EVALUATION OF MANAGEMENT PROGRAMS FOR CONTROL OF POTATO EARLY DIE (PED) AND SENSITIVITY OF *HELMINTHOSPORIUM SOLANI* TO THREE CLASSES OF FUNGICIDES

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ABSTRACT

EVALUATION OF MANAGEMENT PROGRAMS FOR CONTROL OF POTATO EARLY DIE (PED) AND SENSITIVITY OF *HELMINTHOSPORIUM SOLANI* TO THREE CLASSES OF FUNGICIDES

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The potato production industry has been severely impacted by a wilt disease; Potato Early Die (PED) complex (*Verticillium dahliae* Kleb. and *Pratylenchus penetrans*) and a storage disease; silver scurf (*Helminthosporium solani*). Experimental field trials were conducted in 2018 and 2020 to evaluate chemical management programs for PED. Vydate C-LV treatment reduced *Pratylenchus penetrans* populations in soil. In vitro studies were established to evaluate three methods to estimate in vitro sensitivity of *Helminthosporium solani* to three classes of fungicides. Sensitivity expressed in mg/L was defined as the effective fungicide concentration at which 50% of the fungal growth or spore germination is inhibited (EC_{50}). *Helminthosporium solani* isolates were most sensitive to pydiflumetofen, difenoconazole and benzovindiflupyr. The relative germination method is recommended for screening succinate dehydrogenase inhibitors. The relative growth method using a spiral gradient dilution may also be used but is recommended for screening demethylation inhibitors and phenylpyrrole fungicides due to their mode of action.

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1.1.1 ORIGIN AND GLOBAL IMPORTANCE

The potato (*Solanum tuberosum* Linnaeus) has been cultivated for at least 8,000 years and is indigenous to the Andes mountains of South America (115). When Spanish explorers arrived at Peru in the mid-1500s, potato was a primary food source for the indigenous people (115). Thereafter, the potato traveled around the world, developing into a valuable resource (115). Potato has influenced migration patterns of humans, influenced wars and lead to improvements in human health (67). In terms of human consumption, potatoes are the most important non-cereal crop and the third most important food crop in the world (18). Annually, over 1.5 billion people consume the crop. In comparison, potatoes yield significantly more calories per acre than maize, rice, soybean, and wheat, producing 80% more protein per unit of land area than rice and >50% more than wheat (98). Potatoes are considered a good source of antioxidants, including ascorbic acid (vitamin C), that serve as free radical receptors (3). Potatoes also provide potassium, phytochemicals, fiber and are fat free (120). It has been estimated that potato production must double by 2050 to ensure global food security (98). The global importance of potato extends beyond a food source, it is also used in the production of paper, adhesive, textile goods, highly absorbent biodegradable material (used in disposable diapers), cosmetics (lipsticks and creams), and water purification systems (114).

1.1.2 GROWTH AND DEVELOPMENT

Potatoes are typically grown from vegetative seed tubers, either as whole or sections that contain meristematic tissue known as buds or eyes (98) or they can be propagated from true potato seed (TPS). However, unlike seed tubers, individual TPSs are genotypically diverse (51). The

genetic diversity of TPS can be used by potato breeders to improve the crop's quality, yield, and disease resistance. Moreover, due to their ability to be maintained in storage conditions in contrast to seed tubers, they are used to commercially grow potatoes in developing countries (51). True potato seed stored in low relative humidity at room temperature can remain viable up to approximately two years, and at 4^{o}C, up to several years without losing germinability (51). Seed tubers require refrigeration (4^{o}C) and are only viable for approximately ten months (51). In developed countries, seed tubers are used to commercially produce potatoes because their identical genetics have been bred for quality, yield, pest, and regional environmental adaptability (98). Seed tubers are temporarily stored at cold temperatures (~4^{o}C) and sprouts on the seed tuber are then brought out of dormancy by a thermal induced hormonal response that initiates continuation of meristematic leaf development on the sprout initials (76). After planting, sprouts elongate between internodes and emerge from the soil (21). As the potato plant matures, numerous lateral underground stems, or stolon's, form at the base of the main stem. Under favorable conditions (e.g. photoperiod length, temperature, and nitrogen limitations), tubers form at the stolon's and are a modified stem with a broadened and shortened axis (21). After approximately 100 days, the potato plant begins to die, resulting in wilting foliage and thickening of potato tuber's skin (48). When potato tuber skin has set, mechanical harvest occurs, and tubers are shipped to fresh markets for sale, storage or chipping manufacturers. The tubers may be sold quickly after harvest or stored in bins with temperature and humidity control for typically 2-5 months (39).

1.1.3 GLOBAL PRODUCTION, PRODUCTION IN THE US, AND PRODUCTION IN THE STATE OF MICHIGAN

The global production of potatoes in 2018 was 368.2 million t, with the US ranked 5th in production, producing 20.6 million t (24). From 1997 to 2007, the production of potatoes in

developing countries increased by 25% (18). Potatoes are the most widely produced vegetable crop in the US, harvesting 1.05 million acres of potatoes in 2017 (17). The top three potato producing states are Idaho, Washington, and Wisconsin, producing 7.2, 5.3 and 1.5 million t, annually (25). The state of Michigan is ranked 6th in the US for potato production, accounting for 1.0 million t, annually (25). The Michigan potato industry contributes $1.24 billion to the state's economy and provides over 3,000 jobs (137). Michigan is ranked first in the US for potato production for the chipping industry, accounting for over 70% (136).

1.2 Verticillium Wilt

1.2.1 IMPORTANCE OF VERTICILLIUM WILT OF POTATO

Verticillium wilt of potatoes is incited by either of the two fungal pathogen *Verticillium* spp., *Verticillium dahliae* Kleb. or *Verticillium albo-atrum* (73). In 1879, Verticillium wilt of potato was described (78). In 1916 similar symptoms were termed "early dying", and in 1968 the *Verticillium* fungus was first reported as an important causal agent of the previously described "early dying" on potato (78). *Verticillium dahliae* is a major yield reducer of potato production across the world and of special importance in warmer and dryer climates, more specifically temperate zones (104). *Verticillium dahliae* is a greater concern than *Verticillium albo-atrum* under higher temperatures because of each pathogen's cardinal temperatures (60). Among ten *Verticillium* spp. negatively impacting agriculture, *Verticillium dahliae* Kleb. is globally the most destructive (134). The importance of *Verticillium dahliae* in multiple provinces in China has increased, resulting in yield losses up to 50% (80). Until 2016, *Verticillium albo-atrum* was the only reported causal agent of Verticillium wilt of potatoes in Korea. However, proof-of-pathogenicity testing of infected *Solanum tuberosum* 'Superior' in Jeongseon-gun, Korea

confirmed infection by *Verticillium dahliae* (75). A study by Powney et al. 2005 revealed approximately one third of all commercial processing potato crops were infected with *Verticillium dahliae* (109). Another study by Harding and Wicks reported that *V. dahliae* is the only pathogen severely affecting potatoes grown in Australia (50). The optimal temperature for *V. albo-atrum* infection is about 20^{o}C, and about 27 ^{o}C for *V. dahliae* (120). The increase in *V. dahliae* infection may be a result from average temperatures increasing globally due to climate change. Verticillium wilt disease causes overall wilting and early vine maturity, resulting in reduced nutrients available for tuber production and therefore, reduced yields and quality (70). *Verticillium dahliae* is also a causal agent of stem-end defect resulting in dark coloring along the vasculature and adjacent tissue in the interior of the potato tuber at the stem-end, a serious quality concern for the US chipping industry (133). There have been reports that *V. dahliae* microsclerotia can survive in the soil for approximately ten years, with or without a host, making disease management difficult (36). The Verticillium wilt disease of potato threshold can be less than 10 colony forming units per gram (CFU/g) of soil (36). Approximately 41% of commercial seed lots in North America contained *V. dahliae* propagules with populations in a single seed lot containing as high as 10^6 CFU/g of plant debris and soil (36).

1.2.2 *VERTICILLIUM DAHLIAE,* CAUSAL AGENT OF VERTICILLIUM WILT

1.2.2.1 Taxonomy

Verticillium dahliae (*V. dahliae*) Klebahn (Kleb.) was identified by Nees von Esenbeck in 1816 and was described based on conidiophore morphology (69). The *Verticillium* genus was previously in the Deuteromycota phylum, without a known teleomorph, but gene sequencing resulted in the genus *Verticillium* being placed in the Ascomycota phylum as anamorphic (11, 129). The sexual stage of *V. dahliae* is unknown; it produces asexual hyphae, microsclerotia,

conidia and yeast cells (129). Approximately 190 *Verticillium* species have been described since 1816 (68). The *Verticillium* species complex share distinctive conidiophore morphology consisting of narrow flask-shaped spore-forming phialides assembled into whorls (verticils) and attached along a main axis (69, 105). Species deviation is determined by the type of resting structure and may include dark mycelium, chlamydospores, and microsclerotia (69, 105, 68).

In 1879, *Verticillium albo-atrum* Reinke & Berthold was first described as the causal agent of vascular wilt of potato (68). Diseased tuber and stem potato tissue turned dark brown to black due to melanization of the fungal hyphae. In addition, septation in the hyphae increased while short cells were formed that gradually increased in width, resulting in melanized hyphae varying in shape and size in diseased tissue (69). In 1913, Klebahn first isolated *V. dahliae* from *Dahlia* sp. cv. Geiselher (11). Klebahn characterized the main point of difference between *V. dahliae* and *V. albo-atrum* as *V. dahliae* formed microsclerotia while *V. albo-atrum* formed resting mycelium as their respective resting structures (11). The microsclerotia developed from irregular multilateral septation and budding of cells of one hypha or of a number of adjacent hyphae (69). Initially, controversy began due to Klebahn's publication of the new *Verticillium* species, which differentiated *V. dahliae* from *V. albo-atrum*. However, *V. albo-atrum* and *V. dahliae* can be differentiated based on resting structures, optimal pH and temperature range, geographic distribution, host range, conidial size, and conidiophore shape (68, 69, 105, 119). Recently, the species differentiation has been confirmed with molecular markers (105, 42, 68, 93, 103).

1.2.2.2 Host range

Over 400 plant species, including weeds, are hosts to *V. dahliae* and *V. albo-atrum,* resulting in a wide distribution of disease occurrence (12). In general, monocotyledons are not susceptible, except barley, but *V. dahliae* can colonize hosts including grasses and mustards, as an

endophyte (60, 129, 134). *Verticillium dahliae* and *V. albo-atrum* are pathogenic on over 200 dicotyledon plant species such as mint and potato (35). More recently *V. dahliae* infection is now recognized as a serious problem for artichoke (*Cynara cardunculus* var. *scolymus*) in Eastern Spain (102). In Michigan, the dominant source of Verticillium wilt is *V. dahliae*, but under favorable conditions, *V. albo-atrum* has been found to cause disease in northern Michigan (78). Potato wilt is more likely to be caused by *V. dahliae* in temperatures near 27°C, while infection by *V. albo-atrum* is favored at temperatures near 20°C (12). Therefore, warmer climates contribute to increased disease incidence from *V. dahliae*, while *V. albo-atrum* is more pervasive in cooler climates (120).

1.2.2.3 Fungal morphology

Verticillium dahliae has vegetative mycelium that is hyaline, septate, and multinucleate with haploid nuclei (12). The conidia are ellipsoid or ovoid, typically single-celled (2-4 x 4-11 µm) and borne on phialides (specialized hyphae formed in a whorl surrounding each conidiophore, carrying a mass of conidia) (12, 120). The overwintering or long-term survival fungal structure is a small, black to brown, variably shaped but typically spherical structure, referred to as microsclerotia (66). *V. dahliae* Kleb. produces chains of thick microsclerotia 30-60µm in diameter and conidiophore that are septate, have side branches, swollen at the base, and arranged in verticillate whorls (120). Conidia can be in collected heads on the sterigmata tips and there are one to eight primary whorls of branches (120). The conidiophores may be from 100-300µm in length and the terminal branch of the conidiophore is 15-60 µm long (120). The name Verticillium is derived from the whorled or “verticillate” arrangement of the phialides (69). In comparison to *Verticillium albo-atrum, V. dahliae* has lighter hyphae.

1.2.2.4 Vegetative compatibility groups

Groups that are genetically diverse within *V. dahliae* have been determined using vegetative compatibility testing. This is useful because *V. dahliae* has no known sexual stage (114). Isolates of *V. dahliae* can be divided into vegetative compatibility groups (VCGs) based on their ability to undergo hyphal anastomosis with other isolates and form a stable heterokaryon (72). Sub-specific groups have been defined and they contain host-adapted pathotypes that are highly virulent and prevalent on specific hosts (12). Researchers used nitrate-non-utilizing (*niit*) mutants to classify four major *V. dahliae* VCGs (VCG 1, VCG 2, VCG 3, VCG 4) and VCG 2 and 4 are further divided into subgroups (2A and 2B, 4A and 4B) established based on differential interactions between isolates (29). Isolates of *V. dahliae* collected from potato stems, tubers and fields in the USA, indicate that the most prevalent VCGs belong to VCG 4A and VCG 4B, with VCG 4A isolates having higher virulence on potatoes in contrast to VCG 4B and other VCGs (72, 36). Potato is primarily associated with VCG4B and VCG2 outside of North America (36). It was reported by Alkher et al. 2009, that isolates from potato were aggressive on potato and sunflower (*Helianthus annus* L.), while isolates from sunflower were aggressive on sunflower but not always on potato (2).

1.2.3 INTERACTION WITH ROOT-LESION NEMATODE

The potato early die (PED) complex is the interaction between certain fungi and nematodes causing premature death of potato (6). Nematodes pose an additional threat to fungal disease severity because they are wounding agents that alter host tissue and modify the surrounding rhizosphere (121). *Verticillium dahliae* (Kleb) and *V. albo-atrum* are the most severe pathogens associated with the potato early die complex. However, other pathogens such as *Pectobacterium carotova*, *Rhizoctonia* spp., *Fusarium* spp., and *Colletotrichum coccodes* have been reported to be

associated with PED (118). The potato early die complex primarily involves the interaction of the predominant fungus species, *Verticillium dahliae* and the root lesion nematode (*Pratylenchus* spp.) in particular, *Pratylenchus penetrans* (82, 123). *Pratylenchus* spp. identification is difficult morphologically but, can be confirmed by Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) of the Internal Transcribed Spacer region (94, 132). The nematode, *P. penetrans* is polyphagous and a migratory endoparasite (94). *Pratylenchus penetrans* juveniles and adults penetrate host root tissue and move throughout the root cortex, feeding on cortical cells ultimately causing necrosis (94). The necrotic tissue results in reduced water and nutrient uptake, and transport efficiency (53, 78). *Pratylenchus* spp. do not alter the host as drastically as sedentary nematode parasites but, nematode feeding does disrupt tissue when they migrate between feeding sites (65). The host range *P. penetrans* includes potato, alfalfa, corn, strawberries, small grains, celery, and mint (53). The increased *V. dahliae* incidence in the presence of *P. penetrans* is hypothesized to be due to biochemical changes rather than mechanical damage due to lack of evidence of mechanical damage (129). It was hypothesized that *P. penetrans* feeding, biochemically altered the potato plant which allowed for *V. dahliae* to colonize the host for a period without triggering a host immune response (129). Once the host plants root system is occupied by soil microorganisms the environment is unfavorable for *P. penetrans* and they migrate to the rhizosphere (94). After harvest, they feed on remaining roots and weeds where 15-86% of the *P. penetrans* population may decline during winter (94).

1.2.4 LIFE CYCLE AND EPIDEMIOLOGY

The overwintering structure (multicellular melanized microsclerotia) and conidia are stimulated to germinate in the spring by root exudates from host or non-host plants (135). *Verticillium wilt* is a monocyclic pathogen with microsclerotia as the source of inoculum (6).

Germinated *Verticillium dahliae* microsclerotia or conidia colonize the potato on the root surface by producing hyphae that directly penetrate the root tips (138). The hyphae colonize the root cortex and xylem vessels, where conidia are produced and further spread through the plant by the xylem (117). It has been observed that when the potato plant undergoes physiological changes at the time of flowering that *V. dahliae* conidia are produced and move acropetally through the xylem, resulting in obstructions and restriction of evapotranspiration (135). Once *Verticillium* wilt has colonized the vascular system, symptoms begin to appear (52). *V. dahliae* has a hemibiotrophic lifestyle and is biotrophic while infecting the roots but, transitions into a necrotrophic state after infecting the vascular system (34). The lack of water and nutrient transport by the clogged xylem tissue in infected hosts' may be a result of *V. dahliae* mycelium and/or an accumulation of Tyloses or host tissue breakdown products (63, 128). Additionally, *Verticillium* toxins (*Vd* toxins) are a major contribution in the development of disease in the host (96). At the time of senescence, microsclerotia develop in all colonized tissues: vascular, cortical, and epidermal tissue of potato stems, leaves, petioles stolon's, and in epidermal, cortical, and phloem tissues of roots (135). Late in the growing season, any microsclerotia remaining in the soil, or in plant material, can stay dormant in the soil for approximately ten years or more without a viable host present (134). *Verticillium dahliae* is not mobile and is transported through wind, water or within soil on equipment and persons (95). It has been reported that an inoculum density of 10-20 *V. dahliae* colony-forming units (cfu) per gram of soil is sufficient to cause economic loss from reduced potato yields (30). Optimal air temperature for infection is between 24 and 28°C and high soil temperatures of 22-27°C favor disease development (111, 10). However, disease pressure is not reported in regions of elevated temperatures, because *V. dahliae* development is considered inhibited at temperatures above 30^{o}C (129, 114).

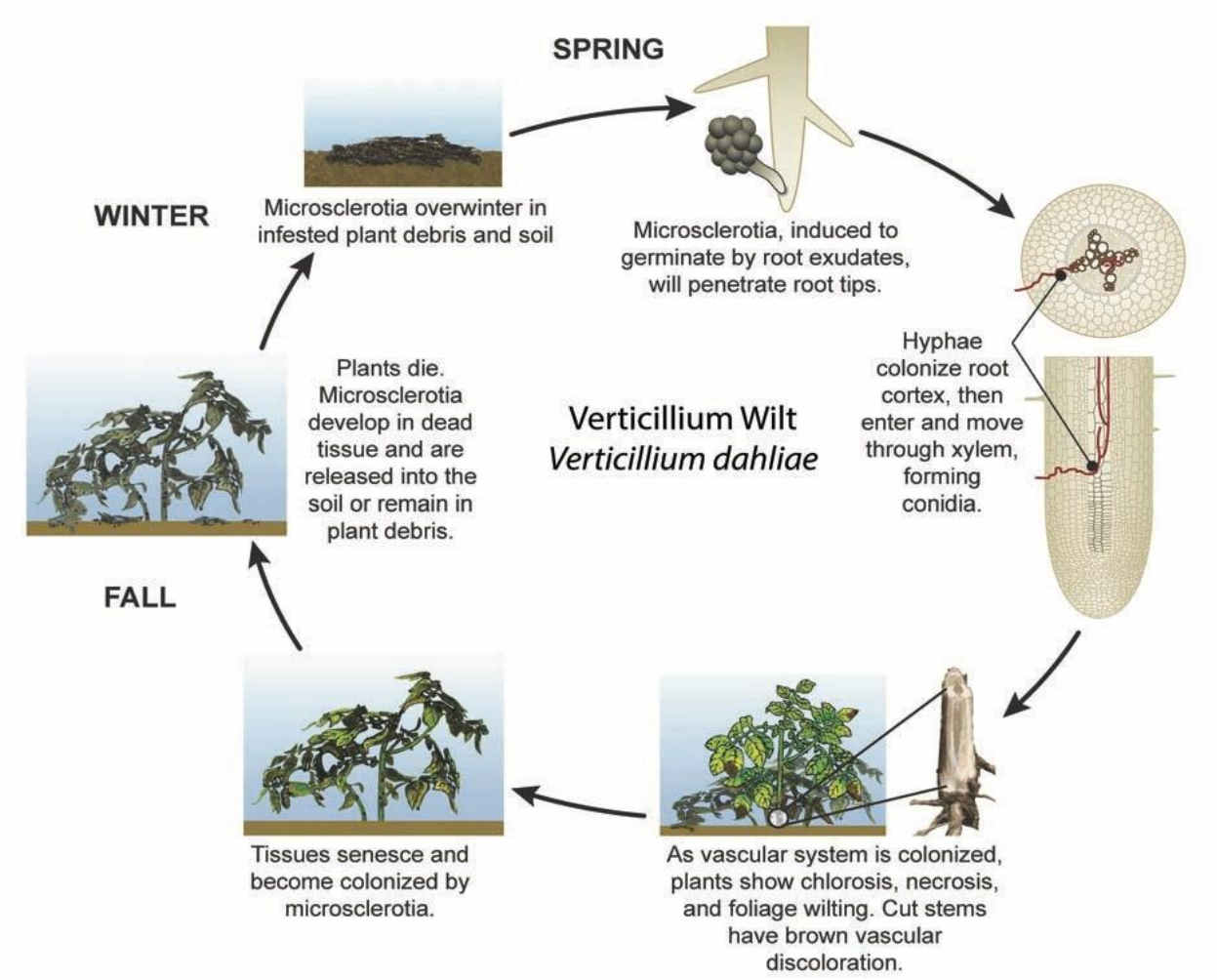

Figure 1.1. The disease cycle for Verticillium wilt shows direct penetration of the root cortex that leads to blocked vascular tissue and plant fatality. The remaining necrotic plant tissue serves as an overwintering structure for microsclerotia. Image is reproduced with permission, from Michigan State University Extension Bulletin E-3207 © 2015 Michigan State University. All rights reserved.

1.2.5 VERTICILLIUM WILT DISEASE SYMPTOMS

Potatoes grown in soil infested with *V. dahliae* will not have visual symptoms until approximately 60-70 days after planting or at flowering (58). Nonvisual symptoms such as reduced net photosynthesis and transpiration, and increased leaf surface temperatures in contrast to healthy plants, will begin up to several weeks prior to visual symptoms (108). Infection by *V. dahliae* effects gas exchange of the potato plant by decreasing carbon assimilation rate, stomatal conductance, transpiration, apparent intercellular CO_2 concentration, and increased leaf temperature (14). Potatoes infected with *Verticillium* wilt express a number of symptoms, beginning with progressive foliar chlorosis leading to necrosis that causes an overall wilting appearance (97). A common Verticillium wilt symptom that is indistinguishable from natural senescence is acropetal progression of chlorosis and necrosis of foliage followed by premature (4-6 weeks) defoliation (69, 82, 108, 113). Uneven chlorosis on leaflets is possible, beginning on the lower section of stems (74). In some cases, the symptoms appear only on individual leaves or on one side of the plant, potentially resulting in one remaining vertical stem (also referred to as 'flagging') (108, 58). Flagging is reported to be the result of individual vascular bundles being colonized at variable times (120). Chlorosis and necrosis of potato foliage can also be the result of numerous plant pathogens and/or environmental stresses. Other wilt diseases such those caused by *Fusarium* spp. and *C. coccodes* have similar symptoms as *V. dahliae* but, in contrast, plants infected with *V. dahliae* tend to die erect (113). Symptoms will appear more severe in the afternoon on warmer and sunnier days (108). In severe cases, the vascular tissue in the stems may be brown with necrotic tissue and the plant may be stunted (36, 47). The potato may respond to *V. dahliae* infection with the production of Tyloses in the xylem to block the spread of conidia throughout the vascular system, ultimately resulting in a clogged vascular system and necrotic stem (128).

Some potato varieties will develop vascular ring browning in tubers (10). Overall, *Verticillium* wilt symptoms on potato consist of yellowing and browning of the foliage, wilting of the foliage, and browning of the vascular tissue in the stems and tubers.

1.2.6 VERTICILLIUM WILT DISEASE MANAGEMENT

Management of *Verticillium dahliae* can be complex due to the longevity of its microsclerotia. Site selection is important to consider when managing *Verticillium* wilt (13). Soil that has not been previously farmed with a host of *Verticillium* wilt or farmed at all, is ideal (73) due to longevity of microsclerotia in the soil (78). To design the best management strategy when the soil being used to grow has recently been farmed with a host of *V. dahliae*, it is recommended to estimate the inoculum density which can completed by submitting a soil sample to a diagnostic lab to estimate the quantity of colony forming units of *V. dahliae* in the soil (104). The amount of *V. dahliae* inoculum present is proportional to disease severity (107). Due to the potato early die complex involving *V. dahliae* and *P. penetrans*, control of the nematode population with chemical and biological controls, good farm sanitation, crop rotation and nematode-free seed is crucial to managing disease severity (53). The soil can be treated with fluopyram, a nematicide (Velum® Prime, Bayer Crop Sci, Greensboro, NC) and/or fumigation (e.g. metam sodium) (129). *V. dahliae* can also be partially managed with seed treatments used to manage Rhizoctonia disease or Fusarium dry rot (73). Registered fungicides for the suppression of *V. dahliae* are limited but include in-furrow (Elatus®) and foliar (Aprovia®) applications (126). In addition to chemical control applications, fumigation is another management strategy (111). The soil can be fumigated with 1,3-dichloropropene, related hydrocarbons, and/or chloropicrin for significant reduction in *Verticillium* disease without reducing the pathogen population in the soil (62). Conducive weather conditions, not solely inoculum levels in the soil, may be an important factor in disease

development (64). A common source of inoculum is potato seed tubers. Therefore, planting certified disease-free seed tubers is preventative management (97, 116). Another management strategy includes rotation with leguminous crops and green manure applications (e.g. sudangrass, rapeseed, broccoli, winter pea, corn, oat, rye, and buckwheat (79)) as a biological control of *V. dahliae* (59, 111). Long-term crop rotations of 3-4 years with potato and a cereal crop and/or hay (avoid peppers, tomatoes, brussel sprouts, cabbage, cauliflower, eggplants, or fields with a history of raspberries or strawberries) have also been reported to decrease disease severity caused by *V. dahliae* (106, 58). Crop rotations with some species in the *Brassicaceae* family including broccoli, canola, turnip, radish, various mustards, and rapeseed can reduce disease incidence and populations of nematodes and fungal pathogens in the soil (79). While some species in the *Brassicaceae* family reduce Verticillium wilt diseases incidence, a few species are host to *V. dahliae* and should be avoided including brussel sprouts, cabbage, and cauliflower (58). Green manure applications and crop rotations with some species from the *Brassicaceae* family reduce *V. dahliae* disease incidence through bio fumigation; the production of volatile compounds that are toxic to many soil microorganisms, nematodes and weed seed through the breakdown of plant metabolites in soil (79). In addition, changes in the soil microbial community that are not related to toxic metabolite levels, are also responsible for decreased *V. dahliae* incidence (79). Long rotations with non-hosts such as sugar beets, will not add inoculum to the soil (129). Applications of *Trichoderma harzianum* have been used in managing *Verticillium dahliae* on potato, resulted in increased yield (20, 31). To help manage the movement of *Verticillium* wilt inoculum, good sanitation practices in the field are required, including sanitizing machinery, boots, vehicles, and other farm equipment between fields (6). Overall good plant health through fertilizer and irrigation applications can help to reduce the severity of disease and limit yield reduction (40, 33).

Susceptible varieties including ‘BelRus’, ‘Russet Norkotah’, ‘Shepody’ and ‘Superior’ should be avoided. ‘Russet Burbank’ is moderately resistant (107). Other less popular potato cultivars resistant to *V. dahliae* include ‘Red Dale’, ‘Ranger Russet’ and ‘Defender’ (44). The development of genetically stable tolerant or resistant cultivars is desirable for Verticillium wilt management (130).

1.3 Silver Scurf of Potato

1.3.1 IMPORTANCE OF SILVER SCURF OF POTATO

Helminthosporium solani Dur. & Mont. is the causal agent of silver scurf on potatoes (129). It has been an economically important disease since the early 1990s, particularly in temperate regions (84). *Helminthosporium solani* is of economic importance due to the reduced marketability of infected tubers as a result of surface cosmetic symptoms and signs, especially for fresh market production (10). The disease does not cause a loss of yield at harvest but does result in weight loss in storage (48). *Helminthosporium solani* is a major storage disease of potato (16) and infection in storage is more economically damaging than field infection (98). In storage, water loss through areas infected with *H. solani* can result in up to 13% yield reduction, affecting growers’ profits, regardless of the production sector (i.e. fresh vs processed) (46). Due to silver scurf compromising the integrity of the tuber’s skin and increasing water loss, length of storability is greatly reduced (48, 57). In Michigan, where approximately 70% of potato production is for chip stock, long-term storability is crucial for growers. In California, it has been reported that after 7 months of storage, 95% of tubers were infected with silver scurf (27). Silver scurf infection can toughen the tuber’s skin, resulting in peeling difficulty during processing (9). There has been a shift in the potato production industry for pre-washed and packaged tubers, with high-quality skin (58). It has been

reported that tuber blemish diseases (i.e. silver scurf, common scab, and black dot) are a major component of wasted product in the British potato industry and the potential cost savings by reducing tuber blemish diseases could amount to £9 million per year (46). A study completed in 1989-1990 reported that 86% of the potato crop assessed in the UK was infected with *H. solani* (57).

Development of thiabendazole (TBZ) fungicide resistance in *H. solani* isolates from England was first reported in 1988, and was subsequently reported from Sweden, Canada, and the US throughout the 1990s (9). Thiabendazole resistance was a result of its routine use as a postharvest treatment of *Fusarium* spp. (Fusarium dry rot) and non-target management of silver scurf (88). Fungicide resistance in *H. solani* continues to be of economic importance and results in an increase in disease incidence, a decrease in storability, and complicated disease management (9, 88). Therefore, effective chemistries for managing *H. solani* are essential coupled with determining the risk of reduced sensitivity to available fungicides labeled for *H. solani* to increase their time span of efficacy.

1.3.2 *HELMINTHOSPORIUM SOLANI*, CAUSAL AGENT OF SILVER SCURF

1.3.2.1 Taxonomy

The genus *Helminthosporium*, belongs in the Ascomycota phylum as an anamorphic fungus (38), belonging to the family Massarinaceae, order Pleosporales (8). It has had a complex taxonomic past (132). The sexual stage of *Helminthosporium solani* is not known, but it produces hyphae and multicellular conidia (129). Classification of *Helminthosporium* species is mainly determined by conidia morphology, mode of conidiophore and conidium formation (81) (99). The genus *Helminthosporium* was heterogenous and was divided into subgenera *Cylindro-Helminthosporium* and *Euhelminthosporium*, however, neither of those two subgenera included

the generic type, therefore, subgenus *Helminthosporium* was established based on *H. velutinum* (81). The subgenus *Helminthosporium* differed from the subgenera *Cylindro-Helminthosporium* and *Euhelminthosporium* because subgenus *Helminthosporium* conidial germ tubes are percurrent, the conidiophore proliferations, if present, are percurrent, and the conidiophores are typically grouped on a stroma (81). The three subgenera were similar due to conidia produced at the tips and along the sides of the conidiophores, however, their development of conidia differ (81). In *Cylindro-Helminthosporium* and *Euhelminthosporium* the conidia are acrogenous and develop to pseudopleurogenous by lateral proliferation of the conidiophore tip between each successive conidium, while the subgenus *Helminthosporium,* has pleurogenous conidia (81). Approximately 700 taxa have been assigned to the *Helminthosporium* genus, but after careful morphological analyses, species with acrogenous conidia were placed in the genera *Corynespora* and *Exosporium* (132). Many other *Helminthosporium* species were assigned to different genera including but not limited to; *Bipolaris, Curvularia* and *Exserohilum* (132). Most plant pathogens that were formerly classified in the genus *Helminthosporium* are pathogenic on grasses (e.g. *Setosphaeria, Cochliobolus*) (100). Currently, species in the genus *Helminthosporium* have pleurogenous, distoseptate conidia with conidial scars consisting of simple, flat-ringed pores and conidia are acropleurogenously borne on septate, erect conidiophores which terminate growth after development of terminal conidia (132). As of December 2016, the MycoBank reported an accepted 46 species to the genus *Helminthosporium*, however, little sequence data are available on these species (132).

1.3.2.2 Host range

Species in the genus *Helminthosporium* compose a small group of pathogens that mainly infect woody substrates (81). *Helminthosporium solani* is reported to only infect potatoes (26, 129, 100, 9). A field and growth room study reported that of 12 weed and 19 crop species tested, potato was observed to be the only host of *H. solani* (9).

1.3.2.3 Fungal morphology

Helminthosporium solani produces vegetative hyphae known as mycelium, and septate conidiophores with conidia produced in whorls that may be visible to the naked eye (66). An extracellular sheath surround *H. solani* hyphae developing over the surface of tubers (83). Conidia are phragmosporous, acropleurogenous, 7-11 x 24-85 µm, dark brown to black, two to eight septate, with a distinct dark scar at the base and tapered at the apex (66, 81). *In vitro*, pure isolates of *H. solani* appear black in color (110). Pure cultures of *H. solani* may still have saltation (sectoring or disassociation) in the culture and the appearance of morphologically different sectors of the fungal colony (86). Saltation can be the result of difference in compaction or thickness of medium causing differences in topography, sporulation, and colony growth rate (86).

Most isolates of *H. solani* grown *in vitro* have consistent white sectoring and rings, differential coloration, and reduced sporulation (110). It was originally reported that the saltation in culture was natural fungal morphology (92). The cause was later identified by scanning electron microscopy, which confirmed the presence of a mycoparasite, *Acremonium strictum* (*A. strictum*) (110). Additional hyphal tip isolation techniques are required to separate *H. solani* from *A. strictum*, in vitro.

1.3.4 LIFE CYCLE AND EPIDEMIOLOGY

The seasonality of *H. solani* is characteristically polycyclic (56) and may complete several disease cycles with favorable conditions in storage (28). Disease may spread in the field due to conidia from lesions on progeny-tubers infecting other progeny-tubers (46). However, the role of soilborne inoculum on disease incidence is inconclusive (46). *Helminthosporium solani* is a saprotroph (1) with a necrotrophic feeding relationship and only infects the periderm and cortex of the tuber tissue (77, 129, 100, 28). *Helminthosporium solani* enters the tuber primarily *via* hyphae but, germ tubes are capable of direct penetration (83). Infected host cell walls are lysed at the point of fungal entry indicative of mechanical and enzymatic processes that are responsible for periderm penetration (83). *Helminthosporium solani* hyphae were intracellularly present in the periderm and cortical cells 9 hours after tuber inoculation (ATI), host cells are invaded and neighboring cells show signs of necrosis (e.g. absence typical organelles, collapsed peridermal cells, and disrupted cytoplasm) not observable in healthy tubers 2 days ATI, the infection cycle is completed with conidiophores emerging from peridermal cells by direct eruption of the host cell walls 4 days ATI (83). When scanning electron microscopy was used to observe the development of *H. solani* on excised potato tuber periderm incubated in darkness and high humidity at 20-24^{o}C spore germination occurred within 16 h with appressoria formation after 2 d, penetration of the periderm after 4 d, conidiophores with early conidia within 7 d and mature conidia after 9 d post inoculation (55). Histological studies reveal *H. solani* hyphae in the phellem, phelloderm, and cortex of infected potato tubers (55). *Helminthosporium solani* is considered a storage disease because this is when infection is most severe but, a majority of infections occur prior to harvest (55). Infection of the tuber by *H. solani* may occur during the growing season due to contaminated seed tubers or from inoculum in the soil (86). When an infected seed tuber is planted, conidia from the *H. solani*

lesions may spread to the progeny-tubers, with infection occurring as soon as 10-11 weeks after planting (46, 43). Tubers become increasingly susceptible to *H. solani* infection during maturation of the periderm after vine death (49). There are high rates of infection for daughter tubers of infected seed pieces (77). Infection begins with conidia arriving on the tuber periderm through wind, soil, or water (131). High humidity in the fall can result in higher disease incidence of silver scurf (10). It has been reported that elevated total precipitation results in a higher total number of *H. solani* infected tubers and infections are thus more severe (127). Silver scurf infection also increases when soil temperature decreased at a depth of 10cm and is more apparent on lighter rather than heavier soils (127). Infection may begin or worsen in storage due to secondary inoculum created from the circulation of conidia and additional sporulation in storage bins (10). In North Dakota, spore samplers were placed in seed and table stock storages (4^{o}C) with 0 to 12,000 conidia collected each day, and in processing storages (10^{o}C) with 0 to 24,000 conidia collected each day (112). *Helminthosporium solani* conidia can survive in storage bins until the following season if they are not thoroughly cleaned and sanitized (98). When free moisture is present on the tuber, conidia enter through tuber lenticels or direct penetration of the periderm (83). Storage conditions capable of causing infection include humidity levels are greater than 90% (especially 95%) and temperatures above 3°C (49). The optimal temperature for infection in storage is 15-20^{o}C (129). The percentage of tuber surface infected by *H. solani* can increase from 3.5% to 35.5%, while the number of lesions can increase from 6% to 35% during six months of storage (28). *Helminthosporium solani* can overwinter in naturally infested soil for at least one year and subsequently can result in 61% of the tubers harvested infected through natural soilborne inoculum (88). Infection of *H. solani* has been observed at harvest on pathogen free seed-tubers planted in fields that had no history of potato in the previous 1 to 4 years (9). Moreover, *in vitro* colonization

and sporulation of *H. solani* was observed on senescent tissue of various crops (e.g. alfalfa, sorghum, rye, oats, corn, and wheat) and only colonized senescent tissue of rapeseed, red clover, and buckwheat (9).

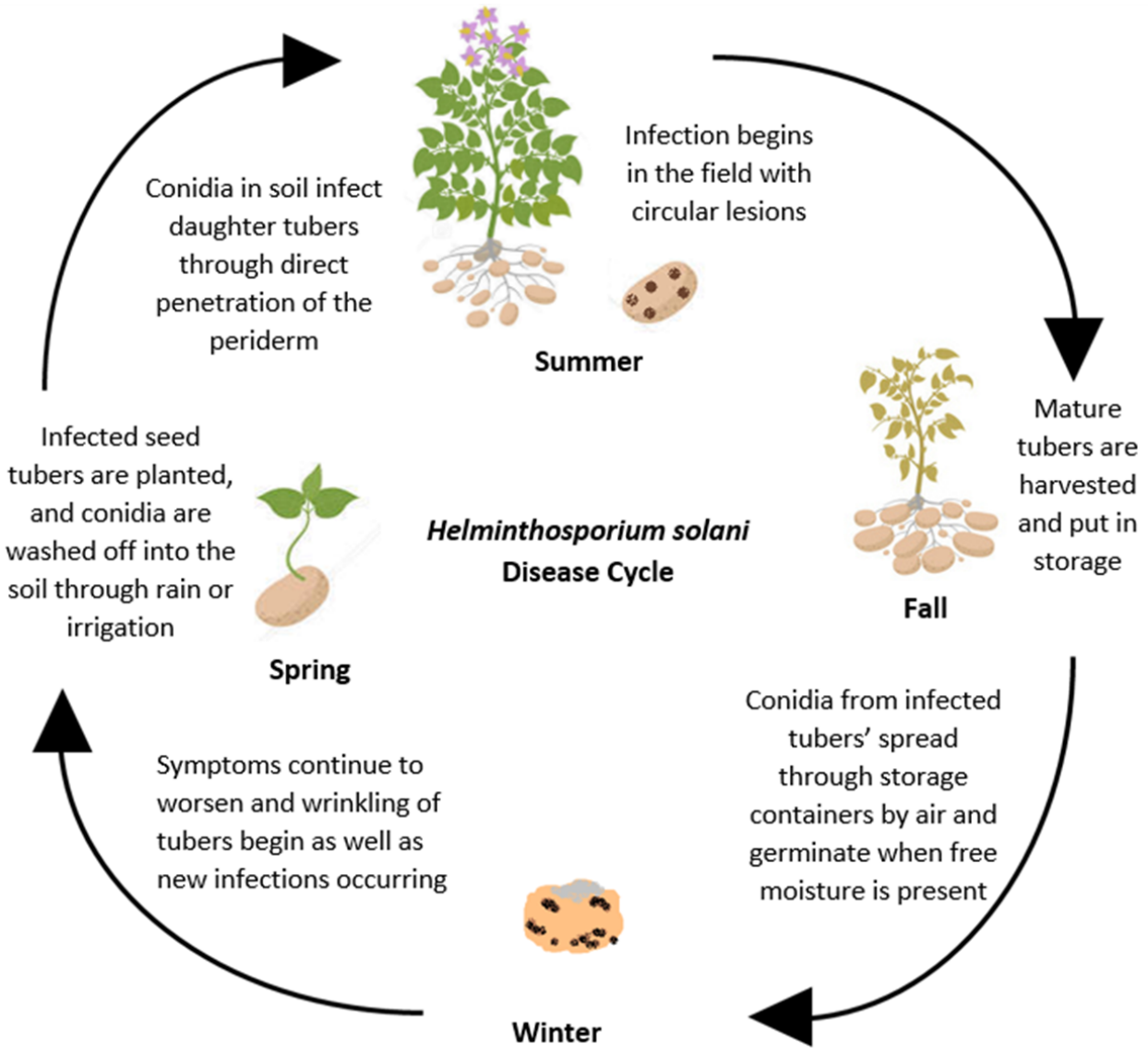

Figure 1.2. The disease cycle for *Helminthosporium solani* shows field infection initiated by direct penetration of the tuber periderm, followed by worsening symptoms of diseased tubers in storage bins. Secondary inoculum (conidia) develops on previously infected tubers and spread throughout the storage bin. Conidia germinate in the presence of free moisture and disease develops while prior symptoms worsen. Infected seed tubers are then planted in the spring, continuing the disease cycle by spreading inoculum through soil, wind, and water.

1.3.5 SILVER SCURF SYMPTOMS

Symptoms in the field begin to appear quickly after tuber initiation on progeny tubers (27). *Helminthosporium solani* infects the tuber periderm by direct penetration with the assistance of cell wall degrading enzymes (19). Infection results in superficial tan to silvery gray to occasional black lesions on the tuber periderm; lesions may appear black and sooty when the pathogen is sporulating (57). Lesions on red varieties may appear as bleached blotches (13). Initial field infection results in slowly developing lesions (27) that are separate and circular or irregular with a defined margin and tend to grow toward the stolon end of the tuber (49). Individual lesions may cover most of the tuber periderm and will enlarge in storage, until they coalesce (58, 9). When infection occurs in storage, lesions develop faster than in the field (27) and are typically more circular in shape (125). The common name, silver scurf, comes from the appearance of mature lesions on tubers that appear silvery when moist (10, 38). The silver appearance is a result of light being reflecting by air trapped under the loosened periderm tissue (101). *Helminthosporium solani* feeds on the tuber periderm, leaving space between the periderm and tuber cortex, resulting in a silvery appearance (13). The silvery appearance can also be a result of loss of pigmentation and/or suberin deposits (41). Severe infections can cause complete loss of pigmentation in the periderm, especially in red and purple colored varieties (13). In severe infections, colonization of the periderm and cortex cells causes cortex cell death and results in water loss from tuber medullary cells (28). Sloughing off of the periderm may also occur as a result of degradation to the underlying tissue (cortex) by cell wall degrading enzymes including glycoside hydrolases (84, 91). The loss of water and healthy tuber periderm can result in yield losses of up to 13% (90). Increased dehydration and severe symptoms typically appear around three months of storage (49). Overall,

H. solani infection can result in lesions on the tuber's periderm, sloughing off of the periderm and moisture loss with shriveling and wrinkling (48, 38, 28).

1.3.6 FUNGICIDE RESISTANCE IN *HELMINTHOSPORIUM SOLANI*

In 1968, thiabendazole (TBZ) was labeled for disease management on potatoes, including *H. solani* (77). Thiabendazole is applied as a seed and post-harvest treatment for a range of pathogens on many plant species and was found to be effective on silver scurf (32, 48). Resistance was reported in the UK in 1988, and subsequently in the US and Canada (85). Therefore, management strategies for *H. solani* are difficult due to the development of insensitivity to thiabendazole (TBZ) and other benzimidazole fungicides. The mode of action (MOA) of benzimidazole is through the binding of the β-tubulin protein in the fungus and thus prevents microtubule function to support and shape cells and provide a route for organelles to move within the cytoplasm (71). The mutation in the β-tubulin gene causes substitution within the β-tubulin molecule due to a SNP in codons 198 or 200 (71). There have also been reports that *H. solani* has reduced sensitivity to demethylation inhibitors (DMI), phenylpyrroles (PP) and, succinate dehydrogenase inhibitors (SDHI) fungicides (77). Reduction in efficacy of fungicides may be compounded due to improper application timing, poor spray coverage, use of ineffective fungicides, and/or a combination of these (89). The effective concentration to inhibit 50% growth (EC_{50}) of 'Highly Resistant' isolates have been reported to be greater than 1000 mg/L (37). Fungicide resistance may develop at varying rates depending on the fungicide properties (chemistry and mode of action), pathogen characteristics (reproductive rate and genetic adaptability), and ecological features (host traits and environmental conditions) (54).

Demethylase inhibitor fungicides are used for the management of a wide spectrum of pathogens including: Ascomycetes; Basidiomycetes; Deuteromycetes, and Oomycetes (32, 54).

This class of fungicides is site-specific, and its MOA disrupts sterol biosynthesis in membranes, with a target site of sterol 14α-demethylase (CYP51), an essential regulatory enzyme in the ergosterol biosynthetic pathway (54). The DMI fungicide difenoconazole is used for management of *H. solani*. Prior to widespread use, the mean sensitivity of *H. solani* isolated from seed tubers from Russia and Germany to difenoconazole was EC_{50}<0.12mg/L (37). Subsequently after widespread use of difenoconazole, *H. solani* isolates recovered from 2013-2016 from Russia, Germany, and the Netherlands had a range of EC_{50} values of 0.05 to 0.13mg/L (37). Reduced sensitivity to DMI fungicides has been reported in *Blumeria graminis,* the causal agent of powdery mildew, and *Venturia inequalis,* the causal agent of apple scab (32). The development of resistance to DMI fungicides is slow due to directional selection. Therefore, close monitoring of pathogen sensitivity is important to prolong fungicide effectiveness (32). However, difenoconazole is still highly effective for management of *H. solani* (37, 77).

A common PP fungicide is fludioxonil and is one of few registered pesticides for the management of dry rot caused by *Fusarium* spp. in potatoes, resulting in its overuse (98). Fludioxonil is also labeled for use on *H. solani* (e.g. CruiserMaxx Potato seed treatment) (124). Fludioxonil is site-specific, and its MOA is in signal transduction, with a target site of MAP/Histidine-Kinase in osmotic transduction (*os*-2, *HOG*1) (23). The interaction of fludioxonil and the target site is poorly defined but, involves group III hybrid histidine kinase (HHK), sensor kinases that adjust environmental stress response pathways and the high osmolarity glycerol (HOG) pathway (15). Fludioxonil does not act directly on HHK but activates the conversion of the kinase to a phosphatase, resulting in dephosphorylation of Ypd1 that constitutively activates HOG signaling, resulting in cell-cycle arrest, glycerol accumulation, cell swelling, and finally rupture (15).

Recently, a class of fungicides known as succinate dehydrogenase inhibitors (SDHI) were commercialized. Prior to 2003, SDHIs were only used to control basidiomycetes but, replacing the 1,4-oxathiin ring by a pyridine moiety and simultaneously introducing a phenyl group in the 2' position of the anilide ring allowed for its activity on various ascomycete fungi (71). This class of fungicides is site-specific, its MOA is in the inhibition of mitochondrial respiration, with a target site of complex II: succinate dehydrogenase (SDH), and the site of action is the ubiquinone binding pocket (Q-site) (71). Complex II is part of the mitochondrial respiration chain and is a link between mitochondrial respiration and the Krebs cycle (56, 71). Targeting the succinate dehydrogenase complex blocks the citric acid cycle and ATP production required for fungal respiration (7). The SDH complex contains a flavoprotein (SDH subunit A), an iron-Sulphur protein (SDH subunit B) and two membrane-anchoring proteins (SDH subunits C and D) (7). Succinate dehydrogenase inhibitors bind to a highly conserved active site containing residues found in subunits B, C and D, where ubiquinone (coenzyme Q_{10}) is reduced to ubiquinol (61). Two registered SDHI fungicides, benzovindiflupyr (Solatenol^{TM}) and sedaxane, are primarily used for management of silver scurf on potato. Non-labeled registered and experimental SDHI fungicides, are also being evaluated for effectiveness at managing silver scurf on potato.

In some fungi, known target site mutations in the succinate dehydrogenase (sdh) gene (5) cause single-site mutations in subunits B, C and D of the SDH complex and are associated with resistance to SDHI fungicides (7). The SDHI fungicides have multiple target site resistance mechanisms in numerous fungal species (e.g. *Botrytis cinerea, Corynespora cassiicola, Alternaria alternata, Alternaria solani, Didymella bryoniae, Podosphaera xanthii* and *Sclerotinia sclerotium* field isolates) (71, 4). Some of the target site resistance mechanisms directly impact the binding behavior of SDHIs while other mutations have been found associated with structural rearrangement

in the transmembrane region of complex II (71). Overall, some mutations have led to full resistance to all labeled SDHI fungicide chemistries, while others have only conferred partial resistance.

Last revised in 2012, the Fungicide Resistance Action Committee has not reported *H. solani* resistance to DMI, PP or SDHI fungicides (22). The baseline sensitivity of pydiflumetofen, benzovindiflupyr, difenoconazole, fludioxonil, and sedaxane, in *H. solani* fungal has not been reported. However, due to the risk of fungicide resistance and the significant economic impact of *H. solani* on potatoes (45), fungicide sensitivity assays are important for the disease management and monitoring of *H. solani* resistance. Fungicide resistance can be managed through the use of fungicides with different MOAs to enable disruptive selection that decreases the probability of resistant mutants and/or population (54).

1.3.7 SILVER SCURF DISEASE MANAGEMENT

Planting certified disease-free seed is the first step in avoiding a common source of *H. solani* inoculum, infected seed tubers (13). Site selection is also an important factor in managing silver scurf and ground that has not grown potatoes for at least 3 years is recommended (125). Good sanitation practices can help manage silver scurf and includes sanitizing all farm equipment and clothing between fields, and storage bins to limit the amount of inoculated soil moving around (112, 87). Storage management practices influence the amount of *H. solani*, and loose soil in the storage bin should be limited to reduce secondary inoculum (101). Maintaining storage bins at the lowest practical temperature, ~3°C for seed and ~6°C for table stock, can assist in managing silver scurf (13, 49). Humidity favors disease development in storage and should be minimized (48, 49). Limiting when stored potatoes are handled or transported out of storage can impact the spore count of *H. solani* significantly in the storage bin (43). *Helminthosporium solani* is primarily exposed to fungicides containing the active ingredients azoxystrobin, thiabendazole, imazalil, prochloraz,

manganese chloride, thiophanate-methyl (TPM) with mancozeb, captan with mancozeb, benomyl, difenoconazole, fludioxonil, sedaxane and benzovindiflupyr (56, 16, 46). Fungicide applications as seed treatments reduce sporulation of infected seed tubers at/after planting and post-harvest treatments extend tuber storability (121). Syngenta® has registered fungicides for management of *H. solani* including seed (CruserMaxx® Potato Extreme, Maxim®PSP, Maxim®MZ PSP, Maxim®D, and Vibrance®Ultra Potato), in-furrow (Elatus®), foliar (Aprovia™ and Quadris®), and post-harvest (Stadium™) treatments (125). Fungicide resistance to benzimidazole and Thiophanate-methyl has been reported in *H. solani* and their use should be avoided to decrease the risk of additional resistant populations (27). In place of fungicides or in addition, soil inoculated with antagonistic bacteria, *Trichoderma viride* (Z 17) and *Bacillus polymyxa* S-13, can significantly reduce silver scurf incidence (45). Overall good plant health also reduces the severity of symptoms and can be accomplished through fertilizer, irrigation and weed management (33). Harvesting potatoes as soon as the skin is set after vine killing can help reduce silver scurf disease incidence (10). Resistant potato varieties, often russet varieties, where resistance develops from production of phenolic compounds in the periderm of the tuber, can also be used (13). However, there are limited consistently effective management strategies and industry accepted and adopted resistant cultivars, resulting in an increase in silver scurf incidence in commercial potato production (57).

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CHAPTER 2: EVALUATION OF CHEMISTRIES ON MANAGEMENT OF VERTICILLIUM WILT IN MICHIGAN POTATO PRODUCTION SYSTEMS

Abstract

The potato production industry has been severely impacted by the wilt disease that decreases tuber yield and quality, Potato Early Die (PED) complex (*Verticillium dahliae* Kleb. and *Pratylenchus penetrans*). The disease is of global importance and is an issue for potato growers in the US. Potatoes in nontreated fields may develop severe infections resulting in marketable yield losses up to 50%. The high cost associated with management, limited availability of effective disease management strategies, and the need for high quality tubers has focused research on managing Verticillium wilt in potato in Michigan and the US. Experimental field trials were conducted in 2018 and 2020 to evaluate chemical management programs of PED applied at five application times: pre-planting/pre-plant incorporated, seed treatment, in-furrow at planting, 2 in emergence, and 7 Days after 2 in emergence. *Verticillium dahliae* propagules in the rhizosphere, root-lesion nematode (RLN) levels in the soil, and disease severity were evaluated. Plots treated with Vydate C-LV reduced populations of *Pratylenchus penetrans* in soil.

2.1 Introduction

Verticillium dahliae Kleb., the primary causal agent of Verticillium wilt and one of the most important soilborne diseases in the world results in loses of billions of dollars across numerous crops and ornamental plants (20). When environmental conditions are favorable, root exudates stimulate microsclerotia (fungal resting structures) or conidia to germinate in rhizosphere soil or its vicinity, penetrate the host roots, then further colonizes the xylem vascular system (43).

Verticillium wilt disease causes overall wilting and early vine maturity, resulting in a reduction in nutrients available for tuber production (e.g. water and water-soluble nutrients) and therefore, reduced yields and quality (114). Due to *Verticillium dahliae* being soilborne and monocyclic, the initial inoculum density of the soil (microsclerotia per g of soil) is an important factor in resulting disease severity and how management practices are implemented (43, 33). On potato (*Solanum tuberosum* L.), quality, tuber size, and specific gravity can be significantly reduced by *V. dahliae* infection (7). *Verticillium dahliae* infection can result in up to 50% reduction in potato yields (21, 2).

The most severe infections of *V. dahliae* on potato may be a result of the potato early die (PED) complex, which is the interaction of the plant pathogen, *Verticillium dahliae*, and the Root Lesion Nematode, *Pratylenchus penetrans* (26). The relationship between *P. penetrans* and *V. dahliae* is considered synergistic due to juvenile and adult nematodes feeding on potato root surfaces and cortex (32), resulting in necrotic tissue that reduces nutrient/water uptake and transport efficiency (13, 19). However, disease severity is hypothesized to be primarily increased by biochemical changes by *P. penetrans* because of lack of evidence for mechanical damage increasing disease severity (40). Verticillium wilt symptoms may be further exacerbated during environmental stresses such as high temperatures and rate of evapotranspiration, drought and lack of nutrients (primarily nitrogen and phosphorous) in the soil (16, 7). When tubers with severe *V. dahliae* infection are processed and fried for chips it can result in a stem-end chip defect which is dark coloring of the vasculature and adjacent tissues at the tuber stem end (42). Potato early die is a major concern because chipping is important in the US, where 21% of potato production is used for chips (4) and of particular importance in Michigan, the largest producer of potatoes for chipping in the county (30).

Management of Verticillium wilt is difficult due to the wide host range of the pathogen, limited effective pesticides, and the production of microsclerotia, a durable resting structure that may persist in soils up to ten years even in the absence of a host (20). Integrated disease management strategies involving cultural strategies (green manure applications (20)), induced resistance and/or resistant potato cultivars, and practices (e.g. crop rotations, soil fertility and irrigation) have not been successful in effectively reducing the amount of *V. dahliae* microsclerotia in the soil and Verticillium wilt disease incidence (1, 2). Soil fumigation with production containing methyl isothiocyanate, 1,3-dichloropropene, or trichloronitromethane, alone or in combination effectively reduced the *V. dahliae* population in the soil (33); however, applications are expensive and negatively impact human and environmental health (28). The combined or sole use of nematicides and/or fungicides at variable application timings may reduce the *P. penetrans* population and quantity of *V. dahliae* microsclerotia in the soil, subsequently reducing Verticillium wilt symptoms and increasing tuber yield. There are few registered fungicides for the suppression of Verticillium wilt (37).

The efficacy of non-systemic fungicides is dependent on accurate placement of the fungicide in relation to the pathogen (9). Therefore, the most effective management strategy for *V. dahliae* would be fungicide applications to the soil. To reduce the density *V. dahliae* inoculum, systemic fungicides that are translocated via xylem or phloem may be necessary (9). Foliar applications of systemic fungicides in the beginning of the growing season may increase yields and provide protection against vascular diseases (9).

Over two growing seasons, chemistries and application placements and timings were evaluated for their ability to reduce *V. dahliae* microsclerotia and *P. penetrans* populations in the soil, decrease the incidence of vascular discoloration of the stem-end of the tuber, and increase

plant stand and marketable tuber yield. Management programs including seed treatment, in-furrow, and foliar applications of fungicides and/or nematicides were tested. An effective management strategy for *V. dahliae* and *P. penetrans* that does not include fumigation, which is environmentally and financially damaging, would be a significant advance for potato growers in Michigan and around the world.

2.2 Materials and Methods

2.2.1 EVALUATION OF VERTICILLIUM WILT MANAGEMENT PROGRAMS

2.2.1.1 Field preparation, planting, and maintenance

A field trial was established at Michigan State University (MSU) Clarksville Research Center (CRC), Clarksville, MI (Capac loam soil); 42°52'29.2"N and longitude - 85°15'09.3"W to evaluate selected in-furrow and foliar fungicides, and nematicides for early die control (Table 2.1 and 2.2). The experimental field trial was established at CRC because the soil type is similar to that of which commercial potatoes in Michigan are grown and the history of potato cultivation and other *Verticillium dahliae* host's provided adequate inoculum density for natural infestation. 'Superior' seed tubers were mechanically cut into approximately 2 oz seed pieces around one week prior to planting and allowed to heal. These trials were conducted using the potato cultivar 'Superior' due to its susceptibility to Verticillium wilt (31) and its commercial use throughout the state of Michigan and the Midwest US potato growing region. Tubers were planted on 2 June 2018 into four-row by 7.62-m plots (~25.4-cm between plants to give a target population of 60 plants at 86.36-cm row spacing) replicated four times in a randomized complete block design. A 1.54-m alley separated the two-row beds. Treatment application timings included: Pre-planting/pre-plant incorporated (A); Seed treatment (B); In-furrow at planting (C); 2-inch emergence (D); and 7 days

after 2-inch emergence (E) (Table 2.3). In-furrow, at planting applications of fungicide were delivered with a hand-held R&D spray boom delivering 10 gal/A (50 p.s.i.) and using one XR8002VR nozzle per row. A non-treated control was compared with 9 different treatment programs to evaluate their efficacy in controlling potato early die (PED) based on application time (Table 2.1). The active ingredients evaluated in this study included: fluensulfone; oxamyl; penflufen + prothioconazole; *Bacillus subtilis*; fluopyram; pyrimethanil + fluopyram; thiamethoxam + fludioxonil; azoxystrobin + benzovindiflupyr, and benzovindiflupyr (Table 2.1). The non-treated control treatment included no in-furrow treatment at planting. Foliar applications were applied with a calibrated backpack sprayer (R & D Inc., Opelousas, LA) in 234 L water/ha at ~550 KPa at 2 in emergence and 7 days after 2 in emergence.

Fertilizer was applied to plots before planting, formulated according to results of soil tests. Additional nitrogen (final N 28 lb/A) was applied to the developing crop with irrigation 45 DAP (days after planting). Bravo WS 6SC 1.5 pt/A was applied on a seven-day interval, for a total of eight applications, for foliar disease control. Weeds were controlled by cultivation, hilling, and with S-metolachlor (Dual II Magnum; Syngenta Crop Protection) at 2.24 L/ha 10 DAP and sethoxydim (Poast; BASF Corporation) at 1.75 L/ha 58 DAP. Vines were killed with diquat dibromide (Reglone 2EC; Syngenta Crop Protection Inc.) applied at 1.2 L/ha ~120 DAP. All plot maintenance applications were applied with a tractor mounted spray boom (R&D Inc. Opelousas, LA) delivering 234 L/ha (550 kPa) and using three XR11003VS nozzles (TeeJet® Technologies. Louisville, KY) per row. Plots were irrigated to supplement precipitation to about 0.63 cm/ha/4 d period with overhead irrigation.

Plots were harvested in early October (~130 DAP) and individual treatments were weighed, graded and tuber interiors evaluated for *Verticillium* symptoms. Meteorological variables were

measured with a Campbell weather station (Campbell Scientific Inc., Logan UT) located at each farm from 1 June to 14 October. Weather data were provided by MSU Enviroweather.

Table 2.1. Products evaluated for effect on Verticillium wilt of potato caused by *Verticillium dahliae* in 2018 study including product name, active ingredient, mode of action, chemical group name, and FRAC code group

Product Name	Active Ingredient (s)	Mode of Action (MOA)	Group Name	FRAC[a] Code
Nimitz	fluensulfone	unknown	fluoroalkenyl thioether group	nematicide N/A
Vydate C-LV	oxamyl	acetylcholinesterase (ACHE) inhibitor	carbamate	1A (insecticide)
Emesto Silver	penflufen + prothioconazole	respiration + sterol biosynthesis in membranes	succinate dehydrogenase inhibitors (SDHI) + demethylation inhibitors (DMI)	7 + 3
Serenade Soil	QST 713 strain of *Bacillus subtilis**	host plant defense induction	microbial	P 06
Velum Prime	fluopyram	respiration	succinate dehydrogenase inhibitor (SDHI)	7
Luna Tranquility	pyrimethanil + fluopyram	amino acid and protein synthesis + respiration	anilinopyrimidine (AP) + succinate dehydrogenase inhibitor (SDHI)	9 + 7
CruiserMaxx Potato	thiamethoxam + fludioxonil	nicotinic acetylcholine receptor (NACHR) competitive modulators + nucleic acids metabolism + signal transduction	neonicotinoids + phenyl amides (PA) + phenylpyrroles (PP)	4A (insecticide) + 4 + 12
Elatus	azoxystrobin + benzovindiflupyr	respiration + respiration	quinone outside inhibitors (QoI) + succinate dehydrogenase inhibitors (SDHI)	11 + 7
Aprovia	benzovindiflupyr	respiration	succinate dehydrogenase inhibitors (SDHI)	7

[a] FRAC = Fungicide Resistance Action Committee

*Contains a minimum of 1 x 10^9 cfu/g

Table 2.2. Products evaluated for effect on Verticillium wilt of potato caused by *Verticillium dahliae* in 2020 study including product name, active ingredient, mode of action, chemical group name, and FRAC code group

Product Name	Active Ingredient(s)	Mode of Action (MOA)	Group Name	FRAC[a] Code
Vydate C-LV	oxamyl	acetylcholinesterase (ACHE) inhibitor	carbamate	1A (insecticide)
Nimitz	fluensulfone	unknown	fluoroalkenyl thioether group	nematicide N/A
CruiserMaxx Potato	thiamethoxam + fludioxonil	nicotinic acetylcholine receptor (NACHR) competitive modulators + nucleic acids metabolism + signal transduction	neonicotinoids + phenyl amides (PA) + phenylpyrroles (PP)	4A (insecticide) + 4 + 12
Elatus	azoxystrobin + benzovindiflupyr	respiration + respiration	quinone outside inhibitors (QoI) + succinate dehydrogenase inhibitors (SDHI)	11 + 7
Aprovia	benzovindiflupyr	respiration	succinate dehydrogenase inhibitors (SDHI)	7
Emesto Silver	penflufen + prothioconazole	respiration + sterol biosynthesis in membranes	succinate dehydrogenase inhibitors (SDHI) + demethylation inhibitors (DMI)	7 + 3
Velum Prime	fluopyram	respiration	succinate dehydrogenase inhibitor (SDHI)	7
Luna Tranquility	pyrimethanil + fluopyram	amino acid and protein synthesis + respiration	Anilinopyrimidine (AP) + succinate dehydrogenase inhibitor (SDHI)	9+7
Serenade Soil	QST 713 strain of *Bacillus subtilis**	host plant defense induction	microbial	P 06

[a] FRAC = Fungicide Resistance Action Committee.

*Contains a minimum of 1 x 10^9 cfu/g

Table 2.3. Treatment programs used in the evaluation of Verticillium wilt management programs trial, times of each application, and year applied

Treatment and Rate	Time of Application	Year Applied
Non-treated Control		2018 & 2020
Nimitz 3.5 pt/a	Pre-planting/pre-plant incorporated	2018
Nimitz 7 pt/a	Pre-planting/pre-plant incorporated	2018
Nimitz 3.5 pt/a	In-furrow at planting	2018 & 2020
Nimitz 7 pt/a	In-furrow at planting	2018 & 2020
Vydate C-LV 725 g ai/ha Vydate C-LV 362.4 g ai/ha	In-furrow at planting 2-inch emergence & 7 days after 2-inch emergence	2018 & 2020
CruiserMaxx Potato 0.31 fl oz/cwt Elatus 7.7 oz/A	Seed treatment 2-inch emergence	2018 & 2020
CruiserMaxx Potato 0.31 fl oz/cwt Aprovia 0.75 l/a	Seed treatment In-furrow at planting	2018 & 2020
Emesto Silver 0.31 fl oz/cwt Serenade Soil 1 qt/a Velum Prime 237.5 g ai/ha Luna Tranquility 409 g ai/ha	Seed treatment In-furrow at planting In-furrow at planting 2-inch emergence	2018
Emesto Silver 0.31 fl oz/cwt Velum Prime 237.5 g ai/ha Luna Tranquility 409 g ai/ha	Seed treatment In-furrow at planting 2-inch emergence	2018
Emesto Silver 0.31 fl oz/cwt Velum Prime 237.5 g ai/ha Luna Tranquility 409 g ai/ha	Seed treatment In-furrow at planting 2-inch emergence & 7 days after 2-inch emergence	2020

Table 2.3. (cont'd)

Emesto Silver 0.31 fl oz/cwt	Seed treatment	2020
Serenade Soil 1 qt/a	In-furrow at planting	
Velum Prime 237.5 g ai/ha	In-furrow at planting	
Luna Tranquility 409 g ai/ha	2-inch emergence & 7 days after 2-inch emergence	
Emesto Silver 0.31 fl oz/cwt	Seed treatment	2020
Vydate C-LV 1450 g ai/ha	In-furrow at planting	
Velum Prime 237.5 g ai/ha	In-furrow at planting	
Luna Tranquility 409 g ai/ha	2-inch emergence & 7 days after 2-inch emergence	
Emesto Silver 0.31 fl oz/cwt	Seed treatment	2020
Vydate C-LV 1450 g ai/ha	In-furrow at planting	
Vydate C-LV 724 g ai/ha	2-inch emergence	
Luna Tranquility 409 g ai/ha	2-inch emergence & 7 days after 2-inch emergence	

2.2.1.2 Soil sampling and *Verticillium dahliae* colony forming units (CFU) in soil

Soil samples were taken from each plot at planting in 2018 and 2020, with an additional sample taken at ~60 DAP in 2020. Five samples from each plot row (ten total) were collected with a 25 mm JMC soil corer (Clements Assoc., Newton, IA) to a depth of 100 mm and blended in a one-gallon sample bag for total of ~1000 g soil per sample. Soil samples were sent to MSU Plant and Pest Diagnostics to determine microsclerotia populations of *Verticillium dahliae* colony forming units (CFUs) per gram of soil in each plot.

2.2.1.3 Percent plant emergence, rate of emergence, yield

Plant stand was rated ~28, 35, and 42 DAP and percent plant emergence was expressed as a percentage of the target population of 120 plants/100ft. row from a sample of 1 x 25 ft rows per plot. Rate of emergence was calculated as the Relative Area Under the Emergence Progress Curve [RAUEPC from ~0–42 DAP, maximum value = 1.00]. Plots (1 x 25-ft row) were machine-harvested ~130 DAP. Individual treatments were weighed for a total, graded into US#1 and B sizes then weighed, respectively. Weights taken in pounds were transformed for yield (CWT).

2.2.1.4 Progression of *Verticillium* wilt and disease severity rating

Plots were not inoculated and relied on natural soil infestation of *Verticillium dahliae* for disease establishment. Severity of Potato Early Die (PED) was measured using the Horsfall-Barratt rating scale of 0 (no infection) to 11 (all foliage and stems dead). Severity of PED was rated ~70, 77, 84, 91, 98, 105, and 112 DAP and the relative rate of disease progression was calculated as the Relative Area Under the Disease Progress Curve [RAUDPC from 0–111 DAP, maximum value = 1.00].

2.2.1.5 Nematode sampling and extraction

Soil and root samples were collected at planting and ~60 DAP in 2018, but only soil samples were collected in 2020. Samples were sent to MSU Plant and Pest Diagnostics to

determine population levels of Root-lesion nematode (RLN) (*Pratylenchus penetrans*) prior to and after in-furrow and foliar applications. Samples were collected and stored using protocols established by the Michigan State University Plant Diagnostic Clinic.

2.2.1.6 Vascular discoloration evaluation and trial repetition

Following harvest, 10 tubers from each plot were evaluated for stem-end vascular discoloration. The tubers were sliced in half and 0.5 cm from the stem end and opposite end to expose the vascular ring. Tubers severely diseases with *Verticillium* wilt develop stem-end discoloration. The total number of tubers from each plot were collected to determine an average of *V. dahliae* tuber symptoms (%) present for each treatment. This trial was repeated in 2020 within the same trial area with the same potato cultivar and crop maintenance. In 2020, five of the same treatments were replicated from 2018 and, two treatments were similar but, with an additional application of Luna Tranquility at application timing (E), and novel two treatments (Table 2.3).

2.2.1.7 Data collection and analysis

Data were subjected to analysis of variance (ANOVA). Means separation was calculated using Fisher's Least Significance Difference (LSD α=0.10). The data analysis for this paper was generated using SAS software.

2.3 Results

2.3.1 EVALUATION OF VERTICILLIUM WILT MANAGEMENT PROGRAMS

The initial trial was established at CRC in 2018. Weather was conducive for disease (Table 2.4). No treatments differed significantly in % emergence (Table 2.6). No treatment differed significantly in RAUEPC (0-46 DAP) (Table 2.6). Evaluation of *V. dahliae* CFU/g soil sampled at planting ranged from 1.4 to 6.1 CFU/g soil with no significant differences (Table 2.6). Analysis of root-lesion nematode (RLN) populations in soil sampled at planting revealed treatments: Nimitz 7 pt/a (A); Emesto Silver (B) + Velum prime (C) + Luna Tranquility (D); and CruiserMaxx Potato (B) + Aprovia (C); were significantly lower compared to the NTC (Table 2.6). No treatment differed significantly from the NTC in root-lesion nematode (RLN) populations in soil at the sampling time of 14 days after time (D) applications (~60 DAP). No treatment differed significantly from the NTC in root-lesion nematode (RLN) populations in roots at the sampling time of 14 days after time (D) applications (~60 DAP). Analysis of disease severity (wilt symptoms) on a 0-11 Horsfall-Barrett scale revealed that no treatments were significantly different at 69 and 76 DAP (Table 2.5). At 83 DAP treatment: CruiserMaxx Potato (B) + Elatus (D) had significantly lower disease severity compared to the NTC (Table 2.5). At 90 DAP treatments: CruiserMaxx Potato (B) + Elatus (D); and CruiserMaxx Potato (B) + Aprovia (C); had significantly lower disease severity compared to the NTC. At 97 DAP treatments: Nimitz 7 pt/a (A); Emesto Silver (B) + Serenade Soil (C) + Velum prime (C) + Luna Tranquility (D); CruiserMaxx Potato (B) + Elatus (D); and CruiserMaxx Potato (B) + Aprovia (C); had significantly lower disease severity compared to the NTC. At 104 DAP the treatments that had significantly lower disease severity compared to the NTC included: Nimitz 7 pt/a (A); Emesto Silver (B) + Serenade Soil (C) + Velum prime (C) + Luna Tranquility (D); Emesto Silver (B) + Velum prime (C) + Luna

Tranquility (D); CruiserMaxx Potato (B) + Elatus (D); and CruiserMaxx Potato (B) + Aprovia (C);. At 111 DAP treatments that had significantly lower disease severity compared to the NTC included: Emesto Silver (B) + Serenade Soil (C) + Velum prime (C) + Luna Tranquility (D); and CruiserMaxx Potato (B) + Elatus (D);. Evaluation of the RAUDPC (0-111 DAP) revealed that treatments with significantly lower rate of disease compared to the NTC included: Emesto Silver (B) + Serenade Soil (C) + Velum prime (C) + Luna Tranquility (D); and CruiserMaxx Potato (B) + Elatus (D); (Table 2.5). Analysis of yield in CWT revealed that no treatments differed significantly in total, US#1, or B-size yield (Table 2.6). Evaluation of percentage of tubers with vascular discoloration revealed no significant differences (Table 2.6).

The trial was repeated at CRC in 2020. Weather data was conducive for disease (Table 2.7). No treatments differed significantly in % emergence (Table 2.9). No treatment differed significantly in RAUEPC (0-39 DAP) (Table 2.9). Evaluation of *V. dahliae* CFU/g soil sampled at planting ranged from 3.9 to 16.0 CFU/g soil with no significant differences in treatments (Table 2.9). Evaluation of *V. dahliae* CFU/g soil at the sampling time of 14 days after time (D) applications (~60 DAP) ranged from 4.6 to 15.1 CFU/g soil with no significant differences in treatments. Analysis of root-lesion nematode (RLN) populations in soil sampled at planting revealed treatments were significantly higher compared to the NTC included: Vydate C-LV (C, D & E); Nimitz 3.5 pt/a (C); CruiserMaxx Potato (B) + Aprovia (C); and Emesto Silver (B) + Velum prime (C) + Luna Tranquility (D & E); (Table 2.9). No treatments differed significantly in root-lesion nematode (RLN) populations in soil at the sampling time of 14 days after time (D) applications (~60 DAP). Analysis of disease severity (wilt symptoms) on a 0-11 Horsfall-Barrett scale revealed that no treatments differed significantly at any interval after planting (Table 2.8). Evaluation of the RAUDPC (0-109 DAP) revealed that no treatments differed significantly (Table

2.98. Analysis of yield in CWT revealed that total, US#1, and B size yield for all treatments was numerically lower in 2020 compared to 2018. Analysis of yield in CWT also revealed that no treatments differed significantly in total or US#1 size yield, but all treatments except for: CruiserMaxx Potato (B) + Aprovia (C); and Emesto Silver (B) + Vydate C-LV (C) + Velum prime (C) + Luna Tranquility (D & E); were significantly higher in B size yield compared to the NTC (Table 2.9). Evaluation of percentage of tubers with vascular discoloration revealed no significant differences (Table 2.9).

Table 2.4. Mean daily air temperature, mean relative humidity, mean daily soil temperature, and total monthly precipitation data for Clarksville Research Center (Clarksville, MI) in 2018

	Mean Daily Air Temp. (ºC) [Days>30ºC]	Mean Daily Relative Humidity[a] (%)	Mean Daily Soil Temp. (ºC at 10-cm Depth)	Total Monthly Precipitation (cm)
May	20.2 [0]	70.6	20.6	5.2
June	22.6 [2]	66.8	27.3	3.1
July	21.7 [5]	72.2	24.9	13.8
August	17.9 [0]	76.9	22.4	6.6
September[b]	13.4 [0]	80.9	15.7	11.7

[a] % of field capacity

[b] Value for September through October 14 respectively

Table 2.5. Effects of in-furrow, at planting, and foliar treatments on severity of *Verticillium* wilt and rate of disease progression data for Clarksville Research Center (Clarksville, MI) in 2018

Treatment and rate[a]	PED[b] 10 Aug 69 DAP[c]	PED 17 Aug 76 DAP	PED 24 Aug 83 DAP[d]	PED 31 Aug 90 DAP	PED 7 Sep 97 DAP	PED 14 Sep 104 DAP	PED 21 Sep 111 DAP	RAUDPC[e] 0 – 111 DAP
Non-Treated	2.68	9.37	15.81 abc	26.58 bcd	60.94 abc	87.11 a	95.01 ab	0.418 a-d
Nimitz 3.5 pt/a (A)	5.60	9.37	15.49 abc	40.85 ab	64.45 ab	91.80 a	97.15 a	0.477 ab
Nimitz 7 pt/a (A)	4.68	3.51	13.55 bcd	18.87 cde	32.81 de	56.25 b-e	93.16 ab	0.300 cde
Nimitz 3.5 pt/a (C)	4.68	15.23	16.09 abc	37.00 abc	52.35 bcd	69.14 a-d	89.16 abc	0.416 a-d
Nimitz 7 pt/a (C)	4.68	17.56	22.47 ab	43.64 ab	58.20 abc	69.15 a-d	93.42 ab	0.448 abc
Vydate C-LV 725 g ai/ha (C) Vydate C-LV 362.4 g ai/ha (D) Vydate C-LV 362.4 g ai/ha (E)	2.68	5.27	15.81 abc	34.14 abc	54.68 bcd	81.25 ab	95.97 ab	0.413 bcd
Emesto Silver 0.31 fl oz/cwt (B) Serenade Soil 1 qt/a (C) Velum prime 237.5 g ai/ha (C) Luna Tranquility 409 g ai/ha (D)	1.38	4.10	6.68 cd	15.81 de	23.44 e	45.31 de	79.78 c	0.228 e
Emesto Silver 0.31 fl oz/cwt (B) Velum prime 237.5 g ai/ha (C) Luna Tranquility 409 g ai/ha (D	4.68	11.72	15.81 abc	26.58 bcd	39.06 cde	50.00 cde	91.33 abc	0.321 cde
CruiserMaxx[f] 0.31 oz/CWT (B) Elatus 7.7 oz/A (D)	2.22	2.93	5.60 d	11.31 e	19.92 e	42.97 e	82.01 c	0.209 e
CruiserMaxx 0.31 oz/CWT (B) Aprovia 0.75 l/a (C)	2.68	5.86	11.95 bcd	11.52 e	22.26 e	60.94 b-e	88.12 bc	0.284 de
Pr>F	0.28	0.16	0.04	<0.01	<0.01	0.02	0.02	<0.01

[a]Application time; A=Pre-planting/pre-plant incorporated; B=Seed treatment; C=In-furrow at planting; D=2" emergence; E=7 Days after 2" emergence.

Table 2.5 (cont'd)

[b] PED=Potato Early Die severity rated on a Horsfall-Barratt scale of 0 (no infection) to 11 (all foliage and stems dead). Ratings were converted to percentages.

[c] DAP = days after planting on 2 June.

[d] Means followed by same letter are not significantly different at $P = 0.10$ (Fishers LSD).

[e] RAUDPC=relative area under the disease progress curve from planting to 111 days after planting.

[f] Cruisermaxx=Cruisermaxx potato seed treatment.

Table 2.6. Effects of in-furrow, at planting, and foliar treatments on percent plant emergence, rate of emergence, total and marketable yield in hundred-weight per acre, vascular discoloration of tubers, *Verticillium dahliae* colony forming units (CFU) in soil, and root lesion nematode population data for Clarksville Research Center (Clarksville, MI) in 2018

			Yield (CWT)					RLN[g]		
Treatment[a]	**Plant stand[b] 46 DAP[c] (%)**	**RAUEPC[d] 0 – 46 DAP**	**Total**	**US #1**	**B Size**	**VD[e] (%)**	**Average CFU/g of Soil[f] 30 May**	**Soil 30 May[h]**	**Soil 1 Aug**	**Root 1 Aug**
Non-Treated	70.5	0.677	329.0	260.8	67.3	33	5.7	10.6 ab	8.2	1.5
Nimitz 3.5 pt/a (A)	76.1	0.721	388.0	312.3	75.1	58	2.3	4.4 bcd	3.5	0
Nimitz 7 pt/a (A)	74.2	0.715	345.0	274.4	67.2	55	5.6	2.2 d	3.8	1.5
Nimitz 3.5 pt/a (C)	77.4	0.736	282.3	218.8	63.3	53	4.5	10.4 ab	7.3	1
Nimitz 7 pt/a (C)	81.5	0.782	304.0	232.8	70.4	53	4.1	8.5 abc	5.7	0
Vydate C-LV 725 g ai/ha (C) Vydate C-LV 362.4 g ai/ha (D) Vydate C-LV 362.4 g ai/ha (E)	72.4	0.690	327.1	268.8	58.1	43	7.6	15.7 a	2.7	0.5
Emesto Silver 0.31 fl oz/cwt (B) Serenade Soil 1 qt/a (C) Velum prime 237.5 g ai/ha (C) Luna Tranquility 409 g ai/ha (D)	69.4	0.665	292.6	228.4	63.9	65	3.8	2.2 a	3.9	0
Emesto Silver 0.31 fl oz/cwt (B) Velum prime 237.5 g ai/ha (C) Luna Tranquility 409 g ai/ha (D	72.3	0.691	295.0	239.7	54.2	38	1.7	1.6 d	3.3	0.5
CruiserMaxx[i] 0.31 oz/CWT (B) Elatus 7.7 oz/A (D)	69.2	0.670	363.0	291.8	69.7	63	5.1	4.1 bcd	2.7	0
CruiserMaxx 0.31 oz/CWT (B) Aprovia 0.75 l/a (C)	64.7	0.621	285.5	218.2	66.9	55	6.1	2.5 cd	2.3	0
Pr>F	0.81	0.76	0.33	0.24	0.66	0.31	0.76	0.06	0.60	0.34

Table 2.6. (cont'd)

[a]Application time; A=Pre-planting/pre-plant incorporated; B=Seed treatment; C=In-furrow at planting; D=2" emergence; E=7 Days after 2" emergence.

[b] Plant stand expressed as a percentage of the target population of 120 plants/100ft. row from a sample of 1 x 25 ft rows per plot.

[c] DAP = days after planting on 2 June.

[d] RAUEPC=relative area under the emergence progress curve from planting to 46 days after planting.

[e] VD=Vascular discoloration of the stem end; percentage calculated from 10 tubers.

[f] CFU=colony forming units seen on selective *Verticillium dahliae* media.

[g] RLN=root lesion nematode, *Pratylenchus penetrans.*

[h] Means followed by same letter are not significantly different at $P = 0.10$ (Fishers LSD).

[i] Cruisermaxx=Cruisermaxx potato seed treatment.

Table 2.7. Mean daily air temperature, mean relative humidity, mean daily soil temperature, and total monthly precipitation data for Clarksville Research Center (Clarksville, MI) in 2020

	Mean Daily Air Temp. (ºC) [Days>30ºC]	**Mean Daily Relative Humidity[a] (%)**	**Mean Daily Soil Temp. (ºC at 10-cm Depth)**	**Total Monthly Precipitation (cm)**
May	13.1 [2]	66.0	12.5	11.5
June	20.3 [4]	66.7	17.4	9.0
July	23.6 [11]	70.9	22.2	5.2
August	21.4 [8]	70.4	22.2	8.3
September[b]	14.4 [0]	72.7	17.8	9.2

[a] % of field capacity

[b] Value for September through October 14 respectively

Table 2.8. Effects of in-furrow, at planting, and foliar treatments on severity of Verticillium wilt and rate of disease progression data for Clarksville Research Center (Clarksville, MI) in 2020

Treatment and rate[a]	PED[b] 4 Aug 65 DAP[c]	PED 10 Aug 72 DAP	PED 17 Aug 79 DAP	PED 24 Aug 86 DAP	PED 31 Aug 93 DAP	PED 8 Sep 101 DAP	PED 15 Sep 109 DAP[e]	RAUDPC[d] 0 – 109 DAP
Non-Treated	1.38	10.55	13.46	13.46	36.72	90.63	99.70	0.379
Vydate C-LV 725 g ai/ha (C) Vydate C-LV 362.4 g ai/ha (D) Vydate C-LV 362.4 g ai/ha (E)	1.09	3.51	11.52	16.59	46.10	69.73	98.68	0.370
Nimitz 3.5 pt/a (C)	0.54	4.68	7.12	16.19	45.31	83.01	98.68	0.374
Nimitz 7 pt/a (C)	2.81	6.44	18.98	30.25	54.69	92.38	99.85	0.454
CruiserMaxx[e] 0.31 oz/CWT (B) Elatus 7.7 oz/A (D)	2.22	9.96	15.76	28.21	60.55	84.77	99.41	0.482
CruiserMaxx 0.31 oz/CWT (B) Aprovia 0.75 l/a (C)	2.22	8.79	25.60	37.00	63.28	91.80	99.85	0.508
Emesto Silver 0.31 fl oz/cwt (B) Velum prime 237.5 g ai/ha (C) Luna Tranquility 409 g ai/ha (D) Luna Tranquility 409 g ai/ha (E)	1.09	5.86	12.11	14.40	42.58	75.98	98.28	0.393
Emesto Silver 0.31 fl oz/cwt (B) Serenade Soil 1 qt/a (C) Velum prime 237.5 g ai/ha (C) Luna Tranquility 409 g ai/ha (D) Luna Tranquility 409 g ai/ha (E)	0.83	4.68	11.31	15.49	34.38	71.49	99.14	0.340

Table 2.8 (cont'd)

Emesto Silver 0.31 fl oz/cwt (B) Vydate C-LV 1450 g ai/ha (C) Velum prime 237.5 g ai/ha (C) Luna Tranquility 409 g ai/ha (D) Luna Tranquility 409 g ai/ha (E)	1.47	4.10	8.02	11.52	36.72	83.59	99.14	0.347
Emesto Silver 0.31 fl oz/cwt (B) Vydate C-LV 1450 g ai/ha (C) Vydate C-LV 724 g ai/ha (D) Luna Tranquility 409 g ai/ha (D) Luna Tranquility 409 g ai/ha (E)	2.81	17.58	30.25	55.03	67.19	97.66	100.00	0.558
Pr>F	0.79	0.19	0.51	0.42	0.73	0.41	0.53	0.52

[a]Application time; A=Pre-planting/pre-plant incorporated; B=Seed treatment; C=In-furrow at planting; D=2" emergence; E=7 Days after 2" emergence.

[b] PED=Potato Early Die severity rated on a Horsfall-Barratt scale of 0 (no infection) to 11 (all foliage and stems dead). Ratings were converted to percentages.

[c] DAP = days after planting on 2 June.

[d] RAUDPC=relative area under the disease progress curve from planting to 111 days after planting.

[e] Cruisermaxx=Cruisermaxx potato seed treatment

Table 2.9. Effects of in-furrow, at planting, and foliar treatments on percent plant emergence, rate of emergence, total and marketable yield in hundred-weight per acre, vascular discoloration of tubers, *Verticillium dahliae* colony forming units (CFU) in soil, and root lesion nematode population data for Clarksville Research Center (Clarksville, MI) in 2020

Treatment[a]	Plant stand[b] 39 DAP[c] (%)	RAUEPC[d] 0 – 39 DAP	Yield (CWT)			VD[e] (%)	Average CFU/g of Soil[f]		RLN Soil[g]	
			Total	US #1	B Size[h]		5 Jun	4 Aug	5 Jun	4 Aug
Non-Treated	83.9	0.797	124.3	53.8	69.4 f	47.5	9.2	14.8	0.0 d	0.0
Vydate C-LV 725 g ai/ha (C) Vydate C-LV 362.4 g ai/ha (D) Vydate C-LV 362.4 g ai/ha (E)	78.8	0.744	253.1	122.5	127.1 ab	27.5	10.5	4.6	6.0 a	1.8
Nimitz 3.5 pt/a (C)	86.5	0.785	212.3	102.7	103.1 b-e	37.5	8.2	6.0	2.0 abc	1.6
Nimitz 7 pt/a (C)	87.2	0.831	252.2	104.5	139.6 a	35	3.9	5.4	0.7 bcd	0.0
CruiserMaxx[i] 0.31 oz/CWT (B) Elatus 7.7 oz/A (D)	86.9	0.827	204.3	105.3	95.4 cde	40	4.0	8.2	0.5 cd	0.0
CruiserMaxx 0.31 oz/CWT (B) Aprovia 0.75 l/a (C)	77.9	0.728	208.4	121.3	86.3 def	42.5	11.4	9.8	2.4 abc	0.3
Emesto Silver 0.31 fl oz/cwt (B) Velum prime 237.5 g ai/ha (C) Luna Tranquility 409 g ai/ha (D) Luna Tranquility 409 g ai/ha (E)	85.7	0.816	215.2	96.8	114.9 a-d	50	6.6	6.5	3.4 ab	1.1
Emesto Silver 0.31 fl oz/cwt (B) Serenade Soil 1 qt/a (C) Velum prime 237.5 g ai/ha (C) Luna Tranquility 409 g ai/ha (D) Luna Tranquility 409 g ai/ha (E)	83.2	0.784	232.1	115.9	112 a-d	40	10.6	8.5	0.0 d	1.4

Table 2.9 (cont'd)

Emesto Silver 0.31 fl oz/cwt (B) Vydate C-LV 1450 g ai/ha (C) Velum prime 237.5 g ai/ha (C) Luna Tranquility 409 g ai/ha (D) Luna Tranquility 409 g ai/ha (E)	80.9	0.762	215.2	133.5	79.9 ef	50	11.4	9.4	0.5 cd	1.0
Emesto Silver 0.31 fl oz/cwt (B) Vydate C-LV 1450 g ai/ha (C) Vydate C-LV 724 g ai/ha (D) Luna Tranquility 409 g ai/ha (D) Luna Tranquility 409 g ai/ha (E)	86.9	0.789	256.9	127.5	124.6 abc	35	16.0	15.1	0.9 bcd	0.0
Pr>F	0.67	0.80	0.14	0.42	<0.01	0.79	0.67	0.82	0.06	0.45

[a]Application time; A=Pre-planting/pre-plant incorporated; B=Seed treatment; C=In-furrow at planting; D=2" emergence; E=7 Days after 2" emergence.

[b] Plant stand expressed as a percentage of the target population of 120 plants/100ft. row from a sample of 1 x 25 ft rows per plot.

[c] DAP = days after planting on 1 June.

[d] RAUEPC=relative area under the emergence progress curve from planting to 39 days after planting.

[e] VD=Vascular discoloration of the stem end; percentage calculated from 10 tubers.

[f] CFU=colony forming units seen on selective *Verticillium dahliae* media.

[g] RLN=root lesion nematode, *Pratylenchus penetrans.*

[h] Means followed by same letter are not significantly different at $P = 0.10$ (Fishers LSD).

[i] Cruisermaxx=Cruisermaxx potato seed treatment.

2.4 Discussion

The susceptible potato cultivar 'Superior' was used to enhance *V. dahliae* disease development. Environmental conditions were ideal for potato development (18-21^{o}C) (41) and soil temperatures were ideal for *V. dahliae* germination (22-27^{o}C) (34, 3).

In 2018 and 2020, fungicide and nematicide applications were evaluated as an alternative or addition to soil fumigation to reduce the overall environmental and financial risk associated with soil fumigation (6, 27). The *V. dahliae* inoculum density required for infection is reported as 5 CFU/g soil (32), but 10-20 CFU/g soil is required for economic loss from reduced potato yields (6). At-planting sampling and analysis in 2018 resulted in variable levels of CFU/g soil (1.4-7.6 CFU/g soil) across treatments. At-planting sampling and analysis in 2020 resulted in variable but higher levels of CFU/g soil (3.9-16.0 CFU/g soil) compared to 2018. This indicates that treatments are not reducing the overall inoculum density in the soil across years.

In 2018 and 2020 a treatment utilizing a seed treatment and in-furrow application was used; Cruisermaxx Potato (B) + Elatus (D). Cruisermaxx potato seed treatment is reported to be effective against certain insects and seed-borne fungal diseases of potato (e.g. *Helminthosporium solani* and *Rhizoctonia*) (38). Elatus is an in-furrow and foliar fungicide reported to be effective against certain soilborne diseases (e.g. *Rhizoctonia,* black dot, and silver scurf of potato) and has the potential to suppress *Verticillium* levels as an in-furrow at planting treatment (39). In 2018, treatment with; Cruisermaxx Potato (B) + Elatus (D), resulted in significantly lower RAUDPC and numerically higher total yield by ~34 CWT, but numerically approximately double PED disease severity (%) compared to the NTC. In 2020, the same treatment resulted in significantly higher B-size yield, numerically higher total yield by ~80 CWT, and numerically lower VD% compared to the NTC but, between June and August sampling average *V. dahliae* CFU/g soil more than doubled

(4.0 to 8.2 CFU/g soil). Elatus is registered for the suppression of *V. dahliae* (37) but did not provide effective control in 2018 or 2020. Numerical differences in reduction of potato early die symptoms were achieved through most seed, in-furrow, and foliar application of fungicides and nematicides.

In contrasts, in 2018, applications of Vydate C-LV resulted in a reduction in RLN population in soil between sampling dates, and in 2020, applications of Vydate C-LV resulted in effective management of Potato Early Die. Vydate C-LV is an in-furrow and foliar insecticide/nematicide reported to be effective against common pests of potato including Colorado Potato Beetle and RLN (8). In 2018, plots treated with; Vydate C-LV (C, D, & E), resulted in a reduction in RLN population in soil between May and August sampling from 15.7 to 2.7, and had similar PED disease severity (%) and total yield compared to the NTC. Numerical differences in PED disease severity (%) and total yield may be a result of higher average CFU/g soil in plots treated with Vydate C-LV versus the NTC (7.6 vs. 5.7). In 2020, the same treatment resulted in a large reduction in RLN population in soil between June and August sampling from 6.0 to 1.8, the average CFU/g soil between June and August sampling was reduced by more than half (10.5 to 4.6), numerically lower PED disease severity by 20%, significantly higher B-size yield, and a numerically higher total yield by ~130 CWT compared to the NTC. In a study at Upton, Prince Edward Island in 1989, it was found that in-furrow applications of oxamyl at a rate of 2.24 kg a.i. ha^{-1} (almost double what was evaluated in the trials from this thesis (1.45 kg a.i. ha^{-1})) was an effective nematicide and significantly increased tuber yields of potato cultivar ‘Superior’ by 15.5% (18). Vydate C-LV in the form of oxamyl was also evaluated as an alternative to fumigation and was effective in reducing *P. penetrans* populations based on the May to July sampling (29). In 2018, no treatments significantly impacted management of PED compared to the NTC.

In 2018, a treatment was evaluated that utilized: Emesto Silver (B) + Serenade Soil (C) + Velum prime (C) + Luna Tranquility (D). Emesto Silver potato seed treatment is reported to be effective against seed and soilborne diseases, including Rhizoctonia, Fusarium, and Silver Scurf (22). Serenade Soil (QST 713 strain of dried *Bacillus subtilis*) is an in-furrow or soil drench aqueous suspension biological fungicide was reported to be effective against potato soilborne diseases, including Rhizoctonia and black scurf (24). *Bacillus subtilis* has also been reported to have nematocidal properties and may be an effective biocontrol (36). Velum Prime is a broad-spectrum fungicide and nematicide applied as a soil treatment and is reported to be effective against certain endoparasite nematodes and fungal diseases of potato (e.g. Early blight and white mold) (25). Luna Tranquility is a broad-spectrum fungicide applied as an in-furrow or foliar treatment and is reported to be effective against fungal diseases of potato (e.g. White mold, early blight, and black dot) (23). Velum Prime and Luna Tranquility contain the active ingredient fluopyram, which has produced promising results when evaluated as an in-furrow nematicide (10, 14). Fluopyram has also been evaluated as a foliar treatment for management of another soilborne disease (*Fusarium virguliforme)* with positive results (17). In 2018, treatment with; Emesto Silver (B) + Serenade soil (C) + Velum Prime (C) + Luna Tranquility (D), resulted in significantly lower RAUDPC compared to the NTC but, RLN population increased from May to August sampling (2.2 to 3.9), numerically ~double PED disease severity (%), and lower total yield compared to the NTC. This treatment was adapted in 2020 in hopes additional Luna Tranquility applications or the addition of Vydate C-LV would better manage PED. In 2020, the plots treated with Emesto Silver (B) + Vydate C-LV (C & D) + Luna Tranquility (D & E) resulted in reduced RLN population in soil between June and August sampling from 0.9 to 0.0, numerically lower PED disease severity (%), significantly increased B-size yield, and numerically increased total yield by ~132 CWT

compared to the NTC. The addition of Vydate C-LV to Emesto Silver and Luna Tranquility may have improved management due to the effect of Vydate C-LV on RLN population in soil. Treatment with Vydate C-LV alone or in combination were the most effective chemistry evaluated for management of PED in 2020.

In 2020 the NTC had a RLN population of 0.0 at the time of planting and may partially explain why treatments rarely differed significantly from the NTC across multiple data points. A broad-spectrum seed treatment like Cruisermaxx Potato or Emesto Silver is important because potatoes are exposed to many soilborne pathogens that cause similar/additional symptoms and yield reductions. The results obtained from the 2018 and 2020 trial indicate: 1) No tested treatments were effective at significantly reducing VD% compared to no treatment; 2)Vydate C-LV may have some efficacy in managing PED compared to other chemistries applied and 3) Emesto Silver + Luna Tranquility may be more effective at managing PED with the addition of Vydate C-LV. *Pratylenchus penetrans* may increase disease severity by nematode feeding that increases root exudates subsequently resulting in more microsclerotia germinating than in the absence of *P. penetrans* (35). Managing *Pratylenchus penetrans* populations rather than reducing *V. dahliae* CFU/g soil may be the most effective management strategy for PED with the currently registered pesticides available.

2.5 Conclusions

Globally, Potato Early Die complex is an economically important disease for potato growers (35, 5). A management strategy that is effective, with low risk to the environment, and cost effective for producers has not been identified (35, 27, 12). In part, this is due to difficult management of microsclerotia; the dense and durable *Verticillium dahliae* resting structure that

can persist in soil for a decade without a host (27). To date, the results obtained show applications of Vydate C-LV are effective at reducing RLN population in soil and Emesto Silver and Luna Tranquility are more effective at managing PED when combined Vydate C-LV. Vydate C-LV is the best nematicide for management of *Pratylenchus penetrans* that was evaluated. The improvement of PED management with the addition of Vydate C-LV may be related to reduction in *P. penetrans* populations. Increased management with Emesto Silver + Luna Tranquility compared to other seed treatments + fungicide is not related to reduction in RLN populations but may be related to reduction in average CFU/g soil, protection of roots from *V. dahliae* propagules in the soil, or other factors that still need additional research. The results from 2018 and 2020 are promising and support previous work indicating that managing PED requires an integrated approach including cultural and chemical methods, and host resistance. Further research is required to obtain an effective, low risk to the environment, and cost conscience management strategy for PED.

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CHAPTER 3: EVALUATION OF SILVER SCURF SENSITIVITY TO THREE CLASSES OF FUNGICIDES

Abstract

The potato production industry has been negatively impacted by the storage disease, silver scurf, incited by *Helminthosporium solani*, that decreases tuber quality and storability. Silver scurf causes cosmetic lesions and shrinkage in stored potatoes which negatively impacts the stored crops' marketability. Effective fungicides are needed to limit silver scurf and extend the storability of potatoes. In vitro studies were established to evaluate three methods used to estimate in vitro sensitivity of *H. solani* to three classes of fungicides. The objectives were to: 1) Determine in vitro the sensitivity of *H. solani* isolates to three classes of fungicides: 2) Compare three methods used to evaluate in vitro sensitivity of *H. solani* to three classes of fungicides. Sensitivity expressed in mg/L was defined as the effective fungicide concentration at which 50% of the fungal growth or germination is inhibited (EC_{50}). The relative growth and germination of spores for all mono conidial *H. solani* isolates were recorded to determine fungicide sensitivity. Results from screening isolates (n=33) collected from CA, ID, MI, NY, and WI were completed. *Helminthosporium solani* isolates were most sensitive to pydiflumetofen, difenoconazole and benzovindiflupyr. The relative germination method is recommended for screening succinate dehydrogenase inhibitors. The relative growth method using a spiral gradient dilution may also be used but is recommended for screening demethylation inhibitors and phenylpyrrole fungicides.

3.1 Introduction

Helminthosporium solani is the causal agent of silver scurf on potato and is of economic importance (7). There is limited research on the baseline sensitivity, efficacy, and reduced sensitivity of *H. solani* to succinate dehydrogenase inhibitors, demethylase inhibitors and phenylpyrrole fungicides (2). This information is important to develop new or improving integrative disease management strategies. The reported inconsistencies in efficacy of seed treatments against *H. solani* infection, may be related to the regional diversity of potato production regions (i.e. temperature, soil moisture, soil microbes, soil inoculum and soil type) (6). The differences in potato production regions (in the US) may result in different selection pressures on *H. solani*, resulting in variations in fungicide sensitivity. There are currently limited control methods for *H. solani* (13). Site-specific fungicides select for rare resistant isolates (12) and are standards for *H. solani* control (10, 4). It is important to screen *H. solani* isolates against SDHI, DMI and PP fungicides to monitor efficacy and determine the rate of resistance. SDHI fungicides target respiration and germination, while DMI and PP fungicides target mycelial growth (9). In 2016-2017, determining the sensitivity distributions of *H. solani* were completed for fludioxonil, difenoconazole, benzovindiflupyr and sedaxane. It is important to determine the sensitivity distributions of *H. solani* to new fungicides.

We phenotypically determined the succinate dehydrogenase inhibitor (SDHI), demethylation inhibitors (DMI) and phenylpyrroles (PP) fungicide sensitivity using standard dilution plating to estimate the effective fungicide concentration at which 50% of the fungal conidia germination is inhibited (EC_{50}) in vitro for all isolates. Three methods were used to calculate EC_{50} values: relative germination using standard dilution plating, relative radial growth using standard dilution plating, and relative growth using spiral gradient dilution method. The

standard dilution plating method requires preparation of large amounts of agar media, several fungicide stock solutions, and pouring replicate plates for each fungicide concentration amounting to a time-, labor-, and resource-demanding assay (5). The spiral gradient method saves time and money by screening multiple isolates on one plate, but still requires careful pipetting and data collection to avoid observational error (5). The relative germination method is ideal for SDHI fungicides that target spore germination, and relative growth for DMI and PP fungicides that target mycelial growth. The three methods were compared, while considering resource restraints associated with each. This study had two objectives: 1) Determine in vitro the sensitivity of *H. solani* isolates to three classes of fungicides: 2) Compare three methods used to evaluate in vitro sensitivity of *H. solani* to three classes of fungicides.

3.2 Materials and Methods

3.2.1 EVALUATION OF THREE METHODS TO ESTIMATE IN VITRO SENSITIVITY OF SILVER SCURF TO THREE CLASSES OF FUNGICIDES

3.2.1.1 Sample Collection and geographical origin of isolates

Helminthosporium solani isolates were obtained from commercially grown tubers from the 2018 and 2019 growing season. The isolates were collected from potatoes grown in the following production states: California, Idaho, Michigan, New York, and Wisconsin. Representative tubers were randomly selected from freshly harvested and potato storage bins. Tubers were lightly washed to remove soil and placed into 5 lb heavy duty leno mesh poly bags with 1/8 in x 1/8 in openings (GlacierValley Enterprises, LLC, Baraboo, WI) that are used to market fresh potatoes. The bags were placed into perforated cartons of stable corrugated board in the growth chambers and stored for at least 2 weeks at 20°C in the dark, to promote sporulation. Two humidifiers filled

with distilled H_2O were used in the growth chamber to maintain at least 90% relative humidity. After a minimum of 2 weeks, tubers were inspected for *H. solani* sporulation with a compound microscope. Monoconidial isolates from individual tubers were obtained by sub-culturing a single conidium of *H. solani* onto clarified V8 (CV8) media amended with $CaCO_3$ (900 ml of distilled H_2O, 100 ml of CV8, 15 g of Bacto Agar, and 1.5 g of $CaCO_3$). Isolates were parafilmed and stored in the dark in plastic boxes with lids.

To ensure pure colonies of *H. solani* it was necessary to remove the mycoparasite, *A. strictum*. Two-week-old isolates had aerial fungal growth removed. The isolates were observed daily. For most cases, *H. solani* grows at a faster rate than *A. strictum*. Therefore, after the aerial fungal growth was removed, the isolates were observed for *H. solani* growth prior to *A. strictum* growth. Finally, an additional monoconidial transfer was made.

3.2.1.2 In vitro sensitivity by relative spore germination of *Helminthosporium solani* isolates

Pydiflumetofen, benzovindiflupyr, sedaxane and SYN549522 stock solutions of each fungicide were prepared by dissolving commercial-grade fungicides in a sterile solvent. 25 ml of water agar was poured into each dish, to form a layer of water agar with a known constant volume. A standard tenfold agar plate dilution series was used. For relative germination, water agar media was amended with concentrations of 0.0, 0.001, 0.01, 0.1, 1, and 10 mg L-1 for each SDHI fungicide (Pydiflumetofen, sedaxane and benzovindiflupyr). Per 1 L of water agar media: 1000mL distilled H_2O and 15g Bacto-Agar. Two replications, per concentration, for each fungicide, were used. Experiments were set up in a complete randomized design.

3.2.1.3 Spore germination plating assay procedure

Pure cultures of *H. solani* were used. Suspensions were prepared by flooding colony Petri dishes with distilled water (1000 µL per plate) and conidia were scraped free from the agar surface

with a rubber policeman. Conidial suspension concentration was determined using a hemocytometer and adjusted to a concentration of ($1x10^5$). Subsequently, an aliquot of the conidial suspension (100 µL per plate) was spread across each plate evenly. Plates were assessed after incubation under continuous light for 24 h at room temperature.

3.2.1.4 Data collection and analysis of spore germination assay

Germination of 50 conidia was evaluated with a compound microscope at each concentration (two replications). A conidium was determined to have germinated if a normally developing germ tube was at least equal to the total length of the conidium. Isolate sensitivity was expressed as relative germination, which is defined as the ratio of conidia germinating in the presence of fungicide to those germinating in the absence of fungicide x 100%. Isolate sensitivity expressed in mg/liter was determined by the effective concentration in inhibiting spore germination by 50% (EC_{50}). The EC_{50} values were determined by interpolation of the 50% intercept based on the regression of the arcsine of relative germination on the log_{10}-transformed fungicide concentration. *Helminthosporium solani* isolates collected in 2018 and 2019 were then classified phenotypically into four sensitivity categories based on mean EC_{50} values (mg/L): Sensitive (<1): S; reduced sensitive (1-5): RS; moderately insensitive (5-10): MI; and insensitive (>10): I.

3.2.1.5 In vitro sensitivity by relative radial growth of *Helminthosporium solani* isolates

Pydiflumetofen, benzovindiflupyr, sedaxane, SYN549522, difenoconazole and fludioxonil stock solutions of each fungicide were prepared by dissolving commercial-grade fungicides in a sterile solvent. 25 ml of CV8 agar was poured into each dish, to form a layer of CV8 agar with a known constant volume. A standard ten-fold agar plate dilution series was used. For relative growth, CV8 media was amended with concentrations of 0.0, 0.001, 0.01, 0.1, 1, and 10 mg L-1

for each fungicide. Per 1 L of CV8 media: 900mL distilled H_2O, 100mL clarified V8, 15g Bacto-Agar and 1.5g Calcium Carbonate. Two replications, per concentration, for each fungicide, were used. Experiments were set up in a complete randomized design.

3.2.1.6 Relative radial growth plating assay procedure

Pure cultures of *H. solani* were used. Using a #3 cork borer, an inoculum plug of each isolate was placed in the center of agar in a petri plate, using sterile inoculating needles. Plates were assessed after incubation in the dark for four weeks at room temperature.

3.2.1.7 Data collection and analysis of relative radial growth assay

Growth of mycelial plugs was evaluated at each concentration (two replications). Two perpendicular lines were drawn through the center of the growing culture. Using a digital caliper, the four perpendicular radii were measured, and an average was calculated to determine amount of fungal growth. Isolate sensitivities were expressed as relative growth, which is defined as the ratio of fungal growth in the presence of fungicide to fungal growth in the absence of fungicide x 100%. Isolate sensitivity expressed in mg/liter was determined by estimating the EC_{50}. EC_{50} values were determined by interpolation of the 50% intercept based on the regression of the arcsine of relative growth on the $\log_{10}$-transformed fungicide concentration. *Helminthosporium solani* isolates collected in 2018 and 2019 were classified phenotypically into four sensitivity categories based on mean EC_{50} values (mg/L): Sensitive (<1): S; reduced sensitive (1-5): RS; moderately insensitive (5-10): MI; and insensitive (>10): I.

3.2.1.8 In vitro sensitivity by spiral gradient method of *Helminthosporium solani* isolates

Pydiflumetofen, benzovindiflupyr, sedaxane, SYN549522, difenoconazole and fludioxonil stock solutions of 10,000 mg/liter of each fungicide were prepared by dissolving commercial-grade fungicides in a sterile solvent. 50 ml of CV8 agar was poured into each dish, to form a layer of

CV8 agar with a known constant volume, thus when a stock solution was added to the agar it results in a gradient from 0 to 1000 ppm across the surface. Per 1 L of CV8 media: 900mL distilled H_2O, 100mL clarified V8, 15g Bacto-Agar and 1.5g Calcium Carbonate. Two replications per fungicide were used. Experiments were set up in a complete randomized design.

3.2.1.9 Spiral gradient plating assay procedure

A spiral gradient plater (Spiral Plate Biotechnology Inc.) was used to determine isolate EC_{50}. Pure cultures of *H. solani* were used. Conidial suspensions were prepared by flooding colony Petri dishes with 1000 mL distilled water and scraping the conidia free from the surface with a rubber policeman. The conidial suspension (15 μL) was spread across the fungicide gradient plate from edge to center (two replication). Isolates were incubated for four weeks at room temperature.

3.2.1.10 Data collection and analysis

The point coordinates at which the colonies start, and end were recorded and entered into the software program: RStudio (RStudio Team 2020, Boston, MA) which calculates the EC_{50} for each isolate for each of the fungicides. Isolate sensitivity expressed in mg/liter was determined by the EC_{50}. *Helminthosporium solani* isolates collected in 2018 and 2019 were classified phenotypically into four sensitivity categories based on mean EC_{50} values (mg/L): Sensitive (<1): S; reduced sensitive (1-5): RS; moderately insensitive (5-10): MI; and insensitive (>10): I.

Table 3.1. *Helminthosporium solani* isolate list including isolate ID, location in US, and year.

Isolate ID	Location in US	Year
CA4-P	California	2018
CA18P	California	2018
EMNR1	Michigan	2019
HSID3	Idaho	2018
HSID5	Idaho	2018
HSID8	Idaho	2018
HSID9	Idaho	2018
HSID10	Idaho	2018
HSID13	Idaho	2018
HSID17	Idaho	2018
HSID19	Idaho	2018
HSID23	Idaho	2018
HSID24	Idaho	2018
N1	New York	2018
N4	New York	2018
N5	New York	2018
N6	New York	2018
N7	New York	2018
N8	New York	2018
SS1	Wisconsin	2018
SS2	Wisconsin	2018
SS7	Wisconsin	2018
UID2	Idaho	2019
UID3	Idaho	2019
UID4	Idaho	2019
UID5	Idaho	2019
UID6	Idaho	2019
UID10	Idaho	2019
UID11	Idaho	2019
UID32	Idaho	2019
UID33	Idaho	2019
UID34	Idaho	2019
WF-3	Michigan	2019

Table 3.2. The six chemistries used in the evaluation of in vitro sensitivity of *Helminthosporium solani* to three classes of fungicides in 2019 and 2020 including example product name(s), active ingredient, mode of action, chemical group name, and FRAC code group.

Example Product(s) Name	Active Ingredient(s)	Mode of Action (MOA)	Group Name	FRAC[a] Code
Miravis®	pydiflumetofen (Adepidyn™)	respiration	succinate dehydrogenase inhibitors (SDHI)	7
Amistar® Top Aprovia® Top Inspire® Quadris Top® RevusTop® Stadium	difenoconazole	sterol biosynthesis in membranes	demethylation inhibitors (DMI)	3
Stadium Academy Avicta® Cannonball®WG Chairman® CruiserMaxx®	fludioxonil	signal transduction	phenylpyrroles (PP)	12
Clariva® Complete CruiserMaxx® Vibrance® +	sedaxane	respiration	succinate dehydrogenase inhibitors (SDHI)	7
Elatus ® Trivapro® Aprovia® Mazen®	benzovindiflupyr (Solatenol™)	respiration	succinate dehydrogenase inhibitors (SDHI)	7
Experimental	SYN549522	respiration	succinate dehydrogenase inhibitors (SDHI)	N/A

3.3 Results

3.3.1 EVALUATION OF THREE METHODS TO ESTIMATE IN VITRO SENSITIVITY OF SILVER SCURF TO THREE CLASSES OF FUNGICIDES

Helminthosporium solani isolates collected in 2018 and 2019 from commercial potato fields in California, Idaho, Michigan, New York, and Wisconsin were subjected to in vitro fungicide sensitivity screening. A standard dilution series method was used to estimate the fungicide concentration that caused a 50% inhibition of fungal germination (EC_{50}) in vitro. The relative germination method generated preliminary sensitivity data of *H. solani* to one class of fungicide (SDHI). A total of 32 isolates were screened against pydiflumetofen (PFT), sedaxane (SDX), benzovindiflupyr (BVF), and SYN549522 (SYN) respectively and the mean EC_{50} were estimated for each of the fungicides listed above (Table 3.3).

The mean EC_{50} values for PFT, SDX, BVF, and SYN were 0.40, 0.57, 0.33, and 0.46, respectively (Table 3.3). The distribution of EC_{50} values of PFT was between <1 to 6.01 mg/L (Table 3.3). The distribution of EC_{50} values of SDX was between <1 to 3.91 mg/L. The distribution of EC_{50} values of BVF was between <1 to 2.33 mg/L. The distribution of EC_{50} values of SYN was between <1 to 4.00 mg/L.

Isolates were classified phenotypically into four sensitivity categories based on mean EC_{50} values (mg/L): Sensitive (<1): S; reduced sensitive (1-5): RS; moderately insensitive (5-10): MI; and insensitive (>10): I. For PFT, 96.9, 0.0, and 3.1% of isolates were categorized as S, RS, and MI, respectively (Figure 3.1). For SDX, 84.4 and 15.6% of isolates were categorized as S and RS, respectively. For BVF, 93.8 and 6.3% of isolates were categorized as S and RS, respectively. For SYN, 90.6 and 9.4% of isolates were categorized as S and RS, respectively.

Table 3.3. Comparison of mean effective concentration in relative germination reduction by 50% (EC_{50}) for isolates of *Helminthosporium solani* to pydiflumetofen, benzovindiflupyr, sedaxane, and SYN549522.

			EC_{50} (mg/L)[a]		
Active ingredient	**FRAC[b] code**	**Total # of isolates**	**Mean (s.e.)[c]**	**Minimum**	**Maximum**
pydiflumetofen	7	32	0.40 ± 0.18	0.10	6.01
sedaxane	7	32	0.57 ± 0.13	0.10	3.91
benzovindiflupyr	7	32	0.33 ± 0.08	0.10	2.33
SYN549522	NA	32	0.46 ± 0.13	0.10	4.00

[a]EC_{50} values determined for two replications based on mean effective concentration in germination reduction by 50% by relative germination method

[b]FRAC=Fungicide Resistance Action Committee group name based on chemical relatedness and mode of action

[c]s.e.=standard error of the mean

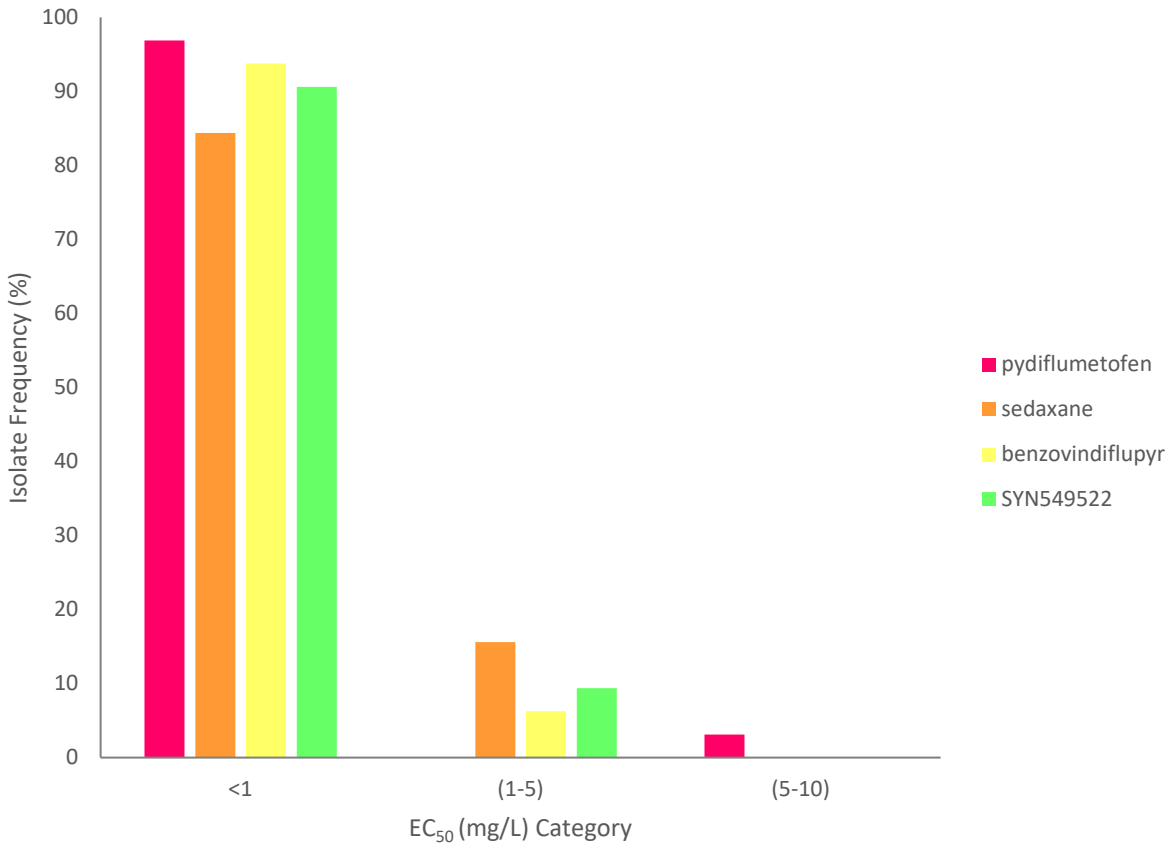

Figure 3.1 Frequency distributions of in vitro sensitivity of *Helminthosporium solani* isolates collected in 2018 and 2019 from potato tubers. Sensitivity expressed as 50% inhibition of fungal germination (EC_{50}) in vitro, fungicide concentration estimate determined by the relative germination method.

A standard dilution series method was used to estimate the fungicide concentration that caused a 50% inhibition of fungal growth (EC_{50}) in vitro. The relative growth method using a standard dilution series generated preliminary sensitivity data of *H. solani* to three classes of fungicides (DMI, PP, and SDHI). A total of 32, 32, 32, 33, 33, and 31 isolates were screened against pydiflumetofen (PFT), difenoconazole (DFZ), fludioxonil (FDL), sedaxane (SDX), benzovindiflupyr (BVF), and SYN549522 (SYN) respectively and the mean EC_{50} values were estimated for each of the fungicides listed above (Table 3.4).

The mean EC_{50} values for PFT, DFZ, FDL, SDX, BVF, and SYN were 0.72, 1.07, 5.18, 3.06, 1.56, and 3.19 respectively (Table 3.4). The distribution of EC_{50} values of PFT was between <1 to 3.34 mg/L (Table 3.4). The distribution of EC_{50} values of DFZ was between <1 to 6.68 mg/L. The distribution of EC_{50} values of FDL was between <1 to 9.97 mg/L. The distribution of EC_{50} values of SDX was between <1 to 9.63 mg/L. The distribution of EC_{50} values of BVF was between <1 to 3.73 mg/L. The distribution of EC_{50} values of SYN was between <1 to 8.83 mg/L.

Isolates were classified phenotypically into four sensitivity categories based mean EC_{50} values (mg/L): Sensitive (<1): S; reduced sensitive (1-5): RS; moderately insensitive (5-10): MI; and insensitive (>10): I. For PFT, 75.0 and 25.0% of isolates were categorized as S and RS, respectively (Figure 3.2). For DFZ, 75.0, 18.8, and 6.3% of isolates were categorized as S, RS, and MI, respectively. For FDL, 18.8, 34.4, and 46.9% of isolates were categorized as S, RS, and MI, respectively. For SDX, 24.2, 60.6, and 15.2% of isolates were categorized as S, RS, and MI, respectively. For BVF, 39.4 and 60.6% of isolates were categorized as S and RS, respectively. For SYN, 25.8, 54.8, and 19.4% of isolates were categorized as S, RS, and MI, respectively.

Table 3.4. Comparison of mean effective concentration in relative radial growth reduction by 50% (EC_{50}) for isolates of *Helminthosporium solani* to pydiflumetofen, difenoconazole, fludioxonil, benzovindiflupyr, sedaxane, and SYN549522.

			EC_{50} (mg/L)[a]		
Active ingredient	**FRAC[b] code**	**Total # of isolates**	**Mean (s.e.)[c]**	**Minimum**	**Maximum**
pydiflumetofen	7	32	0.72 ± 0.16	0.10	3.34
difenoconazole	3	32	1.07 ± 0.28	0.10	6.68
fludioxonil	12	32	5.18 ± 0.69	0.11	9.97
sedaxane	7	33	3.06 ± 0.40	0.10	9.63
benzovindiflupyr	7	33	1.56 ± 0.19	0.30	3.73
SYN549522	NA	31	3.19 ± 0.40	0.40	8.83

[a]EC_{50} values determined for two replications based on mean effective concentration in radial growth reduction by 50% by relative radial growth method using a standard dilution plating series

[b]FRAC=Fungicide Resistance Action Committee group name based on chemical relatedness and mode of action

[c]s.e.=standard error of the mean

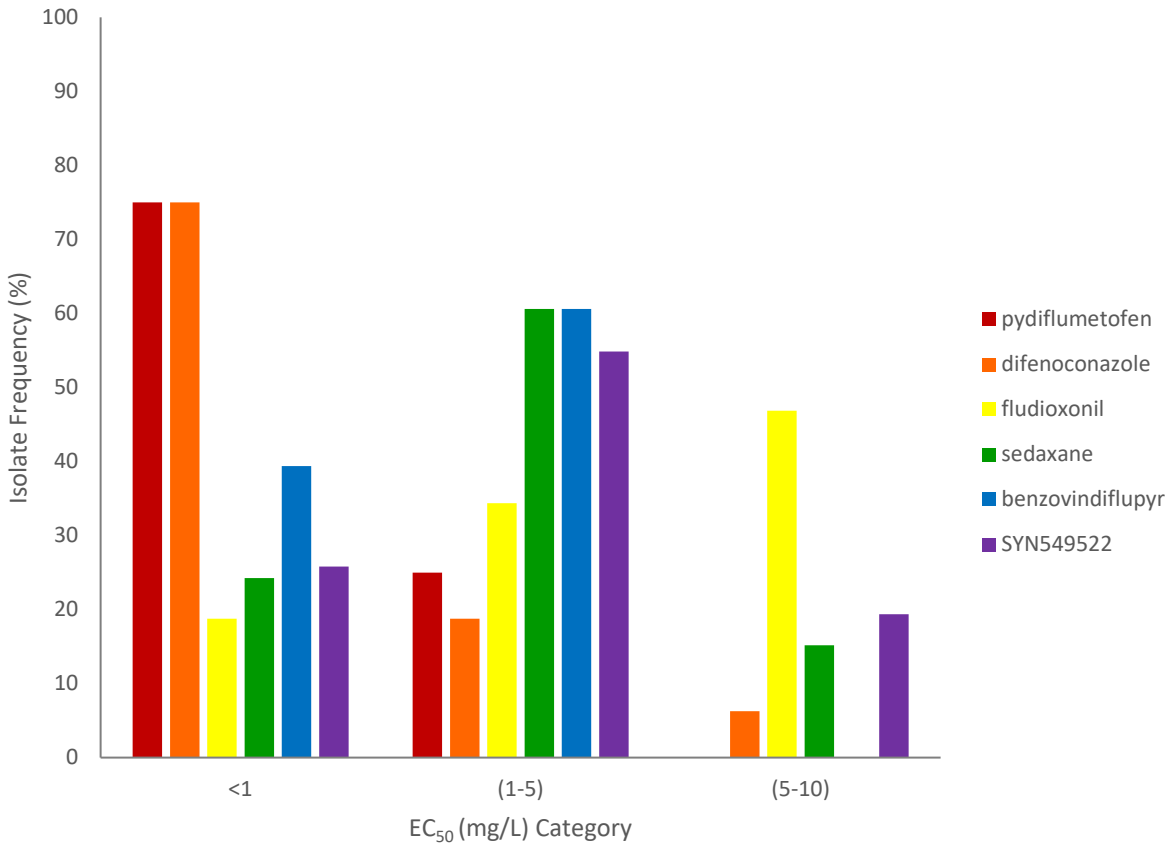

Figure 3.2 Frequency distributions of in vitro sensitivity of *Helminthosporium solani* isolates collected in 2018 and 2019 from potato tubers. Sensitivity expressed as 50% inhibition of fungal growth (EC_{50}) in vitro, fungicide concentration estimate determined by the relative radial growth method using a standard plate dilution series.

A spiral gradient dilution method was used to estimate the fungicide concentration that caused a 50% inhibition of fungal growth (EC_{50}) in vitro. The relative growth method using a spiral gradient dilution generated preliminary sensitivity data of *H. solani* to three classes of fungicides (DMI, PP, and SDHI). A total of 32 isolates were screened against pydiflumetofen (PFT), difenoconazole (DFZ), fludioxonil (FDL), sedaxane (SDX), benzovindiflupyr (BVF), and SYN549522(SYN) respectively and the mean EC_{50} were estimated for each of the fungicides listed above (Table 3.5).

The mean EC_{50} values for PFT, DFZ, FDL, SDX, BVF, and SYN were 0.58, 0.61, 69.15, 0.87, 0.62, and 0.69 respectively (Table 3.4). The distribution of EC_{50} values of PFT was between <1 to 0.58 mg/L (Table 3.5). The distribution of EC_{50} values of DFZ was between <1 to 0.61 mg/L. The distribution of EC_{50} values of FDL was between <1 to 144.2 mg/L. The distribution of EC_{50} values of SDX was between <1 to 2.23 mg/L. The distribution of EC_{50} values of BVF was between <1 to 0.62 mg/L. The distribution of EC_{50} values of SYN was between <1 to 1.63 mg/L.

Isolates were classified phenotypically into four sensitivity categories based mean EC_{50} values (mg/L): Sensitive (<1): S; reduced sensitive (1-5): RS; moderately insensitive (5-10): MI; and insensitive (>10): I. For PFT, 100.0% of isolates were categorized as S (Figure 3.3). For DFZ, 100.0% of isolates were categorized as S. For FDL, 9.4, 40.6, 0.0, and 50.0% of isolates were categorized as S, RS, MI, and I, respectively. For SDX, 87.5 and 12.5% of isolates were categorized as S and RS, respectively. For BVF, 100.0% of isolates were categorized as S. For SYN, 93.8 and 6.3% of isolates were categorized as S and RS, respectively.

Table 3.5. Comparison of mean effective concentration in relative growth reduction by 50% (EC_{50}) for isolates of *Helminthosporium solani* to pydiflumetofen, difenoconazole, fludioxonil, benzovindiflupyr, sedaxane, and SYN549522.

			EC_{50} (mg/L)[a]		
Active ingredient	**FRAC[b] code**	**Total # of isolates**	**Mean (s.e.)[c]**	**Minimum**	**Maximum**
pydiflumetofen	7	32	0.58 ± 0	0.58	0.58
difenoconazole	3	32	0.61 ± 0	0.61	0.61
fludioxonil	12	32	69.15 ± 12.27	0.95	144.2
sedaxane	7	32	0.87 ± 0.07	0.72	2.23
benzovindiflupyr	7	32	0.62 ± 0	0.62	0.62
SYN549522	NA	32	0.69 ± 0.04	0.63	1.63

[a] EC_{50} values determined for two replications based on mean effective concentration in growth reduction by 50% by relative growth method using a spiral gradient dilution

[b] FRAC=Fungicide Resistance Action Committee group name based on chemical relatedness and mode of action

[c] s.e.=standard error of the mean

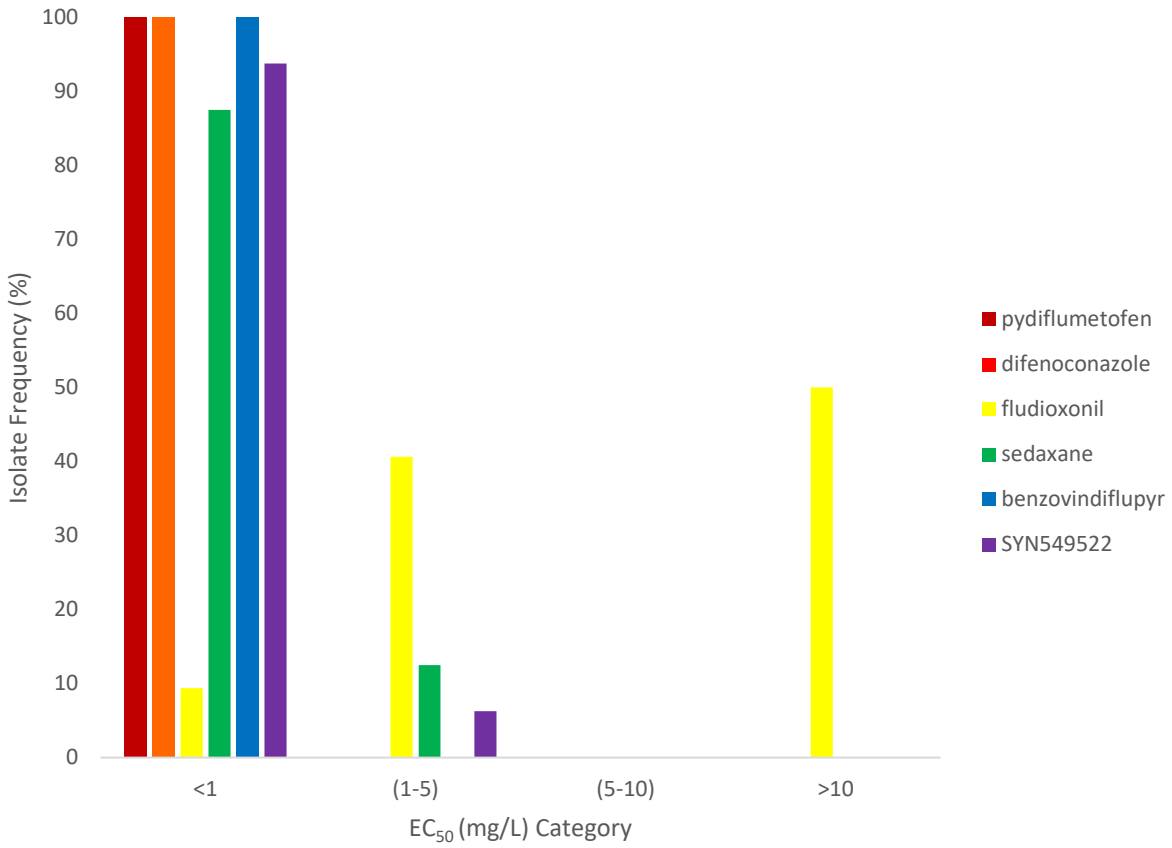

Figure 3.3 Frequency distributions of in vitro sensitivity of *Helminthosporium solani* isolates collected in 2018 and 2019 from potato tubers. Sensitivity expressed as 50% inhibition of fungal growth (EC_{50}) in vitro, fungicide concentration estimate determined by the relative growth method using a spiral gradient dilution.

Table 3.6. Comparison of *Helminthosporium solani* isolate frequency (%) distributions into four sensitivity categories determined by three sensitivity assays to six fungicides.

		Method Used		
Fungicide	**Category[a]**	**Relative Germination**	**Relative Radial Growth[b]**	**Relative Growth[c]**
pydiflumetofen	sensitive	96.9	75.0	100.0
	reduced sensitive	0.0	25.0	0.0
	moderate insensitive	3.1	0.0	0.0
	insensitive	0.0	0.0	0.0
difenoconazole	sensitive		75.0	100.0
	reduced sensitive	-	18.8	0.0
	moderate insensitive	-	6.3	0.0
	insensitive	-	0.0	0.0
fludioxonil	sensitive	-	18.8	9.4
	reduced sensitive	-	34.4	40.6
	moderate insensitive	-	46.9	0.0
	insensitive	-	0.0	50.0
sedaxane	sensitive	84.4	24.2	87.5
	reduced sensitive	15.6	60.6	12.5
	moderate insensitive	0.0	15.2	0.0
	insensitive	0.0	0.0	0.0
benzovindiflupyr	sensitive	93.8	39.4	100.0
	reduced sensitive	6.3	60.6	0.0
	moderate insensitive	0.0	0.0	0.0
	insensitive	0.0	0.0	0.0
SYN549522	sensitive	90.6	25.8	93.8
	reduced sensitive	9.4	54.8	6.3
	moderate insensitive	0.0	19.4	0.0
	insensitive	0.0	0.0	0.0

Table 3.6 (cont'd)

[a]Sensitive (EC_{50}<1 mg/L), Reduced sensitive (EC_{50}:1-5 mg/L), Moderate insensitive (EC_{50}: 5-10 mg/L), and Insensitive (EC_{50}>10 mg/L)

[b]Determined using a standard dilution plating series

[c]Determined using a spiral gradient dilution

Table 3.7. *Helminthosporium solani* combined isolate data including isolate ID and sensitivity expressed as 50% inhibition of fungal germination or growth (EC_{50}) in vitro, fungicide concentration estimate determined by three methods.

	pydiflumetofen			difenoconazole			fludioxonil			sedaxane			benzovindiflupyr			SYN549522		
Isolate	**SS[a]**	**RS[b]**	**SG[c]**	**SS**	**RS**	**SG**	**SS**	**RS**	**SG**	**SS**	**RS**	**SG**	**SS**	**RS**	**SG**	**SS**	**RS**	**SG**
CA4-P	0.20	0.13	0.58	-	5.41	0.61	-	2.53	144.20	0.11	2.40	0.72	0.50	0.49	0.62	0.12	3.39	0.63
CA18P	0.13	0.23	0.58	-	0.21	0.61	-	7.11	2.69	1.07	0.10	0.72	0.18	1.33	0.62	0.12	0.98	0.63
EMNR1	0.25	0.17	0.58	-	0.52	0.61	-	1.16	0.95	0.37	0.79	1.86	0.42	0.57	0.62	0.35	3.01	0.63
HSID3	0.20	0.15	0.58	-	0.33	0.61	-	0.12	144.20	3.91	2.82	0.72	0.11	0.49	0.62	0.16	6.06	0.63
HSID5	6.01	3.34	0.58	-	0.18	0.61	-	8.33	144.20	0.11	6.15	0.72	0.12	2.14	0.62	0.19	0.76	0.63
HSID8	0.13	-	0.58	-	0.36	0.61	-	8.08	144.20	0.34	1.28	0.72	0.15	0.46	0.62	0.48	-	0.63
HSID9	0.18	2.97	0.58	-	1.27	0.61	-	2.52	144.20	0.13	1.92	0.72	0.24	0.30	0.62	0.33	3.11	1.63
HSID10	0.20	0.26	0.58	-	0.23	0.61	-	0.16	73.98	0.17	1.94	0.72	0.10	0.46	0.62	0.15	0.48	0.63
HSID13	0.14	0.23	0.58	-	0.37	0.61	-	0.56	4.25	0.20	2.44	0.72	0.12	0.48	0.62	0.11	0.46	0.63
HSID17	0.66	2.56	0.58	-	0.29	0.61	-	0.17	144.20	0.18	5.75	0.72	0.11	0.88	0.62	0.17	1.53	0.63
HSID19	0.12	0.27	0.58	-	0.30	0.61	-	0.27	75.07	0.10	4.27	0.72	0.10	0.41	0.62	0.14	1.50	0.63
HSID23	0.13	0.24	0.58	-	3.14	0.61	-	2.70	144.20	1.27	3.53	0.72	0.40	2.73	0.62	0.80	0.39	0.63
HSID24	0.88	2.89	0.58	-	6.68	0.61	-	3.24	0.95	0.32	3.22	0.72	1.16	2.95	0.62	4.00	3.48	0.63
N1	0.11	0.18	0.58	-	0.13	0.61	-	6.14	144.20	0.40	4.78	2.07	0.19	0.39	0.62	0.79	0.91	0.63
N4	0.38	0.12	0.58	-	0.18	0.61	-	0.11	4.25	0.15	9.63	0.72	2.33	1.09	0.62	0.10	3.06	0.63
N5	0.10	0.12	0.58	-	0.10	0.61	-	2.34	2.83	0.21	0.97	0.72	0.16	0.62	0.62	0.12	1.05	0.63
N6	0.11	0.10	-	-	-	-	-	-	-	0.23	2.57	-	0.10	1.85	-	0.17	-	-

Table 3.7 (cont'd)

N7	0.24	0.17	0.58	-	0.10	0.61	-	7.99	4.73	2.28	7.63	0.72	0.16	1.73	0.62	0.18	3.15	0.63
N8	0.21	0.20	0.58	-	3.85	0.61	-	4.93	2.83	0.28	2.46	0.72	0.49	1.17	0.62	0.20	0.95	0.63
SS1	0.10	0.17	0.58	-	0.11	0.61	-	2.51	144.20	0.62	3.99	0.72	0.20	0.48	0.62	0.10	0.55	0.63
SS2	0.10	0.26	0.58	-	0.12	0.61	-	2.23	0.95	0.18	7.43	2.23	0.13	2.02	0.62	0.43	6.25	1.63
SS7	0.11	0.18	0.58	-	0.91	0.61	-	9.94	144.20	0.71	0.65	0.72	0.27	1.53	0.62	0.93	4.80	0.63
UID2	0.31	0.42	0.58	-	0.87	0.61	-	9.85	2.45	0.48	2.82	0.72	0.13	2.20	0.62	0.47	4.78	0.63
UID3	0.42	0.24	0.58	-	0.71	0.61	-	9.95	144.20	0.30	3.35	0.72	0.55	2.70	0.62	1.67	4.74	0.63
UID4	0.20	1.46	0.58	-	2.13	0.61	-	9.20	3.38	0.23	1.14	0.72	0.14	3.49	0.62	0.23	8.83	0.63
UID5	0.17	0.75	0.58	-	0.14	0.61	-	9.70	144.20	0.11	3.52	0.72	0.23	1.48	0.62	0.17	2.82	0.63
UID6	0.15	0.48	0.58	-	0.96	0.61	-	3.73	144.20	0.75	3.17	0.72	0.18	2.03	0.62	0.12	5.49	0.63
UID10	0.11	1.04	0.58	-	0.82	0.61	-	9.84	2.83	0.48	3.99	0.72	0.40	2.76	0.62	1.12	5.93	0.63
UID11	0.16	0.82	0.58	-	0.79	0.61	-	9.59	3.00	0.45	3.94	0.72	0.11	3.25	0.62	0.12	4.97	0.63
UID32	0.15	0.46	0.58	-	0.50	0.61	-	9.53	2.83	1.02	0.94	1.31	0.10	1.46	0.62	0.12	3.53	0.63
UID33	-	0.10	0.58	-	0.10	0.61	-	1.20	2.14	-	0.14	0.72	-	0.36	0.62	-	1.01	0.63
UID34	0.42	1.26	0.58	-	1.35	0.61	-	9.94	3.77	0.28	0.94	0.72	0.15	3.32	0.62	0.11	6.00	0.63
WF-3	0.10	1.00	0.58	-	1.11	0.61	-	9.97	144.20	0.67	0.14	0.72	0.94	3.73	0.62	0.51	4.94	0.63

[a]SS= Spore germination method using standard dilution plating

[b]RS=Relative growth using standard dilution plating

[c]SG=Relative growth using spiral gradient dilution

3.4 Discussion

In vitro fungicide sensitivity screening using *H. solani* isolates from commercial potato fields in 2018 and 2019 against three classes of fungicides (DMI: DMZ, PP: FDL, and SDHI: PFT, SDX, BVF, SYN) were chosen for this study. The chemistries chosen are the active ingredient(s) in registered fungicides: DMZ; FDL; SDX and BVF or in the process of getting registered: PFT; and SYN, for the management of silver scurf. Three classes were chosen to determine multiple sensitivity across FRAC codes and four SDHI fungicides were chosen to evaluate any cross-sensitivity within the same FRAC code. The relative radial growth method using a standard dilution plating series was chosen because the assay is designed to be accurate and precise for screening ascomycete fungi against DMI, PP, and SDHI fungicides (5). However, it is time consuming, costly, and requires more fungal mass meaning additional clean isolates are required to complete all the screenings (5). The relative germination method using a standard dilution plating series was chosen because the assay is designed to be accurate and precise for screening ascomycete fungi against SDHI fungicides but, is time-, labor-, and resource-intensive (5). The relative growth method using a spiral gradient dilution was chosen because the assay is more time and resource efficient (5) by screening eight isolates per plate but, the accuracy was questioned because of a high risk of observational error when reading the spiral plate graph. The relative germination method only involved screening *H. solani* to one class of fungicide (SDHI) because they target respiration and spore germination, not mycelial growth (9). The fungicide classes: DMI and PP are designed to target mycelial growth, not germination (9). Determining *H. solani* sensitivity to three classes of fungicides using three methods provided fungicide sensitivity data while allowing for comparison of methods.

Literature on the effectiveness of SDHI fungicides (e.g. PFT, SDX, or BVF) on silver scurf is lacking. Preliminary data generated in 2016 found for BVF and SDX, 71.4 and 45.7% of *H. solani* isolates (out of 33) screened had EC_{50} values <1 mg/L (15). SYN549522 is an experimental SDHI fungicide by Syngenta. Therefore, the sensitivity of *H. solani* to SYN549522 has not been reported. However, there is research showing activity against *H. solani* for another fungicide with the same MOA (respiration), azoxystrobin, which targets complex III: cytochrome bc1 and belongs to the group Quinone outside inhibitors (QoI) (1, 13). Azoxystrobin is effective by binding to the cytochrome b complex III at the Q_0 site in mitochondrial respiration, subsequently inhibiting fungal respiration (16). While the MOA is the same, both target site and chemical or biological group are different from SDHI fungicides. This research evaluated the effectiveness of four SDHI fungicides against *H. solani*.

Relative germination using a standard dilution plating series provided data on the activity of SDHI fungicides against *H. solani*. The mean EC_{50} values ranged from 0.33-0.57 mg/L and the SDHI fungicide performance from best to worst was BVF, PFT, SYN followed by SDX. For all SDHI fungicides evaluated, over 80% of isolates screened against them were categorized as sensitive (EC_{50}: <1 mg/L). These results are promising for the use of SDHI fungicides against silver scurf, but additional testing is needed.

Relative radial growth using a standard dilution plating series evaluated the sensitivity in *H. solani* to SDHI, DMI and PP fungicides. A study from 2017 compared difenoconazole, colloidal silver, azoxystrobin and thiabendazole and resulted in difenoconazole as the most effective fungicide against *H. solani* ($EC_{50} \leq 0.12$mg/l) (10). In 1998 and 1999 fludioxonil was evaluated as a seed treatment for the control of silver scurf and did not significantly reduce disease severity of daughter tubers or increase storability (8). In another study, when fludioxonil was used as a seed

treatment for the management of silver scurf, disease incidence was not significantly reduced in Oregon and Washington (6).

The results from this study estimated the mean EC_{50} values ranged from 0.72-5.18 mg/L and the fungicide performance from best to worst was PFT, DFZ, BVF, SDX, SYN, and followed by FDL. For PFT and DFZ, over 70% of isolates screened against them were categorized as sensitive (EC_{50}: <1 mg/L). Over 50% of isolates screened against SDX, BVF and SYN were categorized as reduced sensitive (EC_{50}: 1-5 mg/L) while 50% of isolates screened against FDL were categorized as moderately insensitive (EC_{50}: 5-10 mg/L). These results indicate activity of SDHI and DMI fungicides to *H. solani.* Conversely, results from sensitivity assays of FDL indicates a lack of activity to *H. solani*.

Relative growth using a spiral gradient dilution evaluated the sensitivity of SDHI, DMI, and PP fungicides while providing insight into whether the method was an alternative for the relative radial growth method using a standard dilution plating series. Mean EC_{50} values ranged from 0.58-69.15 mg/L and the fungicide sensitivity from highest to lowest was PFT, DFZ, BVF, SYN, SDX, and followed by FDL. Frequency distributions of in vitro sensitivity of *H. solani* isolates resulted in 100% of isolates screened against PFT, DFZ, and BVF and over 85% of isolates screened against SDX and SYN categorized as sensitive (EC_{50}: <1 mg/L), while 50% of isolates screened against FDL were categorized as insensitive (EC_{50}: >10 mg/L). These results indicate activity of SDHI and DMI fungicides to *H. solani.* Additionally, results from sensitivity assays of FDL indicates a lack of activity to *H. solani*.

The relative germination method using a standard dilution plating series may be used for estimating the in vitro sensitivity of *H. solani* isolates against SDHI fungicides. The method unfortunately cannot be used to evaluate DMI or PP fungicides because they target mycelial growth

and not spore germination. However, this method is very time and resource consuming to plate/read but does provide results within 24 hours of plating. Therefore, the relative germination method for SDHI fungicides is recommended because the assay is relatively short (24 h vs. 4 wk). The relative radial growth method using a standard dilution plating series is more time-, cost-, and resource- consuming than the relative growth method using a spiral gradient dilution. Therefore, the relative growth method using a spiral gradient dilution is recommended over using a standard dilution plating series for evaluation of DMI and PP fungicides. The results of this study further support reduced sensitivity to FDL in *H. solani*. (8) but DFZ still has activity (10). The SDHI fungicides with the highest sensitivity were PFT and BVF. The other two SDHI fungicides (SDX and SYN) were active against *H. solani* but were inconsistent and should be further evaluated before recommending as a potential management tool for silver scurf. Overall, PFT, BVF, and DFZ should primarily be studied for effectiveness against *H. solani*. The registered postharvest fungicide Stadium has the active ingredients; azoxystrobin, FDL, and DFZ. Therefore, postharvest treatment with Stadium, and other fungicides with the active ingredient FDL, is further increasing the population of *H. solani* with reduced sensitivity to FDL and should be re-evaluated as a means to manage silver scurf. This study provided valuable information including three active ingredients with activity against *H. solani*, evidence of reduced activity of the active ingredient FDL on *H. solani,* and recommended method(s) for screening *H. solani* sensitivity to three classes of fungicides.

3.5 Conclusions

Silver scurf, caused by *Helminthosporium solani* Durieu & Montage, has become increasingly more important to the potato production industry as fungicide resistance in pathogen

populations is reported (6). Resistance to an effective chemistry against *H. solani*, thiabendazole (TBZ), began in the 1990s due to over-use as a treatment for *Fusarium* spp. (6). Resistance in pathogen population has resulted in limited effective chemistries for managing silver scurf (11, 14) and therefore, management requires several control methods (3). Research on effective chemistries or review of methods used to evaluate sensitivity of *H. solani* in vitro is lacking. This research was designed to evaluate *H. solani* sensitivity to three classes of fungicides while comparing three evaluation methods. The study resulted in *H. solani* being the most sensitive to PFT, BVF and DFZ. While screening SDHI fungicides, the relative germination method and/or the relative growth method using a spiral gradient dilution are recommended. The relative germination method provides faster results (24 h versus 4 wk) but is more expensive. The relative radial growth method using a standard dilution plating series is not recommended because it requires 4 wk and costs more than the relative growth method using a spiral gradient dilution. In regard to screening DMI and PP fungicides, the relative growth method using a standard gradient dilution is recommended over the relative radial growth method using a standard dilution plating series because it is less time-, cost- and resource-consuming, while providing similar results. Further studies are required to evaluate PFT, BVF, and DFZ as effective chemistries for silver scurf management in the field and in storage. Additional isolates should also be screened across all labeled and registered fungicides and methods used in this study.

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CONCLUSIONS AND FUTURE DIRECTION

Verticillium wilt caused by *Verticillium dahliae* Kleb. and Potato Early Die (PED) caused by the interaction of *Verticillium dahliae* and *Pratylenchus penetrans*, are annual concerns for potato producers in Michigan and throughout the US. Inoculum levels of *V. dahliae* in the soil are correlated to disease incidence and disease severity is further exacerbated by the presence and level of Root Lesion Nematode (RLN) (*P. penetrans*) populations in soil. Therefore, managing levels of *V. dahliae* CFU/g soil and RLN populations in soil are common management strategies. In the past, PED was primarily managed by costly soil fumigation. However, there are human and environmental health risks associated with soil fumigation. The combination of toxicity and cost subsequently resulted in growers searching for alternative management strategies for PED. The results of experimental field trials from 2018 and 2020 indicated that applications of Vydate C-LV provide the best control of RLN populations in the soil. In 2020, treatment with Emesto Silver (B) + Vydate C-LV (C & D) + Luna tranquility (D & E) reduced the RLN populations in soil, numerically lower VD% in tubers and increased total yield compared to the NTC. Future research could maximize the effectiveness of Vydate C-LV in limiting RLN populations in soil based on application timing, placement, and dosage. Additional treatments could be added to combine three applications of Vydate C-LV with the protection of a seed treatment such as Emesto Silver and the fungicide Luna Tranquility. It may be beneficial to combine Vydate C-LV and Elatus in a research trial because Vydate C-LV effectively controlled *P. penetrans* and Elatus is registered for suppression of *V. dahliae*. It would also be useful to evaluate the added effects of Vydate C-LV by comparing the following two treatments 1) Emesto Silver + Luna Tranquility; and 2) Emesto Silver + Luna Tranquility + Vydate.

Silver scurf caused by *Helminthosporium solani* is of increasing concern to potato growers in Michigan and the US. The disease was primarily controlled by Thiabendazole (TBZ) fungicides until resistance was documented across the globe in the late 1990s. Resistance of pathogen populations to key fungicides has resulted in the need for alternative management strategies. Due to limited research on silver scurf management there is little known about the comparison of methods used to screen *H. solani* isolates for fungicide sensitivity. The results from in vitro sensitivity screening of *H. solani* revealed isolates that were most sensitive to PFT, DFZ, and BVF. To screen SDHI fungicides the relative germination method and/or the relative growth method using a spiral gradient dilution should be used. When screening DMI and PP fungicides, the relative growth method using a spiral gradient dilution should be used over the relative radial growth method using a standard dilution plating series to conserve resources. Future research could include screening additional isolates against SDHI fungicides and DFZ. It could be helpful to incorporate a greenhouse trial that evaluates fungicide efficacy against silver scurf on daughter tubers. Any chemistries that show promise in vitro and/or in the greenhouse could be further tested in a field trial to evaluate their efficacy using disease incidence at harvest and storability. A genetic approach to evaluate isolates with higher EC_{50} mg/L values may be important for monitoring and classifying any observed fungicide resistance.