice bran (11-20 Mg ha^{-1}), ethanol (2-10% v/v), and cover crops such as cowpea, sunn hemp (*Crotalaria juncea* L.), sorghum-sudangrass, and pearl millet (*Pennisetum glaucum* L.) (30 to 80 kg dry weight ha^{-1}), with promising results in managing some soilborne diseases (Strauss and Kluepfel 2015). However, in Virginia, no studies have been conducted examining ASD.

Within the U.S., investigations to manage *S. rolfsii* with ASD have been conducted using C sources such as cowpea, sunn hemp, pearl millet, sorghum-sudangrass, wheat bran, and dry

molasses in greenhouses and field trials with variable results (Butler et al. 2012a; Shrestha et al. 2018; Strauss and Kluepfel 2015). For example, while Shrestha et al. (2018) reported that sclerotial germination was reduced by over 78% using wheat bran or dry molasses, Butler et al. (2012a) noted that the reduction of sclerotial germination ranged from 42% and 82% using cover crops biomass compared to nonamended control, and that sorghum-sudangrass had the higher sclerotial germination without poultry litter amendment but had above 95% inhibition when poultry litter was applied. Additionally, sclerotial colonization by *Trichoderma* sp. or *Fusarium* sp. increased with ASD treatments (Shrestha et al. 2018). However, when *Trichoderma* species were applied prior to ASD treatments, sclerotial colonization by *Trichoderma* sp. did not improve compared to the non-amended *Trichoderma* sp. treatments, likely due to the already promoted indigenous mycoparasites and bacteria by ASD alone (Shrestha et al. 2020).

ASD significantly changes the soil microbial community according to soil type and C source used for the process (Strauss and Kluepfel 2015; Testen and Miller 2018). Some studies have shown an increase of Clostridiales, Acidobacteria, and Burkholderia (Strauss and Kluepfel 2015). Conversely, others have reported that ASD reduced communities of Acidobacteria, Actinobacteria, Chloroflexi, and Planctomycetes, and increased the presence of Firmicutes; which are negatively correlated with root rot and nematodes galling severity in tomato (Testen and Miller 2018). More studies are needed to determine the impact on the soil microbial community according to soil type, region, and C source.

Chemical control

Several strobilurins or quinone outside inhibitors (QoI fungicides; Fungicide Resistance Action Committee (FRAC) group 11) are labeled for control of *S. rolfsii* in vegetables in the

U.S., but none are registered specifically for potato southern blight. Studies have shown they also provide some control in peanut and tomato (Keinath and DuBose 2017; Rideout et al. 2002; Xie and Vallad 2010). In certain crops, succinate dehydrogenase inhibitors (SDHI fungicides; FRAC Group 7) have also been recommended for control of southern blight. Flutolanil applied more than 60 days after planting, resulted in less in-field southern blight incidence compared to chlorothalonil (Culbreath et al. 1992). In vitro studies on tomato stems have shown that fluxapyroxad and penthiopyrad provide some control, but boscalid, which is in the same FRAC group 7 did not reduce sclerotial germination (Keinath and DuBose 2017). In field studies, a sequential application of fluxapyroxad and pyraclostrobin has been found more effective in reducing tomato southern blight than penthiopyrad alone or compared with the nontreated control (Keinath and DuBose 2017). Studies abroad have shown that hexaconazole and tebuconazole (DeMethylation Inhibitors; FRAC group 3) significantly inhibit mycelial growth (Das et al. 2014). Other studies in India noted that carboxin, difenoconazole, and hexaconazole, at all concentrations (0.05%, 0.1%, and 0.2%), and metalaxyl MZ at 0.2% inhibited 100% growth of mycelia, while carbendazim at 0.05% yielded the least inhibition (47%) (Naik 2012). An application at planting of pentachloronitrobenzene (FRAC group 14) is recommended in vegetables (Bost et al. 2014). However, despite the control of *S. rolfsii,* some studies noted secondary effects, such as stunted plants in tomato (Keinath and DuBose 2017). Additionally, in-furrow applications at planting of azoxystrobin have shown no effect on decreasing late-season disease pressure in peanut, and mid-season applications are still need to accomplish acceptable control levels (Rideout et al. 2002).

Contact, non-systemic fungicides such as captan and mancozeb have shown less reduction of *S. rolfsii* mycelial growth (16 and 20%, respectively) than systemic fungicides (Das

et al. 2014). In India, *in vitro* studies have found that thiram and mancozeb at all concentrations (0.1%, 0.2%, and 0.3 %) inhibited 100% of the growth of *S. rolfsii*. Captan and zineb inhibited 100% of mycelial growth only at 0.3% concentration (Naik 2012).

Fumigants, such as methyl bromide, chloropicrin, and metam-sodium, are perhaps the broadest spectrum and reliable mechanism for controlling soilborne pathogens. In a study for managing tomato southern blight Georgia, it was found that metam-sodium, chloropicrin, methyl bromide, and chloropicrin + dichloropropene, considerably decreased southern blight incidence (<37 infection loci) compared to nontreated control (153 infection loci) (McCarter et al. 1976). Methyl bromide and chloropicrin + chloropropene resulted in greater marketable tomato fruit yields (91-100%) compared to nontreated control (55-65%) as a result of the direct effect of controlling root-knot nematode (*Meloidogyne incognita*), bacterial wilt (*Ralstonia solanacearum*), and southern blight (Dickson 1998). However, methyl bromide use has been restricted by the Environmental Protection Agency due to environmental and health concerns (Panth et al. 2020; Rosskopf et al. 2005; Xie and Vallad 2010). Additionally, due to the aggressiveness of the pathogen, the treated areas can get reinfested (Bost et al. 2014). In general, the application of a chemical to soils tends to be costly due to a large amount required thereby decreasing the feasibility (Mullen 2001).

Project objectives

Most research on potato southern blight has mainly focused on pesticides, with results being inconsistent. In addition, even though cultural practices to reduce inoculum are recommended, research within the mid-Atlantic region on potato southern blight and other vegetables is extremely limited. Therefore, the main objective of this PhD research is to assess

potato cultivars responses to *S. rolfsii* and potential cultural control options in managing southern blight to ensure the sustainability of potato and vegetable production in regions impacted by this disease such as the ESVA. This research, under appropriate dissemination, will provide, in a short time, valuable information to the growers and researchers, and this will be achieved by completing the following main objectives:

1. To evaluate the effect of planting date and cultivar selection on southern blight incidence in potato, by establishing field trials examining four different planting dates and 10 cultivars and assessing disease incidence, yield parameters, and tuber quality.
2. To compare cultivar susceptibility to *S. rolfsii* of potato tubers by assessing tuber decay severity of 10 potato cultivars in post-harvest settings.
3. To evaluate different cover crop and other organic materials as carbon sources in anaerobic soil disinfestation and their effect on the management of *S. rolfsii* and impact on the soil and tomato plants.

Literature Cited

Anahosur, K. 2001. Integrated management of potato Sclerotium wilt caused by *Sclerotium rolfsii*. Indian Phytopath. 54:158-166.

Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*: or the status of Rolfs' fungus after 70 years. N. C. Agric. Exp. Stn. Bull. 174:202.

Bark, A., and Khan, A. 1981. Effect of nitrogenous amendments on the incidence of sclerotium wilt of potato. Potato Res. 24:363-365.

Basu, A., and Maiti, M. K. 2006. Role of host nutrition and varieties on the development of stem rot of potato. Ann. of Pl. Protec. Sci. 14:479-480.

Besler, B., Grichar, W. J., and Smith, O. 1997. Reaction of selected peanut varieties and breeding lines to southern stem rot. Peanut Sci. 24:6-9.

Beute, M., and Rodriguez-Kabana, R. 1981. Effects of soil moisture, temperature and field environment on survival of *Sclerotium rolfsii* in Alabama and North Carolina. Phytopathology 71:1293-1296.

Blok, W. J., Lamers, J. G., Termorshuizen, A. J., and Bollen, G. J. 2000. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. Phytopathology 90:253-259.

Bost, S., Hale, F., Hensley, D., and Wszelaski, A. 2014. Crop profile for tomatoes in tennessee. National IPM Database, Regional IPM centers. https://ipmdata.ipmcenters.org/documents/cropprofiles/TNtomato2014.pdf (Accessed 01 Apr 2021).

Bowen, K. 2003. Development of stem rot (caused by *Sclerotium rolfsii*) in peanut in Alabama. Peanut Sci. 30:120-128.

Brittingham, W. H., Bell, F. W., and Christiansen, J. K. 1963. Economic history and present status of potato production in Virginia. Virginia Joint Agric. Pub. 5:6-14.

Bulluck III, L., and Ristaino, J. 2002. Effect of synthetic and organic soil fertility amendments on southern blight, soil microbial communities, and yield of processing tomatoes. Phytopathology 92:181-189.

Butler, D. M., Rosskopf, E. N., Kokalis-Burelle, N., Albano, J. P., Muramoto, J., and Shennan, C. 2012a. Exploring warm-season cover crops as carbon sources for anaerobic soil disinfestation (ASD). Plant Soil 355:149-165.

Butler, D. M., Kokalis-Burelle, N., Muramoto, J., Shennan, C., McCollum, T. G., and Rosskopf, E. N. 2012b. Impact of anaerobic soil disinfestation combined with soil solarization on plant–parasitic nematodes and introduced inoculum of soilborne plant pathogens in raised-bed vegetable production. Crop Protect. 39:33-40.

Butler, D. M., Kokalis-Burelle, N., Albano, J. P., McCollum, T. G., Muramoto, J., Shennan, C., and Rosskopf, E. N. 2014. Anaerobic soil disinfestation (ASD) combined with soil solarization as a methyl bromide alternative: vegetable crop performance and soil nutrient dynamics. Plant Soil 378:365-381.

Carpenter, C. W. 1920. Potato diseases in Hawaii and their control. Hawaii Agr. Exp. Stat. Bull. 45:42.

Chet, I., and Henis, Y. 1975. Sclerotial morphogenesis in fungi. Annu. Rev. Phytopathol. 13:169-192.

Chowdhury, S. 1948. Diseases of Pan (*Piper betle*) in Sylhet, Assam. Pages 227-239 in: Proceedings of the Indian Academy of Sciences-Section B Springer.

Culbreath, A., Minton, N., Brenneman, T., and Mullinix, B. 1992. Response of Florunner and Southern Runner peanut cultivars to chemical management of late leaf spot, southern stem rot, and nematodes. Plant Dis. 76:1199-1203.

Cumagun, C. J. R. 2014. Advances in formulation of Trichoderma for biocontrol. Pages 527-531 in: Biotechnology and Biology of Trichoderma. V. K. Gupta, M. Schmoll, A. Herrera-Estrealla, R. S. Upadhyay, I. Druzhinina and M. G. Tuohy, eds. Elsevier, Waltham, MA.

Das, N., Dutta, B., and Ray, D. 2014. Potential of some fungicides on the growth and development of *Sclerotium rolfsii* Sacc. in vitro. Int. J. Scient. Res. Pub 4:12.

Dickson, D. 1998. Fumigants and non-fumigants for replacing methyl bromide in tomato production. in: Annual International Conference on Methyl Bromide Alternatives, USA.

Edmunds, B. A., Gleason, M. L., and Wegulo, S. N. 2003. Resistance of hosta cultivars to petiole rot caused by *Sclerotium rolfsii* var. *delphinii*. HortTechnology 13:302-305.

Edson, H., and Shapovalov, M. 1923. Parasitism of *Sclerotium rolfsii* on Irish potatoes. Jour. Agr. Res 23:41-46.

Elad, Y., Chet, I., and Katan, J. 1980a. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70:119-121.

Elad, Y., Katan, J., and Chet, I. 1980b. Physical, biological, and chemical control integrated for soilborne diseases in potatoes. Phytopathology 70:418-422.

Errakhi, R., Lebrihi, A., and Barakate, M. 2009. *In vitro* and *in vivo* antagonism of actinomycetes isolated from Moroccan rhizospherical soils against *Sclerotium rolfsii*: a causal agent of root rot on sugar beet (Beta vulgaris L.). J. Appl. Microbiol. 107:672-681.

Farr, D. F., and Rossman, A. Y. 2017. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved December 7, 2017, from https://nt.ars-grin.gov/fungaldatabases/

Fiedler, K. 2014. Integrated approach to understanding tomato sour rot and improving disease management on the Eastern Shore of Virginia. Virginia Tech - Dissertation, Blacksburg, VA.

Fiers, M., Edel-Hermann, V., Chatot, C., Le Hingrat, Y., Alabouvette, C., and Steinberg, C. 2012. Potato soil-borne diseases. A review. Agron. Sustain. Dev. 32:93-132.

Flores-Moctezuma, H., Montes-Belmont, R., Jimenez-Perez, A., and Nava-Juarez, R. 2006. Pathogenic diversity of *Sclerotium rolfsii* isolates from Mexico, and potential control of southern blight through solarization and organic amendments. Crop Protect. 25:195-201.

Franke, M., Brenneman, T., Stevenson, K., and Padgett, G. 1998. Sensitivity of isolates of *Sclerotium rolfsii* from peanut in Georgia to selected fungicides. Plant Dis. 82:578-583.

Gabryś, B., and Kordan, B. 2013. Cultural control and other non-chemical methods. Pages 517-541 in: Insect Pests of Potato. Elsevier.

Garren, K. H. 1958. The stem rot of peanuts and its control. Virginia Agric. Exp. Stn. 144:29.

Garren, K. H., and Duke, G. B. 1958. The effects of deep covering of organic matter and non-dirting weed control on peanut stem rot. Plant Dis. Rep. 42:629-636.

Garren, K. H., and Bailey, W. K. 1963. Comparative responses of a Virginia runner and a Virginia Bunch peanut to cultural control of stem rot. Agron. J. 55:290-293.

Goto, K. 1938. *Sclerotium rolfsii* Sacc. in perfect stage. Ann. Phytopath. Soc. Japan 7:203-220.

Hagan, A., Campbell, H., Bowen, K., and Wells, L. 2015. Seeding rate and planting date impacts stand density, diseases, and yield of irrigated peanuts. Plant Health Prog. 16:63-70.

Hagan, A. K., Weeks, J. R., Bowen, K. L., and Wells, L. W. 2001. Influence of production practices on peanut disease and yield. Alabama Agric. Exp. Sta. Bull. 643.

Harrison, A. L. 1958. Terraclor for the control of southern blight of peanuts. Texas Exp. Sta. Prog. Rep. 2014:3.

Hino, I., and Endo, S. 1940. Trichoderma parasitic on sclerotial fungi. Ann. Phytopath. Soc. Japan 10:231-241.

Holm, D., Twomey, J., and Workman, M. 1987. Ute Russet: An oblong russet potato cultivar with resistance to leafroll net necrosis and *Sclerotium rolfsii*. Am. J. Potato Res. 64:559-562.

Howard, R. J. 1996. Cultural control of plant diseases: a historical perspective. Can. J. Plant Pathol. 18:145-150.

Javaid, A., and Ali, A. 2016. Screening of *Trichoderma* species for their biological control potential against *Sclerotium rolfsii,* the cause of collar rot disease of chickpea. Mycopath. 13:93-96.

Jenkins, S. F., and Averre, C. W. 1986. Problems and progress in integrated control of southern blight of vegetables. Plant Dis. 70:614-620.

Keinath, A. P., and DuBose, V. B. 2017. Management of southern blight on tomato with SDHI fungicides. Crop Protect. 101:29-34.

Keyser, H. A., and Ferreira, J. 1988. Chemical and biological control of *Sclerotium rolfsii* in grapevine nurseries. South African J. of Enol. and Vitic. 9:43-44.

Kirk, W. W., and Wharton, P. S. 2014. Fungal and bacterial disease aspects in potato production. Pages 234-274 in: The potato: botany, production and uses. R. Navarre and M. J. Pavek, eds. CABI, Boston, MA.

Kuhar, T. P., Arancibia, R. A., Hamilton, G. C., VanGessel, M. J., and Wyenandt, C. A. 2019. 2019 Mid-Atlantic commercial vegetable production recommendations. Virginia Cooperative Extension 456-240.

Le, N. 2011. Diversity and biological control of *Sclerotium rolfsii*, causal agent of stem rot of groundnut. Wageningen University, Wageningen, The Netherlands.

Leoni, C., ter Braak, C. J., Gilsanz, J. C., Dogliotti, S., Rossing, W. A., and van Bruggen, A. H. 2014. *Sclerotium rolfsii* dynamics in soil as affected by crop sequences. Appl. Soil Ecol. 75:95-105.

McCarter, S., Jaworski, C., Johnson, A., and Williamson, R. 1976. Efficacy of soil fumigants and methods of application for controlling southern blight of tomatoes grown for transplants. Phytopathology 66:913.

Mehan, V., Mayee, C., Brenneman, T., and McDonald, D. 1995. Stem and pod rots of groundnut. International Crops Research Institute for the Semi-Arid Tropic. Information Bulletin. 44.

Momma, N., Yamamoto, K., Simandi, P., and Shishido, M. 2006. Role of organic acids in the mechanisms of biological soil disinfestation (BSD). J. Gen. Plant Pathol. 72:247-252.

Mullen, J. 2001. Southern blight, southern stem blight, white mold. The Plant Health Instr. DOI: 10.1094. PHI-I-2001-0104-01.

Naik, K. 2012. Integrated management strategy for *Sclerotium rolfsii* causing wilt of potato. Int. J. Plant Protec. 5:124-127.

O'Dell, C. R., Conner, C., White, R., and Snodgrass, H. S. 2009. String trellising of tomatoes to improve quality and profits. Virginia Cooperative Extension 438-017.

Ordentlich, A., Elad, Y., and Chet, I. 1987. Rhizosphere colonization by *Serratia marcescens* for the control of *Sclerotium rolfsii*. Soil Biol. Biochem. 19:747-751.

Ordentlich, A., Elad, Y., and Chet, I. 1988. The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. Phytopathology 78:84-88.

Panth, M., Hassler, S. C., and Baysal-Gurel, F. 2020. Methods for management of soilborne diseases in crop production. Agriculture 10:16.

Peltier, G. L. 1916. A serious disease of cultivated perennials caused by *Sclerotium rolfsii*. Circular (University of Illinois Urbana-Champaign campus). Agric. Exp. Stn. No. 187.

Pethybridge, S. J., Sharma, S., Silva, A., Bowden, C., Murphy, S., Knight, N. L., and Hay, F. S. 2019. Southern Sclerotium root rot caused by *Athelia rolfsii* on table beet in New York. Plant Health Prog. 20:4-6.

Punja, Z., Jenkins, S., and Grogan, R. 1984. Effect of volatile compounds, nutrients, and source of sclerotia on eruptive sclerotial germination of *Sclerotium rolfsii*. Phytopathology 74:1290-1295.

Punja, Z. K. 1985. The biology, ecology, and control of *Sclerotium rolfsii*. Annu. Rev. Phytopathol. 23:97-127.

Ramsey, G. B., Smith, M. A., and Wiant, J. S. 1949. Market diseases of fruits and vegetables: Potatoes. Misc. Pub. 98:43-44.

Reiter, M. S., Phillips, S. B., Warren, J., and Maguire, R. 2009. Nitrogen management for white potato production. Virginia Cooperative Extension. Virginia Tech. Pub. 430-012.

Remesal, E., Lucena, C., Azpilicueta, A., Landa, B. B., and Navas-Cortés, J. A. 2010. First report of southern blight of pepper caused by *Sclerotium rolfsii* in Southern Spain. Plant Dis. 94:280-280.

Richardson, B. 2020. 2019 Virginia potato and vegetable review. VDACS:29. Available at: https://www.vdacs.virginia.gov/pdf/pvr.pdf (Accessed Dec. 14, 2020).

Rideout, S. L. 2002. The influence of environment and host growth for improved fungicide applications for control of southern stem rot of peanut. Research. The University of Georgia-Dissertation, Athens, GA.

Rideout, S. L., Brenneman, T. B., and Culbreath, A. K. 2002. Peanut disease management utilizing an in-furrow treatment of azoxystrobin. Plant Health Prog. 3:6.

Rivard, C., O'Connell, S., Peet, M., and Louws, F. 2010. Grafting tomato with interspecific rootstock to manage diseases caused by *Sclerotium rolfsii* and southern root-knot nematode. Plant Dis. 94:1015-1021.

Roberts, P. D., French-Monar, R. D., and McCarter, S. M. 2014. Southern Blight. in: Compendium of Tomato Diseases and Pest. J. B. Jones, T. A. Zitter, M. Momol and S. A. Miller, eds. American Phytopathological Society, St. Paul, MN.

Roca, L. F., Raya, M. C., Luque, F., Agustí-Brisach, C., Romero, J., and Trapero, A. 2016. First Report of *Sclerotium rolfsii* Causing Soft Rot of Potato Tubers in Spain. Plant Dis. 100:2535-2535.

Rolfs, P. H. 1893. The tomato and some of its diseases. Florida Agr. Exp. Station. 21:38.

Rosskopf, E. N., Chellemi, D. O., Kokalis-Burelle, N., and Church, G. T. 2005. Alternatives to methyl bromide: A Florida perspective. Plant Health Prog. 6:19.

Rubayet, M., and Bhuiyan, M. 2016. Integrated magament of stem rot of potato caused by *Sclerotium rolfsii*. Bangladesh Phytopathological Society 32.

Schonbeck, M., and Morse, R. 2007. Reduced tillage and cover cropping systems for organic vegetable production. Virginia Association of Biological Farming Info Sheet 9-07.

Schumann, G. L., and D'Arcy, C. J. 2010. Essential plant pathology. 2nd ed. APS Press, St. Paul, Minn.

Sennoi, R., Singkham, N., Jogloy, S., Boonlue, S., Saksirirat, W., Kesmala, T., and Patanothai, A. 2013. Biological control of southern stem rot caused by *Sclerotium rolfsii* using *Trichoderma harzianum* and arbuscular mycorrhizal fungi on Jerusalem artichoke (Helianthus tuberosus L.). Crop Protect. 54:148-153.

Shaw, F., and Ajrekar, S. 1915. The genus *Rhizoctonia* in India. Botanical Series. Memoirs of Agriculture Department of India 7:77.

Shrestha, U., Dee, M. E., Ownley, B. H., and Butler, D. M. 2018. Anaerobic soil disinfestation reduces germination and affects colonization of *Sclerotium rolfsii* sclerotia. Phytopathology 108:342-351.

Shrestha, U., Dee, M. E., Piya, S., Ownley, B. H., and Butler, D. M. 2020. Soil inoculation with *Trichoderma asperellum*, *T. harzianum* or *Streptomyces griseoviridis* prior to anaerobic soil disinfestation (ASD) does not increase ASD efficacy against *Sclerotium rolfsii* germination. Appl. Soil Ecol. 147:103383.

Smith, V., Punja, Z., and Jenkins, S. 1986. A histological study of infection of host tissue by *Sclerotium rolfsii*. Phytopathology 76:755-759.

Smith, V., Campbell, C., Jenkins, S., and Benson, D. 1988. Effects of host density and number of disease foci on epidemics of southern blight of processing carrot. Phytopathology 78:595-600.

Smith, V. L., Jenkins, S. F., Punja, Z. K., and Benson, D. M. 1989. Survival of sclerotia of *Sclerotium rolfsii*: Influence of sclerotial treatment and depth of burial. Soil Biol. Biochem. 21:627-632.

Stapleton, J. J., Summers, C. G., Mitchell, J. P., and Prather, T. S. 2010. Deleterious activity of cultivated grasses (Poaceae) and residues on soilborne fungal, nematode and weed pests. Phytoparasitica 38:61-69.

Strauss, S., and Kluepfel, D. 2015. Anaerobic soil disinfestation: A chemical-independent approach to pre-plant control of plant pathogens. J. Integr. Agric. 14:2309-2318.

Tarafdar, A., Rani, T. S., Chandran, U. S., Ghosh, R., Chobe, D. R., and Sharma, M. 2018. Exploring combined effect of abiotic (soil moisture) and biotic (*Sclerotium rolfsii* Sacc.) stress on collar rot development in chickpea. Front. in Plant Sci. 9:1154.

Testen, A. L., and Miller, S. A. 2018. Carbon source and soil origin shape soil microbiomes and tomato soilborne pathogen populations during Anaerobic Soil Disinfestation. Phytobiomes 2:138-150.

Townsend, B. B., and Willetts, H. 1954. The development of sclerotia of certain fungi. Trans. Br. Mycol. Soc. 37:213-221.

Tu, C. C., and Kimbrough, J. W. 1978. Systematics and phylogeny of fungi in the *Rhizoctonia* complex. Botanical Gazette 139:454-466.

USDA-National Agricultural Statistics Service. 2019a. 2017 Census of agriculture-Virginia, State and County data., Washington, D.C. Vol.1. AC-17-A-46.

USDA-National Agricultural Statistics Service. 2019b. Crop Values 2018 Summary. Washington, D.C.

USDA-National Agricultural Statistics Service. 2019c. Crop production 2018 summary. Washington, DC.

USDA-National Agricultural Statistics Service. 2020. Vegetables 2019 summary. Washington, DC.

Weber, G. F. 1923. Potato diseases and insects. Florida Agr. Exp. Station. Bull. 169:123-125.

Weber, G. F. 1943. Southern blight, *Corticium rolfsii* of potato tubers. Phytopathology 33:615-617.

Weindling, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology 22:837-845.

Wells, H. D., Bell, D. K., and Jaworski, C. A. 1972. Efficacy of *Trichoderma harzianum* as a biocontrol for *Sclerotium rolfsii*. Phytopathology 62:442-447.

Xie, C., and Vallad, G. 2010. Integrated management of southern blight in vegetable production. Plant Pathology. University of Florida IFAS Extension. IPN PP272.

Xu, Z., Gleason, M., Mueller, D., Esker, P., Bradley, C., Buck, J., Benson, D., Dixon, P., and Monteiro, J. 2008. Overwintering of *Sclerotium rolfsii* and *S. rolfsii* var. in different latitudes of the United States. Plant Dis. 92:719-724.

Yaqub, F., and Shahzad, S. 2010. Competitive colonization of wheat straw by *Trichoderma* species and *Sclerotium rolfsii*. Pak. J. Bot 42:1983-1989.

CHAPTER 2: Planting date and cultivar selection to manage southern blight in potatoes in the Mid-Atlantic United States

Abstract

Over the past decade, the incidence of southern blight caused by *Sclerotium rolfsii* Sacc. in potato (*Solanum tuberosum* L.) has increased across the mid-Atlantic region. To date, no potato cultivar is resistant to southern blight, and effective management with fumigants and fungicides has been inconsistent and uneconomical. This study evaluated the impact of four planting dates, ten commercial cultivars, and the integrated effects of planting date and cultivar on southern blight incidence, yield parameters, and tuber quality. Disease incidence varied from year to year and was affected by environmental parameters. Less disease incidence in 2019 (25%) than in the preceding seasons (64-79%) was likely due to dry and warm weather. Despite the planting date by cultivar interaction, later plantings generally had greater disease incidence (85-94%) and lower tuber yield (1.8-9.4 Mg ha^{-1}) and quality (47-78%); planting date effects were likely driven by humid, warm weather later in the season that was conducive to disease and detrimental to the potato crop. Though cultivar responses varied across planting date-years, 'Accumulator' consistently had one of the lowest disease incidences (12-71%) and greatest tuber yield among tested cultivars (6.2-37.3 Mg ha^{-1}). Conversely, 'Adirondack Blue' and 'Dark Red Norland' had the overall greatest incidence of *S. rolfsii* (33-100%) but the overall lowest yield was obtained by 'Yukon Gold' (0.3-24.1 Mg ha^{-1}). Potato chipping cultivars tended to be less impacted by *S. rolfsii* than fresh market cultivars; however, additional studies are needed to elucidate potential mechanisms of disease tolerance. This study indicated that early planting dates and cultivar selection were an effective management approach to reduce *S. rolfsii* incidence on potato and to maximize tuber yield.

Introduction

Southern blight of potato (*Solanum tuberosum* L.) is caused by *Sclerotium rolfsii* Sacc. (teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough *)*, a necrotrophic fungal pathogen that attacks over 500 plant species (Farr and Rossman 2017; Mullen 2001). *Sclerotium rolfsii* is present worldwide with more prevalence in tropical and subtropical regions (Aycock 1966; Mullen 2001), and in the U.S. it is known to be more problematic in the southeast (Aycock 1966). The pathogen is mainly spread by sclerotia and mycelia. Mycelia can survive on dead organic material, volunteer plants, or alternate hosts, and sclerotia survive in soil or manure and serve as an overwintering structure (Mullen 2001). *Sclerotium rolfsii* can grow between 8-40°C, but conditions for optimal growth are 27-30°C temperatures coupled with high soil moisture and high humidity (Aycock 1966; Mullen 2001; Punja 1985). When conditions are favorable, *S. rolfsii* produces thick, white mycelial mats on plant parts 3-4 days after infection, and this can occur at any plant host developmental stage, most commonly at the soil line, with fungal growth extending a few centimeters up the stem (Mullen 2001; Roberts et al. 2014).

Over the past decade, *S. rolfsii* has emerged as an economically important soilborne pathogen of vegetables in the mid-Atlantic U.S., and it threatens sustainable production of potato and other vegetable crops. The mid-Atlantic region produced over 281 thousand metric tons of potato tubers in 2018, with a value over $77 million (USDA-National Agricultural Statistics Service 2019a, b). Several diseases limit yield and quality of potato produced in the region, and though in the past there were few reports of southern blight causing economic damage (Brittingham et al. 1963), it is an emerging disease in potatoes and other vegetables such as table beets (*Beta vulgaris* spp. *vulgaris*) in Virginia and the rest of the mid-Atlantic (Pethybridge et al. 2019). Recent increases in southern blight incidence and severity coincide with reductions in fumigation

of potato fields, increasing temperatures and excessive rainfall events, and adoption of reduced-tillage practices that can increase inoculum survival in fields (Bockus and Shroyer 1998; Punja 1985).

The effectiveness of fungicides, cultivar resistance, and cultural practices for management of *S. rolfsii* has been evaluated primarily for crops such as peanut (*Arachis hypogaea* L.) (Garren 1958; Garren and Bailey 1963; Garren and Duke 1958) and tomato (*Solanum lycopersicum* L.) (Bulluck III and Ristaino 2002; Keinath and DuBose 2017; Rivard et al. 2010). Although a few studies have evaluated control of *S. rolfsii* in potato (Browne et al. 2002; Kulkarni 2007; Voss et al. 1984), best management practices for potato southern blight have not been developed. Strobilurins (QoI fungicides, FRAC group 11) are recommended for *S. rolfsii* control in several crops, but few are labeled specifically for southern blight control in the mid-Atlantic region (Kuhar et al. 2019). Fumigation has the potential to reduce *S. rolfsii* inoculum in fields, but high cost, environmental regulations, and toxicity of fumigants to workers and handlers makes this approach uneconomical and impractical for potato production. Due to the lack of developing or identifying potentially resistant cultivars, cultivar selection has not been widely applied to manage southern blight in potato or other vegetable crops (Mullen 2001). However, studies have reported some levels of host resistance in the peanut cultivar 'Florida-07' (Hagan et al. 2015) and the hosta cultivar 'Halcyon' (*Hosta* spp.) (Edmunds et al. 2003). Early studies on potato that screened for cultivar susceptibility to *S. rolfsii* showed no resistance to the pathogen (Aycock 1966; Edson and Shapovalov 1923). In India, two cultivars were rated as moderately resistant (33-47 % incidence), while the other 18 cultivars evaluated were moderate to highly susceptible (>60% incidence) (Anahosur 2001). The Ute Russet cultivar, released in 1986, is considered resistant to *S. rolfsii* (Holm et al. 1987), and Voss et al. (1984) reported that some of the screened

cultivars and breeding lines were at least partially resistant to *S. rolfsii.* Overall, little screening of potato for *S. rolfsii* resistance has been performed in the U.S.

Planting date can be manipulated to increase the chances that a crop will develop during periods when environmental conditions are not conducive for pathogen growth, thereby allowing the crop to escape the disease. Effects of planting date on southern blight have been studied in some crops but influences on disease incidence and severity have been variable. Delayed planting that shifts the period of crop maturation to the fall when cool temperatures are less conducive to disease can reduce southern blight incidence by nearly 50% in peanuts (Hagan et al. 2001) and over 60% in carrots (*Daucus carota* L.) (Jenkins and Averre 1986). One early report on the impact of potato planting date on southern blight indicated that earlier plantings (April) had reduced disease severity by over 50% compared to late (May) planting for at least one of the *S. rolfsii* strains evaluated (Edson and Shapovalov 1923). However, altering planting date as a management practice has not been well documented for potato and no standardized recommendations have been determined.

Since warm, wet conditions favor southern blight development, we hypothesize that by manipulating potato planting date to earlier in the spring, it may be possible to reduce disease incidence and protect tuber yield and quality. In addition, though resistance to southern blight has not been identified in potato cultivars commercially grown in mid-Atlantic region, we hypothesize that cultivars will vary in their response to the disease. Thus, in the present study, our objective was to evaluate the impact of four planting dates, ten commercial cultivars, and the integrated effects of planting date and cultivar on southern blight incidence, yield parameters, and tuber quality.

Materials and Methods

Field experiment establishment

Field experiments were established in 2017, 2018, and 2019 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA (37.584779, -75.821014) on a Bojac sandy loam soil with a 0-2% slope (Natural Resources Conservation Service-USDA 2017). There was no known history of *S. rolfsii* infestation in the experimental fields. Wheat was previously grown in the experimental fields in 2017 and 2018, and corn was grown in 2019. The land was prepared according to mid-Atlantic commercial vegetable production recommendations (Kuhar et al. 2019).

Annual experiments were arranged in split-plot designs with four replications, with planting dates as the main plots and cultivars as sub-plots. Potato tubers were planted on March 7, March 24, April 14, and May 10 in 2017; March 29, April 11, April 24, and May 9 in 2018; and April 3, April 17, May 2, and May 16 in 2019. Initial annual planting dates occurred as soon as soil temperature and moisture allowed for land preparations and included standard planting dates (March 10-April 5) recommended for commercial production of potato the mid-Atlantic region (Kuhar et al. 2019). Ten commercial cultivars (Table 2.1), most of which are planted commercially in the mid-Atlantic, were evaluated for susceptibility to infection by *S. rolfsii*. Each main plot was 7.62 m long x 18.3 m wide with a single guard row on each side and 6.1 m wide buffers between blocks. Sub-plots consisted of two rows per cultivar with 0.9 m between rows. Certified potato seed was hand-cut one to two days prior to planting and dusted with mancozeb (Nubark Mancozeb 6D; Wilbur-Ellis Company LLC, Fresno, CA) immediately after cutting (0.6 g of a.i. kg^{-1} of potato seed). Seed were hand planted 0.25 m apart within rows in preformed split back ridges. Bifenthrin + imidacloprid (Brigadier 2SC at 253 g ha^{-1} a.i.; FMC

Corporation, Philadelphia, PA), azoxystrobin (Quadris 2.08SC at 96.1 g ha^{-1} a.i.; Syngenta Crop Protection, LLC, Greensboro, NC), and mefenoxam (Ridomil Gold 4SL at 190.2 g ha^{-1} a.i.; Syngenta Crop Protection, LLC, Greensboro, NC) were applied in-furrow at planting using a CO2-pressurized sprayer with single XR-80015 nozzle (TeeJet Technologies, Springfield, IL) calibrated to deliver 93.5 L ha^{-1}. Granular fertilizer (10N-10P-10K) at 673 kg ha^{-1} was hand-banded into preformed split back ridges prior planting.

S-metolachlor (Dual II Magnum 7.64EC at 0.96 L ha^{-1} a.i.; Syngenta Crop Protection, LLC, Greensboro, NC) and metribuzin (TriCor 75DF at 420 g ha^{-1}, a.i.; United Phosphorus Inc, King of Prussia, PA) were applied within two days after planting for weed control. In 2018, only the first and second planting dates received herbicide, due to excessive rains that followed planting. Weeding was performed manually as needed as to not confound results. Bed-mounding and cultivation were performed 2-3 weeks after emergence. Nitrogen (100 kg ha^{-1} of urea 46% N) was broadcast evenly at bloom. Plots were periodically scouted for pests, and insect and foliar diseases were controlled with standard practices for the mid-Atlantic (Kuhar et al. 2019). No fungicide applied was known to have activity against *S. rolfsii*.

Field inoculum preparation and application

A composite field inoculum utilizing two isolates of *S. rolfsii* collected from the Eastern Shore of Virginia was prepared by adapting a previously described technique (Vagher et al. 2014). Each isolate was grown in separate batches with 500 cm^3 of millet seed (*Pennisetum glaucum* L.) being placed in aluminum foil pans with 600 ml of deionized water and subsequently covered with aluminum foil and autoclaved twice for 45 min. A 7 day-old *S. rolfsii* culture grown on potato dextrose agar (PDA) (Difco, Becton, Dickson and Company, Sparks,

MD) was placed face-down on the autoclaved sterile millet seed, double-covered with aluminum foil, and incubated in a growth chamber at 30°C in the dark for 8 to 10 days. Infested millet seed was ground (≤ 5 mm pieces) and dried at room temperature. The final composite inoculum consisted principally of mycelia with some sclerotia. Inoculum (40 g) was evenly scattered within each plot at blooming (42 to 73 days after planting). Cultivation was performed immediately after application of inoculum, except for the third planting date in 2019 where cultivation was done three days after application due to rainy weather.

Data collection

Emergence was recorded regularly during the early part of the growing season until stems and leaves were fully developed for over 50% of plants. Disease incidence prior to harvest was assessed by counting the number of diseased plants per plot and converting to a percentage of diseased plants per plot. Plants with wilting (symptoms) and/or mycelia and sclerotia at the base of the stems (signs) were considered diseased (Aycock 1966; Mullen 2001; Weber 1943). Soil and air temperature, relative humidity, and rainfall data were collected hourly in 2018 and 2019 with HOBO micro weather station data loggers (Onset Computer Corporation, Bourne, MA) installed in the field. In 2017, the weather data were retrieved from the ESAREC weather station and relative humidity was not available.

Plots were harvested 93 to 114 days after planting (DAP) in 2017, 90 to 105 DAP in 2018, and 89 to 103 DAP in 2019. Harvest time was determined by an average maturity day of all cultivars or when plants withered or died in later planting dates. Each plot was machine lifted, and potato tubers were manually collected in bins. Tubers were weighed and graded according to USDA standards (Agricultural Marketing Service-USDA 2011). Symptomatic tubers with round,

brown, yellow, or tan sunken lesions; white mycelium; or soft tissue were considered diseased by *S. rolfsii* (Weber 1943). Total and marketable yield data were obtained by weighing potato tubers per plot and converted to Mg ha^{-1}. The percentage of diseased tubers ([weight of diseased tubers /total tubers weight] ×100) and percentage of marketable tubers ([weight marketable tubers/total tubers weight] × 100) were calculated.

Statistical analyses

Due to variation across years, the analysis of variance (ANOVA) for each year was analyzed separately. ANOVA was performed using the statistical procedures of a split-plot design in SAS 9.4 Mixed Model Procedures (SAS Institute, Inc., Cary, NC) (Ott and Longnecker 2010). To meet ANOVA assumptions, arcsine-square root transformation was applied to the final southern blight incidence, percentage of marketable tubers, percentage of diseased tubers, and square root transformation was applied for marketable tuber yield (McCune et al. 2002). Non-transformed means are presented. The main effects of treatments (planting date and cultivars), block, and interactions were included in the model. Block was treated as a random effect, and planting date, and cultivar were treated as fixed effects. The use of the proper error terms to test the main factors and interactions was achieved by using PROC MIXED procedures. When the interaction was significant, test slices were used to determine the effect of the cultivar at each planting date as described by Wludyka (Wludyka 2015, SAS Institute Inc., 2017). The least squares mean comparisons were conducted using the Fisher's protected Least Significant Difference test (LSD) at a 5% significance level. A Spearman correlation analysis was carried out between disease incidence, marketable yield, percentage of marketable yield, and percentage of diseased potato tubers using SAS 9.4 PROC CORR (SAS Institute, Inc., Cary, NC). To

determine the environment influence on *S. rolfsii* incidence, marketable yield, and tuber quality, a simple linear correlation analysis was performed between the means of final disease incidence, marketable yield, and percentage of diseased tubers for each planting date-year combination and mean weather parameters (average temperature, temperature oscillations, and total rainfall) across planting date-year combination using SAS 9.4 PROC CORR (SAS Institute, Inc., Cary, NC).

Due to the complex planting date-cultivar interaction across years, a site regression model (SREG) and biplot analysis of the *S. rolfsii* incidence and the marketable tuber yield were performed to evaluate the potato cultivars' performance across all years and planting dates of the study using SAS 9.4 (SAS Institute, Inc., Cary, NC). The environmental variable consisted of each planting date-year combination (N=12) and 10 cultivars. The SREG model and biplots allow to further study cultivar by environment interaction and the adaptability and stability of cultivars across environments (planting dates) (Castillo et al. 2012; Crossa et al. 2015; Vargas et al. 2015; Yan and Kang 2002). In the SREG model, the cultivar plus the cultivar by planting date-year interaction is included, and the latter is subject to a singular value decomposition (Crossa et al. 2015). Further, the biplot of the first two significant principal components (PC1 and PC2) from the SREG model were used to construct the biplots.

Results

Impact of planting date and environmental parameters on disease incidence

Overall final *S. rolfsii* incidence varied across growing seasons. The mean disease incidence across planting dates and cultivars was greater in 2018 (79%) than in 2017 (64%) or 2019 (25%) ($P<0.001$). Planting date influenced average southern blight incidence; however, the

effect was inconsistent. In 2017, disease incidence main effect, averaged across cultivars, increased significantly from 36% for the 7 March planting date to 94% for the 10 May planting date ($P<0.001$). Conversely, in 2019 a decrease in disease incidence was observed between the 3 April planting date (45%) and the 16 May planting date (7%) ($P=0.002$). A trend regarding planting date was indicated in 2018 ($P=0.228$), where disease incidence numerically increased from 73% for the earliest planting date (29 March) to 85% for the latest planting date (9 May).

Variation in disease incidence was associated with differences in rainfall and temperature across years and planting dates. The 2017 growing season had more accumulated precipitation (636 mm) than 2018 (394 mm) or 2019 (377 mm) (Table 2.2 & Figure S 2.1). Furthermore, in 2017 and 2018 all planting dates received above 362 mm and 296 mm of precipitation, respectively, and precipitation was more evenly distributed across the seasons than in 2019 when precipitation for all planting dates was less than 277 mm except for 17 April planting date (Table 2.2 & Figure S 2.1). The mean temperature across the growing season was greater in 2019 (23.5°C) than 2017 (21.1°C) or 2018 (21.8°C) (Table 2.2). Moreover, temperature progressively increased toward later planting dates in all years, and ranged from 17.6 to 23.8°C, 19.7 to 23.7°C, and 21.6 to 24.8°C in 2017, 2018, and 2019, respectively, between the earlier and later planting dates (Table 2.2). However, there was a positive relationship between mean temperature and mean disease incidence only across planting date-year combinations in 2017 and 2018 ($r=0.80$, $P = 0.0179$). In 2019 the lack of moisture (less rainfall) and warmest temperatures likely limited disease development. For example, the later planting dates in 2019 (May) had the least rain (<271 mm) and warmer average temperatures (24.4-24.8°C) than any other planting date-year combination (Table 2.2). Additionally, a significant negative relationship between temperature oscillation and disease incidence across planting date-year combinations was

observed (r=-0.63, *P*=0.038). The regression analysis indicated that rainfall (β=0.30, *P*=0.023) and temperature oscillations (β =-56, *P*=0.002) explained 73% of final disease incidence variability.

Variation in disease incidence among cultivars and planting dates

The SREG analysis indicated a cultivar (*P*<0.001), environment (*P*<0.001), and cultivar by environment interaction (*P*<0.001) effect on disease. Environment and cultivar explained 74% and 9% of the disease incidence variability, respectively, while 5% was attributed to the environment-cultivar interaction. The first two principal components accounted for 82.39% (PC1 69.05% and PC2 13.34%) of the total interaction variability (Figure 2.1). The length of the vectors represents the influence of environments (N=11) on cultivars, thereby allowing to differentiate cultivar performance against *S. rolfsii*. Thus, the long vector (environment 1B: 24 March 2017) with a greater PC2 score contributed with most of the crossover interaction. This is noted in Table 2.3, where the 24 March 2017 planting date had a wider range of disease incidence (65%) than any other planting date-year and the cultivar ranking changed greatly. Moreover, the last plantings of each year (May) were grouped in the upper right quadrant (higher average temperature environments), meaning they similarly influenced the cultivars' performance. The x-axis (PC1) indicates rank of cultivar disease incidence (cultivars' PC1 values were highly correlated to disease incidence across planting date-years; r=99, *P*<0.001), where greater positive values reflect greater disease incidence (e.g., 'Adirondack Blue' and 'Dark Red Norland'). This suggests that the cultivars on the left quadrants had less incidence of *S. rolfsii* (disease incidence below mean in at least 50% of the environments) (Figure 2.1 & Table 2.3). The y-axis (PC2) indicates the level of instability (Yan 2003); therefore, cultivars with greater values (positive or negative) had a more variable response to *S. rolfsii* among

environments (‘Accumulator’ and ‘Dark Red Norland’). However, ‘Adirondack Blue’, by having a greater PC1 value and low PC2 value, was ranked as the cultivar with the most consistently high disease incidence across planting date-years. The polygon drawn in Figure 2.1 connects the cultivars (‘Accumulator’, ‘Dark Red Norland’, ‘Adirondack Blue’, ‘Snowden’, and ‘Yukon Gold’) located farthest from the biplot origin; therefore, they are among the most responsive to the environments (either greater or less than average incidence of *S. rolfsii*).

When analyzed by year, there was a significant planting date-cultivar interaction in 2017 ($P<0.001$) and 2018 ($P=0.001$), but not in 2019 ($P=0.111$). Thus, means were compared among cultivars by planting dates in 2017 and 2018 (Table 2.3). ‘Accumulator’ had lower than average disease incidence across all planting dates. In addition, it was either the lowest ranked or not statistically different from the lowest ranked cultivar for all years and planting dates (Table 2.3). In contrast, disease incidence for ‘Adirondack Blue’ was above average across all planting dates, and except for the 24 March 2017 planting dates, no other cultivar had greater disease incidence (Table 2.3). In the absence of significant interaction in 2019 and relatively low disease pressure, ‘Snowden’, ‘Accumulator’, ‘Russet Burbank’, and ‘Yukon Gold’ had similarly low disease incidence (10-18%). On the other hand, ‘Adirondack Blue’, ‘Envol’, ‘Superior’, ‘Red Norland’, and ‘Dark Red Norland’ had the greatest (33-37%) disease incidence (Table 2.3).

Impact of planting date and environmental parameters on potato tuber yield

Marketable tuber yield averaged across planting dates and cultivars varied by year with greater yield in 2017 (17.8 Mg ha^{-1}) than 2018 (11.4 Mg ha^{-1}) or 2019 (7.7 Mg ha^{-1}). Though overall yield varied by year, there was a planting date effect on marketable tuber yield in all years (2017, $P<0.001$; 2018, $P=0.005$; and 2019, $P=0.002$). Overall, marketable yield was

reduced by 94% in 2017, 76% in 2018, and 55% in 2019, between the earliest and the latest planting date (Table 2.2). March plantings in 2017 and 2018 had the greatest yields among all planting date-year combinations; which coincided with lower (<21°C) temperatures (Table 2.2).

Temperature was the only measured environmental variable that correlated with yield, with yield decreasing as average temperature for a planting date-year increased (r=-0.91, $P<0.001$). Though the mean temperature progressively increased toward later planting dates in all years, in 2019 temperatures remained above 21°C for all planting dates, while cooler temperatures (<21°C) were present in March and early April planting dates in 2017 and 2018 (Table 2.2).

Variation in marketable tuber yield among cultivars and planting dates

The SREG analysis indicated a cultivar ($P<0.001$), planting date ($P<0.001$), and cultivar by planting date interaction ($P<0.001$) effect on yield. Environment and cultivar accounted for 73% and 11% of the total yield variability, respectively, while 6% was attributed to the environment-cultivar interaction. The first two principal components accounted for 87.8% (PC1 78.57% and PC2 9.23%) of environment-cultivar interaction (Figure 2.2). The description above for the disease incidence biplot applies for the yield biplot, except that greater x-axis values (positive/negative) are cultivars with greater/poorer yield. Thus, cultivars on the left two quadrants (all fresh market) had below-average yield (low yielding) in most environments (Figure 2.2). For example, 'Yukon Gold' had the poorest yield in 6 of the 12 planting dates (Table 2.4). On the other hand, 'Accumulator', by having the greater PC1 value, was the highest yielding cultivar overall. The chipping cultivars 'Atlantic' and 'Snowden', with similar overall yield among them, were the next greatest yielding cultivars. Of the 12 planting date-year

environments, environment 2B (11 April 2018) and 2D (9 May 2018) had the longest vectors indicting greater variability among cultivars (range 17.8-22.4 Mg ha^{-1}). Thus, these environments allowed for greater differentiation of cultivar performance. The last three planting dates in 2019 were clustered on the upper right quadrant meaning they had similar effects on cultivar yield.

Cultivar's marketable tuber yield depend on the planting date in all years (2017, $P<0.001$; 2018, $P=0.033$; and 2019, $P<0.001$). Furthermore, yield varied among cultivars for every planting date in all years (Table 2.4). Despite the planting date by cultivar interaction, 'Accumulator' had greater than average marketable tuber yield across all planting dates, and it grouped with the top-yielding cultivars in 11 of the 12 planting dates. Though slightly less consistent, 'Atlantic' and 'Snowden' were also among the highest yielding cultivars across most planting dates. Conversely, 'Yukon Gold' had lower than average yield for all planting dates and grouped with the lowest-yielding cultivars across all planting date-year combinations evaluated (Table 2.4).

Relationship between disease and yield parameters

An inconsistent correlation between disease incidence and marketable tuber yield was observed. For example, disease incidence was negatively correlated to tuber yield in 2017 and 2018 but had a significant positive correlation in 2019, although it was weaker than the positive correlations observed in the preceding years. Further, the percentage of marketable tubers and diseased tubers were positively and negatively correlated, respectively, with marketable tuber yield in all years (Table 2.5). In addition to reducing the tuber yield, delaying planting date also decreased the quality of the tuber (percentage of marketable tubers averaged across cultivars) by

48% and 23% in 2017 and 2018, respectively, and increased diseased tubers (tuber rot caused by *S. rolfsii*) substantially in both years (Table S 2.1 & Table S 2.2).

Discussion

Over the last decade, southern blight caused by *S. rolfsii* has emerged as an economically important vegetable disease in the mid-Atlantic U.S. that threatens the sustainable production of potato and other crops. Therefore, it is essential to find sustainable approaches to combat southern blight that contribute to potato production resilience. To our knowledge, there have been no previous studies on the effect of planting date and modern potato cultivars on southern blight in the U.S. This three-year study evaluated whether altering the planting date can reduce the incidence of southern blight and risk of tuber yield loss for 10 commercially grown potato cultivars in the mid-Atlantic region. Even though there was a planting date by cultivar interaction, later planting dates generally experienced warmer temperatures, greater disease incidence, and reduced potato tuber yield compared to earlier planting dates. Thus, delaying planting date is likely to have negative impacts on yield which are driven by a combination of environmental parameters and greater disease incidence. Though the ranking of some cultivars for disease incidence and yield varied with planting date, others were consistently the most resistant or susceptible to southern blight and among the highest and lowest yielding potato cultivars. This indicates that a combination of well-timed planting dates and cultivar selection are an effective management approach to reduce *S. rolfsii* impact in fields and maximize potato tuber yield.

The current study demonstrated that it may be possible to manipulate planting date so that environmental conditions are favorable for potato crop growth but unfavorable for growth and

crop infection by *S. rolfsii*. Potato grows best between 16 - 25°C (Thornton 2020) and although the growth period can take 90-160 days across the mid-Atlantic, harvest normally occurs 90-120 days post-planting. Conversely, *S. rolfsii* can grow between 8 - 40°C but grows best at 27-35°C with adequate soil moisture and high relative humidity (Mullen 2001; Punja 1985; Ramarao and Raja 1980). Thereby, earlier planting dates can take advantage of cooler temperatures less conducive to disease but still favorable for crop growth (Browne et al. 2002; Nuñez and Aegerter 2019). This was demonstrated in the current study by a significantly lower incidence of southern blight resulting from earlier planting dates in March 2017, which were followed by the lowest temperatures of any planting dates in the study. Thus, most of the crop growth occurred prior to occurrence of temperatures that were favorable for disease development.

In contrast to earlier planting dates, overall *S. rolfsii* incidence was high (> 80%) following late planting dates in two of the three growing seasons (2017 and 2018). These observations are consistent with previous reports in a potato evaluation two months post-transplanting, where May plantings in Virginia had above 80% *S. rolfsii* incidence compared to 33% incidence for potato planted in April; this was likely driven by warmer temperatures following the later planting dates (Edson and Shapovalov 1923). In contrast, studies in peanut and carrot within the U.S. have generally found that late plantings reduced *S. rolfsii* incidence by 50-60% (Bowen 2003; Hagan et al. 2001; Jenkins and Averre 1986). This difference is because, unlike potato, peanut is a warm season crop, and by delaying planting, the later stages of crop development occur during the fall when cooler temperatures are less conducive for disease development. However, in the current study, harvests occurred before August 13 and prior to temperatures that would be unfavorable for *S. rolfsii*. Thus, it is likely that the high incidence of *S. rolfsii* following late plantings in the current study were driven by the synchronization of most

of the crop growth with humid and warm periods (July through August) as indicated by the positive association between temperatures and disease incidence in 2017 through 2018. Unlike preceding seasons, in 2019 southern blight declined by delaying planting dates. This decline can be attributed to below-average rainfall in 2019 and dry conditions that likely limited the growth of *S. rolfsii*. In California, it has been reported that relatively dry topsoil was associated with low *S. rolfsii* incidence in potato (Browne et al. 2002).

Delaying planting dates negatively impacted potato tuber yield and quality in the current study. The overall yield reduction of more than 76% in 2017 and 2018 and more than 50% in 2019 reveals yield potential is limited when the crop is planted later than mid-April. In our study, the reduction was associated with greater disease incidence and warmer temperatures toward later plating dates. Although in 2019 the disease development was hindered by drier conditions, there was a consistent negative relationship between yield and disease incidence in 2017 and 2018. Moreover, a consistent negative relationship between yield and diseased tubers was observed in all years. Earlier studies have noted similar effects where 75% of the plants in the April planting produced tubers compared to 42% of those in the May plantings (Edson and Shapovalov 1923). Moreover, Aycock (1966) expressed that losses to cool weather crops frequently occur when planting dates are delayed because the most development of the crops is through warm periods. The consistently strong negative correlation observed between yield and temperatures supports this hypothesis. Thus, planting later than mid-April will reduce potato yield, as high temperatures from June through August will hamper the tuber development.

In our findings, no cultivar was completely immune to *S. rolfsii* infection; however, there was significant variation in southern blight incidence among cultivars. While disease severity

was not assessed, the lower disease incidence observed for some potato cultivars suggested they are more resistant to southern blight. Previously, there were no reports of mid-Atlantic-grown cultivar responses to *S. rolfsii*. Though cultivar's responses varied across planting date-years, 'Accumulator' consistently had one of the lowest disease incidences among cultivars across planting date-years. Conversely, 'Adirondack Blue' and 'Dark Red Norland', had the greatest incidence of *S. rolfsii* over the years. This was also depicted in the biplot, where the processing and chipping cultivars were grouped in the left quadrants (in contrast to fresh market cultivars except 'Yukon Gold') and 'Accumulator' was ranked with less southern blight incidence (lower PC1 scores). Therefore, our results suggested that 'Accumulator' is less likely to become infected by *S. rolfsii* than all other cultivars evaluated. Differences in cultivar's responses to *S. rolfsii* have been documented domestically and worldwide. For example, Voss et al. (1984) noted that 'Kennebec', 'White Rose', and several breeding lines showed some resistance compared to 'Centennial Russet', while almost a century ago Edson and Shapovalov (1923) documented that 'Irish Cobbler' and 'Bliss Triumph' were highly susceptible to *S. rolfsii*. Kulkarni (2007) found no differences among cultivars in a study in India, where all 10 cultivars evaluated were labeled as highly susceptible (50-80% incidence), and Anahosur (2001) reported that 18 of 20 cultivars evaluated were moderately to highly susceptible (>60% incidence) 60 days post-planting. Moreover, to our knowledge, there are no studies that directly compare the susceptibility among different potato utilization groups. However, reports have noted that the fresh market cultivar 'Monalisa', was more severely affected than the chipping cultivar 'Hermes' (Garibaldi et al. 2006). In studies by Voss et al. (1984), the greater susceptibility was attributed to 'Centennial Russet', a fresh market cultivar. These studies suggest that the physiology and composition of the tubers likely influences differences in susceptibility between the potato utilization groups.

Cells with high starch content have been implicated in hampering *S. rolfsii* hyphae penetration (Aycock 1966); hence, the high starch (Bond 2014) and lower water content of processing and chipping cultivars (Bond 2014; Nzaramba et al. 2013) could contribute to greater resistance to *S. rolfsii* infection compared to fresh market cultivars. This is also supported by the lower percentage of diseased tubers by *S. rolfsii* observed in 'Atlantic', 'Snowden', and 'Accumulator' (Table S 2.2) but determining specifically the physical and chemical properties that contribute to resistance is beyond the scope of this study.

Though the yield was considerably reduced for the April and May planting dates for all cultivars, 'Accumulator' out-yielded other cultivars in marketable tuber yield and quality. This was also shown in the cultivar-environment interaction biplot, where 'Accumulator' had the greatest yield in eight of the 12 environments (planting dates), thus demonstrating more yield stability across environments. Additionally, the chipping cultivars were grouped on the opposite side of the fresh market cultivars except for 'Envol', which indicates that 'Envol' excelled among the fresh market cultivars. In contrast, 'Yukon Gold' had overall poor yield. Though there was a clear relationship between yield and disease incidence, other factors such as warmer temperatures and inadequate moisture are associated with low yields as reported previously (Aycock 1966). Additionally, the lower yield of 'Yukon Gold' can be attributed to the lower stand count compared to the rest of cultivars (data not shown). However, when crop growing conditions were more suitable and disease pressure relatively low, the chipping cultivars showed greater tuber yield and quality, indicating that under the right conditions, these cultivars are well suited to the mid-Atlantic U.S.

This study investigated potato southern blight management through the manipulation of planting date and cultivar selection. The results demonstrated that variation among planting dates in environmental parameters such as precipitation and temperature is a key factor in determining the cultivar performance and disease development. Although disease incidence varied among planting dates in *S. rolfsii* infested fields, overall 'Accumulator' had the least disease incidence and greatest tuber yield across planting date-years. However, the significant increase in disease incidence and consistent reduction in yield and quality of all cultivars that resulted from delayed planting suggested that cultivar selection alone is not reliable and should be coupled with early planting dates to manage southern blight. Therefore, our results indicated that potato planted after the first week of April in Virginia is at greater risk of being affected by *S. rolfsii* and impacted by warmer temperatures (> 21°C) that were found to be associated with lower yield and greater disease in the presence of adequate moisture. Moreover, although susceptibility was not measured per se, the higher disease incidence observed for cultivars such as 'Adirondack Blue' and 'Dark Red Norland' across planting dates suggested that these cultivars are highly susceptible to southern blight and should not be planted in fields with a history of *S. rolfsii* infestation. Further characterization of the cultivars less affected by southern blight can elucidate sources of resistance for breeding. Furthermore, because of consistently low yields and an intermediate incidence of disease for 'Yukon Gold', this cultivar is considered the least adapted to the mid-Atlantic region. Since yield is suppressed by high disease incidence and warmer temperatures, in fields with a history of *S. rolfsii* infestation, mid-Atlantic potato growers should avoid planting later than dates when the forecast indicates that the crop will be exposed to an average air temperature above 21°C that favor southern blight development.

Literature Cited

Agricultural Marketing Service-USDA. 2011. United States standards for grades of potatoes. Retrieved from https://www.ams.usda.gov/sites/default/files/media/Potato_Standard%5B1%5D.pdf. Accessed Oct. 10, 2019.

Anahosur, K. 2001. Integrated management of potato Sclerotium wilt caused by *Sclerotium rolfsii*. Indian Phytopath. 54:158-166.

Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*: or the status of Rolfs' fungus after 70 years. N. C. Agric. Exp. Stn. Bull. 174:202.

Bockus, W., and Shroyer, J. 1998. The impact of reduced tillage on soilborne plant pathogens. Annu. Rev. Phytopathol. 36:485-500.

Bond, J. 2014. Potato utilization and marketing. Pages 50-70 in: The Potato: Botany, Production and Uses. R. Navarre and M. J. Pavek, eds. CABI, Boston, MA.

Bowen, K. 2003. Development of stem rot (caused by *Sclerotium rolfsii*) in peanut in Alabama. Peanut Sci. 30:120-128.

Brittingham, W. H., Bell, F. W., and Christiansen, J. K. 1963. Economic history and present status of potato production in Virginia. Virginia Joint Agric. Pub. 5:6-14.

Browne, G., DeTar, W. R., Sanden, B., and Phene, C. J. 2002. Comparison of drip and sprinkler irrigation systems for applying metam sodium and managing stem rot on potato. Plant Dis. 86:1211-1218.

Bulluck III, L., and Ristaino, J. 2002. Effect of synthetic and organic soil fertility amendments on southern blight, soil microbial communities, and yield of processing tomatoes. Phytopathology 92:181-189.

Castillo, D., Matus, I., del Pozo, A., Madariaga, R., and Mellado, M. 2012. Adaptability and genotype x environment interaction of spring wheat cultivars in Chile using regression analysis, AMMI, and SREG. Chil. J. Agric. Res. 72:167-174.

Crossa, J., Vargas, M., Cossani, C. M., Alvarado, G., Burgueño, J., Mathews, K. L., and Reynolds, M. P. 2015. Evaluation and interpretation of interactions. Agron. J. 107:736-747.

Edmunds, B. A., Gleason, M. L., and Wegulo, S. N. 2003. Resistance of hosta cultivars to petiole rot caused by *Sclerotium rolfsii* var. *delphinii*. HortTechnology 13:302-305.

Edson, H., and Shapovalov, M. 1923. Parasitism of *Sclerotium rolfsii* on Irish potatoes. Jour. Agr. Res 23:41-46.

Farr, D. F., and Rossman, A. Y. 2017. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases/.

Garibaldi, A., Gilardi, G., and Gullino, M. L. 2006. First report of southern blight incited by *Sclerotium rolfsii* on potato (*Solanum tuberosum*) in northern Italy. Plant Dis. 90:1114-1114.

Garren, K. H. 1958. The stem rot of peanuts and its control. Virginia Agric. Exp. Stn. 144:29.

Garren, K. H., and Duke, G. B. 1958. The effects of deep covering of organic matter and non-dirting weed control on peanut stem rot. Plant Dis. Rep. 42:629-636.

Garren, K. H., and Bailey, W. K. 1963. Comparative responses of a Virginia runner and a Virginia Bunch peanut to cultural control of stem rot. Agron. J. 55:290-293.

Hagan, A., Campbell, H., Bowen, K., and Wells, L. 2015. Seeding rate and planting date impacts stand density, diseases, and yield of irrigated peanuts. Plant Health Prog. 16:63-70.

Hagan, A. K., Weeks, J. R., Bowen, K. L., and Wells, L. W. 2001. Influence of production practices on peanut disease and yield. Alabama Agric. Exp. Sta. Bull. 643.

Holm, D., Twomey, J., and Workman, M. 1987. Ute Russet: An oblong russet potato cultivar with resistance to leafroll net necrosis and *Sclerotium rolfsii*. Am. J. Potato Res. 64:559-562.

Jenkins, S. F., and Averre, C. W. 1986. Problems and progress in integrated control of southern blight of vegetables. Plant Dis. 70:614-620.

Keinath, A. P., and DuBose, V. B. 2017. Management of southern blight on tomato with SDHI fungicides. Crop Protect. 101:29-34.

Kuhar, T. P., Arancibia, R. A., Hamilton, G. C., VanGessel, M. J., and Wyenandt, C. A. 2019. 2019 Mid-Atlantic commercial vegetable production recommendations. Virginia Cooperative Extension 456-240.

Kulkarni, V. R. 2007. Epidemiology and integrated management of potato wilt caused by *Sclerotium rolfsii* Sacc. PhD (Agri) Thesis. Univ Agric. Sci., Dharwad.

McCune, B., Grace, J. B., and Urban, D. L. 2002. Data transformations. Pages 67-80 in: Analysis of ecological communities, vol. 28. MjM software design, Gleneden Beach, OR.

Mullen, J. 2001. Southern blight, southern stem blight, white mold. The Plant Health Instructor. doi: 10.1094. PHI-I-2001-0104-01.

Natural Resources Conservation Service-USDA. 2017. Web Soil Survey. https://websoilsurvey.sc.egov.usda.gov/. Accessed Nov. 20. 2018.

Nuñez, J., and Aegerter, B. J. 2019. UC IPM pest management guidelines-potato. University of California, ANR/Communication Services 3463.

Nzaramba, M. N., Scheuring, D. C., Koym, J. W., and Miller, J. C. 2013. Relationships among antioxidant activity, total phenolic content and specific gravity in several potato (*Solanum tuberosum* L.) cultivars grown in different environments. Am. J. Potato Res. 90:541-550.

Ott, R. L., and Longnecker, M. T. 2010. Split-plot, repeated measures, and crossover designs. Pages 1091-1134 in: An Introduction to Statistical Methods and Data Analysis (6th ed.). Brooks/Cole, Belmont, CA.

Pethybridge, S. J., Sharma, S., Silva, A., Bowden, C., Murphy, S., Knight, N. L., and Hay, F. S. 2019. Southern sclerotium root rot caused by *Athelia rolfsii* on table beet in New York. Plant Health Prog. 20:4-6.

Punja, Z. K. 1985. The biology, ecology, and control of *Sclerotium rolfsii*. Annu. Rev. Phytopathol. 23:97-127.

Ramarao, P., and Raja, U. 1980. Effect of soil moisture on development of foot and root rot of wheat and on other soil microflora. Indian J. Mycol. Plant Pathol. 10:17-22.

Rivard, C., O'Connell, S., Peet, M., and Louws, F. 2010. Grafting tomato with interspecific rootstock to manage diseases caused by *Sclerotium rolfsii* and southern root-knot nematode. Plant Dis. 94:1015-1021.

Roberts, P. D., French-Monar, R. D., and McCarter, S. M. 2014. Southern blight. in: Compendium of Tomato Diseases and Pest. J. B. Jones, T. A. Zitter, M. Momol and S. A. Miller, eds. American Phytopathological Society, St. Paul, MN.

SAS Institute Inc. 2017. SAS/STAT® 14.3 User's guide. Cary, NC: SAS Institute. P. 512-515.

Thornton, M. 2020. Potato growth and development. in: Potato Production Systems, J. C. Stark, M. Thornton and P. Nolte, eds. Springer, Cham, CH.

USDA-National Agricultural Statistics Service. 2019a. Crop values 2018 summary. Washington, D.C.

USDA-National Agricultural Statistics Service. 2019b. Crop production 2018 summary. Washington, DC.

Vagher, T., Fenwick, A., and Panella, L. 2014. Preparation of inoculum of *Rhizoctonia solani* Kühn for an artificially inoculated field trial. Pages 137-144 in: Meeting Proceedings.

Vargas, M., Glaz, B., Alvarado, G., Pietragalla, J., Morgounov, A., Zelenskiy, Y., and Crossa, J. 2015. Analysis and interpretation of interactions in agricultural research. Agron. J. 107:748-762.

Voss, R., Weinhold, A., Bowman, T., Dennett, C., and Guerard, J. 1984. Potato cultivars differ in susceptibility to *Sclerotium rolfsii* (Abstract). The Potato Association of America 61:541-541.

Weber, G. F. 1943. Southern blight, *Corticium rolfsii* of potato tubers. Phytopathology 33:615-617.

Wludyka, P. 2015. Using ANOM slicing for multi-way models with significant interaction. J. Qual. Technol. 47:193-203.

Yan, W., and Kang, M. S. 2002. GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. CRC press, Boca Raton, FL.

Table 2.1 Description of the potato cultivars evaluated in field study.

Cultivar[z]	Flesh color	Utilization	Maturity
Superior	White	Early fresh & chipping	Early-medium
Envol	White	Early fresh	Very early
Red Norland	White	Fresh	Early
Dark Red Norland	White	Fresh	Early
Russet Burbank	White	Fresh & processing	Late
Yukon Gold	Yellow	Fresh	Early
Adirondack Blue	Purple	Fresh Specialty	Early-medium
Atlantic	White	Chipping	Medium
Snowden	White	Chipping	Late
Accumulator	White	Chipping	Medium-late

[z] All potato cultivars were purchased from certified potato seed producers

Table 2.2 Summary of *Sclerotium rolfsii* incidence, marketable yield, and the weather parameters measured during the growing seasons in all plantings and years of the study [z]

Year	Planting date	Avg. temp (°C)[y]	Osc.[x]	Total rain (mm)[y]	Dis. incid (%)[w]	Mkt. tuber yield (Mg ha^{-1})[v]	Diseased tubers (%)[v]
2017	7-Mar	17.6	10.3	433	36	30.1	3
	24-Mar	20.3	10.2	382	33	30.0	10
	14-Apr	22.5	9.7	362	93	9.4	45
	10-May	23.8	9.8	423	94	1.8	33
2018	29-Mar	19.7	9.2	296	73	20.9	19
	11-Apr	20.9	9.4	298	78	13.9	37
	24-Apr	22.8	9.0	348	80	8.6	28
	9-May	23.7	8.9	319	85	4.9	35
2019	3-Apr	21.6	9.7	277	45	10.6	16
	17-Apr	23.2	9.9	315	24	8.3	15
	2-May	24.4	10.0	271	.	7.0	17
	16-May	24.8	10.1	264	7	4.8	13

[z] Data were measured using Hobo Micro Station installed within the field where experiments were conducted in 2018 and 2019. For 2017, weather data were retrieved from the Eastern Shore Research Station database at Painter, Virginia.
[y] Weather data were not recorded from May 9 to May 20, 2019.
[x] Osc. = Oscillation. Average daily temperature oscillation determined as the difference between the max and the min daily temperature.
[w] Final mean *Sclerotium rolfsii* incidence across planting dates and cultivars. Data not collected for the 3rd planting date in 2019.
[v] Marketable tuber yield and percentage of diseased tubers averaged across planting dates and cultivars.

Table 2.3 Variation in southern blight incidence caused by *Sclerotium rolfsii* among potato cultivars and planting dates.

	2017				2018				2019[z]			
Cultivar	7 Mar	24 Mar	14 Apr	10 May	29 Mar	11 Apr	24 Apr	9 May	3 Apr	17 Apr	16 May	Mean[y]
	Southern blight incidence (%)[x]											
Superior	53 ab[w]	48 b	97 ab	98 a-c	89 a	95 a	89 ab	83 bc	57	36	7	33 ab
Envol	44 bc	32 bc	92 bc	100 a	60 cd	77 b-d	81 bc	90 a-c	66	30	14	37 a
Red Norland	54 ab	32 bc	99 ab	99 ab	74 b	91 ab	95 a	93 ab	69	27	8	35 ab
Dark Red Norland	51 ab	77 a	98 ab	100 a	71 bc	90 ab	92 a	91 a-c	68	29	1	33 ab
Russet Burbank	25 de	19 cd	95 a-c	96 bc	75 bc	84 bc	72 cd	86 a-c	28	19	6	18 c
Yukon Gold	20 de	12 d	96 ab	100 a	65 bc	57 ef	60 d	86 a-c	35	17	3	18 c
Adirondack Blue	59 a	49 b	99 a	99 ab	89 a	94 a	91 a	95 a	59	38	17	38 a
Atlantic	13 e	17 d	89 c	93 c	76 bc	64 de	74 cd	81 cd	36	17	11	21 bc
Snowden	28 cd	15 d	92 bc	94 bc	81 ab	76 cd	77 c	93 ab	16	15	7	13 c
Accumulator	17 de	27 cd	71 d	66 d	52 d	48 f	67 cd	67 d	22	13	1	12 c
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	-	-	-	0.002

[z] Data were not collected for the third planting date of 2019.

[y] There was no significant interaction between cultivar and planting date in 2019. Thus, cultivars were compared across planting dates.

[x] Percentage of plants out of 30 per plot presenting wilting and/or mycelia and sclerotia at the base of the stems prior to harvest (85-112, 78-82, 61-77 days after planting in 2017, 2018, and 2019, respectively). Arcsine-square root transformation was applied prior to data analysis. Non-transformed means are presented.

[w] Column means followed by different letters are significantly different from each other based on ANOVA and Fisher's LSD test at $P \leq 0.05$.

Table 2.4 Variation in potato marketable tuber yield among potato cultivars and planting dates in *Sclerotium rolfsii* infested fields.

	2017				2018				2019			
Cultivar	7 Mar	24 Mar	14 Apr	10 May	29 Mar	11 Apr	24 Apr	9 May	3 Apr	17 Apr	2 May	16 May
	Marketable tuber yield (Mg ha^{-1})[z]											
Superior	29.8 b-e[y]	29.5 b-e	6.3 d	2.8 ab	17.3 de	8.5 ef	5.9 cd	2.4 d	8.8 c	5.1 d	1.3 c	1.1 d
Envol	32.7 a-c	30.7 b-d	11.4 bc	2.4 a-c	15.9 de	16.2 bc	9.8 bc	6.3 bc	12.2 b	6.7 cd	5.2 b	4.8 bc
Red Norland	30.1 b-d	27.6 d-f	5.0 d	1.6 bc	18.8 cd	13.7 cd	7.3 b-d	3.7 b-d	9.5 c	7.7 c	5.8 b	6.7 ab
Dark Red Norland	32.4 bc	26.3 ef	6.0 d	2.0 a-c	17.7 c-e	11.6 de	8.6 b-d	3.5 cd	7.9 c	6.5 cd	6.1 b	4.3 bc
Russet Burbank	25.7 ef	32.3 a-c	9.2 c	0.3 d	17.9 c-e	8.1 ef	5.7 d	1.8 d	8.4 c	6.7 cd	5.1 b	1.7 d
Yukon Gold	24.1 f	23.7 f	6.3 d	1.4 c	13.4 e	6.2 f	2.9 e	0.3 e	8.4 c	6.1 cd	4.4 b	2.6 cd
Adirondack Blue	27.4 d-f	28.3 c-e	6.7 d	1.5 c	23.0 bc	14.3 cd	7.9 b-d	3.3 b-d	7.3 c	5.3 cd	5.5 b	6.0 ab
Atlantic	33.9 ab	33.0 ab	11.4 bc	1.6 a-c	26.6 ab	20.8 b	9.3 bc	5.2 bc	13.9 ab	12.2 b	11.5 a	7.9 a
Snowden	28.7 c-e	32.5 a-c	12.7 b	2.9 a	27.3 ab	18.6 bc	9.7 b	6.2 b	14.7 a	16.2 a	11.8 a	6.7 ab
Accumulator	37.3 a	36.6 a	19.6 a	2.4 a-c	33.4 a	28.6 a	21.5 a	18.1 a	15.4 a	10.2 b	13.2 a	6.2 ab
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z] Data were obtained by grading and weighing according to USDA standards (Agricultural Marketing Service-USDA 2011). To meet ANOVA assumptions, square root transformation was applied prior to data analysis only for 2017 and 2018. Non-transformed means are presented.

[y] Column means followed by different letters are significantly different from each other based on ANOVA and Fisher's LSD test at $P \leq 0.05$.

Table 2.5 Relationships between percent diseased tubers, southern blight incidence, marketable yield, and percent marketable tubers for potatoes grown in field experiments in Virginia from 2017-2019.

	2017		2018		2019	
Variable[z]	Correlation coefficient[y]	*P*-value	Correlation coefficient	*P*-value	Correlation coefficient	*P*-value
INC vs. DT (%)	0.81	<0.001	0.34	<0.001	0.45	<0.001
INC vs. Mkt Yld	-0.79	<0.001	-0.41	<0.001	0.24	0.009
INC vs. Mkt (%)	-0.82	<0.001	-0.36	<0.001	-0.23	0.010
DT (%) vs. Mkt Yld	-0.76	<0.001	-0.70	<0.001	-0.48	<0.001
DT (%) vs. Mkt (%)	-0.90	<0.001	-0.98	<0.001	-0.82	<0.001
Mkt Yld vs. Mkt (%)	0.87	<0.001	0.75	<0.001	0.65	<0.001

[z] Variables are abbreviated as follows: DT (%) = Percentage of diseased tubers, INC = final southern blight incidence, Mkt (%) = percentage of marketable tubers, Mkt Yld= marketable yield.
[y] Spearman's correlation coefficients.

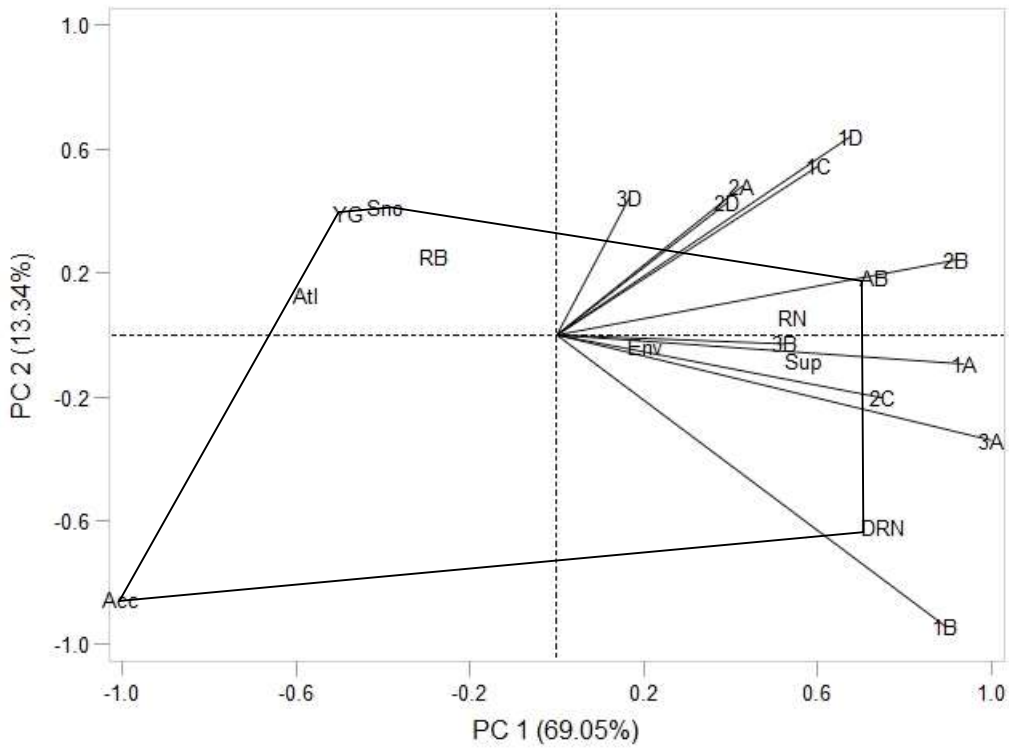

Figure 2.1 Biplot of the first two components (PC 1 & PC2) of the site regression model (SREG) for *Sclerotium rolfsii* incidence on 10 potato cultivars across 11 environments (planting dates) from 2017 to 2019. Cultivars are as follow: Sup=Superior, RN=Red Norland, DRN=Dark Red Norland, Env=Envol, Atl=Atlantic, Sno=Snowden, YG=Yukon Gold, RB=Russet Burbank, AB=Adirondack Blue, Acc=Accumulator. Numbers in environment vectors represent the year 1=2017, 2=2018, and 3=2019, while letters represent the first (A), second (B), third (C), and fourth (D), planting date within a year. The length of the vectors represents the influence of environments on cultivars, thus allowing to differentiate cultivars performance against *S. rolfsii.* PC1 values are correlated to cultivar incidence (greater values=greater susceptibility) and PC2 values to stability.

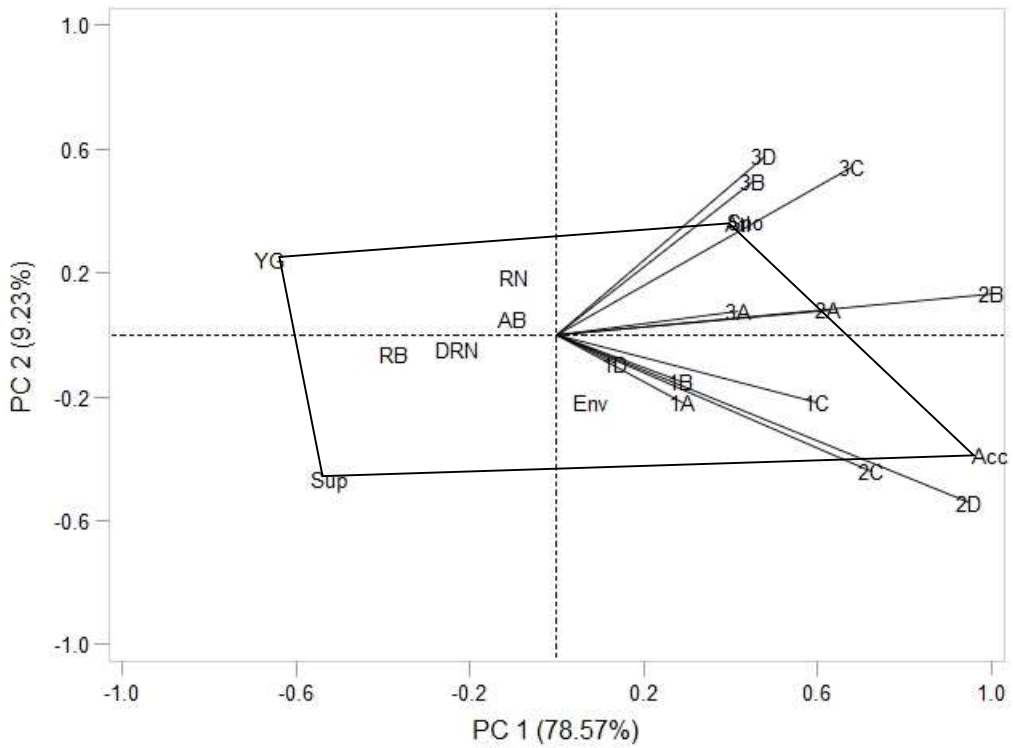

Figure 2.2 Biplot of the first two components (PC 1 & 2) of the site regression model (SREG) for marketable tuber yield of 10 potato cultivars across 12 environments (planting dates) from 2017 to 2019. Cultivars are as follow: Sup=Superior, RN=Red Norland, DRN=Dark Red Norland, Env=Envol, Atl=Atlantic, Sno=Snowden, YG=Yukon Gold, RB=Russet Burbank, AB=Adirondack Blue, Acc=Accumulator. Numbers in environment vectors represent the year 1=2017, 2=2018, and 3=2019, while letters represent the first (A), second (B), third (C), and fourth (D), planting date within a year. The length of the vectors represents the influence of environments on cultivars, thus allowing to differentiate cultivars performance against *S. rolfsii.* PC1 values are correlated to tuber yield (greater values=greater yield) and PC2 values to stability.

Supplemental Data

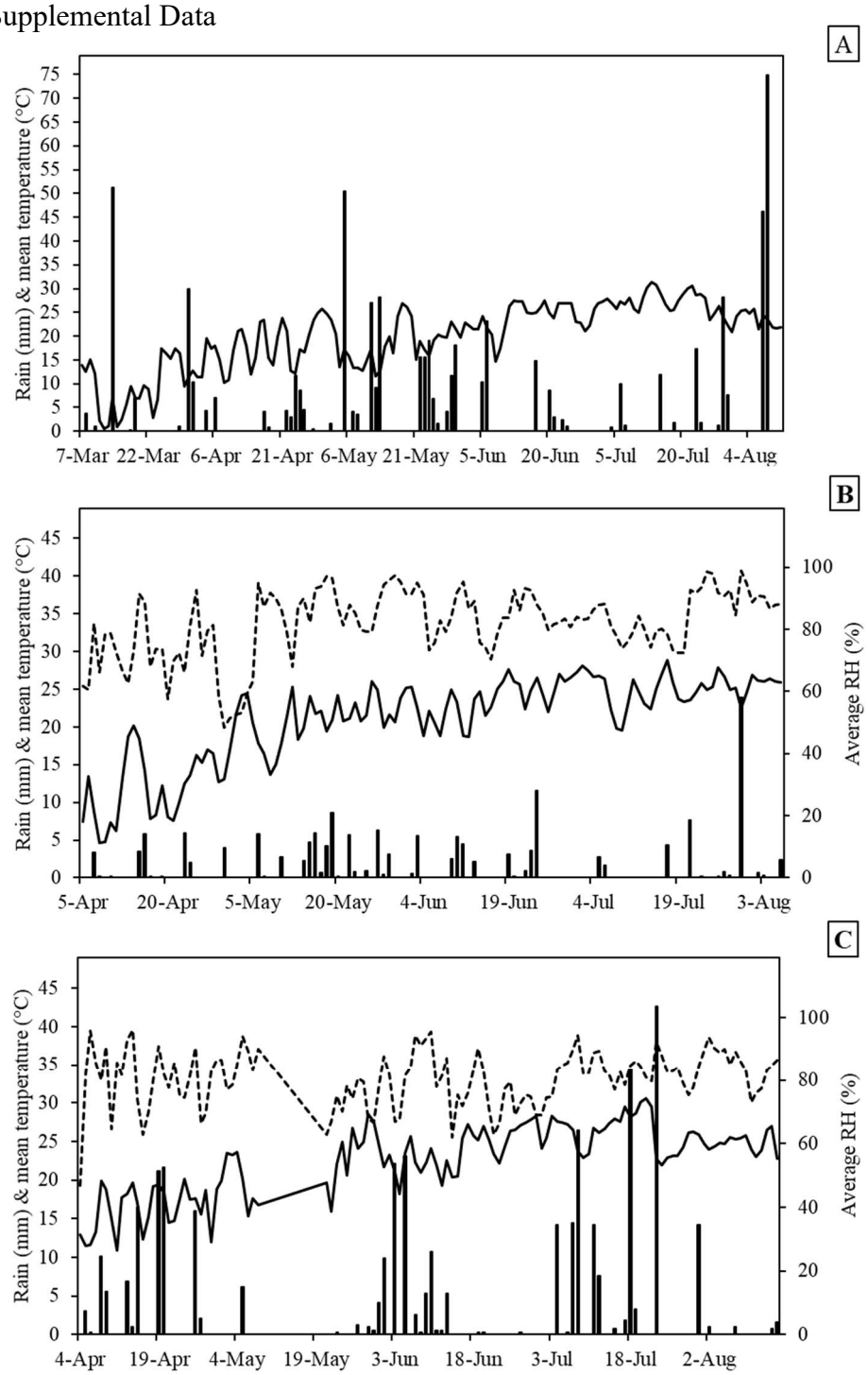

Figure S 2.1 Precipitation (mm)(▬), mean daily air temperature (——), and percent relative humidity (RH) (-----) during 2017 (A), 2018 (B), and 2019 (C) field seasons. For 2017, weather data were retrieved from the Eastern Shore Research Station database at Painter, Virginia. Relative humidity data were not available for 2017. For 2018 and 2019, data were measured using Hobo Micro Station installed within the field where experiments were conducted. Weather data were not recorded from May 9 to May 20, 2019.

Table S 2.1 Variation in the percentage of potato marketable tubers among potato cultivars and planting dates in *Sclerotium rolfsii* infested fields.

	Planting date 2017				Planting date 2018				Planting date 2019			
Cultivar	7 Mar	24 Mar	14 Apr	10 May	29 Mar	11 Apr	24 Apr	9 May	3 Apr	17 Apr	2 May	16 May
	Marketable tubers (%)[z]											
Superior	94 a[y]	84 d-f	50 cd	48 b-d	79 b	55 de	69 b-e	61 bc	79 a-c	74 b	58 e	57 e
Envol	95 a	90 b-d	58 bc	58 a-c	69 bc	77 b	76 a-c	71 b	89 a	79 b	74 cd	72 cd
Red Norland	95 a	81 ef	31 f	45 d	65 c	55 de	56 f	54 cd	73 bc	76 b	73 cd	83 a-c
Dark Red Norland	97 a	77 f	40 e	48 cd	65 c	49 d-f	65 c-f	55 cd	70 c	75 b	77 b-d	67 de
Russet Burbank	84 b	87 c-e	53 cd	12 e	72 bc	47 ef	80 ab	60 bc	74 bc	59 c	73 cd	74 b-d
Yukon Gold	94 a	86 c-e	44 de	61 a	66 c	38 f	59 ef	31 e	76 bc	76 b	71 d	73 b-d
Adirondack Blue	83 b	82 ef	44 de	46 d	89 a	61 cd	62 d-f	47 d	69 c	72 bc	82 a-d	76 b-d
Atlantic	95 a	90 bc	58 bc	43 d	90 a	67 bc	73 a-d	64 bc	82 ab	83 b	84 a-c	91 a
Snowden	96 a	96 a	65 ab	59 a	94 a	71 bc	70 b-e	69 b	88 a	96 a	87 ab	91 a
Accumulator	96 a	92 ab	71 a	58 ab	95 a	87 a	82 a	88 a	89 a	78 b	92 a	86 ab
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z] Data were obtained by dividing the marketable tuber weight by total weight. To meet ANOVA assumptions, Arcsine-square root transformation was applied prior to data analysis. Non-transformed means are presented.

[y] Column means followed by different letters are significantly different from each other based on ANOVA and Fisher's LSD test at $P \leq 0.05$.

Table S 2.2 Variation in the percentage of *Sclerotium rolfsii* infected potato tubers among potato cultivars and planting dates.

	2017				2018				2019			
Cultivar	7 Mar	24 Mar	14 Apr	10 May	29 Mar	11 Apr	24 Apr	9 May	3 Apr	17 Apr	2 May	16 May
	----------Diseased tubers (%)[z]----------											
Superior	4 b[y]	14 ab	48 cd	34 bc	19 b	41 b-d	29 b-d	34 cd	18 a-c	24 a	35 a	30 a
Envol	2 bc	7 cd	40 de	35 bc	31 ab	22 f	24 de	25 d	9 de	16 ab	22 b	19 ab
Red Norland	3 bc	17 ab	66 a	55 a	34 a	43 bc	42 a	44 bc	24 ab	21 ab	23 b	11 bc
Dark Red Norland	2 bc	22 a	60 ab	39 b	34 a	50 ab	33 a-d	42 bc	27 a	21 ab	17 bc	20 b
Russet Burbank	1 c	5 de	37 e	15 d	24 ab	51 ab	16 e	23 d	11 c-e	8 cd	16 bcd	10 bcd
Yukon Gold	4 b	11 bc	53 bc	26 c	32 a	62 a	39 ab	68 a	22 ab	19 ab	24 b	19 ab
Adirondack Blue	11 a	15 ab	56 bc	38 b	9 c	36 c-e	35 a-c	51 b	25 ab	22 ab	12 cd	14 b
Atlantic	1 c	6 c-e	34 ef	49 a	8 cd	31 d-f	26 c-e	32 cd	16 b-d	14 bc	13 cd	6 cde
Snowden	2 bc	2 e	34 ef	29 bc	5 cd	28 ef	28 b-d	28 d	9 de	5 d	9 d	4 e
Accumulator	1 c	5 de	27 f	15 d	3 d	11 g	16 e	7 e	6 e	5 d	3 e	4 de
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z] All potato tubers were inspected visually and symptomatic tubers with *S. rolfsii* infections were considered diseased. To meet ANOVA assumptions, arcsine-square root transformation was applied prior to data analysis. Non-transformed means are presented.

[y] Column means followed by different letters are significantly different from each other based on ANOVA and Fisher's LSD test at $P \leq 0.05$.

CHAPTER 3: Susceptibility of potato tubers from different cultivars to infection by *Sclerotium rolfsii*

Abstract

Southern blight caused by *S. rolfsii* is on the increase in the mid-Atlantic region of the United State. Potato (*Solanum tuberosum* L.) cultivar responses to *S. rolfsii* infection are not well understood and to date, limited studies for *S. rolfsii* resistance in potato have been performed in the U.S. Thus, the objective of this study was to characterize potato cultivar's susceptibility/resistance through laboratory experiments examining tuber response to *S. rolfsii* infection. Ten commercially available cultivars harvested from field trials in 2017, 2018, and 2019 were used in the experiment. Tubers were aseptically inoculated with *S. rolfsii* isolate 16-S2 collected from a potato field in 2016 and incubated in a growth chamber at 30°C. All cultivars had relatively similar external mycelial growth assessed 11 days in 2017 and 8 days in 2018 and 2019 post-inoculation. None of the cultivars tested were completely resistant to tuber disease severity measured by pathogen penetration and weight of tissue diseased. However, 'Adirondack Blue', followed by 'Red Norland', and 'Dark Red Norland', had generally greater pathogen penetration (16.4 -19.9 mm) and diseased tissue (12-17.5 g) across years. Conversely, 'Atlantic' followed by 'Accumulator' had relatively lower tuber rot severity across years (3.7-12.6 g of diseased tissue). Though all cultivar tubers were affected by *S. rolfsii* infection, our results indicated that tubers of the fresh market cultivars 'Adirondack Blue', 'Red Norland', and 'Dark Red Norland' are more likely to be negatively impacted by the pathogen in post-harvest settings, while a relatively lower risk is indicated by the chipping cultivars 'Atlantic' and 'Accumulator'.

Introduction

Potato (*Solanum tuberosum* L.) is an important crop nationally and plays an important role in Virginia's agriculture industry. With more than 404 thousand hectares planted in 2018 in the U.S., potato was the most cultivated vegetable crop when ranked according to area planted (Parr et al. 2019). Additionally, potato is the most consumed vegetable in the U.S. averaging 22.4 kg per person per year (ERS-USDA 2020; Kantor and Blazejczyk 2020). In Virginia, 1,943-ha were planted in 2018, ranking Virginia 21st in the U.S. for potato production. In total for 2018, 47 thousand metric tons with a value in excess of $16.5 million were harvested in the Commonwealth (USDA-National Agricultural Statistics Service 2019a, b, 2020). Approximately 80% of the potato production for Virginia is located on the Eastern Shore of Virginia (ESVA), which constitutes the southern part of the Delmarva Peninsula (USDA-National Agricultural Statistics Service 2019c).

Sclerotium rolfsii Sacc (teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough) is a necrotrophic soilborne fungal pathogen that causes southern blight on potato and many other crops and is included among the most important pathological agents in the basidiomycete group (Schumann and D'Arcy 2010). The pathogen is mainly spread by sclerotia and mycelia. Mycelia can survive on dead organic material, volunteer plants, or alternate hosts, and sclerotia serve as an overwintering structure that survives in soil (Mullen 2001). *S. rolfsii* produces a thick mat of white mycelium 3-4 days after infection under warm (27-30 °C) and high moisture conditions (Aycock 1966; Mullen 2001). Infection occurs at any life stage of the host, commonly at the soil line with mycelia extending a few centimeters up the stem (Mullen 2001; Roberts et al. 2014). Besides stem and foliage, *S. rolfsii* can infect potato tubers (Aycock 1966; Edson and Shapovalov 1923). Lesions on tubers commonly appear on the stem and lenticels. Initial lesions are small, round, and sunken, with a brownish margin; later, they become deeply sunken and turn yellow or tan. A network of

mycelia often adheres to the tuber surface. As the disease progresses, the tissue becomes soft and disintegrates, producing odorless white, opaque, cheesy material, and sclerotia are produced in the cavities (Aycock 1966; Weber 1943).

The occurrence of southern blight in Virginia has been reported since the beginning of the 20th century. There are few reports of the disease in potato (Brittingham et al. 1963), but recently, southern blight has been identified as an emerging disease on this crop on the ESVA (Rideout, personal comm.) and the rest of the mid-Atlantic region (Pethybridge et al. 2019). In India, up to 25% loss in storage due to tuber rot caused by *S. rolfsii* has been reported (Anahosur 2001). Tactics used for control of the disease include fungicides and fumigants, but results are inconsistent. Moreover, with the phase-out of methyl bromide, and the high cost and toxicity of other available fumigants, it is necessary to find southern blight management alternatives for potato and other vegetables (Butler et al. 2012; Rosskopf et al. 2005).

Due to the lack of resistant cultivars, cultivar selection has not been widely adopted to manage *S. rolfsii* (Mullen 2001). Early century field studies showed high susceptibility of two Irish potato cultivars ('Irish Cobbler' and 'Bliss Triumph') to *S. rolfsii* (Aycock 1966; Edson and Shapovalov 1923). It has also been stated that the 'Ute Russet' cultivar, released in 1986, has some resistance to *S. rolfsii* (Holm et al. 1987). In a study that screened potato tuber susceptibility to infection and rot by *S. rolfsii*, 'Centennial' showed greater susceptibility compared to 'Kennebec' and 'White Rose' (Voss et al. 1984). Despite research showing that differences in cultivar susceptibility to *S. rolfsii* are probable, little screening for *S. rolfsii* resistance in potatoes has been performed in the U.S.

To our knowledge, there are no studies in the U.S. investigating the susceptibility of modern commercial potato cultivars to *S. rolfsii*. Therefore, specific cultivar responses to *S. rolfsii* infection are not well understood. We hypothesized that cultivars vary in susceptibility to *S. rolfsii* infection and subsequent tuber rot. Therefore, the objective of the current study was to characterize potato cultivars' susceptibility/resistance to *S. rolfsii* through laboratory experiments.

Materials and Methods

Molecular characterization of *S. rolfsii* 16-S2 isolate. For these studies, an *S. rolfsii* isolate (designated as 16-S2) was collected from a Northampton Co, VA potato field with severe southern blight in 2016. The DNA of fresh 7-day old *S. rolfsii* mycelia grown on potato dextrose agar (Difco, Becton, Dickson and Company, Sparks, MD) was extracted using a DNeasy PowerWater Kit (QIAGEN, Hilden, Germany) following manufacturer's instruction. The complete internal transcribed spacer (ITS) rDNA region of the fungus was amplified using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Pravi et al. 2014; White et al. 1990) using a thermal cycler (Bio-Rad iCycler 852 BR, Hercules, CA). The final volume of the PCR mixture was 25 µl consisting of 12.5 µl of GoTaq Green Master Mix (Promega, USA), 1 µl of forward and reverse primers (100 µM), 8.5 µl of nuclease-free water, and 2 µl of DNA template. The thermal program consisted of maintaining temperatures at 95°C for 5 min, followed by 40 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1min, followed by a final extension at 72°C for 10 min. The PCR product was run in 1.5% agarose gel containing 0.5 µg ml^{-1} ethidium bromide with a 1-kb DNA ladder and visualized on imager (Bio-Rad Chemidoc XRS Gel Imager, Bio-Rad Laboratories, Inc, Hercules, CA). Subsequently, the amplified DNA was purified using a ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA) kit per manufacturer's instructions and sequenced (Eurofins Genomics, Louisville, KY). The forward and reverse sequences were aligned

using Sequencher software (Gene Codes, Ann Arbor, MI) and the quality was reviewed in the Sequecher chromatogram. The sequence identification was compared using Nucleotide Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Potato tuber bioassay. Laboratory experiments were conducted in 2017, 2018, and 2019 at Virginia Tech's Eastern Shore Agricultural Research and Extension Center (ESAREC), in Painter, VA. Potato tubers from 10 cultivars ('Superior', 'Envol', 'Red Norland', 'Dark Red Norland', 'Russet Burbank', 'Yukon Gold', 'Adirondack Blue', 'Atlantic', 'Snowden', and 'Accumulator') harvested from field experiments conducted in each year were used in these studies. Establishment, maintenance, and harvest of field plots was described previously (see Chapter 2). Twenty-four healthy and undamaged potato tubers of each cultivar were selected randomly in 2017 and 2018 (except for 'Yukon Gold' in 2018 where only 18 tubers were collected due to insufficient yield), and 18 tubers from each cultivar in 2019. The tubers were washed in tap water and dried with a paper towel before storing in paper bags at 5°C for 1-2 weeks. Before inoculation, the tubers were pre-washed with tap water, then surface disinfested for 1 min with commercial bleach diluted in deionized water (DI) to 10% (v/v) (≈ 0.6 % NaOCl). Lastly, potato tubers were submerged in DI water for 1 min and dried with a paper towel.

Tuber inoculation and experimental design: *Sclerotium rolfsii* inoculations were conducted aseptically by wounding each tuber to a 5 mm depth using a 5-mm cork borer disinfected in ethanol (100%) and flamed. A 5 mm mycelial plug of a 7– 8 day-old culture was transferred onto each tuber at the wound site. Similarly wounded but non-inoculated tubers of each cultivar were used as negative controls. A total of 50 inoculated tubers (five tubers per cultivar) were randomized within a moistened plastic tray previously disinfected with ethanol (70%), and were subsequently

incubated at 30°C with high relative humidity (measures were taken to increase relative humidity by daily atomizing the plastic tray cover with sterilized deionized water) (Daami-Remadi et al. 2010) in an Environmental Growth Chamber (EGC TC2, Chagrin Fall, Ohio) in 2017 and a Percival growth chamber (MFG CO., Boone, IA) in 2018 and 2019. All non-inoculated tubers were grouped separately from inoculated tubers within a tray to avoid cross contamination, but they were incubated under the same conditions as the inoculated tubers. The experimental design was a randomized complete block design with four blocks in 2017 and 2018 and three blocks in 2019 with five replications (single tubers) per block. The blocks were represented by each growth chamber run in time.

Tuber assessment

External growth of *S. rolfsii* mycelia was measured at 11 days post-inoculation in 2017 and 3-days post-inoculation in 2018 and 2019. Differences in days assessing external mycelial growth was due to the poor growth observed in 2017, therefore a different growth chamber was used in 2018 and 2019. Pathogen external growth was assessed by taking two perpendicular measurements using a caliper and averaging the measurements to obtain the average diameter of the external mycelial growth.

The tuber rot severity was assessed by measuring pathogen penetration and percentage of necrotic tissue at 11 days post-inoculation in 2017 (coinciding with mycelia measurements) and 8-days post-inoculation in 2018 and 2019. The number of days for assessment was selected to allow pathogen to create tuber infection as has been shown in previous studies and based upon observed infection symptomology (Daami-Remadi et al. 2010). Pathogen penetration was estimated by making a longitudinal cut at the inoculation point. Subsequently, with a caliper, maximum lesion

width (w) and depth (d) were measured, and pathogen penetration (P) was calculated with the formula: P (mm) = (w/2 + [d-5])/2 (Daami-Remadi et al. 2010; Lapwood et al. 1984). The diseased tissue weight was estimated as the difference in potato tuber wight before (Wi) and after (Wf) cutting them and removing with spatula the diseased tissue (Diseased tuber (g) = Wi-Wf) (Daami-Remadi et al. 2010).

Statistical analysis

All wounded, non-inoculated tubers had no sign of *S. rolfsii* infection, therefore only data from inoculated tubers were subject to statistical analysis. Six tubers in 2017 and 2019 and 5 in 2018 were discarded for pathogen penetration and weight of diseased tissue due to bacterial disease development at locations different from the inoculated point. Therefore, analysis of variance (ANOVA) by year was performed on means of five replicates averaged within blocks of external mycelial growth and disease tuber severity using JMP Pro 15.0 (SAS Institute, Inc., Cary, NC). Treatments (cultivars) and blocks were entered in the model. Means were compared using the Fisher's protected Least Significant Difference test (LSD) at a 5% significance level. Pearson's (r) correlation analysis was conducted to assess the relationship between the pathogen external growth, pathogen penetration, and weight of diseased tissue.

Results

Amplification of the 16-S2 isolate DNA yielded a product between 643-bp.The obtained sequence of the 16-S2 strain revealed a 99.84% identity of the ITS region to various *S. rolfsii* isolates in GenBank (i.e. accessions KY175225.1, MF425542.1, KU128903.1); which confirmed our isolate to be *S. rolfsii* Sacc. (teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough).

White filamentous, radial-growing mycelium, adhering to the potato tuber surface was observed on all inoculated tubers, whereas no mycelium was observed on non-inoculated tubers (Figure 3.1). External *S. rolfsii* mycelial growth on inoculated tubers varied by cultivar in 2018 but not in 2017 or 2019 (Table 3.1). There was greater external mycelial growth on tubers in 2018 and 2019 than in 2017, likely due to a different growth chamber used in 2017 than the succeeding years, which could have resulted in less humid conditions in 2017. Mean diameter of external mycelial growth across cultivars was 18.5 mm, 59.4 mm, and 47.5 mm in 2017, 2018, and 2019, respectively. In 2018, mycelia grew best on 'Russet Burbank' and 'Accumulator', while 'Superior', 'Red Norland', and 'Yukon Gold' cultivars exhibited the least growth (Table 3.1).

Potato tuber disease severity was assessed by quantifying pathogen internal growth (lesion depth) and weight of diseased tissue (Figure 3.1). A soft odorless white opaque tissue, characteristic of *S. rolfsii* potato tuber infection, was observed in all inoculated tubers. No signs or symptoms of *S. rolfsii* infection were observed on non-inoculated tubers. There was a significant cultivar effect in all years for pathogen internal growth (Table 3.2). Mean pathogen penetration across cultivars was 15.2 mm, 15.9 mm, and 18.2 mm, in 2017, 2018, and 2019, respectively. All cultivars tested showed internal pathogen growth for all years, and 'Adirondack Blue', followed by 'Red Norland' and 'Dark Red Norland' had the largest lesions across years. Conversely, although the cultivars with the least pathogen penetration varied among years, 'Atlantic' was consistently grouped with the cultivars with lower pathogen penetration across the three years. The response of 'Russet Burbank' was variable among years. It was the least affected in 2019, but in 2018 had the greatest pathogen penetration, while an intermediate response was noted in 2017 (Table 3.2).

Diseased tuber tissue weight varied among cultivars in all years (Table 3.3). The mean weight diseased tuber tissue across cultivars was 10.7 g, 11.1 g, and 13.6 g in 2017, 2018, and 2019, respectively. Cultivars possessing greatest diseased tissue varied among years. However, 'Dark Red Norland', 'Red Norland' and 'Adirondack Blue' were one of the top diseased tubers in all years. 'Adirondack Blue', 'Russet Burbank', and 'Red Norland' exhibited the greatest amount of diseased tuber tissue (range 16.8 g to 17.5 g) in 2017, 2018, and 2019, respectively. In contrast, 'Atlantic' and 'Accumulator' were generally lower in diseased tuber tissue (range 3.7 g to 12.6 g) across all years, while intermediate responses were observed from the rest of the cultivars (Table 3.3).

Pearson's correlation analysis showed a statistically significant positive relationship between pathogen penetration and diseased tuber tissue in all years (Table 3.4). The analysis did not provide enough evidence to support a relationship between external mycelium growth and pathogen penetration in 2017, but it was significant positively correlated in 2018 and 2019. Though external mycelium growth had a significant negative correlation with the weight of diseased tuber tissue in 2017, there was a positive no significant relationship in 2018, and a positive significant relationship in 2019 (Table 3.4).

Discussion

The current study demonstrated that under controlled laboratory conditions, potato cultivars vary to some extent in susceptibility to tuber infection and rot by *S. rolfsii*. Southern blight is an emerging disease of potato, but to date, little screening of potato cultivars for susceptibility/resistance to *S. rolfsii* has been conducted. In our study, susceptibility of tubers to infection and post-harvest rot by *S. rolfsii* was compared by measuring external mycelial growth and tuber disease severity (pathogen penetration and weight of diseased tuber tissue). *S. rolfsii* infects the

potato tuber by disintegrating the tissue and generating an odorless white, opaque, cheesy material (Aycock 1966). While differences in external mycelial growth among cultivars were minimal, the tuber disease severity assessments showed that cultivars react differently to *S. rolfsii* under controlled environment inoculation, thereby allowing for identification of cultivars that will likely be more negatively impacted by the pathogen.

Though external mycelial growth did not consistently vary among cultivars, there were more noticeable differences in the pathogen penetration and weight of diseased tuber tissue among cultivars. None of the cultivars were completely resistant to tuber infection caused by *S. rolfsii* but the study revealed some degree of resistance of some cultivars. Similar reports have been noted in Tunisia, where the cultivars 'Tango' and 'Daysi' had the greatest and least pathogen penetration, respectively, while intermediate reaction was observed for the other nine cultivars evaluated (Daami-Remadi et al. 2012). In the present study, 'Accumulator' and 'Atlantic' had less internal pathogen progress and diseased tissue in 2017 and 2018, suggesting relatively more resistance to *S. rolfsii* among cultivars. On the other hand, 'Adirondack Blue' followed by 'Red Norland', and 'Russet Burbank', were the cultivars with greater pathogen penetration, indicating relatively greater susceptibility. Pathogen hyphae internal progress occurs inter- and intracellularly producing oxalic acid and pectinolytic and cellulolytic enzymes that degrade host tissue (Le 2011; Leoni et al. 2014; Punja 1985). Processing cultivars generally have greater starch content (Bond 2014), and researchers have reported that hyphae had difficulty penetrating host cells with high starch content (Aycock 1966). Thus, this may be one reason why 'Atlantic' and 'Accumulator', two chipping cultivars, had relatively less tuber rot severity, while the cultivars with greater susceptibility were among the fresh market cultivars. However, determining the specific physical and chemical traits constraining pathogen penetration is beyond the scope of this study.

The consistent relationship between pathogen penetration and the weight of diseased tuber tissue is not surprising since more damaged tissue is expected if the pathogen has greater internal progress in the potato tuber. However, the inconsistent relationship between *S. rolfsii* external mycelium growth and the weight of diseased tuber tissue or the pathogen penetration suggests that tuber rot severity cannot be entirely determined by just assessing the external pathogen progress. *S. rolfsii* is a ubiquitous soilborne pathogen that possesses a wide host range (Saraswathi and Reddy 2012) and can easily produce mycelial mats to scavenge for food on dead or decaying organic matter promoted by warm temperatures (27-30°C), high moisture, and low levels of CO_2 (1.2%) (Punja 1985). Thus, this was probably one of the reasons why there was no difference between cultivar tubers external mycelia growth in 2017 and 2019. Previous studies in peanut (*Arachis hypogaea* L) indicated that aboveground disease assessment (presence of *S. rolfsii* mycelium) was inconsistently correlated with yields, limiting pod disease severity assessment and likely compromising fungicide effectiveness (Rideout et al. 2002). Although the conditions in the current study were different, it can be observed by the correlation analysis that a higher susceptibility/resistance is determined by the destructive evaluation of the tuber rather than only by the pathogen external growth.

In our study, despite none of the cultivars being completely resistant to *S. rolfsii*, they varied in susceptibility to tuber rot. Our results suggested that growers need to be cautious when selecting potato cultivars to be planted in a field with significant *S. rolfsii* inoculum present because it will likely increase losses due to tuber infections. Cultivars such as 'Adirondack Blue', 'Dark Red Norland", or 'Red Norland' have a higher risk of being affected by *S. rolfsii* as revealed by the greater severity of tuber rot, thus they are likely to cause greater losses in both preharvest settings and post-harvest storage when conditions are favorable for fungal growth. On the other hand, 'Atlantic' and 'Accumulator'; which had relatively lower tuber rot severity, are less likely to

deteriorate in storage due to infection by *S. rolfsii*. The rest of cultivars with intermediate reaction to *S. rolfsii* indicated they are of less importance to manage southern blight by cultivar resistance.

Literature Cited

Anahosur, K. 2001. Integrated management of potato Sclerotium wilt caused by *Sclerotium rolfsii*. Indian Phytopath. 54:158-166.

Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*: or the status of Rolfs' fungus after 70 years. N. C. Agric. Exp. Stn. Bull. 174:202.

Bond, J. 2014. Potato utilization and marketing. Pages 50-70 in: The Potato: Botany, Production and Uses. R. Navarre and M. J. Pavek, eds. CABI, Boston, MA.

Brittingham, W. H., Bell, F. W., and Christiansen, J. K. 1963. Economic history and present status of potato production in Virginia. Virginia Joint Agric. Pub. 5:6-14.

Butler, D. M., Kokalis-Burelle, N., Muramoto, J., Shennan, C., McCollum, T. G., and Rosskopf, E. N. 2012. Impact of anaerobic soil disinfestation combined with soil solarization on plant–parasitic nematodes and introduced inoculum of soilborne plant pathogens in raised-bed vegetable production. Crop Protect. 39:33-40.

Daami-Remadi, M., Jabnoun-Khiareddine, H., Sdiri, A., and El Mahjoub, M. 2010. Effect of temperature on *Sclerotium rolfsii* mycelial growth and rot severity on potato tubers. Tunisian Plant Science and Biotechnology II. African J. Plant Sci.Biotechnol. 4:54-58.

Daami-Remadi, M., Jabnoun-Khiareddine, H., Sdiri, A., and El Mahjoub, M. 2012. Comparative reaction of potato cultivars to *Sclerotium rolfsii* assessed by stem rot and tuber decay severity. Potato Pathology. Pest Technol. 6:54-59.

Edson, H., and Shapovalov, M. 1923. Parasitism of *Sclerotium rolfsii* on Irish potatoes. Jour. Agr. Res 23:41-46.

ERS-USDA. 2020. Food availability (Per capita) data system. Economic Research Service. https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/.

Holm, D., Twomey, J., and Workman, M. 1987. Ute Russet: An oblong russet potato cultivar with resistance to leafroll net necrosis and *Sclerotium rolfsii*. Am. J. Potato Res. 64:559-562.

Kantor, L., and Blazejczyk, A. 2020. Food availability and consumption. in: United States Department of Agriculture Economic Research Service https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=58340.

Lapwood, D. H., Read, P. J., and Spokes, J. 1984. Methods for assessing the susceptibility of potato tubers of different cultivars to rotting by *Erwinia carotovora* subspecies *atroseptica* and *carotovora*. Plant Pathol. 33:13-20.

Le, N. 2011. Diversity and biological control of *Sclerotium rolfsii*, causal agent of stem rot of groundnut. Wageningen University, Wageningen, The Netherlands.

Leoni, C., ter Braak, C. J., Gilsanz, J. C., Dogliotti, S., Rossing, W. A., and van Bruggen, A. H. 2014. *Sclerotium rolfsii* dynamics in soil as affected by crop sequences. Appl. Soil Ecol. 75:95-105.

Mullen, J. 2001. Southern blight, southern stem blight, white mold. Plant Health Instr. doi: 10.1094. PHI-I-2001-0104-01.

Parr, B., Bond, J. K., and Minor, T. 2019. Vegetables and pulses outlook. VGS-362. U.S. Department of Agriculture, Economic Research Service, May 6, 2019.

Pethybridge, S. J., Sharma, S., Silva, A., Bowden, C., Murphy, S., Knight, N. L., and Hay, F. S. 2019. Southern sclerotium root rot caused by *Athelia rolfsii* on table beet in New York. Plant Health Prog. 20:4-6.

Pravi, V., Jeeva, M., and Archana, P. 2014. Rapid and sensitive detection of *Sclerotium rolfsii* associated with collar rot disease of *Amorphophallus paeoniifolius* by species-specific polymerase chain reaction assay. Mol. Biotechnol. 56:787-794.

Punja, Z. K. 1985. The biology, ecology, and control of *Sclerotium rolfsii*. Annu. Rev. Phytopathol. 23:97-127.

Rideout, S., Brenneman, T., and Stevenson, K. 2002. A comparison of disease assessment methods for southern stem rot of peanut. Peanut Sci. 29:66-71.

Roberts, P. D., French-Monar, R. D., and McCarter, S. M. 2014. Southern blight. in: Compendium of Tomato Diseases and Pest. J. B. Jones, T. A. Zitter, M. Momol and S. A. Miller, eds. American Phytopathological Society, St. Paul, MN.

Rosskopf, E. N., Chellemi, D. O., Kokalis-Burelle, N., and Church, G. T. 2005. Alternatives to methyl bromide: A Florida perspective. Plant Health Prog. 6:19.

Saraswathi, M., and Reddy, M. 2012. Phenolic acids associated with *Sclerotium rolfsii* in groundnut (*Arachis hypogaea* L.) during pathogenesis. Int. J. Plant Pathol. 3:82-88.

Schumann, G. L., and D'Arcy, C. J. 2010. Essential plant pathology. 2nd ed. APS Press, St. Paul, Minn.

USDA-National Agricultural Statistics Service. 2019a. Crop production 2018 summary. Washington, DC.

USDA-National Agricultural Statistics Service. 2019b. Crop values 2018 summary. Washington, D.C.

USDA-National Agricultural Statistics Service. 2019c. 2017 Census of agriculture-Virginia, state and county data., Washington, D.C. Vol.1. AC-17-A-46.

USDA-National Agricultural Statistics Service. 2020. Vegetables 2019 summary. Washington, DC.

Voss, R., Weinhold, A., Bowman, T., Dennett, C., and Guerard, J. 1984. Potato cultivars differ in susceptibility to *Sclerotium rolfsii* (Abstract). The Potato Assocation of America 61:541-541.

Weber, G. F. 1943. Southern blight, *Corticium rolfsii* of potato tubers. Phytopathology 33:615-617.

White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR protocols: a guide to methods and applications, vol. 18. M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White, eds. Academic Press Inc., New York.

Table 3.1 External *Sclerotium rolfsii* mycelial diameter on inoculated potato tubers of 10 cultivars[z].

	External mycelial growth (mm)[y]		
Cultivar	2017	2018	2019
Superior	16.8 a[x]	55.2 c	45.1 a
Envol	16.0 a	58.4 abc	45.7 a
Red Norland	18.2 a	57.2 bc	45.0 a
Dark Red Norland	17.8 a	55.8 c	47.5 a
Russet Burbank	20.1 a	64.3 a	49.8 a
Yukon Gold	18.4 a	59.8 c	48.1 a
Adirondack Blue	19.7 a	63.8 ab	52.7 a
Atlantic	15.8 a	56.9 bc	47.7 a
Snowden	19.9 a	57.7 abc	46.4 a
Accumulator	18.9 a	64.6 a	47.2 a
P-value	0.096	0.038	0.247

[z] Potato tubers were incubated at 30°C in a growth chamber at >60% relative humidity.
[y] Mycelium growth was assessed by taking two perpendicular measurements at 11 days in 2017- and 3-days post-inoculation in 2018 and 2019. Measurements were averaged to obtain the external growth diameter.
[x] Means values in a column followed by same letter do not significantly differ ($P = 0.05$) as determined by Fisher's Protected LSD test.

Table 3.2 *Sclerotium rolfsii* penetration on inoculated potato tubers of 10 cultivars[z].

	Pathogen penetration (mm)[y]		
Cultivar	2017	2018	2019
Superior	14. 9 de[x]	15.4 abc	17.2 cd
Envol	15.5 bcd	15.4 abc	17.3 cd
Red Norland	17.3 abc	17.0 a	20.2 a
Dark Red Norland	17.5 ab	16.4 ab	19.0 ab
Russet Burbank	15.6 bcd	17.1 a	16.3 d
Yukon Gold	15.4 cd	16.1 abc	18.3 bc
Adirondack Blue	18.7 a	17.0 a	19.9 a
Atlantic	13.0 ef	14.3 c	17.3 cd
Snowden	13.3 ef	15.3 abc	18.3 bc
Accumulator	11.4 f	14.7 bc	17.8 bc
P-value	<.0001	0.0302	0.0002

[z] Potato tubers were incubated at 30°C in a growth chamber at >60% relative humidity.
[y] Lesion depth and width was estimated by making a longitudinal cut at the inoculation point 11 days post-inoculation in 2017- and 8-days post-inoculation in 2018 and 2019.
[x] Means values in a column followed by same letter do not significantly differ ($P = 0.05$) as determined by Fisher's Protected LSD test.

Table 3.3 Diseased tuber tissue caused by *Sclerotium rolfsii* of 10 cultivars[z]

	Diseased tuber tissue (g)[y]		
Cultivar	2017	2018	2019
Superior	10.1 b	10.8 bcd	11.0 d
Envol	10.3 b	9.9 cde	12.7 cd
Red Norland	15.3 a	12.5 abc	17.5 a
Dark Red Norland	15.1 a	12.0 abc	15.7 ab
Russet Burbank	10.9 b	13.6 a	10.6 d
Yukon Gold	10.0 b	11.0 a-d	14.7 bc
Adirondack Blue	16.8 a	13.1 ab	17.3 a
Atlantic	6.3 cd	7.5 e	11.0 d
Snowden	8.2 bc	10.1 cde	12.7 cd
Accumulator	3.7 d	9.3 de	12.6 cd
P-value	<.0001	0.0019	<.0001

[z] Potato tubers were incubated at 30°C in a growth chamber at >60% relative humidity.
[y] The diseased tissue was estimated as the difference in tuber wight before and after diseased tissue was removed with spatula 11 days post-inoculation in 2017 and 8 days post-inoculation in 2018 and 2019.
[x] Means values in a column followed by same letter do not significantly differ ($P = 0.05$) as determined by Fisher's Protected LSD test.

Table 3.4 Pearson's correlations between *Sclerotium rolfsii* external growth, diseased tuber tissue, and pathogen penetration for potato tuber inoculation laboratory experiments from 2017 -2019.

Variable	2017[z]		2018[y]		2019[y]	
	Diseased tuber tissue (g)	*S. rolfsii* ext. growth (mm)	Diseased tuber tissue (g)	*S. rolfsii* ext. growth (mm)	Diseased tuber tissue (g)	*S. rolfsii* ext. growth (mm)
	----------Pearson's Correlation Coefficient----------					
	---------- (*P*-value) ----------					
S. rolfsii external growth (mm)	-0.36	-	0.26	-	0.57	-
	(0.024)[x]		(0.109)	-	(0.001)	-
Pathogen penetration (mm)	0.95	-0.24	0.86	0.47	0.96	0.60
	(<0.001)	(0.134)	(<0.001)	(0.002)	(<0.001)	(<0.001)

[z] Assessments were performed 11 days post-potato tuber inoculation.
[y] Assessments were performed 3 days post-potato tuber inoculation.
[x] Pearson's' correlation (upper number) and level of significance for correlation (lower number).

Figure 3.1 Potato tuber disease severity and external mycelium growth caused by *Sclerotium rolfsii* on laboratory inoculated tubers.

CHAPTER 4: Exploring cover crops biomass as carbon sources in anaerobic soil disinfestation to manage *Sclerotium rolfsii*

Abstract

Anaerobic soil disinfestation (ASD) is a pesticide-free alternative to managing soilborne plant pathogens and its effectiveness relies upon carbon sources. ASD growth chamber and greenhouse experiments were established to evaluate the effectiveness in inducing soil anaerobicity for six cool-season cover crop biomasses and three locally sourced organic materials compared to nonamended aerobic soils (noncovered) and anaerobic soils (covered) controls. Moreover, the carbon source's effect on reducing *Sclerotium rolfsii* viability and impact on soil fertility and tomato (*Solanum lycopersicum* L.) plant height and shoot dry biomass were assessed. Legumes, grasses, and brassicas biomass, plus poultry litter, wood biochar, mushroom compost amendments, along with sclerotial inoculum were incorporated into field-collected soil in pots, covered with polyethylene mulch, saturated with water, and incubated at 15/25°C 12/12-h cycle for three weeks. All carbon sources, except wood biochar and mushroom compost, resulted in anaerobic conditions (33-90 % iron oxide paint removal) compared to nonamended controls (0-1.2%). However, ASD treatments did not reduce sclerotial viability and likely promoted sclerotial germination and reduced colonization by *Trichoderma* sp. compared to the nonamended aerobic control. All carbon source amended soil undergoing ASD induced greater soil microbial activity (0.7-2.0 % CO_2 in vol), and poultry litter promoted the most microbial activity among treatments (2.0 % CO_2). Soil pH was generally increased by ASD treatments. Soil nitrate accumulation post-ASD varied, but mushroom compost, poultry litter, Austrian winter pea (*Pisum sativum* subsp. *arvense*), and spring oat (*Avena sativa* L.) increased nitrate concentration in at least one of the experiments compared to nonamended aerobic control. Poultry litter, followed by Austrian winter pea, resulted in greater yield and shoot dry biomass

and was associated with the effect of ASD on soil nutrient concentrations. Though more studies are needed to determine the parameters to successfully decrease *S. rolfsii* viability with ASD in plasticulture systems in the mid-Atlantic region, cool-season cover crops biomass can serve as carbon source in ASD.

Introduction

With the phase-out of methyl bromide, and the high cost, toxicity, and inconsistent results of other available fumigants, it is essential to find sustainable alternatives to manage soilborne plant pathogens (Butler et al. 2012a; Rosskopf et al. 2005). Anaerobic soil disinfestation (ASD) is a recent approach developed in the early 2000s to manage soilborne pathogens (Blok et al. 2000; Momma et al. 2006). Over the last decade, ASD research has been performed throughout the U.S., with promising results in managing soilborne fungal pathogens such as *Verticillium dahliae*, *Fusarium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pyrenochaeta lycopersici*, *Colletotrichum coccodes*, some oomycetes, nematodes, plant pathogenic bacteria, and even certain weeds (Butler et al. 2012a; Butler et al. 2012b; Shrestha et al. 2018; Strauss and Kluepfel 2015; Testen and Miller 2018).

ASD consists of applying and incorporating carbon sources to the soil, immediately covering with plastic polyethylene film, and then irrigating to field water capacity for generally three weeks (Blok et al. 2000; Butler et al. 2014b; Strauss and Kluepfel 2015). Anaerobic conditions are created when oxygen is depleted by soil microflora (Blok et al. 2000) shifting soil microbial communities to facultative and obligate anaerobic respiration, releasing organic compounds such as acetic, butyric, propionic acids, and volatile compounds that are toxic to most soilborne pathogens (Butler et al. 2012a). Though exact ASD processes have not been completely elucidated, it has been documented that ASD impacts soil chemical properties. In

aerobic soils, bacteria mineralize organic nitrogen (N) producing ammonia that can be further converted to nitrate (Baldwin and Mitchell 2000). However, under anaerobic conditions, nitrate can be reduced to ammonia or denitrified to produce nitrogen gas (Patrick Jr and Tusneem 1972; Pilot and Patrick Jr 1972). Accumulation of soil ammonium following ASD has been reported (Butler et al. 2012b), while lower accumulation of nitrate in ASD treatments compared to conventional soil fumigation has been noted (Di Gioia et al. 2017). Moreover, the soil pH increases during reducing conditions (Buresh and Patrick 1978) and mostly trends toward a neutral pH in flooded soils (De-Campos et al. 2009; Inglett et al. 2005; Ponnamperuma 1972) due to the consumption of protons in the reduction of nitrate, ferrous iron, manganese, and sulfate (Inglett et al. 2005). While some studies have noted marginal increase of soil pH, others have noted some decrease after ASD treatments (Butler et al. 2012b; Runia et al. 2014; Shrestha et al. 2018; Testen and Miller 2018). However, there are still data gaps to understand the direct effect of local carbon sources used in ASD on the chemical properties of the soil in plasticulture production systems.

Sclerotium rolfsii Sacc. (teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough*)* over the last decade has increasingly become problematic in both conventional and organic vegetable production systems in the mid-Atlantic region. This increase has been hypothesized to be associated with climate fluctuations, reduction in the use of fumigants, and increased soil organic matter through conservation practices and cropping system that favor soilborne pathogens with wide host range (Panth et al. 2020; Schonbeck and Morse 2007) . *S. rolfsii* is a ubiquitous necrotrophic soilborne pathogen capable of infecting more than 500 plant species (Mullen 2001). The pathogen is more prevalent in tropical and subtropical regions, and domestically it is more problematic in the southeastern US (Aycock 1966). However, the fungus is capable of surviving

and damaging crops in northern latitudes and increases have been noted in the mid-Atlantic region (Xu et al. 2008). Mycelia and sclerotia are the two means of pathogen survival. While mycelium is a short-living stage, sclerotia, which are merely hyphal strands coalesced forming a small round structure, can persist in the soil for several years (Mullen 2001; Punja 1985).

Though ASD treatments have shown a reduction of sclerotial viability (Butler et al. 2012b; Shrestha et al. 2018), results have been inconsistent. Within the U.S., investigations to manage *S. rolfsii* with ASD have been conducted in greenhouses using carbon sources such as cowpea (*Vigna unguiculata* L. Walp.), sunn hemp (*Crotalaria juncea* L.), pearl millet (*Pennisetum glaucum* L.), and sorghum-sudangrass (*Sorghum bicolor* (L.) Moench x *S. bicolor* var. *sudanese*) (Butler et al. 2012b; Strauss and Kluepfel 2015). For example, sorghum-sudangrass as the carbon source provided less suppression of sclerotia in the absence of poultry litter but produced greater inhibition when poultry litter was incorporated. Cowpea, sunn hemp, and cowpea mixed with pearl millet had more consistent sclerotial suppression regardless of the application of poultry litter (Butler et al. 2012b). Improved sclerotial suppression has been observed by using wheat bran and dry molasses (Butler et al. 2012b; Shrestha et al. 2018) in growth chamber and field settings. For example, in growth chamber studies, sclerotial germination was 0.6 to 8.5% compared to 21-36% in nonamended controls. Additionally, significant (>87%) *Trichoderma* colonization of sclerotia was observed (Shrestha et al. 2018). These studies have used the technique of burying the inoculum (sclerotia or mycelium) in packages; however, it is not known what results would be obtained when the inoculum is mixed with the soil, which better mimics a soil naturally infested with *S. rolfsii*. Additionally, the use and feasibility of specific cover crops and prevalent soil types vary among regions. Therefore, local studies are needed in Virginia and the mid-Atlantic region to determine ASD effectiveness

under local conditions and to explore the use of organic waste/cover crop residue as an option for vegetable growers for managing soilborne pathogens.

While carbon sources such as molasses or wheat bran show positive results in controlling soilborne pathogens, one disadvantage is a large amount of carbon source dry matter needed. Studies have used 14.5 Mg ha^{-1} of molasses (Butler et al. 2012b) and 20.2 Mg ha^{-1} of wheat bran (Testen and Miller 2018), which require intense labor to apply and a large source of these products for larger commercial operations. Studies utilizing cover crops, such as sunn hemp, sorghum-sudangrass, or pearl millet, the amount of dry matter required (mainly the seed) (34 kg DW ha^{-1}) is much less than with molasses and wheat bran (Strauss and Kluepfel 2015).

We hypothesized that the use of cool-season cover crop biomass, woo biochar, pelletized poultry litter, and spent mushroom compost as a carbon source will induce anaerobic conditions in ASD treatments, thereby decreasing the survival of *S. rolfsii* in the soil and subsequently improving the health of tomato plants. These potential carbon sources are prevalent as agricultural waste or utilized as cover crops in the mid-Atlantic region. The overall goal of the current study was to demonstrate that ASD can be a chemical-independent option to manage soilborne diseases for mid-Atlantic vegetable production. The specific objectives were to 1) compare the effectiveness of induction of anaerobic conditions among cover crops and organic waste as carbon sources in ASD treatments, 2) evaluate the effectiveness of cover crops and organic waste in reducing *S. rolfsii* sclerotial viability through ASD treatments, and 3) assess post-ASD treatment effects on soil fertility and tomato plants.

Materials and Methods

Growth chamber and greenhouse pot experiments were performed at Virginia Tech's Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, Virginia. A

Bojac sandy loam soil was collected on August 2019 (experiment 1) and May 2020 (experiment 2) from a field at ESAREC up to 15-cm depth and sieved through 5-mm aluminum mesh and stored in plastic containers at 4°C until used. Tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L) were grown in the two preceding seasons with no fumigants used in the last five years. The field had no history of *S. rolfsii* presence and inoculum density assessment indicated <0.01 sclerotia per gram of soil. Sclerotial density naturally present in the soil was estimated by following the methanol procedure (Njoroge et al. 2008; Rodriguez-Kabana et al. 1980). Three hundred grams of soil were air-dried overnight at room temperatures in aluminum foil pan 23 cm x 33 cm. Subsequently, the soil was evenly spread in a layer no thicker than 2 mm and moistened with 75 ml of methanol 1.33% (v/v) and placed in clear polyethylene bags and incubated at 30°C for 3 days. Number of sclerotia germinating was recorded. No assessments of other soilborne pathogens were performed. The soil pH and nitrate content prior to ASD initiation were 5.29 and 65 ppm and 5.37 and 9.4 ppm, respectively for experiments 1 and 2. The experiments were arranged in a completely randomized design with four replications. The first experiment was initiated on 9 July 2020 (experiment 1) and the second experiment on 24 September 2020 (experiment 2). Treatments consisted of nine carbon sources covered with polyethylene mulch and two controls (nonamended noncovered [aerobic] and nonamended covered [anaerobic]) (Table 4.1).

Carbon sources and pathogen inoculum preparation

Cool-season cover crops (Table 4.1) were hand-seeded in plastic pots measuring 11 cm x 11 cm x 9.5 cm (width, length, and depth, respectively) laid on 15-cell sheets using peat soil. Subsequently, the trays were placed in a growth chamber at 22°C set on a 12/12 h day/night light cycle. After 45 days, aboveground cover crop biomass was collected and cut into 5-cm pieces

and stored at -18°C. A subsample of the carbon sources was air-dried to determine moisture content and cover crop biomass was milled and passed through a 20-mesh screen prior total C and total N analysis by combustion (Bremner 1996) using an elemental analyzer (Vario El Cube, Germany).

Sclerotium rolfsii sclerotia produced by an isolate collected from a potato field in Northampton Co., VA in 2016, was selected to be used in ASD experiments. Sclerotia were produced as follows: 500 cm^3 of millet seed were placed in aluminum foil pans and mixed with 600 ml of deionized water and subsequently covered with aluminum foil and autoclaved for 45 min. Millet was then stirred and autoclaved for an additional 45 min. A 7 day-old culture of *S. rolfsii* grown on potato dextrose agar (PDA) (Difco, Becton, Dickson and Company, Sparks, MD) was placed face down on the autoclaved millet, double wrapped with aluminum foil, and incubated in a growth chamber for 8 to 10 days at 30°C (Vagher et al. 2014). Infested millet seed was ground and dried aseptically at room temperature and sclerotia were collected after 16 to 18 days and stored at room temperature in sterilized glass vials until used. Inoculum packets containing 10 sclerotia each were prepared in sterilized 255-micron nylon 5 x 10 cm mesh bags (Component Supply Company, Sparta, TN). An additional 20 sclerotia to be mixed with the soil were collected in sterilized glass vials and stored at room temperature until used.

Experimental setup

Carbon sources listed in Table 4.1 and 20 sclerotia were thoroughly mixed with 1,200 g of soil in 0.9 L plastic pots. Carbon source rates were determined based upon previous studies on cover crop rotation at the ESAREC (Wolters 2020). A rubber stopper was inserted in the top of the container to retrieve air samples. A 10-cm indicator of reduction in soil (IRIS) tube was buried in each pot. IRIS tubes consist of a PVC tube coated with Fe oxide paint, and when

exposed to anaerobic conditions, soil microbes use the Fe oxide as an alternative electron acceptor in the respiratory process thereby removing paint from the tubes, allowing for assessment of soil reducing conditions by the quantification of paint removal (Rabenhorst 2008). One inoculum packet per pot and two thermometers (HOBO Pendant data loggers, Onset Computer Corp., Bourne, MA) per treatment were buried at 7-cm depth. Temperature readings were recorded every hour. Pots were saturated with tap water, allowed to drain, and immediately covered (except the nonamended aerobic control) with white 31.8-µm white-faced polyethylene mulch. Covered pots were sealed with rubber bands and electric tape and all pots were incubated in a growth chamber for three weeks in a 12/12-h day/night cycle at 25/15°C. Temperatures were set to mimic average spring temperatures in the mid-Atlantic region according to historical weather data from the ESAREC (Shennan et al. 2014; Shrestha et al. 2018).

Soil surface carbon dioxide (CO_2) efflux, as an indirect indication of soil microbial activity, was measured at 5-day intervals by passing a 100-ml air sample through Dräger tubes (Dräger Safety, Lubeck, Germany). The air sample was retrieved using a 150-ml syringe inserted through the rubber stopper. The length of the coloration measured in the calibrated scale of the Dräger tube was recorded and indicated the percentage of CO_2 in volume of air sampled.

Post ASD assessment

To assess reducing conditions in soils, the IRIS tubes were retrieved from soil and washed at ASD treatment termination. The percentage of paint removed was quantified by wrapping the tube with clear acetate and hand-painting the reduced areas (Coffin 2012). Subsequently, the acetate was scanned and imported to ImageJ 1.53a software (National Institutes of Health, USA) to estimate the percentage of reduced area.

Soil samples were collected immediately after ASD termination and air-dried at room temperature for seven days. Soil pH was determined in a 1:1 volume dry soil/water mixture using a pH meter (basic pH meter 840087, Sper Scientific Ltd., Scottsdale, AZ). In the same sample, a solution filtered with Whatman No. 1 filter paper was collected to measure colorimetrically soil nitrate (NO_3^-) content using Nitrate-Nitrite test strips (Hach, Loveland, CO) (Soil Quality Institute 2001).

Assessment of sclerotia

After ASD termination, packets with sclerotia were removed, washed with tap water, dried for 24 h at room temperature, and stored at 4°C until examined. Sclerotia were carefully removed from packets, washed with tap water to remove any adhering soil, surface-disinfested by submersion in 10% commercial bleach for 1 min. followed by 1 min in 10% ethanol, and rinsed with sterilized deionized water. Sclerotia were plated onto half strength potato dextrose agar (PDA) (Difco, Becton, Dickson and Company, Sparks, MD) amended with rifampicin at 10 mg L^{-1} (Alfa Aesar by Thermo Fisher Scientific, Ward Hill, MA) and incubated at room temperature for 24 to 27 days. The percentage of sclerotia germinating and/or colonized by other fungi was determined by the number of sclerotia with mycelial growth divided by the number of sclerotia recovered. The identification of sclerotial germination and colonization by other fungi was determined based on morphological characteristics observed under a dissecting and compound microscope.

Tomato crop bioassay

To assess the survival of *S. rolfsii* and the impact of ASD on crop performance, one 25-day old dwarf tomato plant (*Solanum lycopersicum* L. cv. Micro-Tom) grown in peat soil in a polystyrene 34 cm x 66 cm seedling tray was transplanted into each pot (10 August 2020,

experiment 1; 23 October 2020, experiment 2) 7 days post-ASD termination and moved to a greenhouse. No fertilizer was applied throughout the growing period and hourly temperature and relative humidity readings were recorded by installing two HOBO Pro v2 unit data loggers (Onset Computer Corp., Bourne, MA). After 46 days (experiment 1) and 53 days (experiment 2) severity of lesions characteristic of *S. rolfsii* infection, plant height, shoot weight, fruit fresh weight, and signs of phytotoxicity were recorded. On tomato, *S. rolfsii* stem lesions start as small irregular light brown water-soaked lesion at the base of the plant, and as the pathogen progresses, the cortex is decayed a few centimeters up and below the soil line with often white mycelia present (Aycock 1966; Xie and Vallad 2010). Lesion severity was visually assessed using the following scale: 1- no stem lesion, 2-small stem lesion (<25% of stem circumference), 3-moderate stem lesion (26-50% of the stem circumference), 4-large stem lesion (>51% of stem circumference), and 5-dead plant (stem completely girdled) (Fery and Dukes Sr 2002). Chlorophyll content of the newly developed leaves on each plant was estimated weekly using a chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc, Japan). Above-ground plant biomass was dried for 36 days at 65°C. Samples were ground and passed through a 20 mesh sieve prior to total C and total N analysis by combustion (Bremner 1996) using an elemental analyzer (Vario El Cube, Germany).

Statistical analysis

A combined analysis of variance (ANOVA) which included experiments, carbon sources, and experiments-carbon sources interaction as factors, was performed using JMP Pro 15.0 (SAS Institute, Inc., Cary, NC). When interaction was significant, each experiment was analyzed separately using one-way ANOVA, and means were compared using the Fisher's protected Least Significant Difference test (LSD) at a 5% significance level. Arcsine-square root transformation

and square root transformation were applied to the percentage of IRIS tube paint loss and tomato fruit fresh weight, respectively, prior to data analysis to satisfy normality assumptions. Carbon dioxide and estimated chlorophyll content were analyzed using a mixed-model ANOVA, with carbon source as a between-subject (plants) factor and time as a within-subject factor. Plants were treated as a random effect. When interaction was significant, the effect of treatments was analyzed at every time point, and mean comparisons were conducted using the Fisher's protected Least Significant Difference test (LSD) at a 5% significance level. A Pearson (r) correlation analysis was conducted to determine the relationship between the percent of sclerotial germination and other variables measured (stem-lesion index, IRIS paint loss, CO2, and pH); as well as between stem-lesion index and IRIS paint loss; and between tomato biomass dry weight and nitrate, SPAD chlorophyll values, and IRIS paint loss.

Results

Effect of ASD carbon sources on soil pH and soil nitrate

The overall mean soil temperatures during ASD treatments were generally higher in experiment 2 than in experiment 1. Mean day temperature among treatments ranged from 24.2 to 24.9°C and 24.4 to 26.0°C in experiment 1 and experiment 2, respectively, while mean night temperatures ranged from 19.1 to 20.0°C and 20.0 to 21.4°C in experiment 1 and experiment 2, respectively.

There was a significant effect of carbon sources on soil pH post-ASD treatments in experiment 1 and experiment 2 (Table 4.2). All carbon amendments resulted in greater pH compared to control treatments (nonamended aerobic and anaerobic) in experiment 1, with poultry litter having the highest soil pH (5.93). However, in experiment 2, all anaerobic treatments (including the nonamended anaerobic control), except spring oat and mushroom

compost had greater pH compared to the aerobic control. The soil amended with spring oat had the lowest pH (5.37) while wood biochar had the highest soil pH (6.02) (Table 4.2).

There was a significant effect of carbon amendment on post-ASD nitrate-N concentration, and experiment 1 (70 ppm) had overall greater nitrate concentrations than experiment 2 (14 ppm) (Table 4.2). Mushroom compost followed by the nonamended aerobic control had the greatest nitrate concentrations in experiment 1, with wheat, rapeseed, and hairy vetch amendments having the lowest nitrate. Conversely, in experiment 2, poultry litter had the greatest nitrate concentration followed by Austrian winter pea, while the anaerobic control and wood biochar amendment resulted in the least nitrate.

Effect of carbon amendment on anaerobic conditions and sclerotial germination

There was no time effect or treatment-time interaction in soil CO_2 efflux measured at 5, 10, 15, and 21 days after ASD initiation; therefore, the percent of CO_2 was averaged across time. There was an effect of carbon sources on soil respiration (% CO_2 in vol.) in both experiments (Table 4.2). Concentrations of CO_2 among carbon amendments ranged from 0.6 to 1.8% and 0.7 to 2.0% in experiments 1 and 2, respectively, but CO_2 averaged across amended treatments was slightly greater in experiment 1 (1.1%) than experiment 2 (0.9%) (P=0.004). All carbon sources resulted in greater CO_2 concentration than the control treatments (nonamended aerobic and nonamended anaerobic) except wood biochar in experiment 1. Poultry litter had the greatest CO_2 concentration in both experiments (Table 4.2).

Carbon sources affected soil reducing conditions based on the percentage of iron oxide paint loss of the IRIS tubes (soil reduction indicator). All carbon sources except wood biochar and mushroom compost produced significantly greater reducing conditions compared to the nonamended control treatments in both experiments (Table 4.2). Wood biochar and mushroom

compost did not induce anaerobic conditions and were similar to control treatments. Austrian winter pea and poultry litter produced the greatest reducing conditions in experiments 1 and 2, respectively (Table 4.2). Iron oxide paint loss had a positive relationship with concentration of CO_2 averaged across time in experiment 1 (r=0.49, $P<0.001$) and experiment 2 (r=0.59, $P<0.001$), and paint loss was positively correlated with soil pH in experiment 1 (r=0.79, $P<0.001$) but negatively correlated in experiment 2 (r=-0.36, $P=0.017$).

Germinated sclerotia percentage ranged from 73-100% in experiment 1 with no significant differences among treatments detected. Sclerotial germination among carbon sources ranged from 80 to 95% in experiment 2. Though there was a significant effect of treatments in experiment 2, the aerobic control resulted in significantly less sclerotial germination (greater suppression) than any of the anaerobic treatments, while all carbon sources except hairy vetch were not significantly different from the nonamended anaerobic control (Table 4.2). Additionally, there was not a significant relationship between the percentage of sclerotial germination with iron oxide paint loss, CO_2, or soil pH in either experiment. Moreover, sclerotial colonization by other fungi, except *Trichoderma*, was minimal (data not presented). Sclerotial colonization by *Trichoderma* ranged from 0 to 17% in experiment 1, with no significant differences among treatments ($P=0.319$), and only the nonamended aerobic control showed significantly greater *Trichoderma* colonization (28%) in experiment 2 ($P<0.001$). Moreover, there was a negative correlation between sclerotial germination and *Trichoderma* colonization across experiments (r=-0.66, $P<0.001$).

Effect of ASD carbon sources on tomato plants

The mean daily air greenhouse temperature was generally higher across the growing period in experiment 1 than experiment 2. However, both temperature and relative humidity

were more variable across experiment 2 compared to experiment 1 (Figure 4.1). The effect of ASD on stem lesions caused by *S. rolfsii* was not significant in either experiment (Table 4.3). Furthermore, there was no evidence of a relationship between stem lesion index and reducing conditions (IRIS paint loss) across experiments (r=0.003, *P*=0.975).

Signs of phytotoxicity were not observed with any of the treatments. The effect of carbon sources on the tomato leaf chlorophyll content estimated by SPAD values changed over time in experiment 2 *(P*<0.001*)* but not in experiment 1 (*P*=0.233). SPAD values averaged across treatments differed over time in experiment 1 (*P*<0.001). While no significant differences were observed between 7 and 14 days after planting (46.5 and 45.6 SPAD value, respectively), estimated chlorophyll content progressively decreased at 21 and 28 days (42.8 and 34.2 SPAD value, respectively). When averaged across time, nonamended aerobic control and mushroom compost amendment resulted in significantly greater SPAD values in experiment 1 while the nonamended anaerobic control had the lower SPAD values (Table 4.4). In experiment 2, due to a significant treatment-time interaction, the effect of carbon sources was analyzed at each time point (Table 4.4). The mean SPAD values differed between treatments at 14, 21, and 28 days, but not at 7 days after planting (Table 4.4). From 14 through 28 days, legumes, spring oat, and poultry litter had statistically similar SPAD values compared to the aerobic control and had the greatest SPAD value among treatments. Austrian winter pea and poultry litter had the greatest SPAD values at 21 and 28 days after planting, while nonamended aerobic control had the greatest SPAD values at 14 days. Estimated chlorophyll content generally remained constant from 14 to 28 days for treatments, except for the anaerobic control, rapeseed, wood biochar, and mushroom compost, which had a SPAD value decrease (Table 4.4).

Tomato plant height differed among treatments in experiment 1 only (Table 4.3). Austrian winter pea, poultry litter, mushroom compost, and aerobic control treatments produced the greatest plant heights. Additionally, there was an effect of carbon sources on shoot dry biomass in both experiments (Table 4.3). Moreover, there was a significant positive correlation between IRIS paint loss and plant height across experiments (r=0.30, P=0.005). Also, aerobic control and mushroom compost treatments produced greater dry biomass in experiment 1, while poultry litter followed by Austrian winter pea had greater dry biomass in experiment 2. Wood biochar produced consistently less dry biomass compared to most treatments in both experiments and was similar to wheat in experiment 1 and the anaerobic control in experiment 2. Tomato shoot dry biomass was positively correlated with soil nitrate in experiment 1 (r=0.50, P<0.001) and experiment 2 (r=0.69, P<0.001), and positively correlated to SPAD values in experiment 1 (r=0.71, P<0.001) and experiment 2 (r=0.69, P<0.001). IRIS tubes paint loss across experiments, an indicator of soil reducing conditions, was weakly positively correlated with shoot dry biomass across experiments (r=0.24, P<0.026). Furthermore, carbon sources significantly influenced total fruit weight (Table 4.3). Averaged across experiments, soil amended with poultry litter produced the greatest yield, while wheat and wood biochar soil amendment resulted in the least fruit yield (Table 4.3).

A model to predict tomato shoot dry biomass based on IRIS paint loss, soil pH, soil nitrate-N, and stem-lesion index was constructed using multiple linear regression analysis (Table 4.5). Each of the predictor variables had a significant partial effect on the full model and explained 54% of the variability in final shoot dry weight biomass (P<0.001). A similar analysis predicting the fruit fresh weight was constructed (Table 4.5). All the predictors, except soil pH,

had a significant contribution to the model and they accounted for 41% of the variability in fruit fresh weight ($P<0.001$).

Discussion

There has been increased interest in anaerobic soil disinfestation in the last decade as a pesticide-free alternative for managing soilborne plant pathogens; however, studies on ASD across the mid-Atlantic region are limited. Optimized ASD utilizes large amounts of carbon sources that are unfeasible or unavailable for larger operations in the mid-Atlantic region to import. Thus, we investigated the effectiveness of ASD using local sources of cool-season cover crops' fresh biomass and other readily available organic materials that farmers were accustomed to growing or were easily obtainable. We also aimed to use practical rates for sources to ensure that test data was directly relatable to current farming operations. In addition, we assessed the impact of ASD treatments using local carbon sources on *S. rolfsii*, a major pathogen of tomato. Anaerobic conditions measured by IRIS tube paint loss percentage were consistently achieved by most of the carbon amendments. However, none of the amended soils decreased sclerotial germination compared to nonamended controls or reduced *S. rolfsii* lesion development in tomato plants post-ASD treatment in the current study. The greatest anaerobic conditions were achieved in soils amended with Austrian winter pea or poultry litter. Although no significant inhibition of *S. rolfsii* was observed, the overall net impact of ASD on the tomato crop was positive based on soil pH and soil nitrate accumulation by some amendments; which positively impacted crop biomass and yield.

All carbon sources evaluated in the current study, except wood biochar and mushroom compost, effectively induced anaerobic conditions utilizing procedures to achieve ASD at our prescribed application rates. Anaerobic conditions were quantified by the percentage of iron

oxide paint loss on IRIS tubes; which is an alternative method that was approved by the National Technical Committee for Hydric Soil to determine reducing soil conditions, and > 25% paint removal is a positive indicator of reducing conditions (Rabenhorst 2008). IRIS devices consist of polyvinyl chloride (PVC) tubes coated with iron oxide paint that, when inserted into saturated soils, where the microbial oxidized organic matter depleting soil ambient oxygen, resulting in reducing conditions where Fe (III) oxide painted in the tubes becomes the electron acceptor by anaerobic microbial respiration which became soluble and removed from the IRIS tube. In this study, the anaerobicity levels obtained by all the cover crops (>33% iron oxide paint loss), suggest that cover crops could be grown in-situ and incorporated into the soil prior to raised bed formation early in the spring for vegetable plasticulture production in the mid-Atlantic region. These results agree with studies in Tennessee, where cool and warm season cover crops had greater accumulation of anaerobic conditions compared to nonamended controls (Butler et al. 2014a; Butler et al. 2012b). In contrast, when using locally available organic waste, anaerobic conditions were only achieved with poultry litter, and the non-reducing conditions in the presence of wood biochar and mushroom compost suggest that these are not recommended carbon sources for ASD settings under our study conditions. The non-anaerobic conditions that resulted from the soil amended with wood biochar are not surprising considering the high C:N ratio (280:1). The optimum C:N ratio for decomposing organic residues applied to the soil is 25:1 - 30:1 (Pace et al. 1995), and greater C:N ratio and high cellulose and lignin materials will considerably slow the decomposition by soil microbes due to the low N availability and low biological degradation (Brady and Weil 2017). Moreover, anaerobic conditions were not achieved in soils amended with mushroom compost, despite the lower C:N ratio, likely attributed to the slow breakdown of the lignocellulosic materials (straw, hay, corn cobs, and corn fodder)

from which mushroom compost is fabricated. Therefore, the two foregoing carbon sources were not suitable as microbial food sources to induce anaerobic conditions in ASD under our study settings and applicated rates.

Despite the non-induction of anaerobic conditions by mushroom compost and wood biochar, all carbon sources increased soil microbial activity as indicated by the CO_2 efflux throughout the ASD incubation. Higher CO_2 efflux indicates higher microbial activity (Brady and Weil 2017; Sanabria-Velazquez et al. 2019). Thus, in our study, the greater CO_2 efflux by all carbon sources compared to the nonamended controls suggested that ASD treatments examined in this study enhances soil microbial activity. Furthermore, poultry litter produced the greatest CO_2 efflux, indicating that this carbon source promoted the greatest microbial activity among all treatments. An increase of CO_2 efflux has been previously reported during ASD treatments (Runia et al. 2014; Sanabria-Velazquez et al. 2019). However, the consistent levels of CO_2 detected over time suggest that some alcoholic fermentation pathway could have occurred. Shifting soil microbial respiration to facultative and obligate anaerobic pathways has been found to release organic compounds such as acetic, butyric, propionic acids, and volatile compounds that are harmful to most soilborne pathogens (Butler et al. 2012a)

Though anaerobic conditions were achieved by most carbon sources, ASD treatments did not affect sclerotial germination. The anaerobic conditions induced by the carbon sources were not detrimental for sclerotia and no relationship between reducing conditions and sclerotial germination was observed. Our results differed from previous reports that ASD reduces *S. rolfsii* sclerotial germination (Shrestha et al. 2018). However, studies by Shrestha et al. (2018) used dry molasses and wheat bran, while more variable effects on sclerotial germination have been observed when using cover crop carbon sources (Butler et al. 2012b). Therefore, the dissimilarity

of our results might reflect the difference in the biochemical properties of the organic residues used. The lower C:N ratio and higher moisture of the biomass might not have produced enough detrimental compounds to produce a reduction in sclerotial germination, and instead, the application of cover crops could have enhanced the survival as indicated by the greater suppression of sclerotial germination in the nonamended aerobic control in experiment 2. Moreover, the lower germination of sclerotia in the aerobic control in experiment 2, and the overall negative association between *Trichoderma* colonization and sclerotial germination indicates that the carbon sources might have hindered the survival of beneficial fungi such as *Trichoderma* species. Our results did not support previous findings where ASD promotes sclerotial colonization by *Trichoderma* sp. and other fungi (Shrestha et al. 2018). Additionally, in the previous study by Shrestha et al. (2018) daily temperatures exceeded 28°C, while in our studies average temperatures were 20°C/25°C during the night/day, which were not deleterious for *S. rolfsii* sclerotia. Therefore, a synergy between anaerobic conditions and temperature is likely needed for ASD to successfully suppress *S. rolfsii*. Previous studies noted that anaerobic conditions at low temperature (15°C) were not detrimental for *V. dahliae*, but it did reduce viability in pots incubated at 25°C (Shennan et al. 2018). *S. rolfsii* sclerotia can survive extreme weather conditions and previous studies noted that consistent reduction of the pathogen was achieved by applying 38°C/27°C day/night regimes in soils amended with poaceous (e.g., wheat [*Triticum aestivum* cv. Yolo] and barley [*Hordeum vulgare* cv. UC337]) in bioreactor experiments (Stapleton et al. 2010). This could limit the successful application of ASD to suppress soilborne pathogens early in the spring in the mid-Atlantic, where mean temperatures did not exceed 26°C before mid-May during the last five years (ESAREC weather database).

Thus, further studies are needed to determine treatment conditions that can inhibit *S. rolfsii* by using fresh cover crop biomass in mid-Atlantic crop production systems.

It has been previously proposed that assessment of *S. rolfsii* viability in soils using inoculum packets might differ from results obtained by mixing sclerotia with carbon sources (Butler et al. 2012b). In this study, the effect of ASD on disease severity (*S. rolfsii* stem-lesion index) of tomato plants post-ASD treatment was highly variable. Although most carbon source treatments resulted in diseased tomato plants, there was a high amount of variability in lesion severity within treatments. Additionally, as for sclerotial germination, there was not a significant relationship between reducing conditions and disease severity, and results suggested that disease incidence could be greater in amended soils compared to nonamended soils. This could be attributed to the enhancement of sclerotial germination by the organic compounds released by decomposition of the organic matter (Flores-Moctezuma et al. 2006; Punja 1985).

Although the soil pH was affected by carbon sources, none of the treatments changed the soil pH to a crop detrimental level (5.37 – 6.02). In our study, soil pH was generally raised by the ASD treatments and this is logical considering that anaerobic conditions tend toward neutral pH in flooded soils due to the consumption of protons in the reduction of nitrate, ferrous iron, manganese, and sulfate (Inglett et al. 2005). Previous studies have reported mixed effects of ASD on soil pH. While drastic changes have not been observed, some studies have reported slight reductions in pH (Butler et al. 2012b; Runia et al. 2014; Shrestha et al. 2018), and others have noted slight increases compared to the control (Butler et al. 2014a; Testen and Miller 2018). Additionally, the higher pH that resulted from amending with poultry litter and wood biochar is not surprising, since these materials are recommended as amendments to neutralize

soil acidity (Hue 1992; Mullins et al. 2019), and similar effects with poultry litter have been previously reported (Butler et al. 2012a; Butler et al. 2012b).

The soil nitrate accumulation post-ASD treatment varied among carbon sources. The numerical values of the nitrate content of the nonamended aerobic control were similar to the nitrate content before the ASD treatment; therefore, they can be used as a reference for the change of nitrate content post-ASD treatments. Thus, the consistent decrease of N in the anaerobic control and wood biochar amended soil compared to the aerobic control indicated that soil microbes assimilated soil N to supply the demand of N, thus decreasing soil mineral nitrogen driven by the lack of carbon source in the control or high C:N ratio in the wood biochar (Brady and Weil 2017). Furthermore, the soil nitrate-N accumulation generally decreased by carbon sources compared to the nonamended aerobic control except mushroom compost, poultry litter, Austrian winter pea, and spring oat treatments, which increased nitrate accumulation in at least one of the experiments. Soil microbes decompose organic matter, mineralizing organic-N and thereby releasing ammonia. Although most of the carbon sources had lower C:N ratios which are readily decomposed releasing nitrogen to the soil (Brady and Weil 2017), the lower nitrate present post-ASD in our experiments indicates that not enough nitrification occurred to accumulate nitrate, and likely most N was allocated as ammonia-N or ammonium-N, or denitrification occurred. Significant losses of N through reduction of nitrate have been observed in wet soils compared to dry soils (Pilot and Patrick Jr 1972). The increase of ammonia and ammonium during ASD has been previously reported (Butler et al. 2014b; Butler et al. 2012b; Runia et al. 2014). However, further studies to measure ammonia efflux using the current carbon sources are recommended to confirm this. While the lower nitrate accumulation post-ASD might not contribute to decreased fertilizer inputs needed for a subsequent crop, it represents an

advantage for ecologically sensitive areas (such as within the Chesapeake Bay watershed) where nitrate is prone to run-off. However, some carbon sources such as mushroom compost, poultry litter, or Austrian winter pea indicated that some accumulation of nitrate can also occur.

No signs of phytotoxicity were observed in tomato plants post-ASD treatments, suggesting that ASD using cover crops biomass and local carbon sources does not produce compounds that are detrimental to plant growth. A similar observation has been noted in previous studies (Butler et al. 2012b). The difference in crop performance detected in our study is more linked to the effect of the carbon sources on the concentration of soil nutrients. For example, the dry crop biomass had a consistently positive relationship with the soil nitrate content at ASD termination. For this study, the final effect on the tomato plant, however, might be masked by disease caused by S. *rolfsii*, and this is supported by the significant negative contribution of stem-lesion index when constructing a model to predict dry biomass and tomato yield. Additionally, wood biochar is expected to make less of a contribution to soil nutrients compared to other carbon sources due to its high C:N ratio, which promotes soil mineral nitrogen assimilation and results in less enhancement of crop performance. This was deduced by the lower dry biomass and fruit weight that resulted from the wood biochar among carbon amendments, while poultry litter and legume cover crop amendments resulted in greater yield.

Overall, this study demonstrated that anaerobic conditions can be achieved by using cover crop biomass and carbon sources local to the mid-Atlantic region in ASD settings; however, the non-inhibition of sclerotial germination suggested that control of southern blight will not be achieved with the carbon sources evaluated at the temperatures maintained in this study. Although variable, some amendments such as legumes, poultry litter, and mushroom compost can provide some nutrients for crop growth. The absence of negative impacts on crop

performance in the current study suggests that ASD is unlikely to be detrimental for crops when using cover crop biomass, mushroom compost, or poultry litter. Additional studies with higher temperatures and longer ASD treatment times are needed to optimize conditions for possible control of *S. rolfsii* by ASD.

Literature Cited

Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*: or the status of Rolfs' fungus after 70 years. N. C. Agric. Exp. Stn. Bull. 174:202.

Baldwin, D. S., and Mitchell, A. 2000. The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river–floodplain systems: a synthesis. Regul. Rivers: Res. Manage. 16:457-467.

Blok, W. J., Lamers, J. G., Termorshuizen, A. J., and Bollen, G. J. 2000. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. Phytopathology 90:253-259.

Brady, N. C., and Weil, R. R. 2017. The nature and properties of soils. 15 ed. Pearson Education Ltd. London, EN.

Bremner, J. M. 1996. Nitrogen-total. Methods of soil analysis: Part 3 Chemical methods 5:1085-1121.

Buresh, R., and Patrick, W. 1978. Nitrate reduction to ammonium in anaerobic Soil. Soil Sci. Soc. Am. J. 42:913-918.

Butler, D., Ownley, B., Dee, M., Eichler Inwood, S., McCarty, D., Shrestha, U., Kokalis-Burelle, N., and Rosskopf, E. 2014a. Low carbon amendment rates during anaerobic soil disinfestation (ASD) at moderate soil temperatures do not decrease viability of

Sclerotinia sclerotiorum sclerotia or Fusarium root rot of common bean. Pages 203-208 in: VIII International Symposium on Chemical and Non-Chemical Soil and Substrate Disinfestation 1044.

Butler, D. M., Kokalis-Burelle, N., Muramoto, J., Shennan, C., McCollum, T. G., and Rosskopf, E. N. 2012a. Impact of anaerobic soil disinfestation combined with soil solarization on plant–parasitic nematodes and introduced inoculum of soilborne plant pathogens in raised-bed vegetable production. Crop Protect. 39:33-40.

Butler, D. M., Rosskopf, E. N., Kokalis-Burelle, N., Albano, J. P., Muramoto, J., and Shennan, C. 2012b. Exploring warm-season cover crops as carbon sources for anaerobic soil disinfestation (ASD). Plant Soil 355:149-165.

Butler, D. M., Kokalis-Burelle, N., Albano, J. P., McCollum, T. G., Muramoto, J., Shennan, C., and Rosskopf, E. N. 2014b. Anaerobic soil disinfestation (ASD) combined with soil solarization as a methyl bromide alternative: vegetable crop performance and soil nutrient dynamics. Plant Soil 378:365-381.

Coffin, C. A. 2012. Indicators of Reduction in Soils (IRIS) in various conditions of saturation in south Florida marl. University of Florida.

De-Campos, A. B., Mamedov, A. I., and Huang, C.-h. 2009. Short-term reducing conditions decrease soil aggregation. Soil Sci. Soc. Am. J. 73:550-559.

Di Gioia, F., Ozores-Hampton, M., Zhao, X., Thomas, J., Wilson, P., Li, Z., Hong, J., Albano, J., Swisher, M., and Rosskopf, E. 2017. Anaerobic soil disinfestation impact on soil nutrients dynamics and nitrous oxide emissions in fresh-market tomato. Agric., Ecosyst. Environ. 240:194-205.

Fery, R. L., and Dukes Sr, P. D. 2002. Southern blight (*Sclerotium rolfsii* Sacc.) of cowpea: yield-loss estimates and sources of resistance. Crop Protect. 21:403-408.

Flores-Moctezuma, H., Montes-Belmont, R., Jimenez-Perez, A., and Nava-Juarez, R. 2006. Pathogenic diversity of *Sclerotium rolfsii* isolates from Mexico, and potential control of southern blight through solarization and organic amendments. Crop Protect. 25:195-201.

Hue, N. 1992. Correcting soil acidity of a highly weathered Ultisol with chicken manure and sewage sludge. Commun. Soil Sci. Plant Anal. 23:241-264.

Inglett, P., Reddy, K., and Corstanje, R. 2005. Anaerobic soils. Pages 72-78 in: Encyclopedia of Soils in the Environment. H. Daniel, ed. Elsevier, Oxford.

Momma, N., Yamamoto, K., Simandi, P., and Shishido, M. 2006. Role of organic acids in the mechanisms of biological soil disinfestation (BSD). J. Gen. Plant Pathol. 72:247-252.

Mullen, J. 2001. Southern blight, southern stem blight, white mold. The Plant Health Instr. doi: 10.1094. PHI-I-2001-0104-01.

Mullins, G. L., Alley, M. M., Phillips, S. B., and Maguire, R. 2019. Sources of lime for acid soils in Virginia. VCE Publication 452-510.

Njoroge, S. M., Riley, M. B., and Keinath, A. P. 2008. Effect of incorporation of Brassica spp. residues on population densities of soilborne microorganisms and on damping-off and Fusarium wilt of watermelon. Plant Dis. 92:287-294.

Pace, M. G., Miller, B. E., and Farrell-Poe, K. L. 1995. The composting process. Utah State University, Extension AG-WM 01.

Panth, M., Hassler, S. C., and Baysal-Gurel, F. 2020. Methods for management of soilborne diseases in crop production. Agriculture 10:16.

Patrick Jr, W., and Tusneem, M. 1972. Nitrogen loss from flooded soil. Ecology 53:735-737.

Pilot, L., and Patrick Jr, W. 1972. Nitrate reduction in soils: effect of soil moisture tension. Soil Science 114:312-316.

Ponnamperuma, F. N. 1972. The chemistry of submerged soils. Pages 29-96 in: Advances in agronomy, vol. 24. Elsevier.

Punja, Z. K. 1985. The biology, ecology, and control of *Sclerotium rolfsii*. Annu. Rev. Phytopathol. 23:97-127.

Rabenhorst, M. C. 2008. Protocol for using and interpreting IRIS tubes. Soil Horizons 49:74-77.

Rodriguez-Kabana, R., Beute, M., and Backman, P. 1980. A method for estimating numbers of viable sclerotia of *Sclerotium rolfsii* in soil. Phytopathology 70:917-919.

Rosskopf, E. N., Chellemi, D. O., Kokalis-Burelle, N., and Church, G. T. 2005. Alternatives to methyl bromide: A Florida perspective. Plant Health Prog. 6:19.

Runia, W. T., Thoden, T. C., Molendijk, L., Van Den Berg, W., Termorshuizen, A., Streminska, M., van der Wurff, A., Feil, H., and Meints, H. 2014. Unravelling the mechanism of pathogen inactivation during anaerobic soil disinfestation. Pages 177-193 in: VIII International Symposium on Chemical and Non-Chemical Soil and Substrate Disinfestation 1044.

Sanabria-Velazquez, A. D., Testen, A. L., Enciso, G. A., Soilan, L. C., and Miller, S. A. 2019. Effects of Anaerobic Soil Disinfestation on Sclerotinia sclerotiorum in Paraguay. Plant Health Prog. 20:50-60.

Schonbeck, M., and Morse, R. 2007. Reduced tillage and cover cropping systems for organic vegetable production. Virginia Association of Biological Farming Info Sheet 9-07.

Shennan, C., Muramoto, J., Lamers, J., Mazzola, M., Rosskopf, E., Kokalis-Burelle, N., Momma, N., Butler, D., and Kobara, Y. 2014. Anaerobic soil disinfestation for soil borne

disease control in strawberry and vegetable systems: current knowledge and future directions. Pages 165-175 in: VIII International Symposium on Chemical and Non-Chemical Soil and Substrate Disinfestation 1044.

Shennan, C., Muramoto, J., Koike, S., Baird, G., Fennimore, S., Samtani, J., Bolda, M., Dara, S., Daugovish, O., and Lazarovits, G. 2018. Anaerobic soil disinfestation is an alternative to soil fumigation for control of some soilborne pathogens in strawberry production. Plant Pathol. 67:51-66.

Shrestha, U., Dee, M. E., Ownley, B. H., and Butler, D. M. 2018. Anaerobic soil disinfestation reduces germination and affects colonization of *Sclerotium rolfsii* sclerotia. Phytopathology 108:342-351.

Soil Quality Institute. 2001. Soil quality test kit guide. National Resources Conservation Service, ARS, USDA, Washington, DC.

Stapleton, J. J., Summers, C. G., Mitchell, J. P., and Prather, T. S. 2010. Deleterious activity of cultivated grasses (Poaceae) and residues on soilborne fungal, nematode and weed pests. Phytoparasitica 38:61-69.

Strauss, S., and Kluepfel, D. 2015. Anaerobic soil disinfestation: A chemical-independent approach to pre-plant control of plant pathogens. J. Integr. Agric. 14:2309-2318.

Testen, A. L., and Miller, S. A. 2018. Carbon source and soil origin shape soil microbiomes and tomato soilborne pathogen populations during Anaerobic Soil Disinfestation. Phytobiomes 2:138-150.

Vagher, T., Fenwick, A., and Panella, L. 2014. Preparation of inoculum of *Rhizoctonia solani* Kühn for an artificially inoculated field trial. Pages 137-144 in: Meeting Proceedings.

Wolters, B. R. 2020. To Mix or Not to Mix: Performance and Management of Diverse Cover Crop Mixtures. Virginia Tech.

Xie, C., and Vallad, G. 2010. Integrated management of southern blight in vegetable production. Plant Pathology. University of Florida IFAS Extension. IPN PP272.

Xu, Z., Gleason, M., Mueller, D., Esker, P., Bradley, C., Buck, J., Benson, D., Dixon, P., and Monteiro, J. 2008. Overwintering of *Sclerotium rolfsii* and *S. rolfsii* var. *dilphinii* in different latitudes of the United States. Plant Dis. 92:719-724.

Table 4.1 Total biomass rate, moisture content, total carbon, total nitrogen, and C:N ratio for the carbon sources evaluated for anaerobic soil disinfestation (ASD).

Carbon source	Biomass rate[z] (Mg ha^{-1})	Moisture (%)	Total carbon (Mg ha^{-1})	Total nitrogen (Mg ha^{-1})	C:N ratio
Rye (*Secale cereale* L.)	9.0	87.8	3.08	0.43	7.2
Spring oat (*Avena sativa* L.)	9.0	87.0	3.57	0.41	8.7
Wheat (*Triticum aestivum* L.)	5.6	91.3	2.14	0.24	9.1
Rapeseed (*Brassica napus* L.)	5.6	91.8	2.13	0.28	7.6
Austrian winter pea (*Pisum sativum* subsp. *arvense)*	14.0	88.3	5.73	0.57	10.1
Hairy vetch (*Vicia villosa* Roth)	5.6	80.4	2.18	0.28	7.7
Poultry litter[y]	11.2	3.2	3.02	0.37	8.2
Biochar[x]	10.0	53.8	4.76	0.02	280.5
Mushroom compost	20.0	5.3	6.81	0.67	10.2

[z] Fresh weight
[y] Pelletized poultry litter Coop Poop (Healthy Grow, Pearl City, IL).
[x] Commercial biochar made from wood (Wakefield BioChar, Columbia, MO).

Table 4.2 Mean soil pH, soil nitrate, carbon dioxide content, soil reducing conditions, and sclerotial germination post-anaerobic soil disinfestation (ASD) treatment.

	Soil pH		Soil nitrate-N (ppm)		Carbon dioxide (% in vol.)[z]		IRIS paint loss (%)[y]		Sclerotial germination (%)	
Treatment	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Aerobic control[x]	5.09 e[w]	5.46 de	70.0 b	10.6 c	0.3 e	0.1 e	0.1 d	0.0 e	88 a	61 c
Anaerobic control[y]	5.16 e	5.73 b	38.8 c	1.9 ef	0.7 cde	0.4 de	0.8 d	1.2 e	75 a	98 a
Rye	5.64 bc	5.58 cd	16.3 de	8.1 cde	1.3 ab	1.1 b	38.3 c	70.0 b	85 a	87 ab
Spring oat	5.53 bc	5.37 e	16.9 de	12.5 c	1.2 b	0.8 bcd	49.0 c	78.0 ab	95 a	84 ab
Wheat	5.69 abc	5.63 bc	11.9 e	3.5 def	1.0 bcd	0.7 cd	41.3 c	33.0 d	95 a	95 ab
Rapeseed	5.73 ab	5.55 cd	9.7 e	10.0 c	1.1 bcd	0.7 cd	46.7 c	42.9 d	100 a	93 ab
Austrian winter pea	5.72 ab	5.52 cde	18.1 de	38.8 b	1.2 bc	1.0 bc	89.9 a	76.4 ab	95 a	87 ab
Hairy vetch	5.71 ab	5.49 cde	12.5 e	8.8 cd	1.0 bcd	0.9 bc	76.0 b	56.5 c	93 a	80 b
Poultry litter[v]	5.93 a	5.57 cd	15.9 de	46.3 a	1.8 a	2.0 a	76.5 b	83.2 a	73 a	87 ab
Biochar[u]	5.45 cd	6.02 a	31.3 cd	1.5 f	0.6 de	0.8 bcd	1.5 d	1.1 e	83 a	93 ab
Mushroom compost	5.28 de	5.44 de	92.5 a	6.9 c-f	1.1 bcd	0.9 bc	0.9 d	1.8 e	93 a	89 ab
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.548	0.017

[z] Percentage of CO_2 efflux averaged across time (5, 10, 15, and 21 days after ASD initiation).
[y] Arcsine-square root transformation was applied prior to data analysis. Nontransformed means are presented. Soil reduction indicated by the percentage of iron oxidate paint loss on IRIS tubes.
[x] Nonamended controls.
[w] Means values in a column followed by same letter do not significantly differ ($P = 0.05$) as determined by Fisher's Protected LSD test.
[v] Pelletized poultry litter Coop Poop (Healthy Grow, Pearl City, IL).
[u] Commercial biochar made from wood (Wakefield BioChar, Columbia, MO).

Table 4.3 Mean stem-lesion index, plant height, shoot dry weight (DW), shoot N concentration, fruit numbers, and total fruit fresh weight (FW) of tomato plants grown in greenhouse post-anaerobic soil disinfestation (ASD) treatment.

	Stem-lesion index[z]		Plant ht. (cm)		Shoot wt. (g)		Shoot N conc. (%)	Fruit number		Total fruit wt. (g)[y]
Treatment	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Across Exp.	Exp. 1	Exp. 2	Across Exp.
Aerobic control[x]	1.0 a	1.0 a	10.9 a[w]	8.5 a	1.5 a	0.8 b-e	1.20 c	5.5 a	5.8 cd	12.8 b
Anaerobic control[x]	1.0 a	2.1 a	9.4 bc	8.3 a	0.8 bc	0.5 f	1.07 c	2.8 bcd	3.8 d	7.5 cde
Rye	1.3 a	2.0 a	8.9 c	10.8 a	1.0 bc	0.8 cde	1.21 c	2.5 cd	4.0 d	7.1 de
Spring oat	1.0 a	1.0 a	9.4 bc	9.3 a	0.8 bc	0.9 bcd	1.12 c	1.5 d	5.3 cd	8.0 cde
Wheat	2.0 a	2.8 a	8.5 c	8.6 a	0.6 c	0.7 def	1.13 c	2.0 d	3.8 d	6.1 e
Rapeseed	1.8 a	1.8 a	9.0 c	9.1 a	0.8 bc	0.7 c-f	1.20 c	2.3 d	4.5 cd	8.4 b-e
Austrian winter pea	4.0 a	1.0 a	11.1 a	9.5 a	0.9 bc	1.1 ab	1.52 a	3.8 a-d	10.8 ab	12.7 b
Hairy vetch	2.5 a	1.0 a	10.4 ab	9.1 a	1.2 ab	1.0 bc	1.24 bc	2.5 cd	8.0 bc	11.2 bc
Poultry litter[v]	2.6 a	1.3 a	10.8 a	9.6 a	1.3 ab	1.4 a	1.46 ab	4.8 abc	11.8 a	18.2 a
Biochar[u]	2.0 a	3.0 a	8.8 c	8.8 a	0.7 c	0.5 f	1.20 c	2.8 bcd	2.3 d	6.4 e
Mushroom compost	1.0 a	3.5 a	10.9 a	8.0 a	1.6 a	0.6 ef	1.45 ab	5.0 ab	4.5 cd	10.7 bcd
P-value	0.071	0.113	<0.001	0.139	<0.001	<0.001	0.001	0.022	<0.001	<0.001

[z] Each plant was rated on scale 1-5 as follow: 1-no stem lesion, 2-small lesion (≤25% of the stem circumference), 3-moderate lesion (26-50% of the stem circumference), 4-large lesion (≥51% of stem circumference), 5-deade plant (stem completely girdled).
[y] Square root transformation was applied prior to data analysis. Nontransformed means are presented.
[x] Nonamended controls.
[w] Means values in a column followed by same letter do not significantly differ ($P = 0.05$) as determined by Fisher's Protected LSD test.
[v] Pelletized poultry litter Coop Poop (Healthy Grow, Pearl City, IL).
[u] Commercial biochar made from wood (Wakefield BioChar, Columbia, MO).

Table 4.4 Chlorophyll meter readings (SPAD) of tomato plants at different days after transplanting (DAT) into post-anaerobic soil disinfestation (ASD) treatments.

SPAD value[z]	DAT (Exp. 1)					DAT (Exp. 2)			
Treatment	7	14	21	28	Average[y]	7	14	21	28
Aerobic control[x]	46.2	47.9	46.4	42.4	45.7 a[w]	39.2 a, B	45.3 a, A	42.9 ab, AB	42.1 ab, AB
Anaerobic control[x]	44.1	39.4	35.9	30.0	37.4 d	38.6 a, AB	40.7 cde, A	36.2 cd, B	31.1 e, C
Rye	48.3	47.8	43.9	34.8	43.7 abc	37.4 a, A	41.5 bcd, A	42.5 ab, A	38.8 bc, A
Spring oat	46.2	41.7	37.3	31.0	39.0 bcd	35.7 a, B	42.9 abc, A	43.8 a, A	41.7 ab, A
Wheat	44.4	41.5	37.2	30.3	38.3 cd	36.6 a, A	38.6 ef, A	37.9 c, A	33.7 de, A
Rapeseed	47.2	47.4	46.0	33.7	43.6 abc	35.1 a, C	42.0 bcd, A	39.7 bc, AB	36.3 cd, BC
Austrian winter pea	49.9	47.6	46.1	30.4	43.5 abc	38.3 a, B	43.9 ab, A	45.8 a, A	44.8 a, A
Hairy vetch	47.4	47.7	44.8	35.4	43.8 abc	36.4 a, B	43.2 abc, A	42.8 ab, A	40.8 abc A
Poultry litter[v]	47.4	48.7	45.7	35.7	44.4 ab	38.6 a, B	42.9 abc, AB	44.7 a, A	45.0 a, A
Biochar[u]	43.0	43.0	40.1	31.3	39.3 bcd	36.2 a, AB	37.8 f, A	33.5 d, B	29.3 e, C
Mushroom compost	47.0	49.2	48.4	41.2	46.5 a	36.9 a, A	39.4 def, A	37.0 cd, A	32.1 de, B
P-value					0.034	0.469	0.002	<0.001	<0.001

[z] SPAD value of the newly developed leaves (SPAD-502, Konica Minolta Sensing, Inc, Japan).
[y] SPAD values averaged across times.
[x] Nonamended controls.
[w] Means values in a column (lower case letter) or row (capital letter) followed by same letter do not significantly differ (P = 0.05) as determined by Fisher's Protected LSD test.
[v] Pelletized poultry litter Coop Poop (Healthy Grow, Pearl City, IL).
[u] Commercial biochar made from wood (Wakefield BioChar, Columbia, MO).

Table 4.5 Summary statistics, correlations, and multiple regression predicting tomato shoot dry biomass and yield from stem-lesion index, soil nitrate-N, soil pH, and iron oxidation paint loss (IRIS tubes) post-anaerobic soil disinfestation treatments.

	Pearson correlation coefficient						Shoot DW		Fruit weight	
Variable	Lesion index	Nitrate -N	Soil pH	IRIS paint loss	Shoot DW (g)	Fruit weight (g)	β	*P*-value[z]	β	*P*-value[z]
IRIS paint loss (%)					0.24*	0.29*	0.004	<0.001	0.056	<0.001
Soil pH				0.33*	-0.18	-0.12	0.375	0.018	4.143	0.099
Nitrate-N (ppm)			-0.61*	-0.21	0.59*	0.43*	0.013	<0.001	0.131	<0.001
Lesion index (1-5)		-0.20	0.27*	0.003	-0.31*	-0.33*	-0.058	0.005	-0.998	0.003
							Intercept= -1.4961		Intercept= -16.327	
Mean[x]	1.84	21.93	5.56	39.31	0.9	9.91	R^2=0.54		R^2=0.41	

*P<0.05

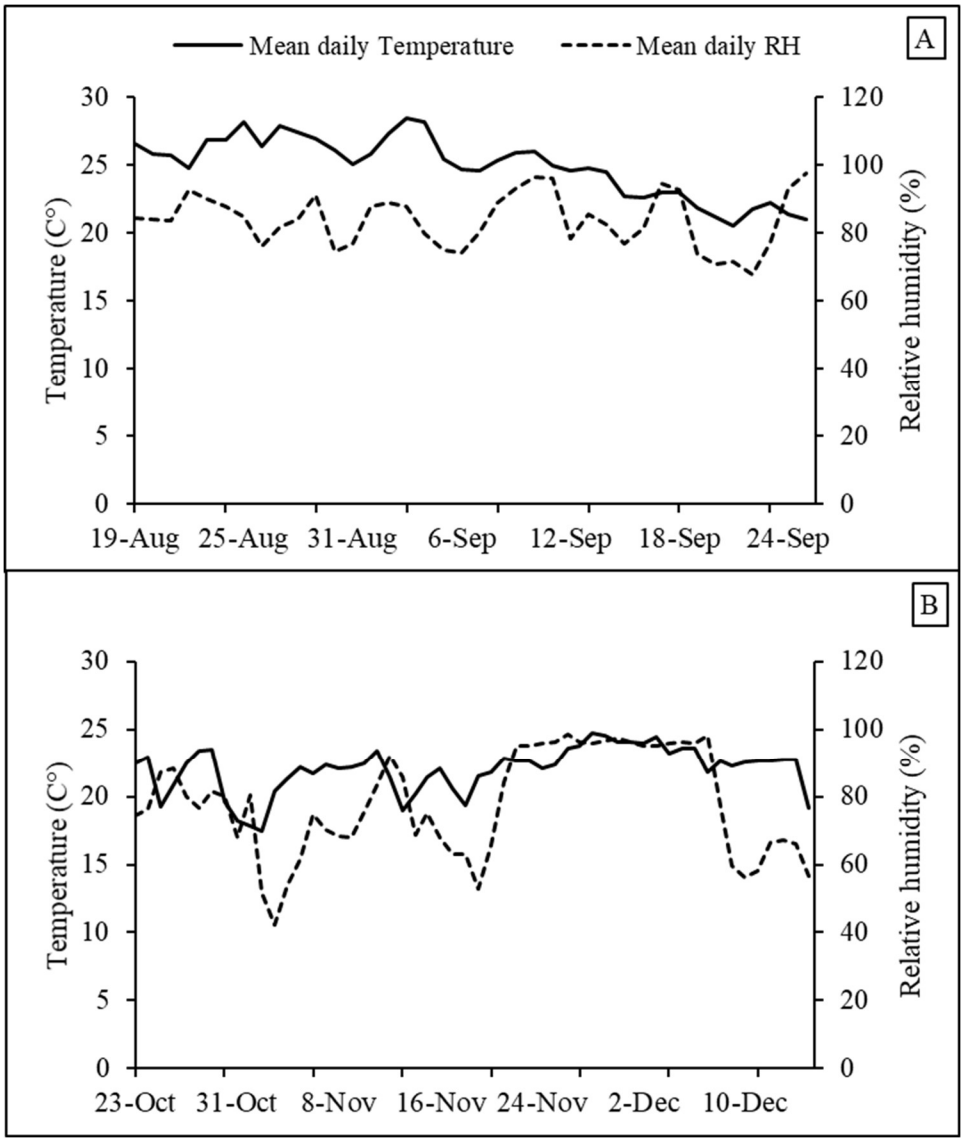

Figure 4.1 Mean daily air temperature and percent relative humidity during the tomato growth period in greenhouse in experiment 1 (A) and experiment 2 (B). Data were recorded by using a HOBO Pro v2 units (Onset Computer Corp., Bourne, MA). Each daily value represents the average of two measurements.