

ORIGINAL ARTICLE

Sclerotinia sclerotiorum (Lib) deBary causes root rot and necrosis in sugar beet in Moorhead, MN, USA

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Abstract

Sugar beet is a major sugar yielding crop in the states of Minnesota (MN) and North Dakota (USA). Sugar beet root samples collected from Moorhead, MN in September 2020 had typical rot symptoms along with whitish mycelia growth and blackish sclerotia on the external surface of the root. Pure, sterile cultures were obtained from infected roots. *Sclerotinia sclerotiorum* was identified based on morphological features and further confirmed molecularly by sequencing of the Internal Transcribed Spacers (ITS) region and matching homology with reported ITS of the fungus. Pathogenicity of *S. sclerotiorum* was confirmed through mycelial inoculation of seeds and roots under laboratory and greenhouse conditions. Inoculated seeds showed a range of symptoms that included pre- and post-emergence damping off, wilting, black discoloration of roots, constricted collar regions and stunted seedling growth. Under laboratory conditions, roots were artificially wounded using a cork borer and inoculated by mycelial plug. This resulted in noticeable root decay and growth of whitish, cottony mycelia and sclerotia externally. Transverse sections of the diseased root showed brown to black discoloration and rotting of internal tissue. Root inoculation of 4-week old sugar beet plants was achieved by depositing pathogen colonized barley grains near roots in the greenhouse, resulting in brown to black lesions and necrosis of root tissue when evaluated at 28 days post inoculation. The *S. sclerotiorum* was re-isolated from inoculated roots showing infection and identical pure isolates of the pathogen were recovered from field samples. These findings could be useful for sugar beet growers in Minnesota, allowing better management of this pathogen under field and storage conditions before its widespread future occurrence.

Keywords: disease management soilborne diseases, fungal pathogen, pathogenicity

Introduction

Root rot diseases are considered to be the most important limiting factors of sugar beet production worldwide. Root rot diseases of sugar beet caused by *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Aphanomyces* sp., *Fusarium* sp., *Pythium* sp., *Phytophthora* sp., and *Rhizopus* sp. result in significant yield losses every year (Berkeley 1994; Jacobson 2006; Khan 2017).

Sclerotinia sclerotiorum (Lib.) de Bary is an important fungal pathogen that affects many broad leaf plant species, including sugar beet (Purdy 1979; Willetts and Wong 1980; Boland and Hall 1994). This pathogen is cosmopolitan and is prevalent in many US states including those in the North Central region (Bradley and Lamey 2005; Bradley *et al.* 2006). *Sclerotinia*

sclerotiorum is responsible for more than US\$ 200 million losses annually in the United States (USDA 2005). In the North Central region it affects several economically important crops like canola, dry bean, soybean, and sunflower. Every percentage unit of disease incidence reduces on average between 0.5 and 0.8% of canola and dry bean yields (del Río *et al.* 2004; del Río *et al.* 2007). In soybean every 10% increase in incidence reduces yields by 133–333 kg · ha⁻¹ (Peltier *et al.* 2012). Moreover, North Dakota (ND) and Minnesota (MN) are two leading sugar beet growing states that produce 57% of the US sugar beet, which results in over \$5 billion in total economic activity. This fungus forms sclerotia, a hard bodied, black colored resting spore. Under adverse environmental conditions, it can survive for many years in nature (Adams and Ayers 1979; Bell and Wheeler 1986).

The primary inocula of epidemics caused by *S. sclerotiorum* on aerial plant tissues are ascospores. However, the fungus is also capable of infecting roots using mycelia that emerge directly from sclerotia. The sclerotia of *S. sclerotiorum* can survive in soil for at least 5 years (Peltier *et al.* 2012). Under favorable conditions, apothecia are developed from sclerotia (Adams and Ayers 1979; Alexopoulos *et al.* 1996). In a shaded, moist and cool (40–60°F; 4–16°C) environment, sclerotia within the top two inches of the soil profile can germinate to produce apothecia (Wu and Subbarao 2008). The apothecia are small (3–6 mm in diameter), tan, cup-shaped mushrooms and produce ascospores (sexual spore) (Abawi and Gorgan 1979; Willets and Wong 1980). Generally, ascospores cannot directly infect living plant tissues (Abawi and Gorgan 1979). Ascospores require exogenous nutrient sources and a film of water to germinate (Bolton *et al.* 2006). Senescing flower parts serve as the primary source of nutrients for ascospores as they fall on leaves, petioles or stems (Inglis and Boland 1990; Turkington and Morrall 1993). Infection is favored by cool to moderate daily temperatures with a maximum of <85°F or 29°C, and moisture from rain, fog, dew, or high relative humidity (Workneh and Yang 2000).

Sclerotinia sclerotiorum causes a wide range of symptoms. It mainly causes stem rot, leaf blight, head rot, stalk rot, root decay and crown rot, etc. At first, symptoms appear as water-soaked lesions on infected roots. Afterwards, the lesions expand and become depressed, and later girdle the root surface. Whitish, cottony mycelia grow on the lesion areas and develop blackish, hard coated, globose, circular, and or variously shaped sclerotia on the external part of the root. These are unique features for morphological identification (Kohn 1979). At advanced stages of disease, the infected tissue becomes necrotic and disintegrates. Sclerotia may be dislodged onto the soil surface by wind or during harvesting and can also be

distributed vertically in the soil profile by land preparation and irrigation (Brown and Butler 1936; Cook *et al.* 1975; Steadman *et al.* 1975). Root rot symptoms due to *S. sclerotiorum* are very similar to those caused by *Rhizoctonia solani* or *Sclerotium rolfsii* root rot. 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; Underwood *et al.* 2020). It has been reported that *Sclerotinia* leaf blight of sugar beet is becoming a serious problem for many areas in ND and MN (Khan *et al.* 2020; Khan *et al.* 2021), and Montana (our observation) in the USA. However, there is no report that *S. sclerotiorum* could infect the roots of sugar beet plants in ND and MN. In this paper, we investigated the pathogenicity of isolates of *S. sclerotiorum* obtained from rotted roots from Moorhead, MN, causing seedling emergence and root rot of sugar beets.

Materials and Methods

Isolation and identification of *S. sclerotiorum* from infected sugar beet roots

During a visit to a commercial sugar beet field in Moorhead MN (46.9190 N, 96.70610 W) in September 2020, the presence of numerous wilting plants was noted. Root samples showing necrotic lesions and the presence of sclerotia were washed with running tap water to eliminate visible dirt. The samples were then surface disinfested by immersing them in an aqueous solution of 10% sodium hypochlorite for 1 min, and in 70% ethanol for 30 s. Disinfested roots were then rinsed thrice with sterile water and placed in a laminar airflow hood to remove excess moisture. Root pieces, approximately 5 mm long, were plated on Potato Dextrose Agar (PDA) media amended with 200 mg · l⁻¹ of streptomycin sulphate and incubated in the dark for 3 days at 22°C in the lab.

Molecular identification

Genomic DNAs of three isolates were extracted using Qiagen's DNeasy plant kit (Qiagen, Hilden, Germany). The Internal Transcribed Spacer Sequence (ITS) ribosomal DNA (rDNA) of the isolates was amplified using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 primers (5'-TCCTCCGCTTAT'TGATATGC-3'), as described by White *et al.* (1990). For polymerase chain reaction (PCR), a 25-µl PCR reaction mixture contained 10 pmol of each primer, 0.5U Taq DNA polymerase (ThermoFisher Scientific, Waltham, MA, USA), 10X PCR buffer, 0.2 mM dNTP and 10 ng of fungal DNA. The cycling conditions were: an initial step at 95°C for 4 min; 30 cycles at 94°C for 30 s; at 63°C for 30 s; and

at 72°C for 30 s; followed by a final extension cycle at 72°C for 5 min (Qin *et al.* 2011). For analyzing the PCR products, 2 µl of the PCR reaction were mixed with 6 µl of the loading buffer [0.25% bromophenol, 30% glycerol in distilled H₂O, fluorescent nucleic acid dye GelRed 15X (Biotium Inc., Fremont, CA, USA)] and were loaded onto 1% agarose gels in 1X TBE buffer (Sambrook and Russell 2001). Purification of a single gel band of PCR product was done using PureLink™ Quick Gel Extraction Kit (ThermoFisher Scientific). The purified PCR products were Sanger sequenced by the Molecular Cloning Laboratories (MCLAB, San Francisco, CA, USA).

Seed inoculation with *S. sclerotiorum* mycelia

To fulfill Koch's postulates, three commercial sugar beet cultivars, C-572, H-9739 and S-655, were inoculated at planting time using mycelial plugs from the *S. sclerotiorum* isolate from Moorhead. Since the commercial seeds were coated with fungicides, the seeds were washed with distilled water, surface disinfested in 70% ethanol, and rinsed thrice with autoclaved water. Groups of 10 seeds per cultivar were sown in plastic trays (25 × 12 × 10 cm) filled with FLX soilless mix (PRO-MIX, Quakertown, PA, USA) amended with osmocote (N-P-K: 15-9-12) fertilizer (Scot Company; Marysville, OH, USA). The pots were arranged in a completely randomized design and the study was conducted twice with three replications and 10 seeds per replicate each time. A similar number of plants from each cultivar were mock-inoculated with an agar plug without mycelia of the pathogen. About 5 mm diameter agar plugs containing actively growing hyphal tips from a 2–3 day old fungal colony were used for seed inoculation. Individual seeds were inoculated by placing one 5 mm diameter mycelial plug on each seed and covered with the soilless mix. Inoculated pots were incubated under humidity at 25°C for 5 days and then transferred to a greenhouse room for observation.

Root inoculation with mycelial plugs and barley grains and disease severity evaluation

The test of pathogenicity by root inoculation was done in two ways: inoculating fungal mycelial agar plugs through a wound under room conditions, and root inoculation with colonized barley grains with fungal mycelia in a greenhouse, respectively. Pathogenicity of the causal agent, *S. sclerotiorum* was attained by placing a fully colonized agar plug over a wound of the same size using a sterile cork borer. Four sugar beet roots (cv. M 504) were inoculated, and an equal number of roots were inoculated with an agar plug without fungal mycelia, serving as a control, and the experiment was repeated. Roots were incubated at room temperature (25°C) for 21 days and disease symptoms were evaluated.

In the second method, 4-week old sugar beet plant (cv. B-8606) roots were inoculated by artificially prepared colonized barley grains. Individual barley grains were placed in close contact with the root 1 inch deep from the soil line and covered with soil. The barley inoculum preparation method was adapted from Noor and Khan (2014) with slight modifications. Three plants of each variety were inoculated by one colonized barley grain. Equal numbers of plants were mock-inoculated with sterile barley grains only and the experiment was repeated. The greenhouse was set to maintain a 14/10 h photoperiod and a 24 h temperature of 25 ± 2°C during the experiment. Plants were regularly watered. Disease symptoms were evaluated at 28 days post inoculation (dpi). Roots were pulled by hand and washed under tap water followed by root rot evaluation.

Results

Field survey, isolation and identification of *S. sclerotiorum* from infected sugar beet roots

A quick survey of the field revealed that the percentage of affected plants was approximately 5–10%. Affected plants had blighted lower leaves and necrotic lesions near the soil line on their taproots (Fig. 1A). Sugar beet root samples with necrotic lesions accompanied by whitish mycelial mats and blackish sclerotia on the infected root surface were collected from a field and were taken to the North Dakota State University (NDSU) Sugar Beet Pathology Laboratory for isolation (Fig. 1B). Sclerotia which were adhered to the root surface measured approximately 4.8 mm in width and 6.3 mm in length (Fig. 2A). About 30–40% of infected root samples displayed necrotic lesions and the presence of sclerotia. The pathogen was isolated from the infected root samples. Hyaline, septate, creamy white mycelia grew on the dishes (Fig. 2B). Hyphal tips were transferred to clean PDA media and allowed to grow as described (Fig. 2C). After 14 days, hard, blackish sclerotia identical to those collected from field samples were observed (Fig. 2D). Based on macroscopic and microscopic observations, the pathogen was identified as *S. sclerotiorum* (Kohn 1979).

Molecular identification

The ITS nucleotide sequences obtained from the isolates MHSS-1, MHSS-2 and MHSS-3 showed 100% identity with the corresponding region of *S. sclerotiorum* GenBank accessions MT393753, MG516658, MW696199 and MW375456. The sequence obtained from one of the isolates retrieved from the Moorhead field was deposited in the NCBI GenBank as accession MW786662.

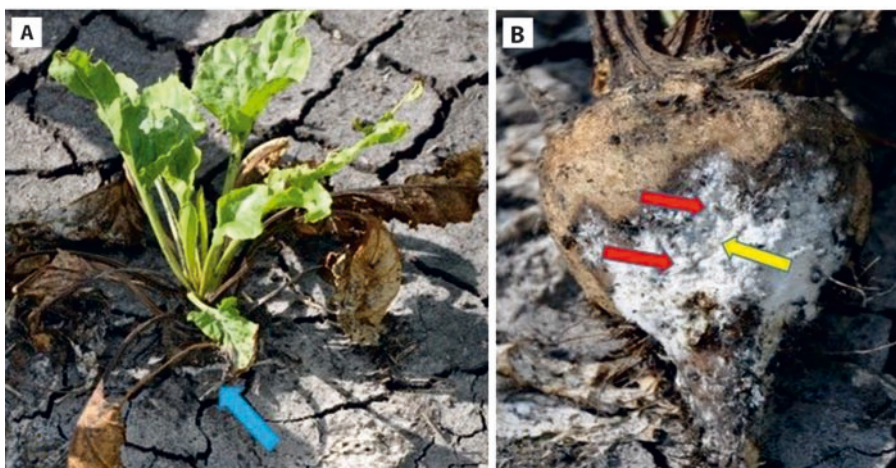


Fig. 1. Plants showing blighted leaves and root rot symptoms near the soil line (blue arrow) in a field at Moorhead, MN. (A) Diseased brown to black beet roots; (B) discolored and necrotic lesions sometimes covered with whitish cottony mycelia (yellow arrow) and blackish sclerotia (red arrows)

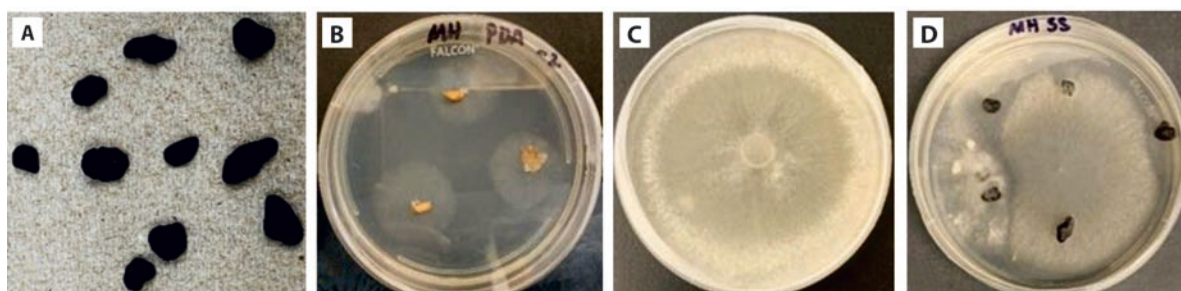


Fig. 2. (A) Sclerotia recovered from sugar beet roots infected by *Sclerotinia sclerotiorum*; (B) creamy white mycelia growing from infected sugar beet root tissues onto PDA; (C) 5-day old culture of *S. sclerotiorum* showing whitish cottony mycelial growth; (D) 14-day old *S. sclerotiorum* culture showing blackish sclerotia developed on a PDA plate

Seed inoculation with *S. sclerotiorum* mycelia

Fourteen days post seedlings from inoculated seeds showed variable symptoms (Fig. 3). Pre-emergence damping off reduced plant emergence by 30 to 40%. Seedlings that emerged frequently showed post-emergence damping off, with water-soaked lesions at the soil line accompanied by wilting and wrinkling of cotyledons and had thin, black discolored hypocotyl compared to healthy asymptomatic seedlings (Fig. 3C and D). Seedlings developed from non-inoculated seeds were healthy and vigorous, and uprooted seedlings had clear roots (Fig. 3A). The fungus re-isolated from infected tissues (Fig. 4A) showed morphology typical of *S. sclerotiorum* recovered from the field samples (Fig. 4B, C, D).

All three cultivars evaluated were highly susceptible to the disease. The pathogen reduced seedling emergence and infected those that emerged, while seedlings developed from non-inoculated seeds were healthy, vigorous and asymptomatic (Fig. 3A). Although the seedling emergence on un-inoculated soil was 100%

for all three varieties (data not shown), the seedling emergence on *S. sclerotiorum* mycelial agar plugs inoculated soil was 98% for S-655, and above 80% for the H-9739, and C-572 cultivars at 6 dpi. Thus the seedling emergence of the three varieties was reduced by about 14% (Fig. 5). At 14 dpi, the average incidence of symptomatic seedlings was 65, 75 and 79% for the cultivars S-655, H-9739 and C-572, respectively.

Root inoculation with mycelial plug and barley grains and disease severity evaluation

After 21 dpi with pathogen mycelial agar plugs, roots showed evident root decay (Fig. 6A) and growth of cottony mycelia and sclerotia had formed externally (Fig. 6B). Transverse sections of inoculated sugar beet roots presented brown to black discoloration and rotting of internal tissues (Fig. 6B). Mycelial growth and sclerotia were also found internally (Fig. 6B). Roots inoculated with sterile agar plugs did not show symptoms



Fig. 3. Pre-emergence and post-emergence damping off. (A) Symptoms observed 14 dpi; (B) sugar beet caused by *Sclerotinia sclerotiorum* on cultivar H-4302, asymptomatic seedlings showed healthy hypocotyls; (C) symptomatic seedling showed necrotic lesions on hypocotyls; (D) non-inoculated seeds were healthy and well-developed cotyledons

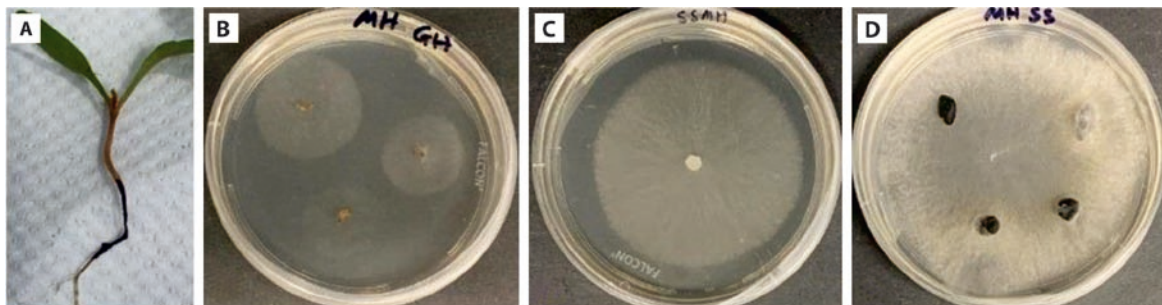


Fig. 4. (A) Infected seedling showing black discolored hypocotyl; (B) *Sclerotinia sclerotiorum* re-isolated from the infected seedling; (C) pure culture growing from mycelia; (D) matured culture of *S. sclerotiorum* with sclerotia

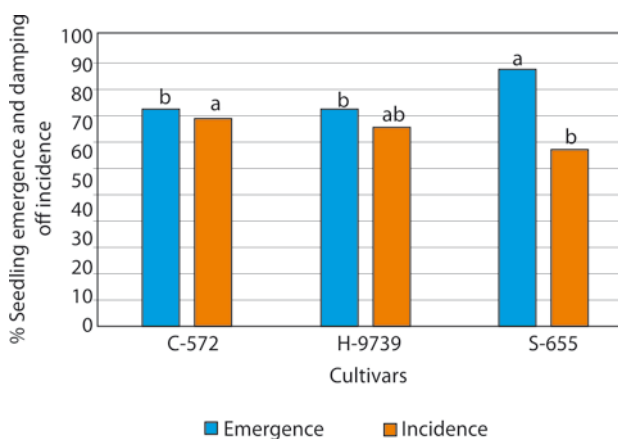


Fig. 5. Percentage of seedling emergence and damping off incidence of three commercial sugar beet cultivars C-572, H-9739, and S-655 inoculated with *Sclerotinia sclerotiorum* mycelial agar plugs at planting. Columns of similar color but different letters are statistically different at $p < 0.05$

(not shown). In the second inoculation test with pathogen colonized barley grains (Fig. 6C), all inoculated roots developed numerous lesions on root surfaces, as well as necrosis of root tissue at 28 dpi (Fig. 6F). The non-inoculated samples had clear roots without any symptoms (Fig. 6E).

Discussion

This paper described the symptomology of sclerotinia root rot of sugar beet and its pathogenicity to sugar beet seeds and roots under room and greenhouse conditions. To the best of our knowledge, this is the first report of morphological and molecular identification of *S. sclerotiorum* causing root rot on sugar beet in

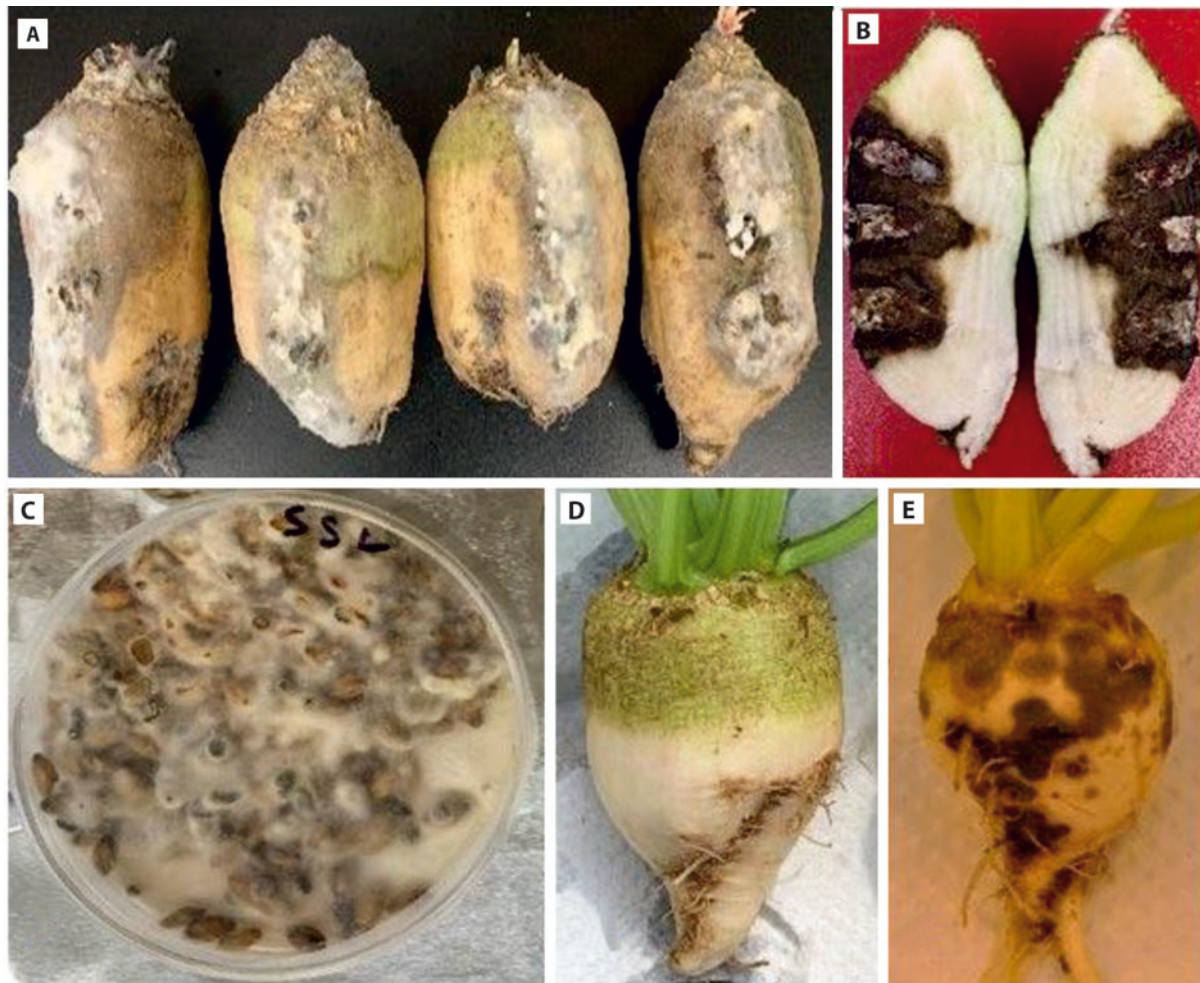


Fig. 6. (Top panels) Sugar beet root inoculation with *Sclerotinia sclerotiorum* colonized agar plugs under laboratory conditions show characteristic root decay at 21 dpi. (A) Whitish cottony growth of mycelia and sclerotia developed on the point of inoculation; (B) transverse section of inoculated beet represented brown to black discoloration, rotting of internal tissues and black sclerotia. (Bottom panels) Sugar beet root inoculation in the greenhouse; (C) pathogen colonized barley grains; (D) mock-inoculated root showing no symptoms; (E) pathogen inoculated roots showing scattered lesions on root surface at 28 dpi

Moorhead, Minnesota, USA. Under greenhouse conditions, the pathogen caused at least 14% pre-emergence damping off and 65% to 79% post-emergence symptoms on three different varieties from the three major sugar beet seed companies. The corresponding author also observed similar root rot of sugar beet in Ada, Foxhome and Hector, MN and Fairmount, ND, in 2020. Sugar beet is stored up to eight months before processing in many production areas in the US and great care is taken to avoid placing infected roots in storage piles. Infected roots in piles can cause millions of dollars in losses and financial penalties by state authorities when juice from piles with diseased beets contaminate surrounding areas and waterways. Growers are highly recommended to integrate multiple approaches, including biocontrol (del Rio *et al.* 2002; Fernando *et al.* 2007) for *Sclerotinia* management in order to curb serious economic losses annually. One commonality among sugar beet fields observed with sclerotinia root rot in 2020

was the presence of soybean and or edible bean crops in the rotation, especially as the preceding crop. The soybean and edible bean crops had significant white mold infestations especially in 2018 and 2019. We did not observe sclerotinia root rot in sugar beet fields where the preceding crop was wheat. As such, it will be useful for growers to use wheat preceding sugar beet to reduce the possibility of root rot infection until more research is done to better understand this pathogen system. In areas such as southern Minnesota where the rotation involves corn, soybean and sugar beet where the two latter crops are host of *S. sclerotiorum*, it may become practical and economical to more regularly apply and incorporate products such as Contans[®] that has *Coniothyrium minutans* to reduce the population of sclerotia. Preliminary laboratory data suggest that succinate dehydrogenase inhibitors such as penthiopyrad, and demethylation inhibitors including difenoconazole, tetraconazole and prothioconazole, particularly

in mixtures, should effectively reduce radial growth of the pathogen. Field research has been initiated to evaluate different fungicides for their efficacy in reducing root and foliar infections by *S. sclerotiorum*. Initial greenhouse screenings of a few commercial sugar beet varieties suggest that they are susceptible to both foliar and root infection by *S. sclerotiorum*. Breeders, who are now becoming aware of a new destructive pathogen of sugar beet, will have to start looking for sugar beet germplasms that are tolerant to *S. sclerotiorum*. It will take an integrated effort including crop rotation, reducing the pathogen population in other host crops where possible, and rapidly finding effective fungicides including biological control agents and determining when and how they should be applied to manage this new threat to the sugar beet industry as breeders work on developing improved tolerant varieties.

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