MANAGING *SCLEROTINIA SCLEROTIORUM*: AN EMERGING THREAT TO THE SUGAR BEET INDUSTRY

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Title

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

MASTER OF SCIENCE

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ABSTRACT

Recent studies have reported *S. sclerotiorum* (Lib.) de Bary as an emerging pathogen of sugar beet (*Beta vulgaris* L.) causing leaf blight, seedling damping-off and root necrosis, becoming a fundamental production problem in the Red River Valley of North Dakota and Minnesota. This study aimed to (1) assess inoculation methods for sugar beet reaction to *S. sclerotiorum*, varietal response and cross-infectivity, and (2) evaluate fungicide efficacy against Sclerotinia diseases in three sugar beet varieties. Disease evaluation was measured as lesion size. Results from this study indicated that barley inoculum was particularly effective in causing leaf blight on sugar beet plants. CrystalM837 variety showed reduced susceptibility to Sclerotinia leaf blight. ACH166 and Beta7029 were moderately susceptible to leaf blight, but for root infection, they showed reduced susceptibility which was significant at all stages during the fungicide efficacy study (P=0.05). Proline and Priaxor provided the most effective control against Sclerotinia leaf blight and root necrosis (P=0.05). These findings offer critical insights into sugar beet variety and fungicide selection for effective control of *S. sclerotiorum*, as well as the cross-infectivity status of major host crops (soybean, sunflower and canola) providing valuable information for crop rotation decisions towards mitigating losses caused by *S. sclerotiorum*.

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DEDICATION

My dedication goes to GOD Almighty, the reason for my existence and all the successes I have achieved thus far, my family who has been my everything, my advisors (Dr. Ajayi, Dr. Biodun-Cole and Prof. Khan) who believed and saw potential in me and presented me a platform to excel. And, to all scientists in the field of plant pathology, whose research works proved resourceful and has contributed to the advancement of global plant health.

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LIST OF ABBREVIATIONS

ACSC .....American Crystal Sugar Company

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AES .....Agricultural Experiment Station

ANOVA .....Analysis of Variance

AOX .....Alternative Oxidase

ARS .....Agricultural Research Service

ATP .....Adenosine Triphosphate

AUDPC .....Area Under the Disease Progress Curve

CLS .....Cercospora Leaf Spot

CP .....Cytochrome Pathway

CWDEs .....Cell Wall Degrading Enzymes

CYTB .....Cytochrome B gene

DMIs .....Demethylation Inhibitors

DPI .....Days Post Inoculation

ERS .....Economic Research Service

FAOSTAT .....Food and Agricultural Organization of the United States

FRAC .....Fungicide Resistance Action Committee

ISO .....International Sugar Organization

LSD .....Least Significant Differences

MBCs .....Methyl Benzimidazole Carbamates

MDFC .....Minn-Dak Farmer Cooperative

MOA .....Mode of Action

MTZ .....Metconazole

NPK.....Nitrogen, Phosphorous and Potassium

NSI.....National Sclerotinia Initiative

OA.....Oxalic Acid

PDA.....Potato Dextrose Broth

PROC GLM/GLIMMX .....General Linear Mixed Model Procedure

QoIs.....Quinone Outside Inhibitors

ROS.....Reactive Oxygen Species

SAS .....Statistical Analysis System

SDHIs.....Succinate Dehydrogenase Inhibitors

SHAM.....Salicylhydroxamic Acid

SMBSC .....Southern Minnesota Beet Sugar Cooperative

SSR .....Sclerotinia Stem Rot

TM.....Thiophanate-Methyl

USDA.....United States Department of Agriculture

USEPA.....United States Environment Protection Agency

1. LITERATURE REVIEW

1.1. The Sugar Beet Industry: History and Development

Sugar beet (*Beta vulgaris* L.) is a dicot in the family Amaranthaceae (formerly Chenopodiaceae) in the order Caryophyllales (McGrath and Townsend 2015). Sugar beet accounts for 14% of the world's sucrose requirement, which is considered the second most important source of sucrose (FAOSTAT, 2022) after sugar cane (*Saccharum officinarum*). As opposed to sugar cane, which is largely cultivated in the tropical and subtropical regions, sugar beet is mostly grown in the colder temperate regions with latitudes between 30° and 60° N, such as Northern America, Europe, Africa, and Asia, which accounts for a total estimate of 50 sugar beet producing countries (Winner, 1993).

The origin of sugar beet can be traced back to 8500 B.C. (Pathak et al. 2022). The ancestors of sugar beet are said to be the wild sea beets (*B. vulgaris ssp. maritima, or B. maritima*) usually found at mean sea level (Winner, 1993). The sugar beet industry started with the domestication of the wild parent species which were unconsciously harvested and eaten by prehistoric men (Stevanato and Panella, 2013). This unconscious selection led to the discovery of *Beta vulgaris,* which became a highly valued commodity in Germany in the 1700s. Its use as a sweetener was discovered by Oliver de Serres, a French agronomist in 1705 (Austin 1928). Andreas Marggraf, a German Chemist, discovered in 1747 that the pulverized sugar beet crystals obtained after crude extraction were similar to that of sugarcane. Thirty-seven years after Andreas Marggraff discovered crystal by-products from sugar beet, his student Franz Carl Archard began cultivar selection for sugar beets with high sugar content and also developed the extraction process of sugar from sugar beet in 1784. Archard initiated the world's first sugar factory and production in 1801 at Kunern, Silesia (Poland) (Austin 1928).

The globalization of the sugar beet industry followed the success of Archard, which caught the attention of Napoleon Bonaparte, the French Emperor and military leader during the European Continental Blockade in the early 19th century. The European blockade caught off access to raw cane sugar supply from the West Indies, the French Empire had to experiment the cultivation and processing of sugar from sugar beet which proved successful resulting to the initial establishment and expansion of sugar beet processing which amounted to 543 processing plants in 1837 (Pathak et al. 2022). By mid-19th century, the sugar beet industry had been well established for sugar production in Europe (Cooke and Scott, 1993). With adequate technical developments and favorable government policies supporting sugar beet cultivation and sugar processing, the sugar beet industry expanded throughout Europe, Asia, Africa, North America, and South America (Francis, 2006).

Sugar beet production in the United States first started in 1838 with two Americans who lived for a while in Paris. Edward Church and David Lee Child built the sugar factory in Northampton, Massachusetts, which unfortunately closed soon after due to low sucrose extraction and sugar production (Winner, 1993). After many challenging attempts to successfully introduce sugar beet cultivation and processing to the United States, Dyer, a businessman, now considered the founder of the sugar beet industry in America, successfully built the first functional sugar beet processing factory in Alvarado, California in 1870 (Coons, 1949). By 1900 the United States had 34 functional sugar beet processing factories (Francis, 2006). The sugar beet industry rapidly expanded in the 20th century with the support of the American trade policy which helped protect the small domestic sugar industry while the implementation of the Jones-Costigan Amendment also known as the Sugar Act taxed imported sugar (Souder, 1971).

Russia, France, Germany, and the United States (U.S.) are ranked consecutively as the top four (4) sugar beet producing nations globally (FAOSTAT, 2017). In a report made by USDA-ERS (2021), the United State produced an estimated 8.4 million metric tons of sugar processed from sugar beet which contributes 55% of the total domestic sugar production. A total of 33 million tons of sugar beet were harvested in 2019 from an estimated 450,000 hectares across ten primary sugar beet-producing states: Colorado, California, Idaho, Michigan, Minnesota, Montana, North Dakota, Nebraska, Oregon, and Wyoming (USDA-ERS, 2019).

The first sugar beet processing factory in the Red River Valley was built in Grand Forks by American Beet Company in 1926, now known as American Crystal Sugar Company (ACSC). Currently, three cooperatives: Minn-Dak Farmer Cooperative (MDFC), Southern Minnesota Beet Sugar Cooperative (SMBSC) and the American Crystal Sugar Company (ACSC) coordinate the seven sugar beet processing factories in North Dakota and Minnesota (Shoptaugh, 1997). North Dakota and Minnesota have consistently held the forefront in sugar beet production, covering 57% of the total sugar beet production in the United States, contributing a significant bi-state's total economic activity estimated up to $6.2 billion (Bangsund and Hodur, 2023; ISO 2020; USDA-ERS, 2021). In the Red River Valley of North Dakota and Minnesota, the economic value and production of sugar beet is limited by many factors including weeds, and insects, with diseases being the most prominent and challenging one. Sugar beet diseases are caused by all pathogenic groups including oomycetes, viruses, bacteria, nematodes, and most importantly fungal pathogens (Jacobsen 2006).

1.2. Diseases Affecting Sugar Beet

In general, the most common root diseases of sugar beet are Rhizoctonia crown and root rot (*Rhizoctonia solani* Kühn), Fusarium yellows (*Fusarium oxysporum f. sp. betae* Snyder &

Hansen), Fusarium yellowing decline (*Fusarium secorum*), Sclerotinia root rot (*Sclerotium rolfsii* Sacc. teleomorph, Athelia rolfsii (Curzi) Tu and Kimbr) and Aphanomyces root rot (*Aphanomyces cochlioides* Drechsler) (Asher and Hanson, 2006, Farhaoui et al. 2023). Rhizomania, caused by beet necrotic yellow vein virus (BNYVV) and transmitted by *Polymyxa betae* Keskin, is also an important viral disease affecting sugar beet production (Canova et al., 2016). Plant-parasitic nematodes as reported in many other crops (Aderoju & Ajayi, 2022) affect sugar beet particularly Sugar beet Cyst Nematode (Nelson et al. 2012). On aerial plant parts, the most important disease is Cercospora leaf spot (CLS), which is caused by *Cercospora beticola* Saccardo. CLS is considered the most economically damaging disease of sugar beet causing a significant yield loss and reduction in recoverable sucrose content (Dexter et al. 1998; Skaracis et al. 2010; Harveson, 2013). *S. sclerotiorum* has been added to the list of fungal pathogens affecting sugar beet. Recent reports have confirmed *S. sclerotiorum* to be an emerging pathogen of sugar beet causing leaf blight and root rot in numerous sugar beet producing states in the United States (Khan et al. 2020; Khan et al. 2021; Bhuiyan et al. 2021). Considering the increasing number of damages caused by the collective effort of these fungal pathogens, there is an urgent need for extensive study on how to manage the already established pathogens, like *C. beticola* and *R. solani*, as well as emerging pathogens, like *S. sclerotiorum.*

1.3. Sclerotinia Leaf Blight and Root Rot Diseases of Sugar Beet

Sclerotinia sclerotiorum (Lib) de Bary generally known as “white mold”, has been added to the list of devastating fungal pathogens of sugar beet amongst others, such as *Cercospora beticola, Rhizoctonia solani*, *Aphanomyces* sp., *Fusarium* sp., *Phytophthora* sp., *Rhizopus* sp., and *Pythium* sp., which causes significant economic annual losses annually (Franc et al. 2001; Jacobsen 2006; Bolton et al. 2010; Secor et al. 2013; Khan et al. 2020; Khan et al. 2021, Bhuiyan

et al. 2021). *S. sclerotiorum* has a broad geographical distribution prevalent in many US states, especially in the North Central region (Bradley et al. 2006), where it has been responsible for significant economic losses. Due to the wide host range of *S. sclerotiorum,* the infection could lead to a total abandonment of fields used for cultivating preferred crops (Purdy, 1979). *S. sclerotiorum* has been associated with 60 names due to its cosmopolitan nature (Purdy, 1979) including cottony rot, watery soft rot, stem rot, drop, crown rot, blossom blight and, most commonly, "white mold". This pathogen causes sclerotinia stem rot (SSR) which is reportedly the most damaging disease of soybean and canola (Willbur et al. 2019; del Río et al. 2007) in North Dakota, and basal stalk root and wilt, and head and mid-stalk rot on sunflower (Mathew et al. 2020).

In Japan, there was a previously unreported case of *S. sclerotiorum* causing Sclerotinia stalk rot in sugar beets in 1983, which was further confirmed by Naito and Sugimoto (1986), after which no other concrete report has been made in sugar beet fields until recently in 2020 in the United States. In July 2019, the incidence of *S. sclerotiorum* causing leaf blight on sugar beet in the United States was first reported in North Dakota (Khan et al. 2020), the infected leaves collected from the sugar beet field in Fairmount, North Dakota, showed signs of light brown to black necrotic leaf lesions with grayish mycelial outgrowth at the center on the commercial sugar beet, which were similar to symptoms on other host plants such as soybean, canola, sunflower, which are known hosts of this pathogen (Heffer Link and Johnson, 2007).

Recent studies have shown *S. sclerotiorum* to be an emerging pathogen of sugar beet causing leaf blight and root rot in numerous sugar beet producing states in the United States due to its cosmopolitan nature. In 2021, *S. sclerotiorum* was reported to cause root rot and necrotic lesions in commercial sugar beet fields in Moorhead, MN (46.9190 N, 96.70610 W). Affected sugar beet plants showed wilting and typical rot symptoms along with whitish mycelia growth and

blackish sclerotia on roots. The isolation, morphological identification, molecular assay, and greenhouse trial carried out further confirmed the pathogenicity of *S. sclerotiorum* as the causative organism of disease (Bhuiyan et al. 2021).

More recently, a first report was made in 2021 for *S. sclerotiorum* causing similar leaf blight on sugar beet in Minnesota (Khan et al. 2021) and in 2022, five fields surveyed around the Sidney Sugar Factory district in Montana also showed obvious symptoms of leaf blight (Khan et al. 2022). Approximately, 40 to 50% of the field plants were affected, and about 70 to 80% of the infected leaf area blighted. The increasing reports of the spread of this pathogen across sugar beet producing states (North Dakota, Minnesota, Montana, Wyoming, and Colorado) are an indication of its potential threat to the sugar industry (Khan et al. 2022).

1.3.1. Description of the Pathogen: *Sclerotinia sclerotiorum* (Lib.) de Bary

S. sclerotiorum (Lib.) de Bary is an ascomycetous class, soil-borne, belligerent, non-specific, ubiquitous, cosmopolitan, necrotrophic plant pathogen affecting more than 500 plant species, majorly dicotyledonous plants consisting of approximately 225 genera and 64 families, as well as a few monocotyledonous plants such as tulip and onion (Purdy, 1979; Boland and Hall, 1994; Sharma et al. 2016a). *S. sclerotiorum* was first referred to as *Peziza sclerotiorum* (Bolton et al. 2006), then *S. libertiana* Fuckel in 1870 (Purdy 1979). To maintain consistency with the International Code of Botanical Nomenclature system, the name was changed to *S. sclerotiorum* (Lib.) de Bary in 1884 (Purdy 1979) and was placed in the Class Leotiomycetes within the Order Helotiales (Heffer Link and Johnson, 2007) and further assigned to the Family Sclerotiniaceae (Whetzel, 1945). A particularly unique feature of *S. sclerotiorum* is its ability to overwinter as sclerotia. The sclerotia are long-term survival structures made of mycelial aggregates surrounded by a rind with high melanin content; melanin which serves as a protective element during adverse

environmental conditions (Bell and Wheeler, 1986). Sclerotia can remain viable for up to 8 years or more in the soil (Brustolin et al. 2016), and at the onset of the growing season under favorable conditions they produce ascospores which serve as sources of inoculum for infection (Whetzel, 1945; Kohn, 1979; Heffer Link and Johnson, 2007).

This fungus alone is responsible for more than $ 200 million annual crop loss in the United States (USDA, 2005). This led the United States Congress to approve the establishment of the National Sclerotinia Initiative in 2004, managed by the USDA, Agricultural Research Service (ARS) which focuses on conducting advanced research on biology, disease epidemics, host resistance and management of diseases caused by *S. sclerotiorum. Sclerotinia sclerotiorum* causes a significant reduction in the quality and yield of several economic crops in the United States including sugar beet and has been reported as a potential threat to sugar beet production in the Red River Valley of North Dakota and Minnesota (Khan et al. 2020; Khan et al. 2021, Bhuiyan et al. 2021).

1.3.2. Taxonomic Classification of *Sclerotinia sclerotiorum*

Kingdom: Fungi

Phylum: Ascomycota

Class: Leotiomycetes

Order: Helotiales

Family: Sclerotiniaceae

Genus: Sclerotinia

Species: *Sclerotinia sclerotiorum* de Bary

(Xia et al. 2019)

1.3.3. Disease Epidemiology and Symptoms of Sclerotinia Diseases

S. sclerotiorum is a highly damaging monocyclic pathogen with no production of secondary inoculum after the completion of the initial infection cycle (Fig. 1.1), it proceeds to form an overwintering survival structure called "sclerotia" which serves as an inoculum source in the soil (Kabbage et al., 2015; Hossain et al. 2023). *S. sclerotiorum* is widely considered a necrotrophic pathogen (Bolton et al. 2006), but recent studies have shown that it acts as a hemi-biotroph exhibiting a double-feeding lifestyle which transits from biotrophy to necrotrophy in a two-phase infection model during the disease cycle (Liang & Rollins, 2018; Wang et al. 2019; Xia et al 2019). The biotrophic phase is characterized by cuticle penetration, development of subcuticular bulbous hyphae, and suppression of the host basal defense. The necrotrophic phase starts with the upregulation of the effector genes which interferes with the host-pathogen recognition and defense mechanism, an extensive growth of the of the already initiated subcuticular hyphae developing into the ramifying hyphae, this colonize the epidermal and mesophyll cells, signaling the production of reactive oxygen species (ROS), oxalic acid (OA) and OA-independent toxins, cell wall degrading enzymes (CWDEs) which cumulatively leads to cell death, tissue rots and eventual plant death (Liang & Rollins, 2018; Ding et al., 2021; Hossain et al. 2023). *S. sclerotiorum* attacks host plants either using ascospores that can be discharged forcibly upwards from apothecia into the air or by mycelium arising from infected tissue or germinated sclerotia (Hartill & Underhill, 1976; Willetts & Bullock, 1992). When ascospores land on susceptible host tissue, they can germinate under favorable conditions and start a new cycle of infection. Under moist and cool conditions, this fungus rapidly grows inside the infected host tissues and develops symptoms of browning, water-soaking, and a white, cotton-like mycelium, which leads to necrosis, stunting, premature ripening, and wilting of the host (Bolton et al. 2006) Upon killing the host, the fungus

grows saprophytically on the dead plant tissue. Sclerotia are later abundantly formed on the host surface and cavities, in plant debris, and in soil, where they can remain dormant for up to 8 years or more (Ayers and Adams, 1979; Brustolin et al. 2016).

S. sclerotiorum can infect all above-ground parts and roots of the plant, including flower petals, leaves, petioles, stems, and pods. The general disease cycle of *S. sclerotiorum* begins with the sclerotia, a melanized structure of mycelia that falls to the soil during harvest. The sclerotia is a key component in the disease epidemiology of *S. sclerotiorum*, it can germinate myceliogenically or carpogenically depending on favorable climatic conditions (Le Tourneau, 1979; Willetts and Wong, 1980; Bardin and Huang, 2001, Clarkson et al. 2003). Hyphae growing from myceliogenic germination is triggered by exposure to high nutrient conditions or direct contact with healthy plants (Le Tourneau 1979) while apothecia formation is the result of carpogenic germination (Clarkson et al. 2003) which is influenced by environmental conditions. Ascospores function as a major source of inoculum (Abawi and Grogan 1979), forcefully dispersed by a mechanism called puffing (Hartill & Underhill, 1976).

Sclerotia buried within 2-3 cm depth is the required soil depth for the germination of viable apothecium (Abawi and Grogan 1979). The optimum temperature for sclerotia germination is 15-25°C and continuous soil moisture for 10 days (Bardin and Huang, 2001; Wu and Subbarao, 2008). Under favorable environmental conditions and optimum water potential for the production of apothecium could vary between -80kPa to 240kPa and sometimes -300kPa, almost close to field capacity depending on adaptation to reduced soil moisture tension (Abawi and Grogan 1979, Clarkson et al. 2004, Nepal, 2009). Sclerotia produce apothecia between 10-30 days, and ascospores are forcibly released by puffing at a height of 10-100 cm height, during 5 to 10 days after maturation (Shwartz, 1977; Abawi and Grogan 1979; Caesar and Pearson 1983; Phillips

1987). The ascospores produce a germ tube which helps in penetration due to mechanical force when it comes in close contact with the plant tissue, while the hyphae penetrate the cuticle layer of the host directly by mechanical force using the infection cushion or by producing enzymes (Lumsden and Dow, 1973; Liang & Rollins, 2018; Chethana et al. 2021). Upon penetration, the hyphae colonize the host tissue through the open stomata, and spread to other areas of the plants, degrading the nutritional status of the plants, and forming sclerotia on the plant surface in the absence of nutrients to feed on (Abawi and Grogan, 1979; Bhuiyan et al. 2021; Khan et al 2022).

Symptoms of *S. sclerotiorum* vary from plant to plant and might be host specific. In most plants like canola, sunflower and many others, infection is first initiated under field conditions at the flowering stage and sometimes dead or senescent plant tissues (Jamaux et al. 1995) like the case of sugar beet plant that does not flower, causing stem rot, leaf blight, head rot, stalk rot, root decay and crown rot, etc. In flowering plants, ascospores initially colonize the flower petals which initiates extensive mycelium growth which progresses towards the healthy green tissues of the plant, infecting the petioles, sometimes pods and in most cases the stem and leaves producing water-soaked lesions. This is influenced by temperature, relative humidity and the concentration of ascospores (Harikrishnan and del Río 2006). Once established, the water-soaked lesions continue to expand rapidly and move through the petiole infecting other parts of the plant. Infected parts with water-soaked lesions appear soft brown to whitish on the leaves and petioles (Shahoveisi and del Río, 2020) and eventually turn white-greyish or appear bleached. The “white mold” which is used synonymously for the description of *S. sclerotiorum* is a distinctive symptom of this pathogen infection on a wide array of crop species, the light greyish to white fungal growth may appear on the lesions during periods of high humidity (Bolton et al. 2006). In some situations, sclerotia formation occurs at the end of the season wherever the lesion exists on the plant

depending on environmental conditions, host resistance and pathogen virulence, the sclerotia serve as overwintering survival structures persisting in the soil as an inoculum source for more than four years (Ayers and Adams, 1979; Brustolin et al. 2016).

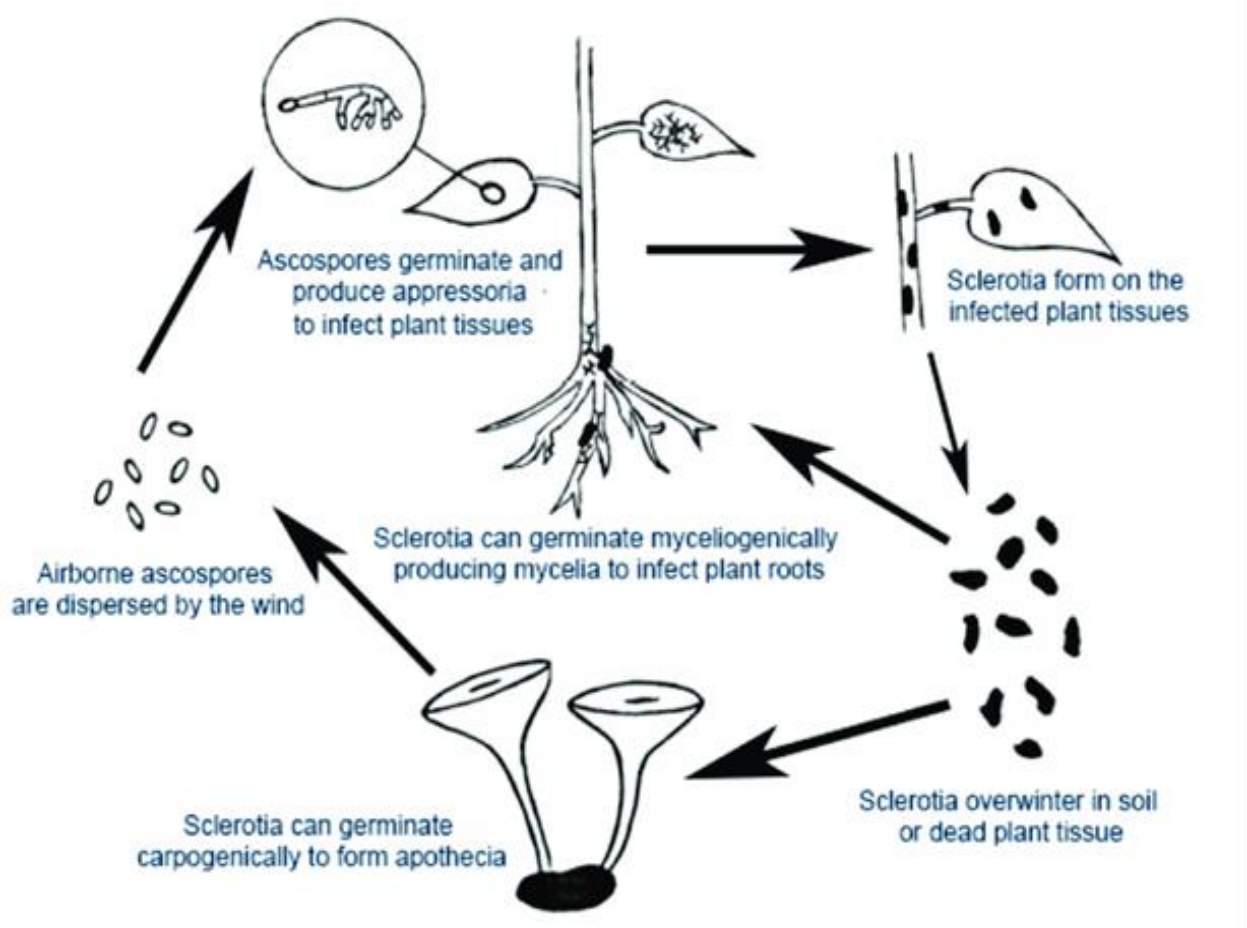

Figure 1.1. Generalized disease cycle of *Sclerotinia sclerotiorum* (Xia et al. 2019)

1.4. General Management of Sclerotinia Diseases

Traditionally, *S. sclerotiorum* can be difficult to control due to its wide host range and ability to survive in the soil as sclerotia which serve as a constant source of inoculum in the field (Purdy, 1979). Also, most plants have a low level of resistance to this pathogen, making it easier for the establishment of diseases caused by *S. sclerotiorum.* According to previous studies, this pathogen requires a multi-approach management strategy combining cultural practices, chemical

fungicides, bio-control agents, and resistant cultivars for effective control (Duncan, 2003). Due to its monocyclic nature, reducing inoculum sources (sclerotia) in the field is the most significant approach to reducing disease pressure, other cultural agronomic practices such as the use of clean seeds (Tu, 1989), plant spacing (Hoes and Huang, 1985), rotation with tolerant or non-host crops (Garcia-Garza et al. 2002). Reduced irrigation reduces the distribution and spread of the sclerotia through the fields and also movement to other fields (Schwartz and Steadman, 1978), while tillage practices could help bury the sclerotia deeper into the soil reducing the germination of the apothecia which is usually not longer than 3cm (Abawi and Grogan 1979), eventually reducing the initial incidence and potential epidemics of the *S. sclerotiorum* diseases. A few bio-control agents have been integrated as an alternative control strategy in the production of many crops like soybean, canola, soybean, and many others (Zeng et al. 2012). Some of them like *Coniothyrium minitans* (Zhao et al. 2020; Chitrampalam et al. 2008), *Trichoderma* species and *Sporidesmium sclerotivorum* (Bardin and Huang; 2001 del Río et al. 2002) function as effective myco-parasitic biocontrol agents. Of them, *S. sclerotivorum* seems to be the most effective with a 95% ability to reduce inoculum density 10 weeks post-application (Ayers and Adams, 1979; del Río et al. 2002), while *C. minitans* which seems to be the only commercially available mycoparasite (Zhao et al. 2020).

The selection of resistant cultivars is the most desirable approach for managing plant diseases because of its positive impact on environmental health. However, there is a limited breakthrough in developing cultivars completely or substantially resistant to *S. sclerotiorum* infection. It is challenging to breed for resistance to *S. sclerotiorum* because it is controlled by multiple genes (Zhao and Meng, 2003); in addition, the lack of host specificity (Sharma et al. 2016b) and absence of a strong host single-gene resistance (Bolton et al 2006) makes it difficult

to improve resistance against *S. sclerotiorum* using classical breeding methods (Wang et al. 2019). With this status, there is a need for continuous research on identifying techniques for breeding cultivars or varieties with improved resistance to diseases in crops (John et al. 2023), most especially sugar beet which is a new host for *S. sclerotiorum* in the United States. In *Brassica* plants, there is a more visible potential for resistance control against *S. sclerotiorum* using marker-assisted selection to find and incorporate physiological resistance into crop cultivars (Bolton et al 2006).

The most practical and significantly effective method for controlling sclerotinia diseases more like any other fungal disease is the appropriate application of fungicides. Optimum timing and effective fungicide application methods are as important as the fungicide's effectiveness for effective chemical control of *S. sclerotiorum* and any other fungal disease. Among several fungicides registered for use in combating this pathogen, azoxystrobin (Quadris, Syngenta Crop Protection, Greensboro, NC), prothioconazole (Proline, Bayer Crop Science, St. Louis, MO), thiophanate-methyl (Topsin, UPL, King of Prussia, PA), and boscalid (Endura, BASF, Research Triangle Park, NC) seems to provide effective control of diseases caused by *S. sclerotiorum* in the United States (Bradley et al. 2006; McMullen and Markell, 2010). However, the excessive use of these fungicides poses a threat to environmental health and could induce resistance from the pathogen. The identification of a potential disease risk and practical use of forecasting models is important to make an economically justified decision for effective fungicide applications.

1.5. Fungicide Use and Resistance

Fungicide application has been the primary approach to managing diseases caused by most plant pathogens. Between 2000 and 2004, boscalid, thiophanate-methyl, prothioconazole, azoxystrobin, tebuconazole, benomyl, iprodione, trifloxystrobin or vinclozolin were widely used

for the control of *S. sclerotiorum* in North Dakota and Minnesota (Bradley et al. 2006). Currently, there are no approved fungicides for the control of *S. sclerotiorum* in sugar beet fields; however, fungicides like QoIs; FRAC group 11 [azoxystrobin (Quadris), pyraclostrobin (Headline)], DMIs; FRAC group 3 [metconazole (Quash), prothioconazole (Proline)], SDHI; FRAC group 7 [boscalid (Endura)], MBCs; FRAC group 1 [thiophanate-methyl (Topsin)] and are registered for use in controlling SSR in canola and many other crops (McMullen and Markell, 2010). Inpyrfluxam (Excalia) is an experimental fungicide while Priaxor which is a mixture of Fluxapyroxad and Pyraclostrobin, are fungicides that have been introduced in controlling foliar and root diseases of sugar beet caused by fungal pathogens (Khan and Hakk, 2017; Khan and Hakk, 2022).

Fungicide efficacy depends on the appropriate application timing to match the environmental condition for infection and the plant developmental stage. (McMullen and Markell, 2010). In case of SSR infection, fungicides are applied at 25% bloom or slightly before the 50% flowering stage (del Río et al. 2007; Markell et al. 2009; Spitzer et al. 2017) In deciding the ideal fungicide for use, it is helpful to choose fungicides with high specificity at low usage rates with systemic and eradicant activity. Using forecasting models by combining weather data, field history and disease status helps drive a more efficient application (Turkington and Morrall, 1993). The forecasting programs determine the risk of infection which are broadcasted to help growers with spraying decisions.

Inappropriate and repeated use of a fungicide or related fungicides with similar chemical/or biochemical mechanisms results in the resistance of fungal pathogens to these fungicides (Brent and Hollomon, 2007). Fungicide resistance could be partial or field resistance depending on the impact of the changes in the pathogen population sensitivity on the efficacy of the fungicides. Field resistance occurs when the field population becomes sensitive under natural conditions. A report

by Lehner et al. (2015) showed that *S. sclerotiorum* showed field resistance to thiophanate-methyl in common bean in Brazil. However, partial resistance occurs when the majority of isolates have some sort of tolerance that leads to observable loss of disease control as shown for *S. sclerotiorum* in response to carbendazim and MBC in oilseed rape fields in China (Wang et al. 2014; Zhou et al. 2014).

Fungicide resistance can be conferred through (I) altered target site, which reduces the fungicide binding activity; (II) alternative enzyme synthesis, capable of substituting the target enzyme; (III) overproduction of the fungicide target; (IV) active efflux or reduced uptake of the fungicide; and (V) metabolic breakdown of the fungicide (Ma and Michailides, 2005; Gisi et al. 2000; Gullino et al. 2000; Fluit et al. 2001; McGrath, 2001). The risk of fungicide resistance is propelled by many factors associated with both the pathogen and the fungicide itself. Using a single-site mode of action (MOA) results in a greater risk of resistance due to selection pressure as compared with a multi-site mode of action (MOA). To manage the excessive risk of fungicide resistance it is important to adjust to mixed or alternative fungicide application approach rather than repeated use of the same fungicide or related fungicides with similar chemical/or biochemical mechanisms for antifungal action. Also, using the information provided by forecasting programs would help improve optimum fungicide application and efficacy, reducing the buildup of resistant populations (Brent and Hollomon, 2007; Secor et al. 2010). To manage fungicide resistance of the *S. sclerotiorum* population in sugar beet fields, the fungicidal sensitivity of *S. sclerotiorum* isolates from sugar beet production areas should be determined continuously monitored. Monitoring fungicide sensitivity is a consistent measure to prevent the risk of fungicide resistance. To this end, conducting a fungicide sensitivity study for *S. sclerotiorum* which is considered an emerging pathogen of sugar beet is of utmost importance.

1.5.1. MBC Fungicides Resistance (FRAC Group 1)

Methyl Benzimidazole Carbamates consists of fungicides in the chemical class of thiophanate and benzimidazoles (Brent and Hollomon, 2007). Thiophanate-methyl (TM) is one of the most successful and widely used members of the MBC group. They inhibit mitosis and ell division by interrupting ß-tubuline assembly (FRAC 2018). Registered as a fungicide in the United States since 1973, its first use as a fungicide for the control of *S. sclerotiorum* in North Dakota was in 2003 (Mueller et al. 2002; USEPA, 2004). Due to its control efficacy and consistent misuse, TM belonging to the benzimidazole group is now considered a fungicide with high risk of resistance (FRAC, 2023). Benzimidazoles resistance is attributed to a change in the β-tubulin protein at multiple target sites, mostly F200Y and E198A/G/K (Brent and Hollomon, 2007).

In 1975, benzimidazole resistance was first recorded in Australia, two years after the commercial use of benomyl for the control of *Monilinia fructicola* (Whan, 1976). After this first incidence, more case of resistance was reported between 1976 to 1982 in Michigan (Jones and Ehret, 1976; Zehr, 1982), New York (Szkolnik and Gilpatrick, 1977; Zehr, 1982), South Carolina (Ogawa et al. 1981; Zehr, 1982), and in California (Ogawa et al. 1981; Zehr, 1982). In 2001, Gossen et al. reported the first case of resistance of *S. sclerotiorum* to benzimidazole on benomyl product (Benlate) in the Canadian prairies, where he observed that some isolates of *S. sclerotiorum* had extremely high EC_{50} values greater than 200 µg/ml. Carbendazim is the primary metabolite for fungicidal activity in benomyl and TM (Vonk and Sijpesteijn, 1971). Pan et al. (1997) conducted a study to establish a baseline sensitivity for *S. sclerotiorum* to carbendazim. From his results, he proposed that isolates with EC_{50} values greater than 2 µg/ml be considered resistant. With continuous studies on benzimidazole resistance, researchers have followed this threshold to separate sensitive thiophanate-methyl isolates from insensitive isolates.

1.5.2. DMI Fungicides Resistance (FRAC Group 3)

Demethylation inhibitors (DMI) is another chemical group that is widely used for the control of many fungal pathogens including *S. sclerotiorum*. DMI fungicides have been around since the early 1970s, with triadimefon, triforine, and imazalil being the first DMI products introduced to the market for commercial use (Brent and Hollomon, 2007), followed by an increasing number of DMI fungicides registered for agricultural use. In 2007, metconazole (MTZ) a triazole in the DMI group was registered in the United State for use in ornamental and turf cultivation (USEPA, 2006). Three years later, in 2010, MTZ was registered for use in North Dakota for control of *S. sclerotiorum* in canola (McMullen and Markell, 2010). In 1982, resistance to DMI fungicides was first reported against powdery mildews on barley and cucurbits after 7 years of commercial use (Brent and Hollomon, 2007). During the 80's, more cases of resistance build-up with fluctuating severity, considered as polygenic resistance, have been reported in *Venturia inaequalis*, *Mycosphaerella fijiensis* var. *difformis* and powdery mildew (Brent and Hollomon, 2007). Vargas et al. (1992) also reported *Sclerotinia homoeocarpa* (a relative of *S. sclerotiorum*) to be resistant to available DMIs at that time including fenarimol (Rubigan), triadimefon (Bayleton), and propiconazole (Banner). With a single-site MOA, there is a varying difference amongst DMIs in their fungicidal activity and resistance mechanisms. DMIs are currently categorized as a fungicide group with medium risk of resistance. DMIs inhibits sterol C-14α-demethylation which functions as a precursor for ergosterol in many fungi. The *CYP51* is the target gene which regulates sterol demethylation in the biosynthesis pathway. DMIs resistance mechanisms is due to alterations in amino acid positions, which could be a Y136F mutation in the 14α-demethylase (*CYP51*) gene caused by a replacement of phenylalanine with tyrosine in *Uncinula necator* (Delye et al. 1997) and *Erysiphe graminis f. sp. hordei* (Delye et al. 1998) or

leucine in *Tapesia acuformis*, suggesting the involvement of leucine in natural resistance to triazoles (Albertini et al. 2003). Overexpression of the *CYP51* gene due to increased copy numbers or transformants *CYP51* (with promoter region) with higher expression level, gradually contributes to DMI resistance development in *Venturia inaequalis* (Schnabel and Jones, 2001), and also ATP-Binding Cassette (ATP) transporters which confers DMI resistance in *Botrytis cinerea.*

1.5.3. QoI Fungicides Resistance (FRAC Group 11)

Quinone outside inhibitors (QoIs) is an innovative fungicidal class which includes strobilurin-derived compounds like pyraclostrobin, picoxystrobin and azoxystrobin. In the 1970s, systemic fungicides with single MOA were introduced to the market, followed by the introduction of sterol demethylation inhibitors (DMIs) as systemic products in the 1980s (Brent and Hollomon, 2007). The development of QoIs, like azoxystrobin, was a game changer because its innovation is based on naturally occurring molecules produced by fungal organisms. Strobilurin A, which was the first QoI molecule identified (Anke et al. 1977), is produced by *Strobilurus tenacellus* and azoxystrobin is a natural fungicidal derivative of β- methoxyacrylic acid (strobilurin A). QoI fungicides interrupt energy production and in this way, they inhibit or reduce spore germination and other stages of development. Azoxystrobin is one of the most important fungicides in the QoI class because of its high stability and activity as a fungicidal compound (Fernández-Ortuño et al. 2008). However, due to their efficacy, they apply strong selection pressure on the fungal population and thus, repeated use promotes resistance buildup (Fernández-Ortuño et al. 2008).

Extensive studies have identified QoI fungicide resistance in *E. graminis f. sp. tritici* (Chin et al. 2001), *M. oryzae* (Ma and Uddin, 2009), *C. beticola* (Malandrakis et al. 2006), *A. solani* (Pasche et al. 2005), *Botrytis cinerea* (Kim and Xiao, 2011), *U. maydis* (Ziogas et al. 2002) and *M. grisea* (Avila-Adame and Koller, 2003). Fungal resistance to QoI fungicides starts with a point

mutation in the mitochondrial cytochrome b gene (CYTB) that promotes a reduction in the ability of the fungicides to bind well. Common mutations that lead to QoI resistance could be a switch in the amino acid from glycine to arginine at position 137 (G137R), from phenylalanine to leucine at position 129 (F129L), and from glycine to alanine at position 143 (G143A) (Gisi et al. 2002). These changes create various levels of resistance, for example, isolates with G143A mutation are considered highly resistant compared to isolates with G137R or F129L mutation which are considered moderately resistant (Fernandez Ortuno et al. 2008). Changes that affect mechanisms, such as efflux transport and alternative respiration, could also promote QoI fungicide resistance (Fernández-Ortuño et al. 2008). During the in-vitro assay, the metabolic pathway provided by alternative respiration helps to circle around the target site of QoI fungicides, providing intoxicated cells with energy (Ziogas et al. 1997). The development of alternative respiration pathways is an endeavor during the transmutation window where isolates that are sensitive become resistant to avoid the toxic effect of QoI fungicides. (Miguez et al. 2003; Wood and Hollomon, 2003). Xu et al. (2013) also showed that alternative oxidase (AOX) respiration affects the mycelium sensitivity of *S. sclerotiorum* to azoxystrobin when it shifts electron efflux from cytochrome pathway (CP) to alternative pathway (AP), also because it can transfer electrons from reduced ubiquinone to oxygen without energy release (Vanlerberghe & McIntosh, 1997; Tamura et al. 1999).

An alternative response pathway provides these organisms with the ability to mask their true sensitivity to QoI fungicides during laboratory toxicity assays. Salicylhydroxamic acid (SHAM) which is known to inhibit the AOX enzyme involved in the alternative respiratory pathway is therefore required to be added to an artificial medium while conducting in-vitro toxicity assays for QoI fungicides (Duan et al 2012; Walker et al 2009; Malandrakis et al 2006). *Ascochyta rabiei* for example is not negatively affected by SHAM even at 100 µg ml^{-1} (Wise et al. 2008)

while on *S. sclerotiorum*, even concentrations of 1 µg ml^{-1} affect spore germination (Muñoz, 2016). However, the use of SHAM for fungicide sensitivity assays is subjected to debate as mentioned by Liang et al (2019). To use SHAM, it is wise to determine the level or concentration that does not interfere with the estimation of the true sensitivity of the organism to QoI fungicide. Accurate precision in determining EC_{50} values within a shorter duration, reduced labor and better accommodation for less space are benefits of conducting in-vitro fungicide sensitivity assays.

1.5.4. SDHI Fungicides Resistance (FRAC Group 7)

Succinate dehydrogenase inhibitors (SDHI) are broad-spectrum inhibitory fungicides that have been widely used for the effective control of many diseases caused by fungal pathogens on grains, fruits, turf, oilseeds, and vegetables (Sang and Lee 2020; FRAC 2018; Sang et al. 2019). SDHI fungicides became prominent following the elevated risk of resistance by fungal pathogens to demethylation inhibitors (DMIs) and quinone outside inhibitors (QoIs). SDHI fungicides target the ubiquinone-binding (Qo) site in complex II of the electron transport chain formed by three succinate dehydrogenase subunits (B, C and D) of fungal pathogens, interrupting mitochondrial respiration (Sierotzki and Scalliet 2013).

According to a recent report by FRAC (2020), SDHIs are considered as fungicides with medium to high resistance risk. More than 20 economically important fungal pathogens including *S. sclerotiorum* (Wang et al. 2015), *A. alternata* (Avenot et al. 2014), *Z. tritici* (Dooley et al. 2016) *B. cinerea* (Fernández-Ortuño et al. 2017) and many others have been reported to exhibit insensitivity and/or field resistance to SDHI fungicides. Boscalid is one of the prominent members of the SDHI class of fungicides registered for use for many fungal pathogens. Unfortunately, the repeated misuse of this fungicide resulted in selection pressure leading to the buildup of fungal populations that are resistant (Avenot and Michailides, 2010). This resistance is caused by a point

mutation in the *sdh* genes (Avenot and Michailides, 2007; Miyamoto et al. 2009; Peng et al 2021). A mutation of a ubiquinone-binding (Qo) site in subunits sdhB and sdhC (Outwater et al. 2019) could cause a change in amino acids from histidine to tyrosine in cases like H272 of sdhB and H146 of sdhC in *B. cinerea* leading to in-sensitivity to SDHI fungicides (FRAC 2018; Sierotzki and Scalliet 2013; Veloukas et al. 2013).

Direct and indirect exposure of fungal pathogens to boscalid and other SDHI fungicides could eventually result in resistance. For example, the continuous indirect exposure of *S. sclerotiorum* to carboxin exerts selection pressure for beneficial mutations leading to SDHI resistance (Wang et al. 2014). Isolates with these mutations have better fitness and survivability (Sierotzki and Scalliet, 2013), increasing their resistance to SDHI fungicides and this becomes a big concern for growers running out of alternative source for fungal disease control.

1.6. Research Importance

Sclerotinia sclerotiorum causes significant reduction in yield and quality of several economic crops in the United States including sugar beet and has been reported as a potential threat to sugar beet production in the Red River Valley of North Dakota and Minnesota. *S. sclerotiorum* has been reported to cause leaf blight, seedling damping-off and root rot/necrosis in commercial sugar beet fields in Montana, Minnesota, and North Dakota between 2020 to 2022. The continuous report of the spread of this pathogen's damaging impact across sugar beet producing states indicates the potential threat to sugar beet growers and the sugar beet industry at large. However, at the moment there is no available information on the management of *S. sclerotiorum* in sugar beet. This begs for proactive actions to be taken to provide practical management options before epidemic outbreaks of this pathogen can occur.

This research aims to provide a better understanding of the characteristic behavior and associated pathogenicity of *S. sclerotiorum* with sugar beet, which is the first step in the development of a management program for this pathogen. The program would also include assessing cultivar response to leaf blight, root rot and seedling damping-off caused by *S. sclerotiorum* in sugar beet. To manage the potential fungicide resistance of the *S. sclerotiorum* population in sugar beet fields, the fungicidal sensitivity of *S. sclerotiorum* isolates from sugar beet production areas should be determined and continuously monitored. Monitoring fungicide sensitivity is a consistent measure to prevent the risk of fungicide resistance. To this end, conducting a fungicide sensitivity study for *S. sclerotiorum* which is considered an emerging pathogen of sugar beet is of utmost importance.

In entirety, the results of this thesis research will equip sugar beet growers with the necessary tools, appropriate information, and management approaches to tackle the potential epidemics that can be caused by this notorious pathogen. This includes answers to what tolerance cultivar to plant, what *S. sclerotiorum* diseases of sugar beet to look out for in the field and most importantly, which fungicides to apply for its control. This would contribute to the sustainable productivity of sugar beet, which is an economically important crop in North Dakota and Minnesota, and the continuous boom of the sugar beet industry.

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2. *SCLEROTINIA SCLEROTIORUM* IN SUGAR BEET: EVALUATION OF INOCULUM SOURCES, VARIETAL RESPONSE AND CROSS INFECTIVITY

2.1. Abstract

Sclerotinia sclerotiorum (Lib.) de Bary is a cosmopolite necrotrophic fungal pathogen causing substantial damage and economic losses in various crops across the United States, notably impacting canola, dry bean, soybean, and sunflower. Recent studies have reported *S. sclerotiorum* as an emerging pathogen of sugar beet (*Beta vulgaris* L.) causing leaf blight, seedling damping-off and root necrosis, becoming a fundamental production problem in the Red River Valley of North Dakota and Minnesota. The continuous spread of this pathogen's damaging impact across sugar beet-producing states necessitates proactive management measures to prevent potential epidemic outbreaks. This study aimed to provide characteristic information about this pathogen by evaluating different inoculation methods of *S. sclerotiorum* on sugar beet, varietal responses, and investigating cross-infectivity across major host crops in the Red River Valley region. Infected plants were evaluated for disease severity. Data collected post-inoculation were expressed as lesion size and AUDPC. Results from this study indicated that barley seed inoculum was particularly effective in causing leaf blight on sugar beet plants, with Crystal M837 demonstrating promising tolerance with reduced susceptibility to *S. sclerotiorum* ($P=0.05$). Furthermore, this study confirmed the cross-infectivity of *S. sclerotiorum* among sugar beet, canola, soybean, and sunflower. These findings offer critical insights into sugar beet cultivar selection and vital information about the cross-infectivity status of major host crops, providing valuable information for crop rotation decisions aimed at mitigating losses caused by *S. sclerotiorum*. This research serves as an essential tool for preventing future damage and ensuring sustainable crop production in regions vulnerable to this pathogen.

2.2. Introduction

Sugar beet (*Beta vulgaris* L.) is a dicot plant and a member of the family Amaranthaceae (formerly Chenopodiaceae) in the order Caryophyllales (McGrath and Townsend 2015; Hamdi et al. 2021). Sugar beet accounts for 14% of the world's sucrose requirement, which is considered the second most important source of sucrose (FAOSTAT, 2017) in the world. The Red River Valley Region of North Dakota and Minnesota combined have consistently been the top producer of sugar beet in the United States with an overall production of 57% of the total annual production. This contributes an estimated $5 billion to the bi-state's total economic activity (Bangsund et al. 2012; ISO 2020; USDA-ERS, 2021).

Sclerotinia sclerotiorum (Lib.) de Bary is a cosmopolite necrotrophic fungal pathogen that affects more than 500 plant species worldwide including sugar beet (Adams and Ayers, 1979; Boland and Hall, 1994; Purdy, 1979; Saharan and Mehta, 2008; Kabbage et al. 2015; Sharma et al. 2016; Liang and Rollins 2018; Khan et al. 2020; Ding et al. 2021). This fungus causes a significant reduction in yield quality and increases yield loss in field or storage condition (Bhuiyan et al. 2021). In 2005, the United States Department of Agriculture (USDA) reported that *S. sclerotiorum* alone is responsible for more than $200 million annual crop loss in the United States (Bolton et al. 2006; US Canola Association, 2022,). This led the United States Congress to approve the establishment of the National Sclerotinia Initiative in 2004 and saddled it with the responsibility of carrying out integrated research that would provide answers to innovative management strategies for the control of diseases caused by *S. sclerotiorum.*

S. sclerotiorum attacks host plants either using ascospores that can be discharged forcibly upwards from apothecia into the air or by mycelium arising from infected tissue or germinated sclerotia (Hartill & Underhill, 1976; Willetts & Bullock, 1992). Ascospores which develop from

carpogenic germination act as a major source of inoculum (Abawi and Grogan 1979), forcefully dispersed by a mechanism called puffing (Hartill & Underhill, 1976). In myceliogenic germination, the hyphae or mycelium growth is triggered by exposure to high nutrient conditions or direct contact with healthy plants (Le Tourneau 1979). The germinating hyphae from overwintering sclerotia can initiate infection in neighboring plants at the soil line as well as underground portions of the plant (Huang and Hoes, 1980; Morrall and Dueck, 1982; Underwood et al. 2020). *S. sclerotiorum* synthesize lytic enzymes viz., cellulase, hemi-cellulase, pectinase, which results in the formation of soft watery lesions (Bolton et al. 2006; Monazzah et al. 2018). The disease progresses with the infection of plant roots or other parts in storage conditions, producing light grey to whitish scurf with cottony mycelial aggregation (Purdy, 1979). Under moist and cool conditions, the infected host tissues develop symptoms of browning, water-soaking, and a white, cotton-like mycelium, which leads to necrosis, stunting, premature ripening, and wilting of the host (Bolton et al. 2006). Upon killing the host, the fungus grows saprophytically on the dead plant tissue, forming sclerotia, a hard bodied, black colored resting structure which overwinters to serve as initial source of inoculum for the next growing season. Under adverse environmental conditions, sclerotia are abundantly formed on the host surface and cavities, in plant debris, and in soil, where they can remain dormant for up to 8 years or more (Adams and Ayers, 1979; Brustolin et al. 2016).

In general, symptoms of *S. sclerotiorum* vary from plant to plant and might be host specific. Disease symptoms are white mold, soft rot, cottony rot, stem rot, canker, damping-off, crown rot, head rot and leaf blight in many crops worldwide. In most plants like canola, sunflower and many others, infection is first initiated under field conditions at the flowering stage and sometimes dead or senescent plant tissues (Jamaux et al. 1995). In sugar beet fields with high severity of below

ground infection, the lesions expand, and the plant become depressed showing symptoms of wilting, and later girdle the root surface producing sclerotia on the external part of the root. Initial root rot symptoms due to *S. sclerotiorum* on roots are remarkably similar to those caused by *Rhizoctonia solani* or *Sclerotium rolfsii* (Bhuiyan et al. 2021). Survival is adversely affected by high soil temperatures and moisture. Optimum temperature for mycelia growth is 25^0C and the optimum pH ranges between 6 and 7 (Jeon et al. 2006; Fagodiya et al. 2017).

In the United States, *S. sclerotiorum* has been reported to cause significant reduction in quality and yield of several economic crops. In the North Central region, it affects several economically important crops like canola, dry bean, soybean, and sunflower (del Río et al. 2007; Peltier et al. 2012). Also, recent reports have identified *S. sclerotiorum* as an emerging pathogen of sugar beet and a potential threat to sugar beet production causing leaf blight and root necrosis in numerous sugar beet producing states in United States (Khan et al. 2020, 2021; Bhuiyan et al. 2021). Diseases caused by *S. sclerotiorum* on sugar beet are becoming a fundamental production problem in the Red River Valley of North Dakota and Minnesota (Khan et al. 2020, Bhuiyan et al. 2021), Montana (Khan et al. 2022) and other sugar beet producing states in the United States including Wyoming and Colorado [M. Khan, personal communication]. The control and monitoring of the progress of development and spread of this fungus is difficult because of the formation of sclerotia which is a dense aggregation of hyphae, hard coated overwintering resting structure (Sousa et al. 2019).

Therefore, effective control of this fungal infection in field and storage condition is complicated and requires integrated management practices including host resistance, biological control, soil and seed treatments, tillage practices, rotation with non-host crops, and other agronomic practices, (Mueller et al. 2002a; Bolton et al. 2006; Hoes and Huang, 1985; Tu, 1989;

Zeng et al. 2012; Garcia-Garza et al. 2002; Schwartz and Steadman1978; Bailey et al. 2001; Bardin and Huang, 2001; del Rio et al. 2002; del Rio et al. 2004; Kamal et al. 2016; Zhao et al. 2020).The immediate and most effective way to control the current developing threat of *S. sclerotiorum* in sugar beet fields is the use fungicides with different mode of actions that have been used on a commercial scale (Bradley et al. 2006; McMullen and Markell, 2010). However, the control is inconsistent because of difficulties in achieving the expected coverage of the spray and timing of the spray in relation to ascospore release (Hunter et al. 1978). Also, excessive use of these fungicides poses a threat to environmental health and could induce resistance from the pathogen (Brent and Hollomon, 2007; Secor et al. 2010). Selection of resistant varieties seems to be the most desirable approach for managing plant diseases because of its positive impact on environmental health. However, there is limited breakthrough in developing varieties completely or substantially resistant to *S. sclerotiorum* infection due to lack of host specificity (Sharma et al. 2016) and absence of a strong host single-gene resistance (Zhao and Meng, 2003; Bolton et al 2006).

The continuous report of the spread of this pathogen's damaging impact across sugar beet producing states indicates the potential threat to sugar beet growers and the sugar beet industry at large. However, at the moment there is no available information on the management of *S. sclerotiorum* in sugar beet. This begs for proactive actions to be taken to provide practical management options before epidemic outbreaks of this pathogen can occur. This research aims to provide a better understanding of the characteristic behavior and associated pathogenicity of *S. sclerotiorum* with sugar beet, which is the first step in developing a management program for this pathogen. This study evaluates different inoculation methods by which *S. Sclerotiorum* could cause leaf blight on sugar beet, varietal response of sugar beet to leaf blight, and also the cross-

infectivity of host specific isolates across four major economic crops produced in the Red River Valley of North Dakota and Minnesota which are known host to this pathogen.

2.3. Materials and Methods

2.3.1. Experiment Location and Conditions

All greenhouse studies conducted in greenhouse rooms were done in the Dalrymple Greenhouse, Agricultural Experiment Station (AES) at North Dakota State University in Fargo, ND. Greenhouse rooms were set to allow for a 16-h photoperiod, and temperature was maintained at 23±2°C (Argus Control Systems Ltd.; British Columbia, Canada). When needed, humidity chambers were set to provide 14/10 hours of photoperiod where light started from 7 am to 9 pm and darkness period started from 9 pm to 7 am with temperature and relative humidity set at 28^0C and > 90 %, respectively. In all studies, beet plants were grown in in 3.5-inch-deep square pots (T.O. Plastics Inc., Clearwater, MN, U.S.A.) containing peat mix (Sunshine mix 1, Sun Gro Horticulture Ltd.; Alberta, Canada) amended with NPK 20-20-20 fertilizer (Osmocote, Scotts-Sierra Horticultural Products Company, Marysville, OH) at planting and 4-leaf stages. Plants were watered daily to maintain adequate moisture for plant growth and disease development.

2.3.2. Inoculum and Plant Materials

The collected sclerotia of all isolates used in this study, WM-031 (an isolate used as a standard in Dr. Del Rio Canola Lab) and MN-22 (selected based on its consistent high EC50 value in our unpublished sensitivity study) were surface sterilized for 3-5 minutes using a 10% NaOCl and water solution (v/v) (Clorox Sales Co., Oakland, CA, USA) and rinsed twice with sterile distilled water and dried with sterile paper (Okabe and Matsumoto 2000). Full strength potato dextrose agar (PDA) medium was prepared with 24 g of Potato Dextrose Broth (Sigma-Aldrich, Co., St. Louis, MO, USA) and 15g Bacto Agar (Becton, Dickinson and Company, Sparks, MD

USA) dissolved in 1000 ml of distilled water, autoclaved at 121^{o}C and 103.4 kPa for 20 minutes. A 25ml of the prepared PDA was poured into a 100 mm petri dish using PourBoy® 4 (Tritech Research, Los Angeles, CA USA). The sterilized sclerotia were inoculated in the PDA medium and incubated 23±2^{o}C to induce mycelial growth until the mycelial colony covered approximately one-quarter of the surface of the plate. Two to three subcultures of hyphal tips were made to obtain pure cultures the isolates before inoculation. Barley inoculum was prepared by mixing the 100g of barley seeds (*Hordeum vulgare* L.) soaked for 24hrs with 100ml of deionized water, 1g of PDB in a 1000ml container and autoclaved at 121^{o}C and 103.4 kPa for 20 minutes (Mueller et al. 2002b). The autoclaved barley seeds were inoculated with plugs from actively growing mycelial culture, the barley inoculum was thoroughly mixed and subsequently shaken for even colonization of the barley seeds and was ready for use after 7 days (Mueller et al. 2002b). In all greenhouse studies, one or more of the following sugar beet varieties were used: ACH 166, Beta 7029, ACH 082, Crystal M837, Sedex 1815, and B-85 (Rhizoctonia-Susceptible). This varieties were selected as a representative of the common commercial sugar beet varieties grown across the Red River Valley region.

2.3.3. *S. sclerotiorum* Inoculation Assay on Sugar Beet

Crystal M837 sugar beet variety and WM-031 *S. sclerotiorum* isolate were used for the inoculation assay. Three inoculation methods, mycelial plug (Ghimire et al. 2019,) barley inoculum (Noor and Khan 2014; Lai et al. 2020) and ascospores (Huzar-Novakowiski and Dorrance, 2018; Clarkson et al. 2003) were evaluated in this study. The concentration of ascospores was estimated using a hemacytometer and adjusted to 5 x 10^4 ascospores ml^{-1}, then the adjusted ascospore concentration were poured into a spray bottle and sprayed on the leaves surface as described by Shahoveisi and del Río Mendoza (2020). A 5-mm diameter plug with actively

growing hyphae from a 3-days old colony was used for the mycelial plug inoculation assay. Barley inoculum was prepared after autoclaving barley seeds soaked for 24hrs. The autoclaved barley seeds were inoculated with plugs from actively growing mycelial culture, the barley inoculum was thoroughly mixed and subsequently shaken for even colonization of the barley seeds and was ready for use after 7 days. The barley inoculum was placed carefully on the leaf surface and held with an adhesive tape. All inoculations were done on the adaxial surface of the sugar beet leaves. Plants were kept at a 16-h photoperiod for 2 weeks in the growth chamber. Lesion size (average of two perpendicular measurement of the lesion in mm) was measured 14 days post inoculation to determine infectivity and efficacy of different inoculation methods (Fig. 2.1).

Figure 2.1. Inoculation of sugar beet leaves (a) inoculum sources, (b) inoculation using mycelial plug for detached leaf assay, (c) disease evaluation for varietal response to *S. sclerotiorum*.

2.3.4. Evaluation of Sugar Beet Varietal Response to *S. sclerotiorum* Infection Using Detached Leaf Assay

The detached leaf assay is a non-destructive procedure that allows any plant whether grown under a controlled environment or in the field to be evaluated for disease response (Wegulo et al. 1998; Kull et al. 2003; Na et al. 2018; El-Mor et al. 2018; Burlakoti et al. 2018; Paczos-Grzęda et al. 2019; Macan et al. 2022; Kirkby et al. 2023). All commercial varieties but ACH 082 were evaluated for their response to *S. sclerotiorum* infection. Six seeds of each variety were germinated in the greenhouse under conditions described in 2.3.2. The youngest leaves of each sugar beet variety at 4 leaf stage were harvested. The experiment for the detached leaf assay was arranged in a completely randomized design with six varieties replicated five times, both inoculated and mock (control) treatment. Each treatment replication had one detached leaf harvested earlier from the 4-leaf stage plant and was placed in a 150mm x 15mm petri dish (Falcon ®) which was laced with a 150mm filter paper (Whatman ®). A 5-mm diameter plug with actively growing hyphae from a 3-days old colony of *S. sclerotiorum* isolate MN-22 (described in 3.3.3) was cut aseptically and inoculated on the leaves already placed in the petri dish and was sprayed subsequently with sterilized water to keep the leaves moist. Each variety treatment had a mock control that was inoculated with agar plugs alone without mycelial serving as the control treatment. Each petri dish containing the inoculated leaves and the mock treatment were incubated in the lab growth chamber at 23±2^{o}C. Data was collected on lesion size every 24hrs until 3dpi. The detached leaf assay was conducted twice.

2.3.5. *In planta* Evaluation of Sugar Beet Varietal Response to Sclerotinia Leaf Blight

All sugar beet commercial varieties were evaluated for their response to leaf blight disease caused by *S. sclerotiorum*. Each treatment had one mature plant (4-leaf stage) replicated three

times and the experiment was conducted twice. A 5-mm diameter plug with actively growing hyphae from a 3-day-old colony of *S. sclerotiorum* isolate MN-22 (described in 3.3.3) was used for the mycelial plug inoculation on the adaxial surface of the sugar beet leaves for the leaf blight evaluation. Each variety treatment had a mock that was inoculated with agar plugs alone without mycelial serving as the control treatment. All plants were kept at 16-h photoperiod in the humidity chamber. The temperature was maintained at 23±2^{o}C. Data was collected on lesion size 3 dpi to determine the disease severity of leaf blight across all sugar varieties.

2.3.6. Evaluation of Cross-Infectivity of *S. sclerotiorum* Isolates Across Four Broad leaf Crops Using Detached Leaf Assay

Four broad-leaf crops: Canola (Westar) (Roy et al. 2024), Sugar beet (Crystal M837), Soybean (Dwight) (Webster et al. 2021), and Sunflower (HA 277) were evaluated for reaction to four host-specific (previously recovered from the particular crop) *S. sclerotiorum* isolates (WM031, MN22, WI-20 and BN166) respectively. Six seeds of each crop type were germinated in the greenhouse under conditions described in 2.3.1. The youngest leaves of each crop were harvested four weeks after planting. The experiment for the detached leaf assay was arranged in a completely randomized design with each crop type replicated 3 times for evaluation of each *S. sclerotiorum* isolate. Each treatment replication had one detached leaf harvested earlier four weeks after planting and was placed in a 150mm x 15mm petri dish (Falcon ®) which was laced with a 150 mm filter paper (Whatman ®). A 5 mm diameter plug with actively growing hyphae from a 3-days old colony of each respective *S. sclerotiorum* isolate was cut aseptically and inoculated on the leaves already placed in the petri dish and was sprayed subsequently with sterilized water to keep the leaves moist. Each inoculated treatment had a mock control that was inoculated with agar plugs alone without mycelial serving as the control treatment. Each petri dish containing the

inoculated leaves and the mock treatment were incubated in the lab growth chamber at 23±2ºC. Data was collected on lesion size every 24hrs until 7 dpi. The detached leaf assay was conducted twice.

2.3.7. *In planta* Evaluation of Cross-Infectivity of *S. sclerotiorum* Isolates Across Four Broadleaf Crops

Similarly, an *in planta* study was conducted to further validate the detached leaf assay using the exact crop type, variety and isolate as described in 2.3.6. Each treatment had one mature plant replicated three times and the experiment had two trials. A 5 mm diameter plug with actively growing hyphae from a 3-days old colony of each respective *S. sclerotiorum* isolate was used for the mycelial plug inoculation on the adaxial leaf surface of each crop for evaluation of *S. sclerotiorum* infection. Each inoculated treatment had a mock control that was inoculated with agar plugs alone without mycelial serving as the control treatment. All plants were kept at 16-h photoperiod in the humidity chamber. The temperature was maintained at 23±2ºC. Disease severity across host crops and aggressiveness all *S. sclerotiorum* isolates were measured 3 dpi as lesion size (mm)

2.3.8. Data Analyses

Temporal lesion expansion data was used to calculate the area under disease progress curve (AUDPC) on different inoculation methods, disease severity of Sclerotinia leaf blight and lesion expansion across the varieties. This was completed using PROC GLM in SAS (SAS Institute, 2013) with replications designated as a random effect and, inoculation method, and sugar beet varieties designated were as a fixed effect. Levene's test for homogeneity of variances was conducted to compare the variance of the two trials to determine whether the trials (within experiment) or experiments can be combined for analysis (Levene, 1960). Comparison between

all isolates across two replications and two trails were made by running Fisher's protected least significant difference value (L.S.D.) at $P = 0.05$. Tukey-Kramer's post hoc test was used to separate means at $P = 0.05$ (Tukey, 1949). Upon confirmation that the variances were similar, a combined analysis of variance ($P<0.001$) was conducted using the general linear model procedure (PROC GLM). All data analysis was achieved using the Statistical Analysis System (Version 9.4, SAS Institute Inc.; Cary, NC, USA).

2.4. Results

2.4.1. Effect of Inoculum Source on Sclerotinia Leaf Blight in Sugar Beet

For the evaluation of the inoculation assay on sugar beet, the two trials were individually analyzed after conducting Levene's test for homogeneity of variances ($P = 0.05$). For both trials, all inoculum sources were able to cause infection on the leaves of the sugar beet plants. Also, the barley seed inoculum source significantly had the highest lesion expansion after inoculation compared to the mycelia plug or ascospore inoculation method which performed the least amongst all inoculation methods tested (Fig. 2.2). This result was consistent for both trials ($P = 0.05$, Table 2.1).

Table 2.1. Effect of inoculum source on lesion size caused by *Sclerotinia sclerotiorum* on leaves of sugar beet cv. Crystal M837.

	Lesion Size[1] (mm)	
Inoculum	Trial 1	Trial 2
Barley	14 a	19 a
Mycelia	6 ab	12 ab
Ascospores	2 b	1 b

Treatments with the same letters in a column are not statistically different according to the Tukey-Kramer post-hoc test with $P = 0.05$. [1] Lesion length was measured 14 days after inoculation. Means represent 4 observations in each trial conducted in greenhouse conditions.

Figure 2.2. Disease evaluation post inoculation for three types of inoculum source, (a) colonized barley seeds, (b) mycelial plug and (c) ascospores for Sclerotinia leaf blight.

2.4.2. Effect of Sugar Beet Varietal Response to Sclerotinia Leaf blight Caused by *S. sclerotiorum*

Levene's test conducted for the evaluation of varietal response to Sclerotinia leaf blight during the *in planta* study and detached leaf assay showed that the two trials for both studies evaluated could be combined ($P = 0.05$). For both studies, the combined glimmix ANOVA was done without the control treatment as there was no disease development on the non-inoculated control. In the *in planta* study, the effect of variety was significant ($P < 0.001$). ACH 082 had the highest tolerance response to leaf blight with the least significant lesion size compared to the other varieties evaluated ($P = 0.05$, Table 2.2). In the detached leaf assay (Table 2.2, Fig. 2.3), the effect of sugar beet varieties, treatments (inoculated and non-inoculated) and the interaction between treatments and varieties were significant ($P = 0.05$). Disease evaluation was analyzed by the least significant mean differences (LSD) to rank the response of all varieties to *S. sclerotiorum* infection

on the leaf using the area under the disease progress curve (AUDPC). Crystal M837 performed significantly better than other varieties with the minimum AUDPC value (P= 0.05).

Table 2.2. Effect of sugar beet varietal response to *S. sclerotiorum* leaf infection measured as lesion size and area under disease progress curve (AUDPC).

	In planta Study	Detached Leaf Assay
Sugar beet Varieties	Lesion Size[1] (mm)	AUDPC[2] (mm)
ACH082	29 b	9353 a
ACH166	48 a	7290 bc
Beta7029	47 a	9298 a
CrystalM837	39 ab	6895 c
B-85	48 a	8635 ab
Sedex1815	41 ab	7954 abc

Treatments with the same letters in a column are not statistically different according to the Tukey-Kramer post-hoc test with P= 0.05. [1] Lesion length was measured 7 days after inoculation. [2] AUDPC readings were collected at 24hr intervals for 7 days. Means represent 6 observations in two trials conducted in the humidity chamber.

Figure 2.3. Varietal response of sugar beet to *S. sclerotiorum* leaf infection using detached leaf assay.

2.4.3. Aggressiveness of *S. sclerotiorum* Isolates on Four Broadleaf Crops

Similarly, Levene's test conducted for the evaluation of cross infectivity of *S. sclerotiorum* isolates across four broad leaf crops (canola, soybean, sunflower and sugar beet) during the *in planta* study and detached leaf assay showed that the two trials for both studies evaluated could be combined ($P = 0.05$). For both studies, the combined glimmix ANOVA was done without the control treatment as there was no disease development on the non-inoculated control. In both the *in planta* study and detached leaf assay, the effect of crop type, isolates and the interaction between crop type and *S. sclerotiorum* isolates was significant ($P < 0.001$).

For the *in planta* study, the main effect of variety showed that the host crops evaluated could be grouped into two, canola and sunflower were significantly more susceptible to *S. sclerotiorum* infection as compared to soybean and sugar beet which were significantly less susceptible ($P = 0.05$, Table 2.3). Main effect of isolates in the *in planta* study showed that three (BN166, MN22, WM031) out of the four isolates evaluated were highly aggressive (Table 2.3). WI-20 was significantly the least aggressive isolate ($P = 0.05$). The interaction between crop type and *S. sclerotiorum* isolates were of magnitude rather than direction (Fig. 2.5). The evaluated amount of infection caused by each isolate varied from one crop to another in the magnitude of lesion size caused and it doesn't follow a particular pattern. BN166 was significantly aggressive on all host crops evaluated while WI-20 was significantly the least aggressive ($P = 0.05$). Sugar beet showed less susceptibility to the *S. sclerotiorum* isolates except for BN166 (most aggressive) and MN22 (an isolate recovered from infected sugar beet field).

The main effect of crop type evaluated in the detached leaf assay showed that sunflower significantly was the most susceptible while sugar beet was the least susceptible based on the AUDPC value ($P = 0.05$, Table 2.3). Again, the main effect of isolates clearly showed that BN166

was significantly the most aggressive isolate while WI-20 was the least aggressive isolate (P= 0.05, Table 2.3, Fig 2.4). Also, as seen in the *in planta* study, the interaction between the crop type and *S. sclerotiorum* isolates were of magnitude rather than direction (Fig 2.6).

Table 2.3. Aggressiveness of *Sclerotinia sclerotiorum* isolates on four broadleaf crops measured as foliar lesion size and area under the disease progress curve (AUDPC).

		In planta Study	Detached Leaf Assay
Factors	Levels[1]	Lesion size (mm)[2]	AUDPC[3]
Crop Type	Canola	45 a	2232 b
	Soybean	23 b	1639 c
	Sugar beet	25 b	1160 d
	Sunflower	42 a	3007 a
Isolates	BN166	43 a	2616 a
	MN22	40 a	2435 b
	WM031	40 a	1788 c
	WI-20	12 b	1199 d

[1] *S. sclerotiorum* isolates isolated from each host crop (Sunflower, Sugar beet, Canola, and Soybean) respectively. [2]Treatments with the same letters in a column are not statistically different according to the Tukey-Kramer post-hoc test with P = 0.05. Lesion length was measured 7 days after inoculation. [3] AUDPC readings were collected at 24hr intervals for 7 days. Means represent 6 observations in two trials conducted in the humidity chamber.

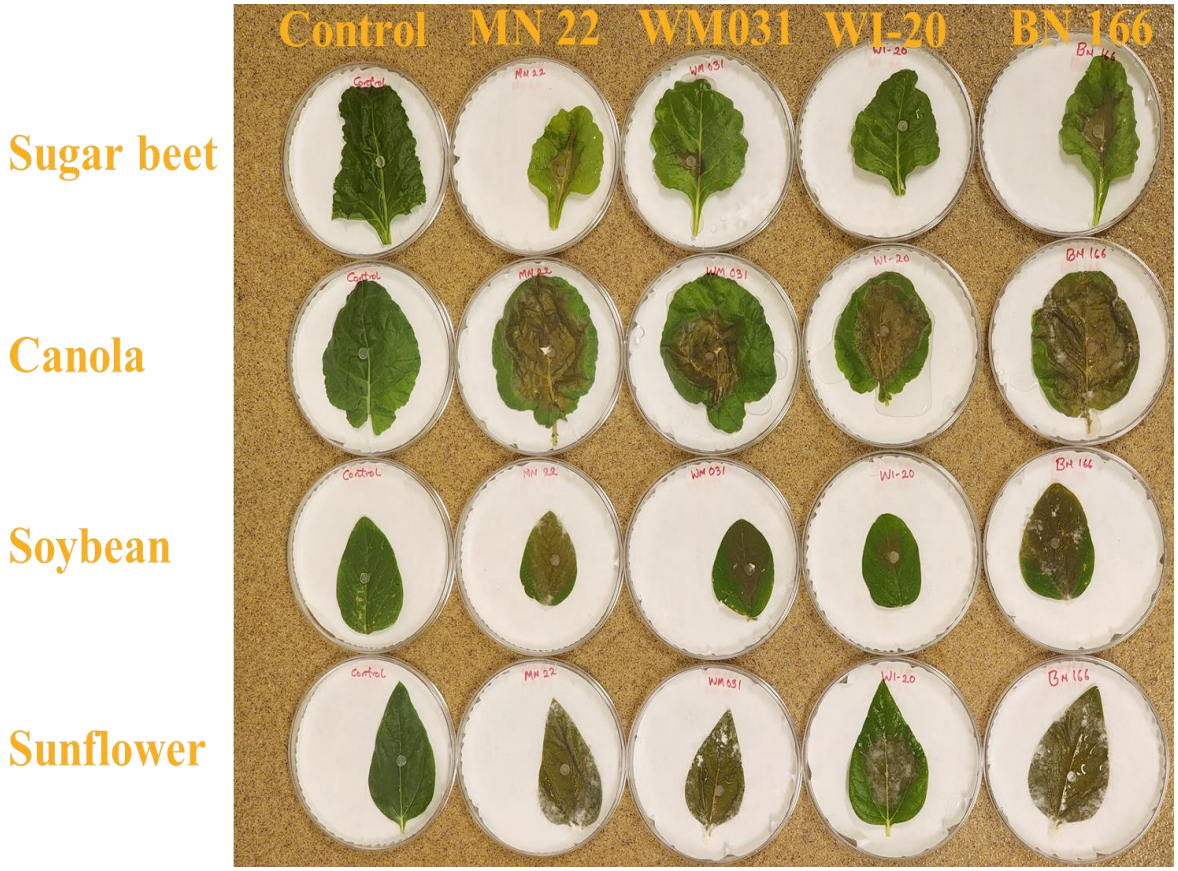

Figure 2.4. Aggressiveness and cross-infectivity of *S. sclerotiorum* isolates on four broadleaf crops using detached leaf assay.

The interaction showed that sunflower consistently had the highest AUDPC (P= 0.05) for all for isolates (BN166, MN22, WI-20 and WM031) evaluated (Fig. 2.4). The AUDPC of BN166, MN22 and WM-031 on sunflower clustered together while WI-20 was visibly separated from others with a reduced AUDPC value. BN166 (sunflower isolate) and MN22 (sugar beet isolates) were consistently aggressive across all host crops. Interestingly, they both had alternating ultimate aggressive effects on their host crop contradicting the predicted host-specificity assumption. This was true for WI-20 (soybean isolate) and WM031 (canola isolate)**.**

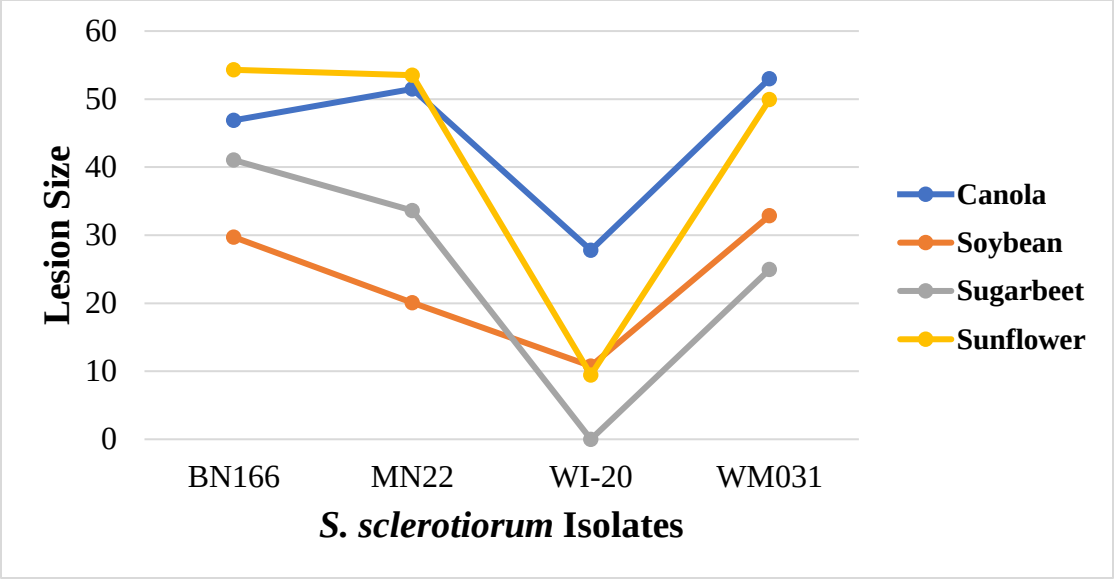

Figure 2.5. Interaction between *S. sclerotiorum* isolates and host crops during *in planta* study.

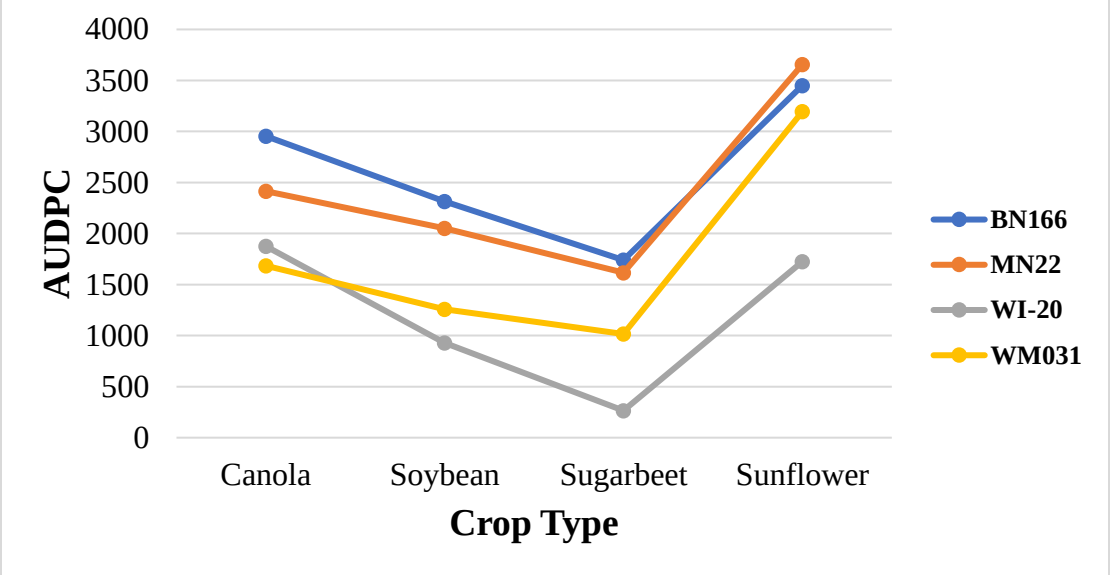

Figure 2.6. Interaction between *S. sclerotiorum* isolates and host crops during detached leaf assay.

2.5. Discussion

This study evaluates the pathogenicity of *S. sclerotiorum* on the leaves of sugar beet plants using different inoculation methods, varietal response to leaf blight infection and also the cross infectivity and aggressiveness of host-specific isolates. The two trials conducted to evaluate the

inoculation method for *S. sclerotiorum* infection on sugar beet leaves showed that barley seed inoculum which was previously demonstrated by Mueller et al. (2002b) and Auclair et al. (2004) as an effective method of evaluating *S. sclerotiorum* infection on soybean in field conditions performed better than the mycelial plug and ascospores as inoculums. Similar results were reported for the seed-based inoculation method for *R. solani* (Noor and Khan 2014) and *Fusarium* (Lai et al. 2020) in sugar beet. This was expected as the barley inoculum contained more mycelium mass and energy base. The barley inoculum was also firmly held to the leaf surface with the aid of adhesive tape in our study, maintaining constant contact between the pathogen and the adaxial surface, allowing continuous and progressive colonization which is very important for significant disease development. However, the mycelia plug inoculation method, which is a more practical inoculation method and exploited for the evaluation of disease infection by many other pathogens (Thompson et al. 2011; Ghimire et al. 2019), performed slightly less but not significantly different from the performance of the barley seed inoculation in terms of lesion size measured across the adaxial leaf surface of the sugar beet plants. This was more evident in the second trial as barley seed and mycelia plug inoculation subsequently had a higher lesion size respectively compared to sugar beet leaves sprayed with ascospores which showed a significantly lesser infection.

Many studies (Abawi and Grogan, 1979; Sutton and Deverall, 1982; Boland and Hall 1994; Bardin and Huang, 2001; Bolton et al. 2006) have reported ascospores as a primary source of inoculum for aerial infection of *S. sclerotiorum* (Huzar-Novakowiski and Dorrance, 2018; Clarkson et al. 2003) in host plants like sunflower, canola and many others. However, our study showed that the ascospores inoculation didn't produce as much lesion expansion as expected. This could be due to the architecture of the leaves of sugar beet (Elliott and Weston, 1993; Misra and Shrivastava, 2022) which is glossier, hardy (at 6 leaf stage) and also wider compared to other host

crops that were previously reported (Bolton et al. 2006; Mathew et al. 2020). This might infer that, for significant disease to occur as a result of aerial ascospore inoculation on sugar beet leaves, it might require higher spore concentration or potentially adapt in a way to overcome the unfavourable conditions presented by the leaf architecture of sugar beet plants. Another hypothesis is that the leaf canopy at 6 leaf stage might not be able to provide warm and wet humidity conditions which is essential for the viability and the establishment of disease infection by ascospore.

The varietal response throughout both the *in planta* and detached leaf assay showed that all varieties were susceptible to *S. sclerotiorum*. However, there were varying responses with respect to susceptibility across the varieties evaluated. ACH 082 was the most tolerant variety during the *in planta* study but turned out to be the most susceptible variety in the detached leaf assay. Beta 7029 was consistently the most susceptible variety in both studies. Crystal M837 was moderately susceptible during the *in planta* study but significantly the most tolerant cultivar in the detached leaf assay. The consistency of Crystal M837 tolerance to *S. sclerotiorum* infection in both studies could be considered a significant step in identifying a tolerant variety that could be recommended to farmers, providing acceptable tolerance to *S. sclerotiorum* leaf infection and potentially against Cercospora Leaf Spot (CLS) which is considered a significant damaging fungal pathogen in the sugar beet industry (Dexter et al. 1998; Malandrakis et al. 2006; Harveson, 2013). We are not aware of any other study that has evaluated the varietal response of sugar beet plants to *S. sclerotiorum.*

The cross-infectivity study evaluated the aggressiveness of host-specific isolates across four broad-leaf crops which are of economic importance in the Red River Valley and known host crops of *S. sclerotiorum.* Both the *in planta* study and detached leaf assay showed that sunflower

was significantly more susceptible to *S. sclerotiorum* infection than any other host crop except canola, while sugar beet was moderately tolerant during the *in planta* study and also had AUDPC value that was significantly lower than any other host crop during the detached leaf assay. This result supports our initial assumption about less disease development in sugar beet which is an emerging host crop for *S. sclerotiorum* (Khan et al. 2020; Khan et al. 2021, Bhuiyan et al. 2021; Khan et al. 2022). Canola, soybean and particularly sunflower have been common hosts for this pathogen for over a decade. Many studies have shown that *S. sclerotiorum* is a known pathogen causing significant damage to these crops (del Río et al. 2007; Mathew et al. 2020; Webster et al. 2021). The performance of the *S. sclerotiorum* isolates evaluated also varied in aggressiveness. Cross infectivity was more pronounced than host specificity as we hypothesized in our study and this result was also in line with what was reported in various studies which have shown the cosmopolitan characteristics of *S. sclerotiorum* to cause disease on a wide range of host crops (Purdy 1979; Boland and Hall, 1994; Bolton et al. 2006).

Also, the interactions between the isolates and host crop were expressed more in terms of magnitude for disease severity measured as lesion size and AUDPC across all isolates with no particular pattern of host specificity. BN166 and MN22, which are both sunflower and sugar beet isolates, were consistently aggressive throughout the study without specificity to their host crops. Interestingly MN22 was more aggressive on sunflower than sugar beet and a similar trend was observed for BN166 with more disease severity expressed as lesion size and AUDPC on Sugar beet. Overall, BN166 (Poudel et al. 2023; Aldrich-Wolfe et al. 2015) was the most aggressive isolate while WI-20 (Webster et al. 2021) was considered the least aggressive isolate based on results from the *in planta* and detached leaf assay. Also, sunflower was the most susceptible to *S. sclerotiorum* while sugar beet was considered the least susceptible host crop. Similar to the result

from our study, Prova et al. (2018) who evaluated the cross inoculation of *S. sclerotiorum* on hyacinth bean, okra and African-American marigold, showed that there is no host specificity of the isolate to any of the crops tested as they all showed symptoms of disease infection. The results from the cross-infectivity study suggest that any of these *S. sclerotiorum* isolate when encountered on the field by any of the host crops would initiate disease but severity might vary in aggressiveness and the susceptibility of the host crops. This information contributes to the potential epidemiological considerations when developing a management program for sugar beet in the future, most importantly when rotating it with sunflower, canola, and soybean that are known hosts for *S. sclerotiorum* (Garcia-Garza et al. 2002), as well as cultural practices to reduce inoculum dispersal (Schwartz and Steadman, 1978; Tu, 1989). Including non-host crops like corn, barley or wheat in this rotation is recommended from the management point of view but this might not be economical as sugar beet is a higher yielding crop in terms of revenue and there is a stricter regulation governing sugar beet production especially in the Red River Valley region. For proper implementation, there should be a consensus from the economic and pathology point of view that can be adopted by the sugar beet cooperatives. Additionally, extensive future research is required for field validation to improve the application and implementation of this study to ensure a sustainable management of *S. sclerotiorum* in sugar beet fields.

Conclusively, this study identified barley seed inoculum to cause the most disease on the leaves of sugar beet plants but due to the difficulty of keeping the barley seed placed on the adaxial leaf surface without the aid of an adhesive, mycelial plug could be considered a more effective method of inoculation when evaluating leaf blight on sugar beet under greenhouse conditions. However, barley seeds are recommended for root inoculation as they wouldn't require additional adhesive like in the case of leaf inoculation. Crystal M837 shows promising results as a tolerant

variety with reduced susceptibility to *S. sclerotiorum.* Also, results from the cross-infectivity showed that any *S. sclerotiorum* isolates would cause disease in sunflower, soybean, sugar beet and canola plants. This information is vital when considering crop rotation on a field with previous history of *S. sclerotiorum*, and this could help prevent unforeseen and future losses that can be attributed to this pathogen on the field.

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3. EVALUATION OF FUNGICIDE EFFICACY, VARIETAL AND AGE RESPONSES FOR THE CONTROL OF SCLEROTINIA DISEASES IN SUGAR BEET

3.1. Abstract

Sclerotinia Sclerotiorum (Lib.) de Bary has been reported to cause seedling damping-off, root necrosis and leaf blight in sugar beet (*Beta vulgaris* L.), becoming an emerging threat to the sugar beet industry. However, at the moment there is no available information on the management of *S. sclerotiorum* in sugar beet. This begs for a proactive need for practical management options for the control of Sclerotinia diseases in sugar beet. The objective of this study was to evaluate common fungicides registered for use in other host crops in the Red River Valley of North Dakota and Minnesota for their control efficacy in managing (1) leaf blight, (2) seedling damping-off and, (3) root necrosis caused by *S. sclerotiorum* in sugar beet. Disease evaluation was measured as lesion size post-inoculation. Results from this study indicated that Crystal M837 was the most susceptible variety, however had the lowest disease severity in terms of leaf blight due to fungicide protection. ACH 166 and Beta 7029 were considered moderately resistant to root infection at all stages ($P = 0.05$). Also, these findings showed that the leaf stage at the time of inoculation plays a significant role in sugar beet susceptibility to root infection ($P = 0.05$). Quadris, Proline and Priaxor respectively showed significant control efficacy in reducing seedling damping-off, and root necrosis, and also performed well in managing Sclerotinia leaf blight ($P = 0.05$). These findings offer critical insights into fungicide selection for effective control of *S. sclerotiorum* in sugar beet. Also, vital information about leaf stages and varietal susceptibility provides valuable insights that could help in the development of a robust and integrated fungicide management program towards mitigating losses caused by *S. sclerotiorum* in sugar beet. This study gives a first look into the

fungicide management of *S. sclerotiorum*, an emerging pathogen and a potential threat to the sugar beet industry.

3.2. Introduction

Sugar beet (*Beta vulgaris* L.) is a dicot in the family Amaranthaceae (formerly Chenopodiaceae) in the order Caryophyllales (McGrath and Townsend 2015; Hamdi et al. 2021). Sugar beet accounts for 14% of the world's sucrose requirement, which is considered the second most important source of sucrose (FAOSTAT, 2017) after sugar cane (*Saccharum officinarum*). North Dakota and Minnesota have consistently held the forefront in sugar beet production, covering 57% of the total sugar beet production in the United States, contributing a significant bi-state's total economic activity estimated at $6.1 billion (Bangsund and Hodur, 2023; ISO 2020; USDA-ERS, 2021). In the Red River Valley of North Dakota and Minnesota, the economic value and production of sugar beet is limited by many factors including weeds, insects, with diseases being the most prominent and challenging one. Sugar beet diseases are caused by all pathogenic groups including oomycetes, viruses, bacteria, nematodes, and most importantly fungal pathogens (Jacobsen 2006).

Sclerotinia sclerotiorum (Lib.) de Bary is an ascomycetous class, soil-borne, belligerent, non-specific, ubiquitous, cosmopolitan, necrotrophic plant pathogen affecting more than 500 plant species. This includes dicotyledonous plants consisting of approximately 225 genera and 64 families, as well as a few monocotyledonous plants such as tulip and onion (Purdy, 1979; Boland and Hall, 1994; Sharma et al. 2016; Kabbage et al. 2015; Liang and Rollins 2018; Ding et al. 2021). The disease cycle of ailments caused by *S. sclerotiorum* begins with its resting structure, sclerotia, which germinates myceliogenically or carpogenically depending on climatic conditions (Le Tourneau, 1979; Willetts and Wong, 1980; Willetts and Bullock 1992; Bardin and Huang, 2001).

Under field conditions, apothecia act as the source of primary inoculum (Abawi and Grogan 1979) that is forcefully dispersed by a mechanism called puffing (Hartill & Underhill, 1976). Upon landing and provided favorable conditions are present, the ascospores produce a germ tube which grows and forms infection cushions to help penetrate plant tissues directly or enter through natural openings. The fungus produces cell wall degrading enzymes (Lumsden and Dow, 1973; Liang & Rollins, 2018; Chethana et al. 2021) that will help access cell contents to feed on it. As the nutritional value of the colonized tissues lowers, the pathogen starts forming overwintering sclerotia (Abawi and Grogan, 1979).

S. sclerotiorum is responsible for more than $200 million in annual crop loss in the United States (USDA, 2005). This led the United States Congress to approve the establishment of the National Sclerotinia Initiative (NSI) in 2004. The NSI is managed by the USDA Agricultural Research Service (ARS) and focuses on conducting advanced research on biology, disease epidemics, host resistance and management of diseases caused by this fungus. *S. sclerotiorum* causes a significant reduction in the quality and yield of several economic crops in the United States. *S. sclerotiorum* has recently been added to the list of devastating fungal pathogens affecting sugar beet and has been reported as a potential threat to the sugar beet industry. Recent reports have confirmed *S. sclerotiorum* to be an emerging pathogen of sugar beet causing leaf blight and root rot in the Red River Valley region of North Dakota and Minnesota and other numerous sugar beet-producing states in the United States (Khan et al. 2020; Khan et al. 2021; Khan et al 2022; Bhuiyan et al. 2021).

The most practical and significantly effective method for controlling Sclerotinia diseases is the appropriate application of fungicides (Mueller et al. 2002; del Río et al. 2007). According to the Fungicide Resistance Action Committee (FRAC), Methyl Benzimidazole Carbamates

(MBCs), Demethylation Inhibitors (DMIs), Quinone outside Inhibitors (QoIs), Succinate dehydrogenase inhibitors (SDHI) are classes of fungicide that have been used for the control of *S. sclerotiorum* FRAC, 2023). For example, in North Dakota where the production of canola, soybean, sunflower and many other host crops is threatened by the damaging impact of *S. sclerotiorum* infection, azoxystrobin (Quadris), metconazole (Quash), prothioconazole (Proline), pyraclostrobin (Headline), picoxystrobin (Approach), penthiopyrad (Vertisan), boscalid (Endura) and thiophanate methyl (Topsin) are fungicide with a single mode of action (MOA) that have been registered for the effective control of *S. sclerotiorum* in various crop fields (Friskop et al. 2024; Brent and Hollomon, 2007). Priaxor which is a combination of two single MOA active ingredients (fluxapyroxad and pyraclostrobin) has been registered for use in canola, soybean, sugar beet, sunflower, corn, dry bean flax, lentils, pea, potato and many other field crops produced in North Dakota (Friskop et al. 2024).

However, the excessive and continuous use of these fungicides poses a threat to environmental health and could induce resistance from the pathogen. Wang et al. (2022) conducted an extensive study which showed the cross-resistance of *S. sclerotiorum* to SDHI fungicides. Similarly, resistance development in *S. sclerotiorum* was reported for MBCs (Gossen et al. 2001; Liu et al 2020), QoIs (Li et al. 2023) and DMI fungicides (Li et al 2014; Zhan et al. 2018). MBCs resistance is attributed to a change in the β-tubulin protein at several target sites, mostly F200Y and E198A/G/K (Koenraadt et al. 1992; Brent and Hollomon, 2007, Hawkins & Fraaije, 2016) and has been reported in Michigan, New York, South Carolina, and California (Jones and Ehret, 1976; Szkolnik and Gilpatrick, 1977; Ogawa et al. 1981; Zehr, 1982). DMIs resistance mechanisms are associated with alterations in amino acid positions, e.g., a Y136F mutation in the 14*α*-demethylase (*CYP51*) gene caused by replacement of phenylalanine with tyrosine (Delye et

al. 1997, 1998) or leucine (Albertini et al. 2003), overexpression of the *CYP51* gene due to increased copy numbers or transformants *CYP51* with promoter region (Schnabel and Jones, 2001) or by the ATP-Binding Cassette (ATP). Fungal resistance to QoI fungicides starts with a point mutation in the mitochondrial cytochrome b gene (CYTB) that promotes a reduction in the ability of the fungicides to bind well, due to a switch in the amino acid from glycine to arginine at position 137 (G137R) or alanine at position 143 (G143A) and from phenylalanine to leucine at position 129 (F129L) (Gisi et al. 2002) resulting in the development of alternative respiration pathways which allow resistant isolates to avoid the toxic effect of QoI fungicides. (Miguez et al. 2003; Wood and Hollomon, 2003). SDHI resistance is caused by a point mutation in the *sdh* genes (Avenot and Michailides, 2007; Miyamoto et al. 2009; Peng et al. 2021) causing a change in amino acids from histidine to tyrosine in cases like H272 of sdhB and H146 of sdhC (FRAC 2018; Sierotzki and Scalliet 2013; Veloukas et al. 2012).

Currently, there is no approved fungicides for the control of *S. sclerotiorum* in sugar beet fields; however, fungicides like azoxystrobin (Quadris ®), pyraclostrobin (Headline ®), metconazole (Quash ®), prothioconazole (Proline ®), boscalid (Endura ®), and thiophanate-methyl (Topsin ®) are registered for use in controlling *S. sclerotiorum* in canola and many other crops (McMullen and Markell, 2010; Friskop et al. 2024). Inpyrfluxam (Excalia) ®) is an experimental fungicide while Priaxor ® which is a mixture of fluxapyroxad and pyraclostrobin, are fungicides that have been introduced in controlling foliar and root diseases of sugar beet caused by fungal pathogens (Khan and Hakk, 2017; Khan and Hakk, 2022; Friskop et al. 2024). However, there is limited information on these fungicides' efficacy specifically for the control of sclerotinia diseases in sugar beet. To this end, approved fungicides commonly used in sugar beet and canola fields were evaluated for their control efficacy in managing diseases caused by *S. sclerotiorum.*

3.3. Materials and Methods

In this study, we conducted multiple experiments to assess the control efficacy of different commercial fungicides against *S. sclerotiorum* infection. Five commercial fungicides were assessed on three sugar beet varieties at field rate for their ability to control or reduce Sclerotinia leaf blight, seedling damping-off and root necrosis.

3.3.1. Fungicides

A total of five (5) commercial fungicides with different modes of action were used in this experiment (Table 3.1.). These compounds are commonly used fungicides in various host crops for controlling diseases caused by *S. sclerotiorum* in the Red River Valley region.

Table 3.1. Fungicide products used in the fungicide efficacy study

Active ingredient (%)	**Product name**	**Company name, Location**	**Field Rate (ml/ha)**	**Group[y] name**
Thiophanate-methyl (45)	Topsin 4.5FL	UPL, King of Prussia, PA	1462	MBCs
Prothiconazole (41)	Proline 480SC	Bayer Crop Science, St. Louis, MO	417	DMIs
Azoxystrobin (23)	Quadris Flowable	Syngenta Crop Protection, Greensboro, NC	1133	QOIs
Inpyrfluxam (31.25)	Excalia SC	Valent U.S.A LLC, San Ramon, CA	219	SDHIs
Fluxapyroxad (14.3) and, Pyraclostrobin (28.16)	Priaxor Xemium SC	BASF, Research Triangle, NC	584	SDHIs + QOIs

[y]FRAC group and names are designated by the Fungicide Resistance Action Committee (https://www.frac.info/, FRAC 2020) as a means of identification for active ingredients with cross-resistance. FRAC Group 1=**MBC** (Methyl Benzimidazoles Carbamates), Group 3= **DMI** (Demethylation Inhibitors), Group 7= Carboxamides, Group 11= **QoI** (Quinone outside Inhibitors).

3.3.2. Evaluation of Fungicide Efficacy on Sugar Beet Seedling Damping-Off Caused by *S. Sclerotiorum*

The experiment was conducted in the Dalrymple Greenhouse Complex of the Agricultural Experiment Station (AES) at North Dakota State University in Fargo, ND in 2023. The greenhouse conditions for this study were similar to that which was described previously in 2.3.1. Similarly, varieties of sugar beet and fungicides described previously in 2.3.2 and 3.3.1 respectively were used in this experiment to evaluate disease severity and fungicide control efficacy against *S. sclerotiorum* causing seedling damping-off. For the seedling damping-off study, five fungicide treatments were evaluated across the three varieties, each treatment had three replications, and ten seeds were planted for each treatment. The experiment included a positive, non-inoculated, control and a negative, inoculated without fungicide application, control. The experiments were conducted twice using MN-22 *S. sclerotiorum* isolate. Barley inoculum was prepared as described by Mueller et al (2002) using barley seeds colonized by *S. sclerotiorum* mycelia. To prepare the inoculum, 100g of barley seeds (*Hordeum vulgare* L.) were soaked for 24hrs in 100 ml of deionized water and then combined with 1g of PDB in a 1000 ml container. The mixture was autoclaved at 121^{o}C and 103.4 kPa for 20 minutes and allowed to cool. Eight hours later, it was autoclaved a second time to ensure complete eradication of all contamination. The autoclaved barley seeds were mixed thoroughly with agar plugs containing hyphal tips from actively growing mycelial cultures and incubated for seven days at 23±2^{o}C (Mueller et al. 2002). Every 24hrs, the inoculum was shaken to promote seed colonization. Fungicide treatments were applied 24hrs before inoculation. The barley inoculum (one colonized barley seed each) was placed carefully close to each sugar beet seed (1:1) at the planting stage (for seedling inoculation) and also at the roots of each sugar beet seedling at 2-leaf growth stage 24hrs after transplanting (for root rot inoculation). All plants were

kept at a 16-h photoperiod for one week in the growth chamber post-inoculation. Temperature was maintained at 23±2ºC. Data was collected on seedling emergence (number of seedlings germinated) and mortality (number of seedlings dead) for sugar beet plants inoculated at planting stage 3 and 7 dpi respectively. This data was later expressed as the percentage of the ten seeds planted. The proportion of seeds that did not produce viable seedlings and the number of seedlings that emerged but were dead by the time of each assessment, were added and expressed as a percentage of plant mortality. Similarly, damping-off (number of plants showing wilting symptoms) and plant mortality rate (3 and 7 dpi respectively) were evaluated on inoculated sugar beet plants at the 2 leaf growth stage and expressed as percentages of the ten seedlings inoculated (Fig 3.1).

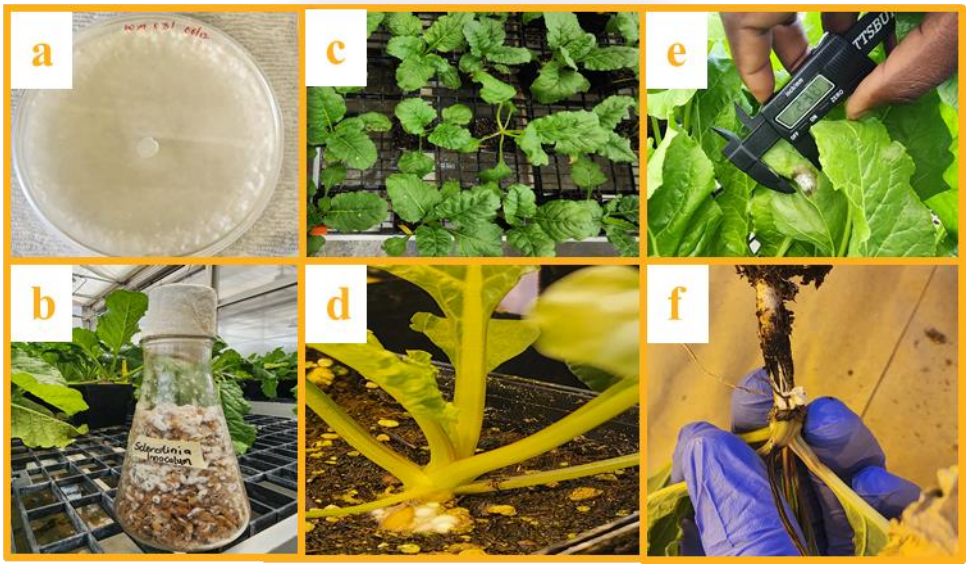

Figure 3.1. Inoculation methods for *S. sclerotiorum* disease assessment. (a), mycelia growth of *S. sclerotiorum* in PDA media. (b), colonized barley seed (c), leaf inoculation with mycelium plugs, (d) root inoculation with colonized barley seeds, (e) disease evaluation of Sclerotinia leaf blight measured as lesion size on inoculated leaves, (f) disease evaluation of root necrosis measured as lesion size.

3.3.3. Evaluation of Fungicide Efficacy of Root Necrosis on Sugar Beet Caused by *S. sclerotiorum*

Fungicide efficacy was also evaluated for the control of root necrosis on sugar beet at the 4 and 6-leaf growth stages. All conditions and treatments described in 3.3.3 were repeated for this experiment in terms of inoculum preparation, fungicides and varieties evaluated. The barley inoculum (one colonized barley seed each) was placed carefully close to the roots of each sugar beet plant for both the 4 and 6-leaf growth stages 24hrs after fungicide application. All plants were kept at a 16-h photoperiod for one week in the growth chamber post-inoculation. Temperature was maintained at 23±2^{o}C. Data was collected on the lesion size at 7 days post inoculation (dpi) as an average of two measurements taken of the lesion diameter at perpendicular angles to each other to determine disease severity of root necrosis and fungicide efficacy across the three varieties.

3.3.4. *In vivo* Efficacy of Fungicides on Sugar Beet Leaf Blight

An *in vivo* study was conducted to assess the protective effect of the five commercial fungicides (Table 3.1) on sugar beet leaf blight caused by *S. sclerotiorum.* Three sugar beet varieties, ACH 166, Crystal M837 (ACH Seeds Eden Prairie, MN) and Beta 7029 (KWS Bloomington, MN), were selected based on their performance in a preliminary varietal response study conducted prior to this experiment (Chapter two, Table 2.2.) against *S. sclerotiorum*. A positive (non-inoculated) and a negative (inoculated without fungicide application) control, were included in this study. Each treatment had one mature plant (6-leaf growth stage) replicated 4 times and the experiments were conducted twice. Fungicides were applied 24hrs before inoculation. All plants were inoculated by placing a 5-mm diameter plug with actively growing hyphae from a 3-day old colony of MN-22, an aggressive isolate of *S. sclerotiorum*, on the adaxial surface of one out of the two youngest leaves of each sugar beet plant for the leaf blight evaluation. All plants

were kept at 16-h photoperiod for one week in the humidity chamber. Temperature was maintained at 23±2^{o}C. Sclerotinia leaf blight was evaluated at the 6-leaf stage. Data was collected on the lesion size at 7 days post inoculation (dpi) as an average of two measurements taken of the lesion diameter at perpendicular angles to each other to determine disease severity of leaf blight and fungicide efficacy across the three varieties.

3.3.5. Data Analyses

All data analyses were achieved using procedures from v9.4 of the Statistical Analysis System (SAS Institute Inc.; Cary, NC, USA) (SAS Institute, 2013). Temporal lesion expansion data was used to calculate disease severity of Sclerotinia leaf blight, seedling damping-off and root necrosis across the varieties. This was completed using the summary procedure (PROC summary). Levene's test for homogeneity of variances was conducted using the general linear model procedure (PROC GLM) to determine whether the variances of the trials were homogeneous and could be combined for analysis (Levene, 1960). When confirmed, the generalized linear mixed model procedure (PROC GLIMMIX) was used to conduct a combined analysis of variance ($P<$ 0.001) with trials and their interaction with treatments being considered random effects and treatments being considered fixed effects. Tukey-Kramer's post hoc test was used to separate treatment means at $P=$ 0.05 (Tukey, 1949).

3.4. Results

3.4.1. Fungicide Efficacy for the Control of Seedling Damping-Off in Sugar Beet

This study was conducted to evaluate the control efficacies of these fungicides on seedling damping-off at planting stage and at the 2-leaf growth stage while root necrosis was evaluated at the 4-leaf and 6-leaf growth stages. Plants inoculated for seedling damping-off were evaluated for seedling emergence at 3 dpi and mortality at 7 dpi. Levene's test for homogeneity of variances

also showed that the two trials conducted for this study could be combined (P=0.05) using GLIMMIX ANOVA. The effect of variety, fungicides and the interaction between the varieties and fungicides tested was significant (P< 0.001) and means were separated using Tukey's post at P = 0.05. The results for this study were expressed as a percentage of seedling emergence and mortality, respectively (Table 3.2). Contrary to what was seen in the case of leaf blight evaluation, the main effect of variety showed that Crystal M837 had the highest (P= 0.05) percentage of seedling mortality as well as the least emergence compared to ACH 166 and Beta 7029. ACH 166 and Beta 7029 performed well having an approximate 69% and 66% seedling emergence, respectively, compared to 54% of Crystal M837.

Table 3.2. Fungicide efficacy for controlling damping-off across three sugar beet varieties at seedling and 2-leaf growth stage.

		Seedling inoculation[1]		Root inoculation[2]	
Factor	Levels	Emergence	Mortality	Damping-off	Mortality
		----------%----------		----------%----------	
Varieties	ACH166	69 a	34 b	35 c	20 b
	Beta7029	66 a	37 b	22 b	24 b
	CrystalM837	54 b	59 a	5 a	37 a
Fungicides	(-) Control	44 e	58 a	52 a	54 a
	(+) Control	97 a	3 d	0 e	3 d
	Excalia	53 cde	57 a	30 bc	34 b
	Priaxor	60 cde	53 ab	6 de	8 cd
	Proline	66 bc	39 bc	13 d	18 c
	Quadris	75 b	35 c	28 c	31 b
	Topsin	47 de	57 a	38 b	40 b

[1]Seedling emergence and mortality were quantified three and seven days after inoculation, respectively. [2]Damping-off quantified seven days after inoculation. Means in both studies represent six observations from two trials conducted in the greenhouse. Treatments with the same letters in a column are not statistically different according to the Tukey-Kramer post-hoc test with P = 0.05.

A similar result was observed for the mortality rate of ACH 166 (34%) and Beta 7029 (37%) which were significantly lower ($P = 0.05$) compared to that of Crystal M837 (59%). For the root inoculation at the 2-leaf growth stage, the varieties behaved similarly which was observed for seedling mortality, Crystal M837 (37%) had the highest ($P = 0.05$) percentage of seedling mortality compared to ACH 166 (20%) and Beta 7029 (24%). However, Crystal M827 performed differently when evaluated for damping-off at the 2-leaf growth stage as it had the least ($P = 0.05$) mortality rate (15%) as compared to Beta 7029 (22%) or ACH 166 (35%) while the latter had the highest percentage of seedling damping-off ($P = 0.05$).

The main effects of fungicides evaluated showed that Quadris increased ($P = 0.05$) seedling emergence by 75%, and also reduced plant mortality to 35% giving a better control efficacy than other fungicides tested during seedling inoculation followed by Proline and Priaxor, respectively. Excalia and Topsin on the other hand performed the least ($P = 0.05$), with a minimal control efficacy on *S. sclerotiorum* infection with regards to seedling emergence (53%, 47%) and mortality (57%, 57%), respectively. An exact trend was observed during the root inoculation study for sugar beet plants at the 2-leaf growth stage, as Priaxor and Proline reduced ($P = 0.05$) seedling damping-off (6%, 13%) and mortality (8%, 13%), respectively. Also, Topsin and Excalia which slightly switched position both significantly had the least control efficacy on seedling damping-off (38%, 30%) and mortality (40%, 34%), respectively ($P = 0.05$).

A significant interaction ($P = 0.001$) between fungicides and varieties was also observed. Quadris increased plant emergence by 330% in Crystal M837 compared to the positive control; this increment was approximately 6 times greater than the increment it caused in ACH 166 and 37 times larger than in Beta 7029 (Fig. 3.2). Similarly, variety Crystal M837 provided the largest response in seedling emergence when treated with Proline and Priaxor (260% and 210%,

respectively) compared to the other sugar beet varieties. Contrary to what was observed with plant emergence, there were varying interactions between the varieties and the fungicides. The variety Crystal M837 did not show the greatest reductions in seedling mortality when treated with Quadris, Proline, or Priaxor. Quadris significantly reduced the mortality rate in ACH 166 by 65% compared to Crystal M837 (35%) and Beta 7029 (26%), while Proline and Priaxor had more reduced mortality in Beta 7029 (42%, 26%) compared to other sugar beet varieties evaluated (P= 0.05, Fig. 3.3).

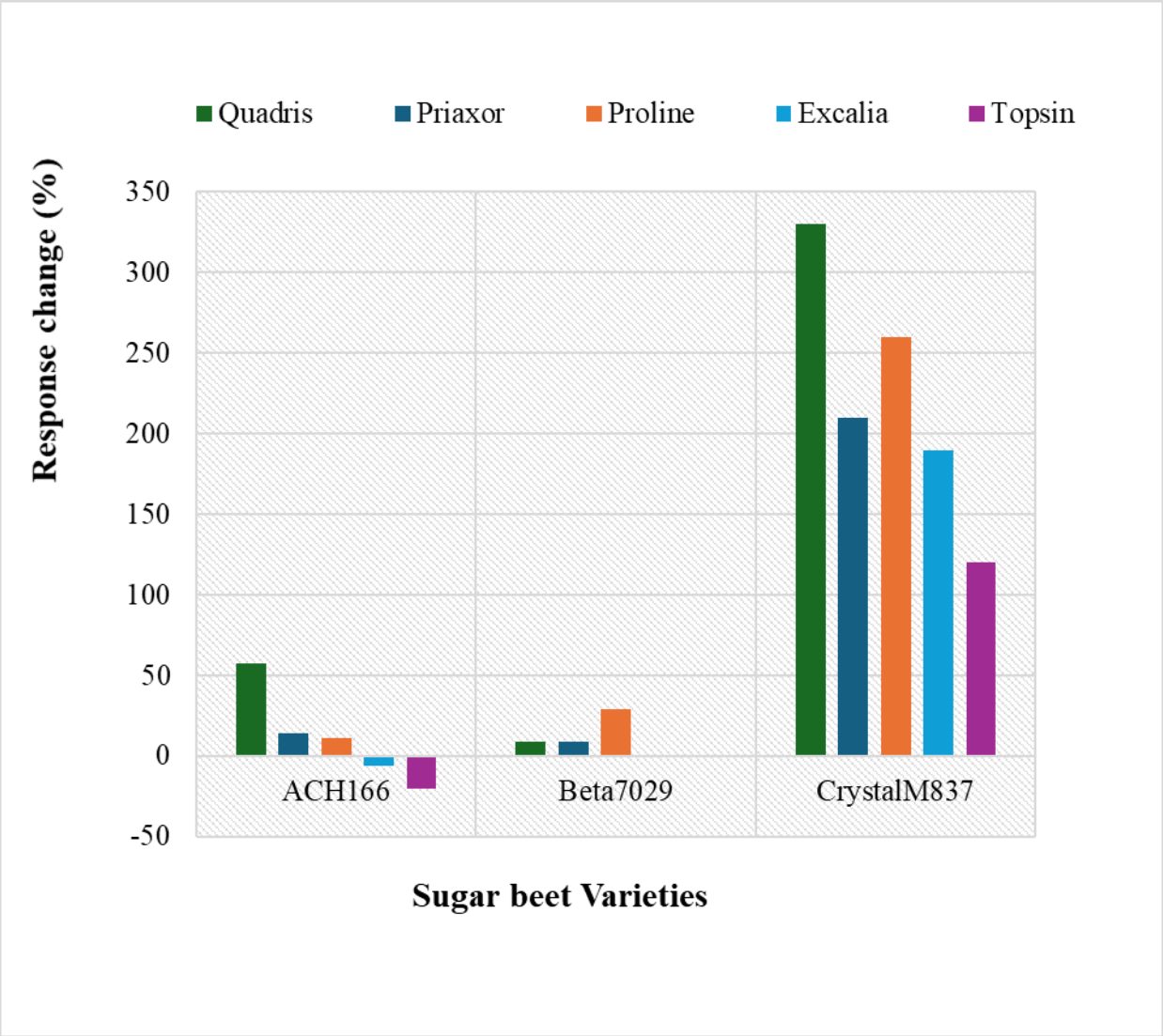

Figure 3.2. Effect of a fungicide application on the percentage of seedling emergence of three sugar beet varieties inoculated with *Sclerotinia sclerotiorum* in greenhouse trials.

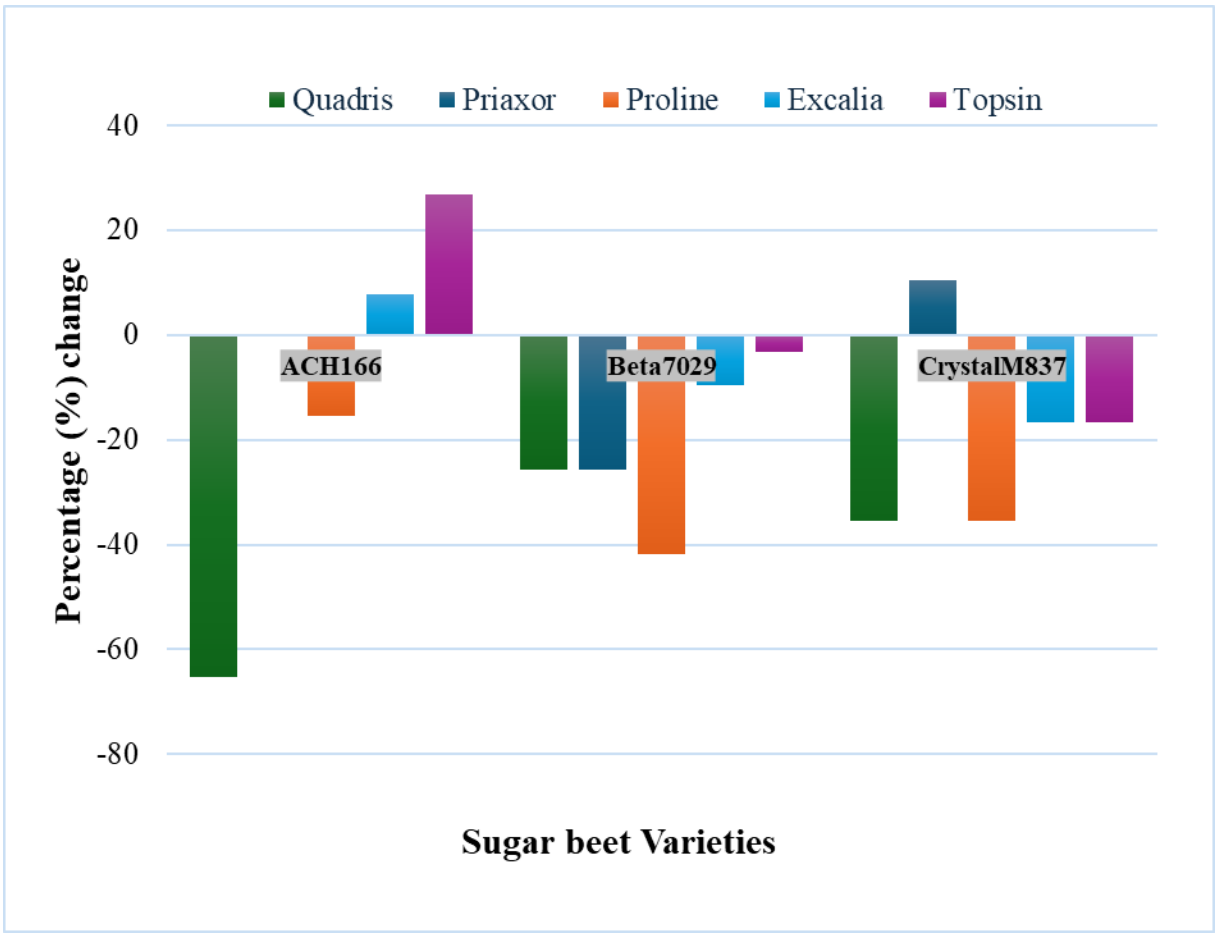

Figure 3.3. Effect of a fungicide application on the percentage of seedling mortality of three sugar beet varieties inoculated with *Sclerotinia sclerotiorum* in greenhouse trials.

For the root inoculation at the 2-leaf growth stage, the interactions between fungicides and varieties evaluated showed that Priaxor consistently provided a significant (P= 0.05) control efficacy by reducing damping-off and mortality in all sugar beet varieties evaluated. The second-best fungicide was Proline, while Topsin was the least efficient in reducing damping-off or mortality across all varieties (Fig. 3.4). There was also a trend of reduced percentage of damping-off and mortality across the sugar beet varieties, respectively, which was lower in ACH 166 followed by Beta 7029 compared to Crystal M837 (P= 0.05).

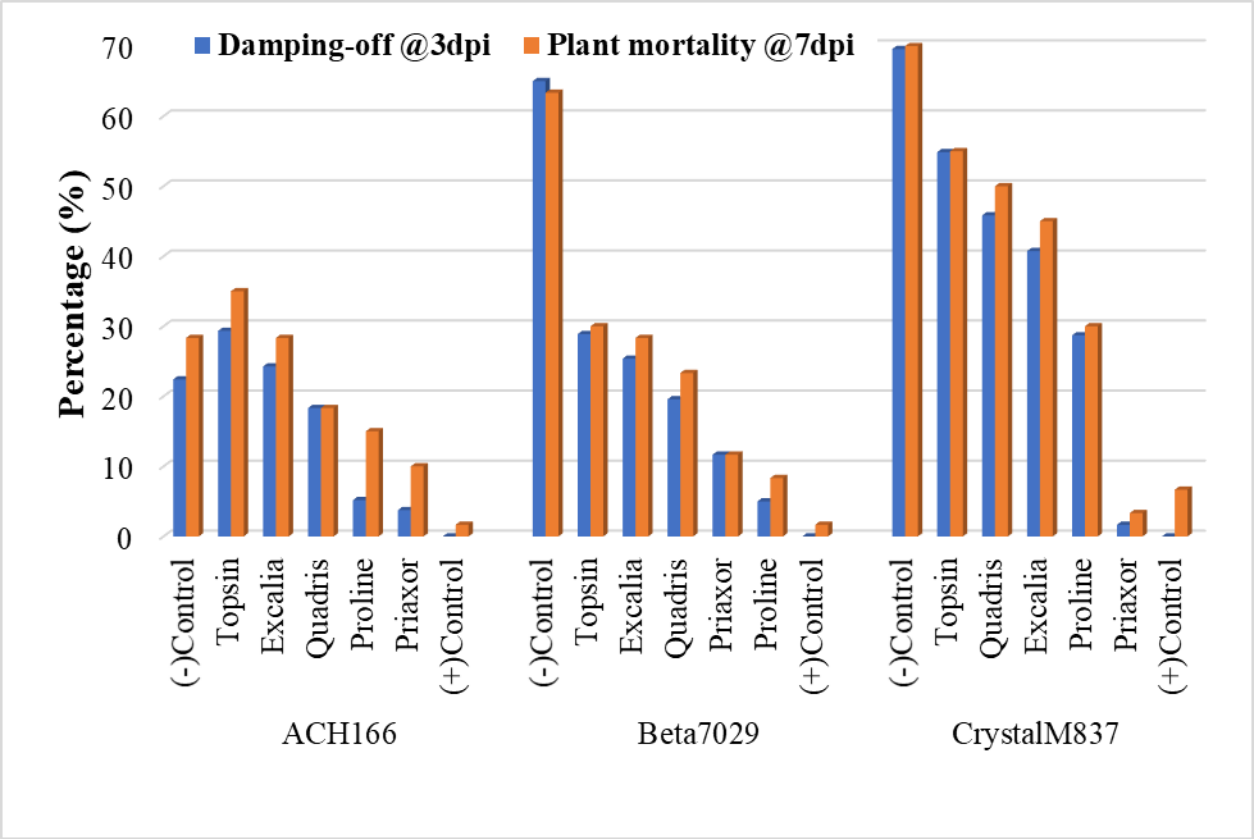

Figure 3.4. Interactions between variety and fungicide response to damping-off (3 dpi) and plant mortality (7 dpi) across sugar beet varieties (at 2-leaf growth stage)

3.4.2. Fungicide Efficacy for the Control of Root Necrosis in Sugar Beet

Also, a study was conducted to evaluate the control efficacy of these fungicides on root necrosis in sugar beet plants at 4 and 6-leaf growth stages and disease severity was measured as lesion size. Levene's test for homogeneity of variances was conducted followed by a combined using GLIMMIX ANOVA (P= 0.05). The effect of leaf stage, variety, fungicides and the interaction between the varieties and fungicides tested was significant (P< 0.001) and means were separated using Tukey's post at P= 0.05.

The combined interactions of varieties and fungicides for both leaf stages were more of magnitude rather than direction. The fungicides behaved similarly across all varieties with Crystal M837 showing the highest disease severity. Priaxor also was distinctively separated from other

fungicides in terms of efficacy in reducing disease severity of necrotic lesions observed in ACH 166, Beta 7029 and even Crystal M837 compared to the other fungicides (Fig. 3.5).

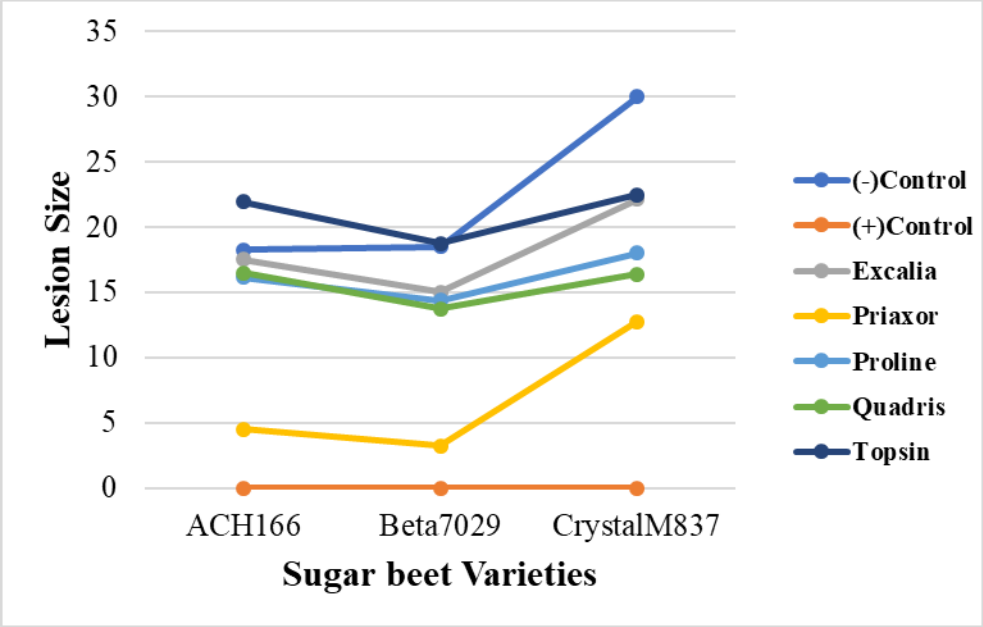

Figure 3.5. Fungicide efficacy on root necrosis measured as lesion size across ACH 166, Beta 7029 and Crystal M837 varieties (4 and 6 leaf growth stages combined).

The results from this study showed also that there was a varying response to disease severity of sugar beet plants based on leaf stages as there was a significant increase in disease severity on the root of sugar beet plants inoculated at 4-leaf growth stage compared to the 6-leaf growth stage (Fig. 3.6).

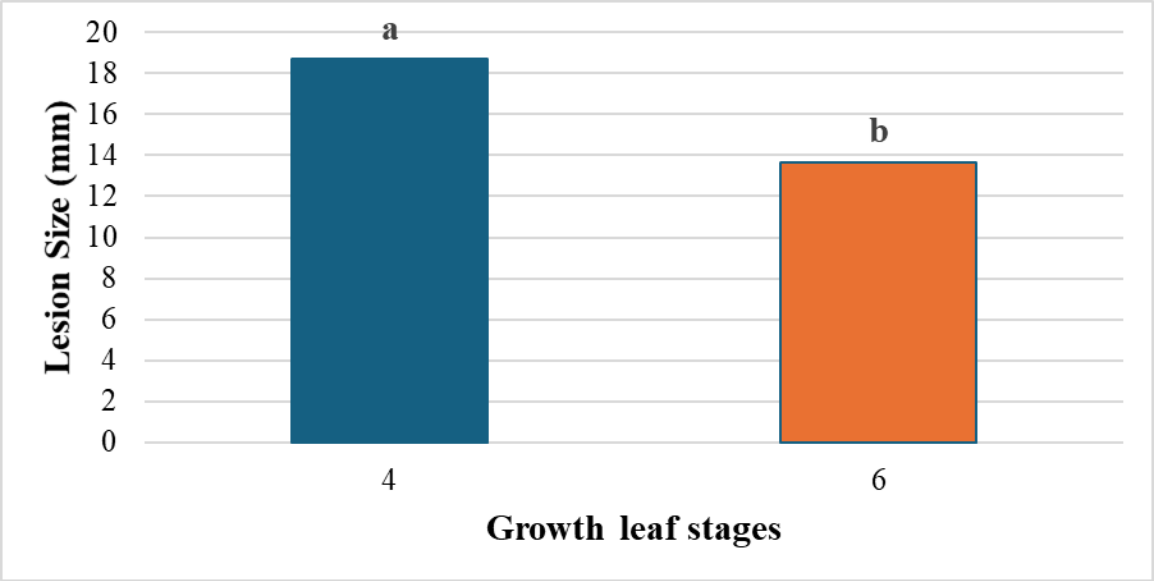

Figure 3.6. Effect of leaf stages on root necrosis expressed as disease severity (lesion size).

The varietal effect also showed that Crystal M837 had a significantly higher disease severity in terms of root necrosis compared to ACH 166 and Beta 7029 (Table 3.3). This was consistent for both 4 and 6-leaf growth stages (P= 0.05). Again, the main effect of fungicides showed that Proline followed by Priaxor were the most efficient fungicides in controlling root necrosis (P= 0.05, Fig 3.7). In both 4 and 6-leaf growth stages Proline significantly had the least disease severity amongst all fungicides evaluated across all varieties at both leaf stages, its efficacy was even more pronounced at the 6-leaf growth stage (P= 0.05). In this case, Quadris and Excalia respectively showed the least efficacy in the control of root necrosis at both leaf stages (P= 0.05).

Table 3.3. Fungicide efficacy for the control of root necrosis across three sugar beet varieties at 4 and 6-leaf growth stages.

		Lesion Size	
Factors	Levels	4-leaf growth stage [1]	6-leaf growth stage [2]
Varieties	ACH166	18 b	12 b
	Beta7029	19 b	13 b
	CrystalM837	20 a	16 a
Fungicides	(-) Control	29 a	20 a
	(+) Control	0 e	0 e
	Excalia	22 b	19 ab
	Priaxor	19 cd	16 c
	Proline	18 d	7 d
	Quadris	22 b	18 abc
	Topsin	21 bc	16 bc

[1] All sugar beet varieties were inoculated at 4 leaf stage. Means represent 8 observations in two trials.[2] Sugar beet varieties inoculated at 6 leaf stage. Means represent 10 observations in two trials. Lesion length was measured 14 days after inoculation. All trials were conducted in greenhouse conditions. Treatments with the same letters in a column are not statistically different according to the Tukey-Kramer post-hoc test with $P = 0.05$.

3.4.3. Fungicide Efficacy for the Control of Sclerotinia Leaf Blight in Sugar Beet.

Levene's test for homogeneity of variances showed that the two trials could be combined ($P = 0.05$) for a more robust analysis using GLIMMIX ANOVA. The results showed that the effect of varieties, fungicides, and the interaction between the varieties and fungicides tested was significant at $P < 0.001$. Means were separated using Tukey-Kramer's post hoc test at $P = 0.05$.

The interaction between varieties and fungicides was one of magnitude but not of direction, as all fungicides evaluated behaved in a similar manner across all varieties (Fig. 3.8). Proline and Priaxor provided the most consistent control as they reduced lesion sizes on sugar beet plants to a mean range of 0-4 mm across all varieties. In contrast, Quadris and Topsin provided better protection to ACH 166 than to the other two cultivars, while keeping lesions to a range of 5-11 mm. Excalia was the least effective fungicide and the protection it offered depended on the

cultivar. Nevertheless, all fungicides evaluated had significant control efficacy compared to inoculated plants that weren't sprayed with any fungicides (negative control treatment).

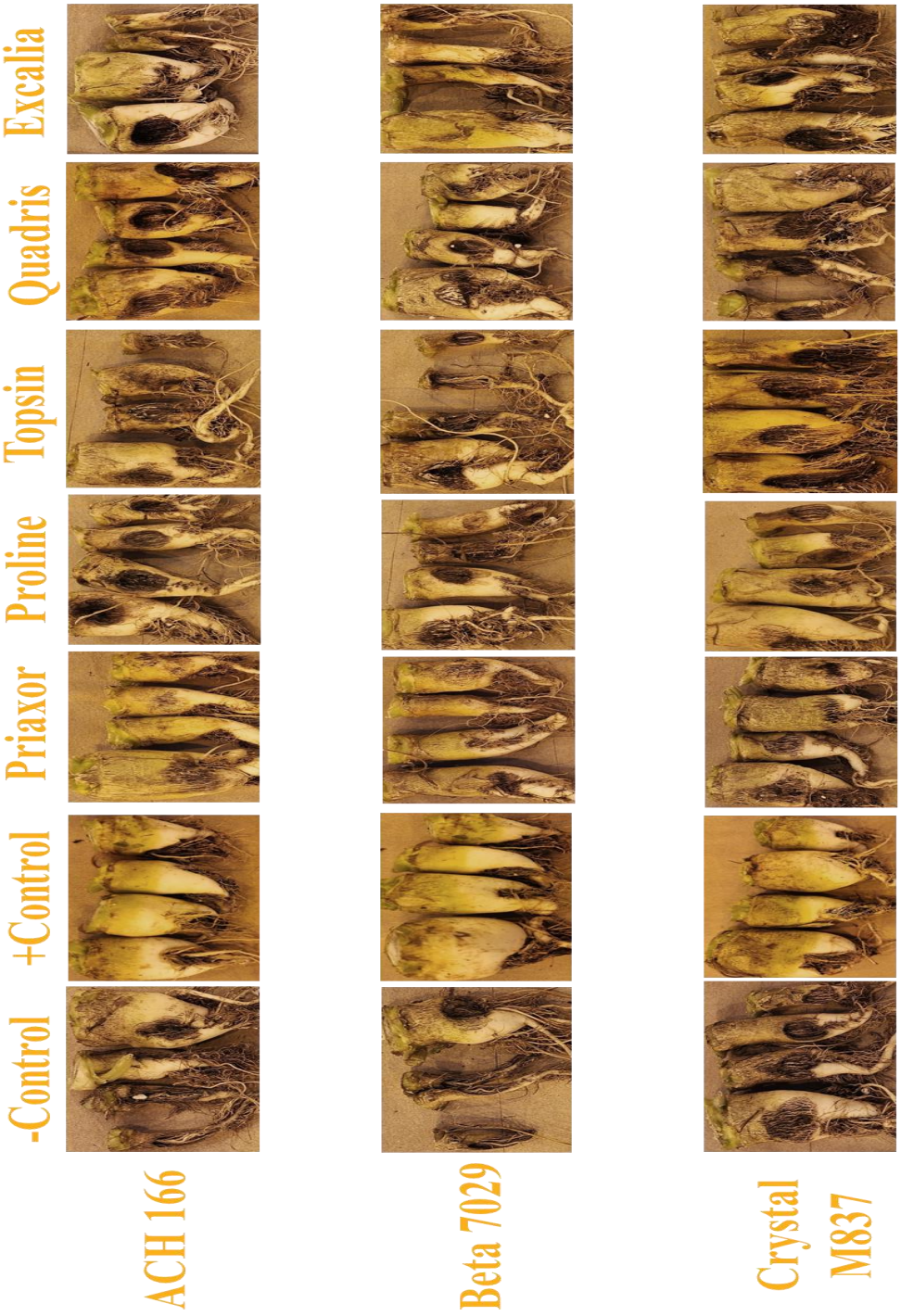

Figure 3.7. Greenhouse experiment showing the control efficacy of fungicide application on root necrosis across three sugar beet varieties. (6-leaf growth stage)

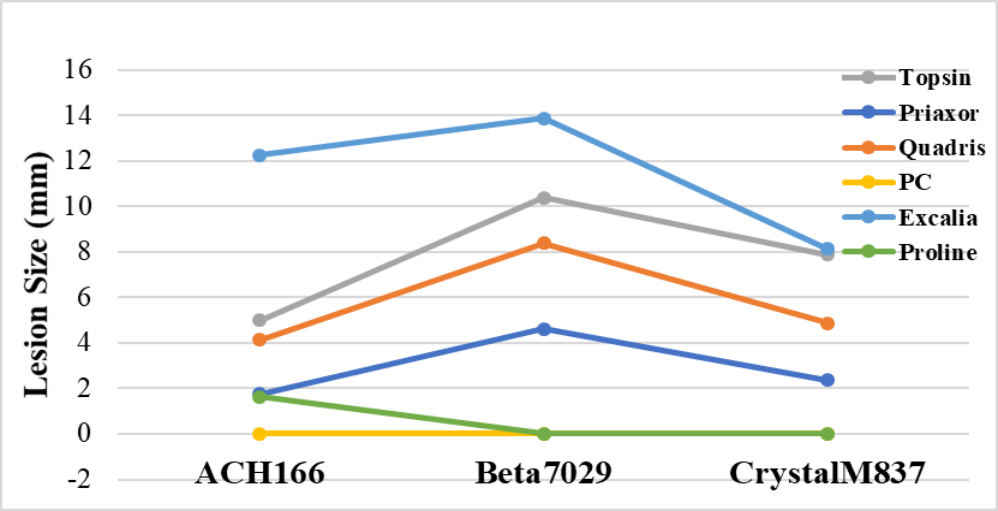

Figure 3.8. Efficacy of fungicides application in reducing Sclerotinia leaf blight measured as lesion size across sugar beet varieties.

The main effect of variety showed that Crystal M837 had reduced disease severity measured as lesion size five times significantly lower compared to other varieties ACH 166 and Beta 7029 (P= 0.05, Fig. 3.9). The main effect of fungicides in the absence of negative control (inoculated but not sprayed) showed that Proline was significantly the most effective fungicide with a disease severity ten times lower than Excalia which has the highest disease severity at P= 0.05 (Fig 3.10). Also, Priaxor after Proline had significant control efficacy on leaf blight across all varieties tested compared to Topsin, Quadris and Excalia.

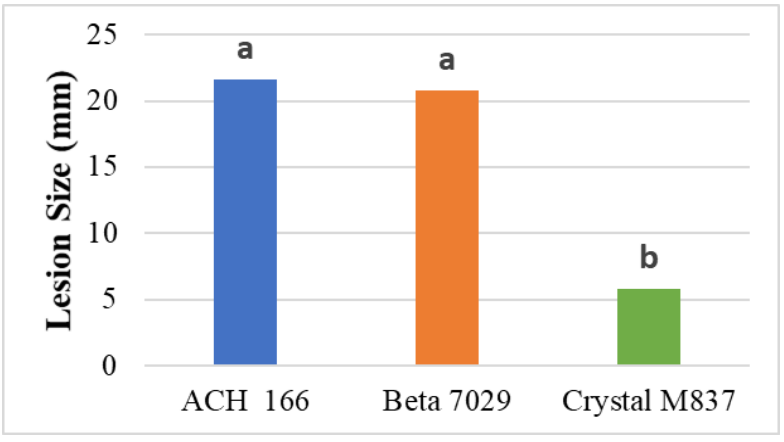

Figure 3.9. Varietal response to leaf blight caused by *Sclerotinia sclerotiorum.*

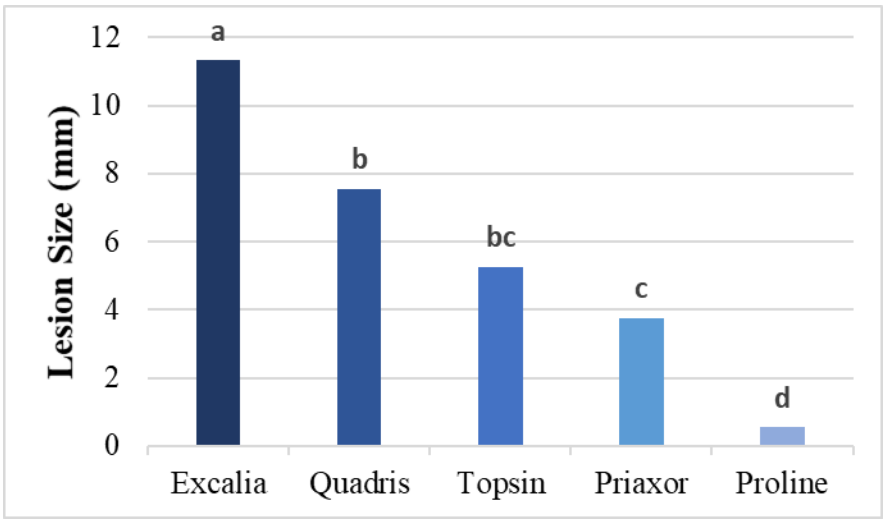

Figure 3.10. Fungicides efficacy in controlling Sclerotinia leaf in sugar beet.

3.5. Discussion

Sugar beet is an economic crop widely grown in the Red River Valley Region of North Dakota and Minnesota (Bangsund and Hodur, 2023; ISO 2020; USDA-ERS, 2021). However, in addition to many fungal pathogens known to limit its production, *S. sclerotiorum* has been reported to cause leaf blight, seedling damping-off and root necrosis in sugar beet Minnesota (Khan et al. 2020; Khan et al. 2021, Bhuiyan et al. 2021). This study provides insights into the efficacy of five commonly used fungicides for the management of Sclerotinia diseases in sugar beet. The results from the fungicide efficacy study on leaf blight showed a significant effect of variety response and fungicide applied on reducing *S. sclerotiorum* infection.

The main effect of variety in this fungicide efficacy study was consistent with the response of Crystal M837 to Sclerotinia leaf blight discussed in Chapter two (section 2.5) which evaluated the varietal response of sugar beet to leaf blight disease caused by *S. sclerotiorum* in the absence of fungicide application. Crystal M837 showed reduced susceptibility, while ACH 166 and Beta 7029 showed increased susceptibility to Sclerotinia leaf blight with or without fungicide application. Crystal M837 demonstrated a significant variety response to *S. sclerotiorum* infection

in terms of reduced lesion size compared to ACH 166 and Beta 7029, this result was similar to what was observed in a study conducted on *R. solani* by Haque and Parvin (2021), they showed that there is a significant varying response to seedling emergence and damping-off across the sugar beet varieties evaluated in the study.

Contrary to what was observed for *S. sclerotiorum* infection on sugar beet leaves, the main effect of varietal response to seedling damping-off and root necrosis showed that Crystal M837 this time was the most susceptible variety to root infection by *S. sclerotiorum* significantly having the lowest mean in terms of seed germination (54%) and mortality (59%) after inoculation at planting stage and also mortality (37%) at 2-leaf growth stage. Crystal M837 which seems to show reduced susceptibility to leaf blight is prone to a significant root damage directly impacting tonnage, recoverable sucrose content and a greater damage if roots of this sugar beet variety with *S. sclerotiorum* infections are stored. Since Crystal M837 has significant susceptibility to root infection which seems to be the energy storage and the major reason for the production of sugar beet, this result hints the possibility that there could be a greater disease severity that is dependent on the part (root) of the plant inoculated. The root inoculation of sugar beet plants for the evaluation of root necrosis at the 4 and 6-leaf growth stages also showed similar results. Liu et al. (2019) observed similar results in a study which shows an age-dependent resistance of sugar beet to *R. solani*.

The evaluation showed that Crystal M837 significantly had the highest disease severity expressed as lesion size across both 4 and 6-leaf growth stages. ACH 166 and Beta 7029 both had similar responses with no significant difference throughout the study, and they both significantly performed better than Crystal M837 throughout the fungicide efficacy study except for ACH 166 which significantly had the highest level of damping-off at the 2-leaf growth stage. The different

response of this sugar beet varieties evaluated in their susceptibility to *S. sclerotiorum* leaf and root infection aligns with results from other studies that have evaluated the response of germplasm lines or cultivars of their host crop to *S. sclerotiorum* (Kim et al. 2000; Mueller et al. 2002; Bradley et al. 2006; Benett et al. 2016; Prova et al. 2018). The efficacy of the fungicides evaluated also varies across infection type (leaf and root inoculation) and leaf stages (seedling, 2, 4 and 6 leaf growth stages) amongst the three varieties evaluated at $P = 0.05$. Bhuiyan et al. (2021) showed in a preliminary study the varying responses of sugar beet genotypes evaluated for root rot caused by *R. solani*. The similarity between the results of these two studies could be tied to the similar lifestyle of the two causative organisms. *S. sclerotiorum* and *R. solani* are both known to be necrotrophic pathogens (Bolton et al. 2006; Mukherjee, 1978), they could similarly interact with the varieties in the same manner as they both causes root infection in sugar beet. Age dependent factor observed in our study for *S. sclerotiorum* in the sugar beet varieties was also reported by Liu et al. (2019), who showed that there is an age dependency factor in resistance response shown in sugar beet cultivars which is more pronounced with reduced susceptibility when sugar beets exceed the 4-leaf growth stage. These results were consistent with our studies which showed reduced susceptibility of sugar beet to *S. sclerotiorum* root infection as the plant age increases from the planting stage to the 2-leaf growth stage, 4 leaf growth stage and then 6-leaf growth stage showed the lowest necrotic lesion at the time of disease evaluation.

All the fungicides showed a level of efficacy in controlling *S. sclerotiorum* for all the diseases evaluated. The main effect of fungicides showed that Proline and Priaxor were the overall best throughout this study ($P = 0.05$). In most cases, either of these two fungicides significantly stood out in their control efficacies while the other followed. In a few cases, they followed behind Quadris like that of the leaf blight evaluation. Proline (DMIs) significantly stood out as the most

efficient fungicide in the leaf blight study and also significantly reduced root necrosis at both the 4- and 6-leaf growth stages (Liu et al., 2019). Priaxor (SDHIs+QoIs) performed the best in reducing damping-off and plant mortality to 6% and 8%, respectively ($P = 0.05$). Quadris (QoIs) was the most efficient fungicide for managing *S. sclerotiorum* infection at the seedling stage increasing seedling emergence by 75% and reducing the mortality rate to 35% ($P = 0.05$) (Mueller et al. 2002). On the other hand, Topsin (MBCs) and Excalia (SDHIs) were the least efficient fungicides among all the fungicides evaluated in this study, providing minimal control of all diseases caused by *S. sclerotiorum* at all stages of inoculation across the three sugar beet varieties. The reduced efficacy of all these respective fungicides could be as a result of the insensitivity of *S. sclerotiorum* to these fungicides which has been continuously used for the diseases caused by *S. sclerotiorum* in crop fields in North Dakota and Minnesota since 2003 (Mueller et al. 2002; Bradley et al. 2006) and is now considered a fungicide with high risk of resistance (Brent and Hollomon, 2007; FRAC, 2023).

The interactive effect between the variety and fungicides in the leaf blight study showed that all fungicides had a control efficacy ranging between 2mm to 12mm (lesion size) across all three varieties except Beta 7029 and Crystal M837 which almost showed no lesion development when sprayed with Proline, a DMI which has been reported to provide significant efficacy against *S. sclerotiorum* infection in many other crops (Mueller et al. 2002; Li et al. 2014). For the root inoculation at the planting stage, the application of Quadris which showed the most control efficacy as described earlier increased plant emergence just by 7% in Beta 7029 and more than 50% when applied on ACH 166. However, when applied on Crystal M387, it increased emergence by more than 300%, showing that Crystal M387 is more susceptible than ACH 166, and similar results were observed for Priaxor (260%) and Proline (210%). This was consistent with the results

from the main effect of fungicides discussed earlier. Also, a report by Zamani-Noor (2021) who evaluated the control efficacy of various fungicides against *S. sclerotiorum* in oilseed rape cultivation showed similar results to what we observed in our study with respect to varying control efficacy of fungicides tested in the oil seed rape cultivation. The interaction between variety and fungicides applied at both 4- and 6-leaf growth stages combined were of magnitude rather than direction. All the fungicides behaved similarly with respect to increasing magnitude across the three sugar beet varieties. More interestingly, this interaction showed a distinct separation of Priaxor from the other fungicides evaluated, the necrotic lesion observed on the roots of the sugar beets evaluated was significantly lower and closer to the positive control particularly for ACH 166 and Beta 7029, while Crystal M837 also had reduced lesion size when Priaxor is applied compared to the other fungicides.

Overall, this study validates that the susceptibility of the varieties, the age of the plants at the time of infection, and the efficacy of fungicides determine the disease severity of *S. sclerotiorum* in sugar beet. Bhuiyan et al. 2021, showed in a preliminary study that there was varying response among the varieties of sugar beet evaluated for *S. sclerotiorum* root infection. Liu et al. 2019 also conducted similar study on *R. solani* in sugar beet with results showing that the response of sugar beet plants to root infection could be impacted by the leaf stage of the sugar beet plant at the time of infection. There have also been previous fungicide control efficacy studies conducted on mitigating the disease severity of *S. sclerotiorum* in other crops like canola, oil rape seed, soybean and many other crops known to be host to this pathogen (Mueller et al. 2002; Bradley et al. 2006; McMullen and Markell, 2010; Li et al. 2014; Zamani-Noor, 2021).

Crystal M837 was promising in its response in terms of reduced susceptibility to Sclerotinia leaf blight. For root evaluation, ACH 166 stood out as the best variety followed by Beta 7029 with

reduced susceptibility to root necrosis caused by *S. sclerotiorum*. Quadris (QoIs) was the most effective fungicide for leaf blight while Proline and Priaxor were both identified as fungicides with the best control efficacy of root necrosis caused by *S. sclerotiorum* in sugar beet (del Río et al. 2007; Friskop 2024). Conclusively, this is the first study to evaluate fungicide efficacy and identify Proline, Priaxor and Quadris respectively as potential fungicides that can be applied to mitigate the potentially damaging impact of Sclerotinia diseases on sugar beet. Hence, this provides an anticipated solution for managing *S. sclerotiorum*, an emerging threat to the sugar beet industry, and this should be further validated with extensive field research.

3.6. References

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