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Review paper

Sclerotinia species in Serbia and possibilities of their control

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SUMMARY

Sclerotinia species are economically important, necrotrophic and aggressive plant pathogens with a broad host range and worldwide distribution. They act as airborne or soilborne pathogens, and can be transmitted by seed. These pathogens can affect crops both during the growing season and after harvest. Yield losses due to Sclerotinia diseases in susceptible crops vary and may be as high as 100%. The most common pathogen from the genus *Sclerotinia* in Serbia is *S. sclerotiorum*. It occurs regularly on sunflower and its incidence may exceed 50% in some years, thus causing economically important crop losses in Vojvodina. Recently, two new species were detected in Serbia: *S. trifolium* in alfalfa and *S. minor* in lettuce plants. Diseases caused by *Sclerotinia* spp. are difficult to control due to the long-term survival of sclerotia in the soil and development of airborne ascospores. As with many other diseases, there is no single treatment that can completely control these pathogens. Implementation of multiple strategies, such as cultural practices (sanitation, crop rotation and tillage), physical, chemical and biological protection, as well as deployment of resistant cultivars, is necessary for effective disease management.

Keywords: plant-pathogenic fungi, *Sclerotinia sclerotiorum*, *S. trifoliorum, S. minor*, identification, plant disease control

INTRODUCTION

Sclerotinia species are some of the most destructive, necrotrophic and aggressive plant pathogens with a broad host range and worldwide distribution (Rothmann & McLaren, 2018). Three closely related *Sclerotinia* species - *Sclerotinia sclerotiorum, Sclerotinia minor* and *Sclerotinia trifoliorum,* are considered the most important species of this genus. Depending on their hosts, species in this genus cause diseases called rot, white mold, Sclerotinia drop rot, late blight or watery soft rot (Farr et al., 1989). *Sclerotinia* spp. cause a wide range of symptoms on above-ground plants parts. Symptoms include yellowing, leaf collapse, and water-soaked lesions with the appearance of white, fluffy fungal threads and black, dormant structures, the sclerotia (Kim & Cho, 2002; Morrall et al., 1972).

Sclerotinia species are capable of airborne or soilborne spread, acting as airborne or soilborne pathogens. In addition, the pathogens can be transmitted by seed, which is an important route of dissemination. Accordingly, there are significant differences in the epidemiology of these two types of diseases caused by *Sclerotinia* spp. regarding the effects of weather conditions on their occurrence (Purdy, 1979). Sclerotia have a major role in disease cycles since they are able to remain dormant in soil for up to 10 years (Adams & Ayers, 1979; Willetts & Wong, 1980). These structures allow the pathogen to persist in the absence of a host and act as a source of infection in following crops. The depth at which sclerotia are buried in soil has a significant impact on their ability to survive. Sclerotia situated deeper in the soil, 10-30 cm, remain alive longer than those in the top 5 cm soil layer. Also, the viability of sclerotia depends on their size - smaller sclerotia have less food resources and are more easily destroyed by soil organisms than larger sclerotia (Ćosić et al., 2012).

Sclerotinia diseases are one of the major causes of agricultural crop losses, despite regular applications of fungicides (Saharan & Mehta, 2008). These pathogens can affect crops both during the growing season and after harvest, causing significant losses to commercially valuable products. Economic losses come from entirely unmarketable collapsed vegetable crops and from decreased grain or oilseed harvests resulting from a decline in seed weight or quality. Yield losses due to Sclerotinia diseases in susceptible crops vary and may be as high as 100% (Purdy, 1979). Every year, Sclerotinia diseases cause hundreds of millions of dollars' worth of yield losses worldwide. *S. sclerotiorum* causes annual crop losses of over $200 million in the United States while an outbreak of Sclerotinia head rot in sunflower in 1999 resulted in crop losses of $100 million (Bolton et al., 2006). Sclerotinia stem rot of soybean has been recently ranked in the top 10 yield-reducing diseases in the USA (Willbur et al., 2017). Losses in peanut production in North Carolina due to Sclerotinia blight have been estimated to reach between $1 and $4 million per year (Smith et al., 2008). Lettuce is also highly susceptible to *Sclerotinia* species. In the United Kingdom, losses in field-grown lettuce are typically 10%, but they may reach 50% under wet conditions (Young et al., 2004). Despite the use of fungicides, losses caused by *S. minor* on lettuce in intensive lettuce-growing areas of Australia are estimated to range from 10 to 45% (Porter et al., 2002).

The most common Sclerotinia disease in Serbia is Sclerotinia wilt, caused by *S. sclerotiorum*. It occurs on sunflower at a rate of 15-20% on average, although the incidence may exceed 50% in some years (Marić et al., 1988). According to Tančić et al. (2011), Sclerotinia wilt causes economically important crop losses in Vojvodina. Recently, two new species have been described in Serbia: *S. trifolium* in alfalfa and *S. minor* in lettuce plants (Mihajlović et al., 2016a; 2022a). There is a lack of data on yield losses caused by *S. trifoliorum* in our country. *S. minor* was described in Serbia in 2020, and up to our knowledge, this pathogen has appeared in three distinct lettuce-producing regions, with an incidence of up to 50% (Mihajlović et al., 2023a).

Sclerotinia sclerotiorum

Taxonomic group: Fungi, Ascomycota, Leotiomycetes, Sclerotiniaceae
Teleomorph: *Sclerotinia sclerotiorum* (Lib.) de Bary 1884

The ascomycete fungus *S. sclerotiorum* is one of the most damaging soilborne fungal pathogens, affecting more than 600 plant species, including many commercially important crops (Liang & Rollins 2018). It is the most important *Sclerotinia* species that has a tremendous economic impact on crop production worldwide (Hao & Subbarao, 2005).

Hosts - *S. sclerotiorum* is a nonspecific plant pathogen. The host range of this pathogen comprises more than 600 plant species, including almost all dicotyledonous and some monocotyledonous plants (Liang & Rollins 2018). It can infect many crops of economic importance, such as sunflowers, beans, soybeans, canola, cotton, potatoes, peas, tomatoes, lettuce and rapeseed, as well as monocotyledonous plants such as tulips and onions (Xu et al., 2015; Liang & Rollins, 2018). Although *S. sclerotiorum* is a widespread, destructive necrotrophic pathogen of dicot plants that causes enormous economic losses every year, it has recently been discovered that not only could it grow as a beneficial endophyte in wheat, rice, barley, corn and other cereal plants, but it also provided protection against Fusarium head blight and wheat rust. To describe microorganisms that may behave as destructive pathogens on one set of plants, while still living mutualistically as endophytes on another group of plants (split nutritional strategy), Tian et al. (2020) coined the term "schizotrophism". Their study indicated that a broad-spectrum pathogen of one group of plants may serve as a biocontrol agent in another group of plants, where they can be utilized as biocontrol agents.

Symptoms - Vegetable crops are susceptible to *S. sclerotiorum* infection at any stage of development. Symptoms typically develop on stems, lower leaves, or

the tops of densely grown crops (Willbur et al., 2019). They include browning, water-soaking lesions, wilting, bleaching, accompanied by distinctive white cotton-like mycelium on infected leaves, stems, fruits, and petioles. Under wet and cold conditions, this fungus rapidly grows inside the infected host tissue and produces symptoms of browning, bleaching, and wilting, which results in necrosis, stunting, premature ripening, and wilting of the host. Later the diseased host tissue becomes soft and watery, which finally results in the crop's complete failure (Figure 1). After destroying the host plant, this fungus develops inside diseased tissue, forming black sclerotia (Liang & Rollins, 2018; Kim & Cho, 2002).

Disease cycle - Sclerotia, the long-lasting survival structures of the pathogen, have a major role in the disease cycle. They have an ability to remain dormant in soil for up to 10 years (Adams & Ayers, 1979; Willetts & Wong, 1980). Depending on environmental conditions, sclerotia can germinate directly by developing mycelium (myceliogenic - asexually) or indirectly (carpogenically - sexually) by producing ascospores that can be released into the air from apothecia and asci and attack the above-ground parts of host plants (Aldrich-Wolfe et al., 2015).

Aerial infection, also known as sclerotial **carpogenic germination**, occurs when sclerotia produce apothecia, fungal structures containing ascospores that are required for dissemination and infection. Carpogenic germination causes most above-ground infections in the field, where 1–20 apothecia can produce up to 2,000,000 ascospores on average (Wu et al., 2007). When ascospores land on susceptible host tissue, they can germinate under favourable conditions and start a new cycle of infection. Sclerotia should be maintained at low temperatures in order to break dormancy and germinate carpogenically, and the most optimal temperature range is between 10 and 20°C. It has been demonstrated that the production of apothecia stopped at temperatures higher than 26°C and the optimum temperature for their production was 21°C (Clarkson et al., 2004, 2007). Viable ascospores have the ability to spread over long distances. Ungerminated ascospores can survive in crop canopy for up to 12 days, depending on location and environmental factors (Willbur et al., 2019). Temperatures over 21°C and exposure to UV light both enhance ascospore mortality. Once the ascosporic mycelium makes contact with a susceptible tissue, it produces an appressorium, and penetration occurs either by mechanical disruption of host cuticle or through natural openings (Johnson & Atallah, 2014).

Unlike carpogenic germination, **myceliogenic sclerotia germination** results in the germination of mycelium directly from sclerotia (O'Sullivan et al., 2021). High humidity is required for germination of *S. sclerotiorum* sclerotia (Huang, 1985). Mycelium can penetrate enzymatically or mechanically by producing appressoria, unless appressorial penetration occurs through stomata (Lumsden, 1979). During myceliogenic germination, sclerotia produce mycelia in the presence of exogenous nutritients. However, in the absence of exogenous nutrients, germination occurs only when the sclerotia are devoid of black pigments, as in the case of immature sclerotia (Smith et al., 2008). Myceliogenic germination is also triggered when normal black sclerotia with crusts are damaged by mechanical means, desiccant treatments or freezing (Bardin & Huang, 2001).

Sclerotinia trifoliorum

Taxonomic group: Fungi, Ascomycota, Leotiomycetes, Sclerotiniaceae
Teleomorph: *Sclerotinia trifoliorum* Eriksson, 1880

S. trifoliorum is a fungal pathogen that primarily affects leguminous crops, and other forage legumes worldwide in countries with temperate climates. This pathogen can cause significant yield losses and impact the productivity and quality of these crops.

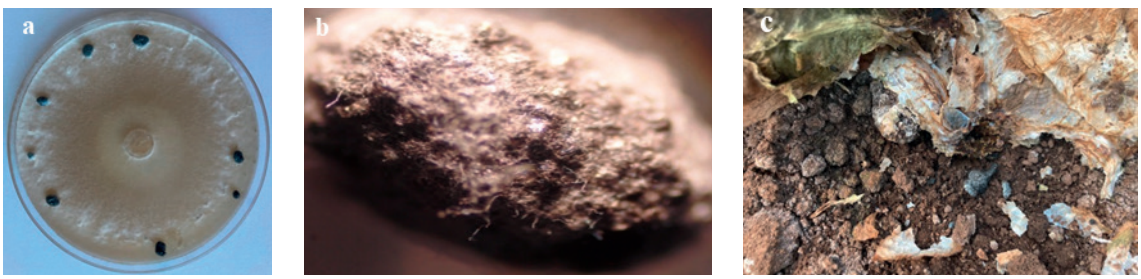

Figure 1. *Sclerotinia sclerotiorum*: a) colony grown on potato dextrose agar for 7 days, b) black sclerotia of *Sclerotinia sclerotiorum* obtained from infected lettuce plants (x10), c) lettuce drop sympthoms in the field

It is the causal agent of Sclerotinia crown and stem rot disease, also known as white mold or Sclerotinia clover rot, which causes one of the major problems in European red clover production (Purdy, 1979). This pathogen was first described in Scandinavia by Eriksson (1880), and there were reports in the early 1880s of a clover rot in England which was in 1897 attributed to *S. trifoliorum*.

Host - The fungus *S. trifoliorum* has a worldwide distribution with a narrow range of hosts mainly limited to species in the family Leguminosae and particularly to forage legumes (Kohn, 1979). In comparison to *S. sclerotiorum* and *S. minor*, *S. trifoliorum* has a relatively limited host range, comprising mostly cool season legumes. The reason for such a limited host range is unknown. *S. trifoliorum* is reported to cause diseases in 21 genera, with major losses occurring mainly in legumes, particularly forage legumes like *Medicago* and *Trifolium* spp. (Willetts & Wong, 1980).

Symptoms - The first symptoms of this disease are yellowing leaves, and hollowed out and collapsed stem. Forage legume rot caused by *S. trifoliorum* occurs as a soft rot of the crown and roots, beginning with brown leaf lesions that progress to stems and shoots (Figure 2). New growth of infected plants starts to collapse and finally dies. White mycelium often covers dead tissues, especially during rainy periods (Barbetti & You, 2014). Even though plants can be infected at any stage of development, losses are typically the highest when infection occurs at the seedling stage. This disease is most commonly observed under cool and humid conditions, often during periods of extended rains or high soil moisture. The fungus produces sclerotia, compact resting structures that can survive in the soil or plant debris for several years, contributing to the persistence of disease in fields (Kanbe et al., 2002).

Disease cycle - *S. trifoliorum* survives in the soil or plant debris as sclerotia (Willetts & Wong, 1980). Precise details of the disease cycle can vary depending on host plant, environmental conditions, and geographic location. In autumn, cool and moist conditions usually induce the formation of apothecia on sclerotia in the soil, which, near the end of October, release large quantities of ascospores into the atmosphere. Ascospores are dispersed by wind and can infect red clover leaves by direct penetration or via stomata. Ascospores are assumed to spread over large distances (Delclos & Raynal, 1995), but no exact data are available.

During winter, when plants are more susceptible due to winter stress, the fungus colonizes the entire plant. New sclerotia start appearing on diseased plants,in early spring. The next fall, sclerotia can generate new ascospore inoculum or they can stay latent in the soil for up to seven years. The mycelium of *S. trifoliorum* can infect various plant organs, including stems, leaves, and crowns. It can enter the plant through wounds, natural openings, or directly penetrate plant tissue. The development of appressoria, which directly penetrate the cuticle with or without enzymatic activity, allows *S. trifoliorum* to infect healthy tissues (Lumsden, 1979). Instances of host stomatal penetration have been documented rarely (Prior & Owen, 1964).

Depending on the weather, the disease can be nearly non-existent or completely absent, with the best conditions being a humid fall, which is required for ascospore germination, and a warm, humid winter with brief episodes of frost. Winters that are cold and dry decrease mycelial development, which stops the disease from progressing. The mycelium of *S. trifoliorum* can infect various plant organs, including stems, leaves, and crowns. It can enter the plant through wounds, natural openings or directly penetrate plant tissues. Once inside the plant, the fungus continues to grow, producing white mycelial mats on the infected tissue. The mycelium can spread within the plant, causing rotting, wilting, and decline of the affected plant parts.

Figure 2. *Sclerotinia trifoliorum*: a) colony grown on potato dextrose agar for 7 days, b) white wilt symptoms on alfalfa, c) irregular patches in alfaalfa field caused by *Sclerotinia trifoliorum*

The fungus may also produce sclerotia within infected tissues. As the disease progresses, the fungus forms sclerotia that can be found on or within infected plant tissues. They serve as survival structures, allowing the fungus to persist in soil or plant debris for future infection cycles (Marum et al., 1994).

Sclerotinia minor

Taxonomic group: Fungi, Ascomycota, Leotiomycetes, Sclerotiniaceae
Teleomorph: *Sclerotinia minor* (Jagger) 1920

S. minor is a widely distributed plant-pathogenic fungus, closely related to *S. sclerotiorum* and *S. trifoliorum* (Melzer et al., 1997). In contrast to the other two species of the genus *Sclerotinia, S. minor* mainly infects plants by myceliogenic germination to produce hyphae which attack plant tissues directly. Apothecia are seen in the field very rarely (Abawi & Grogan, 1979).

Host range – *S. minor* is less common than *S. sclerotiorum* and has narrower host range (Willetts & Wong, 1980). The most susceptible species are dicotyledonous with only three monocotyledonous plant species, namely asparagus, tulip and banana, being reported as hosts (Watson, 2007). *S. minor* primarily infects lettuce crops and is known to cause lettuce drop. However, the host range of *S. minor* is not limited to lettuce and other plant species within the Asteraceae family (endive, chicory, and radicchio). *S. minor* is a pathogen of many economically important crops including soybean, sunflower, common bean, cucumber, lettuce, spinach, cabbage, sweet potato, Irish potato, pepper, tomato, peanut, and many other (Melzer et al., 1997).

Symptoms - Disease symptoms caused by *S. minor* are similar to those caused by *S. sclerotiorum*. Initially, water-soaked lesions appear on the infected stems or leaves (Figure 3). As the disease progresses, fluffy white mycelium may become visible, lesions become bleached and necrotic, while infected stems become shredded and die (Smith et al., 2008). Only plant stems and leaves that are in close contact with soil are susceptible to infection with *S. minor,* whereas *S. sclerotiorum* can also affect upper leaves of the plant by airborne ascospores. After infection establishment, *S. minor* causes a brown, soft decay that will eventually contribute to the destruction of the plant crown tissue, resulting in wilting and collapse of entire plants, making them unsuitable for harvesting.

Disease cycle – *S. minor* has myceliogenic germination, resulting in the development of mycelium directly from sclerotia (O'Sullivan et al., 2021). The mycelium of *S. minor* attacks lower branches and rapidly invades tissue, decomposing plant cells. Plants may be infected at any growth stage from seedling to maturity. Under moist and cool conditions, the fungus rapidly invades host tissues, in which a light brown, watery rot develops, and a white, fluffy mycelial mass forms on tissue surface. Disease is often in aggregated distribution patterns within infested fields. Dispersal and transmission of the pathogen is exclusively by direct contact with germinating sclerotia in order to produce infective hyphae, which colonize plants and eventually produce more sclerotia to recur in the soil. Plant-to-plant spread between diseased and healthy plants can occur by direct contact with infected tissue (Subbarao, 1998). Sclerotia are produced abundantly on dead tissue of the plant as it dies. Some sclerotia may remain on dead plant cells as overwintering inoculum or be excreted from plant tissue into the soil. At the mycelial stage, the fungus infects a healthy plant, and the cycle begins again. After harvest, plant debris infested with *S. minor*, including sclerotia already formed, get into the soil where the inoculum remains dormant until the next planting (Purdy, 1979).

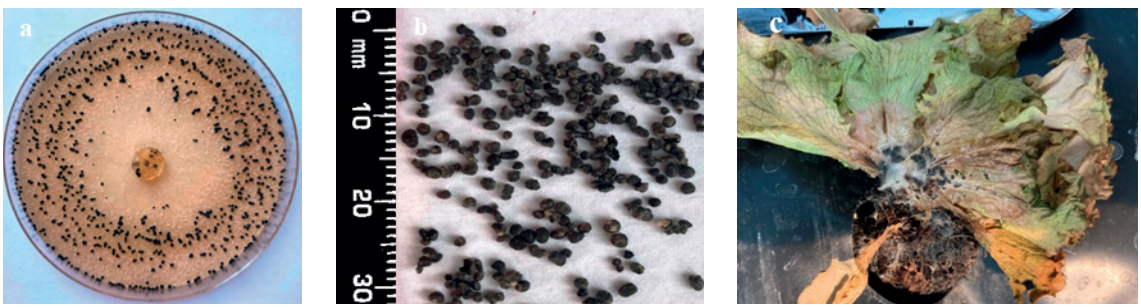

Figure 3. *Sclerotinia minor*: a) colony grown on potato dextrose agar for 7 days b) sclerotia of *Sclerotinia minor* obtained from infected lettuce plants, c) sympthoms on lettuce plant cultivated under greenhouse conditions

IDENTIFICATION

Morphological identification

Traditional morphological traits, such as cultural characteristics, sclerotial size, ascus and ascospore dimensions, and timing of apothecial development in the field, host association, Mycelial Compatibility Test (MCT) and disease symptoms are not always accurate and rapid in differentiating *S. sclerotiorum, S. trifoliorum*, and *S. minor* as distinct species. Furthermore, no differences in hyphal structures of these species have been reported (Kohn, 1979; Willetts & Wong, 1980).

Isolates within a species vary in colony colour, type, and mycelial development. Most isolates have been found to generate white colonies, while the others produced off-white colonies (Rather et al., 2022). Colonies of *S. sclerotiorum* and *S. trifoliorum* on potato dextrose agar (PDA) media consist of white to grey mycelium. The mycelium of *S. minor* is also white to grey, indistinguishable from *S. sclerotiorum* and *S. trifoliorum*, with a lot of sclerotia that are small, globose to irregular and black (Kim & Cho, 2002). *All Sclerotinia species readily produce sclerotia on infected plant material and in culture. Sclerotia dimension could be a preliminary criterion for separating Sclerotinia species (Ekins et al., 2005).* Sclerotia size is one of the differentiating traits, although it is especially unreliable for species identification because of overlapping sclerotia dimensions and shape under certain conditions. This might make it difficult to distinguish between species (Willetts & Wong, 1980). In spite of that, these characters are often used for diagnostic purposes. *S. sclerotiorum* and *S. trifoliorum* produce large sclerotia - the size of peas, whereas *S. minor*, on the other hand, produces small, sesame-sized sclerotia (Sharma et al., 2015). Moreover, compared to *S. minor*, *S. sclerotiorum and S. trifoliorum* produce fewer sclerotia (Morrall et al., 1972).

Morphological differentiation of *S. sclerotiorum* and *S. trifoliorum* relies on the size of ascospores within the ascus. *S. trifoliorum* shows dimorphism in ascospore size (two different-sized ascospores within a single ascus), whereas the ascospores of *S. sclerotiorum* and *S. minor* are monomorphic (Kohn, 1979; Uhm & Fujii, 1983).

The Mycelial Compatibility Test is a technique used to evaluate the genetic relationship of fungal isolates by examining the behaviour of their mycelia upon coming into contact with each other (Aldrich-Wolfe et al., 2015). Incompatibility occurs between mycelia when there is a clean space that has not been colonized between them. In between these two extremes, there are numerous other types of intermediate responses that can occasionally be observed macroscopically, but very often, microscopic studies are required to determine the interaction between the hyphae of various mycelial types. Wong and Willetts (1975) investigated the effects of mycelial interactions between *S. minor*, *S. trifoliorum*, and *S. sclerotiorum*. Based on these findings, it was proposed that *S. minor*, *S. trifoliorum*, and *S. sclerotiorum* be classified as three distinct species. *S. trifoliorum* was first distinguished from the other two by incompatibility line (Loveless, 1951). According to Tariq et al. (1985), the mentioned classification of mycelial interactions is in agreement with their findings. However, they asserted that the only incompatible interaction was a reaction which resulted in a visible zone of separation between the mycelia of different species. The authors additionally verified that mycelial interactions can serve as a means of distinguishing the three species, although only when combined with some other criteria.

Molecular identification

For fast, reliable and accurate identification of these species, the use of molecular methods, primarily the PCR technique, is necessary. Different genes and regions are used for *Sclerotinia* species identification (Table 1). Analyses based on 18S rDNA or the ITS region, the common methods for fungi identification, have revealed that species belonging to the family Sclerotiniaceae have almost identical sequences, so that the methods are not reliable for species identification (Freeman et al., 2002).

For identification of *Sclerotinia* spp., genes for β-tubulin, calmodulin, aspartyl protease, and glyceraldehyde 3-phosphate dehydrogenase are used in standard PCR protocols (White et al., 1990; Njambere et al., 2010; Powers et al., 2001; Cho et al., 2013; Staats et al., 2005).

Based on the sequence variation of laccase 2 (*Lcc2*), aspartyl protease (*Aspr*) and calmodulin (*Cad*) genes, Abd-Elmagid et al. (2013) developed specific primers to rapidly identify *Sclerotinia* species. A multiplex PCR assay developed for specific detection of *S. sclerotiorum*, *S. minor*, *S. trifoliorum*, and *S. homoeocarpa* is a useful, fast and reliable technique that ensures precise identification of isolates in only one reaction (Abd-Elmagid et al., 2013).

Table 1. Targets, primer codes, primer sequence, expected size of polymerase chain reaction products for *Sclerotinia* spp. identification

Target region	Primer code	Primer sequence (5' - 3')	Expected size (bp)
ITS[a]	ITS1/ITS4	TCCGTAGGTGAACCTGCGG TCCTCCGCTTATTGATATGC	540 *S. sclerotiorum* *S. trifoliorum* *S. minor*
ITS[a]	ITS5/ITS4	GGAAGTAAAAGTCGTAACAAGG TCCTCCGCTTATTGATATGC	560 *S. sclerotiorum* *S. minor* 1000 *S. trifoliorum*
18 S[a]	NS5/NS6	AACTTAAAGGAATTGACGGAAG GCATCACAGACCTGTTATTGCCTC	250 *S. sclerotiorum* 600 *S. trifoliorum*
18 S[b]	NS1/NS8	GTAGTCATATGCTTGTCTC TCCGCAGGTTCACCTACGGA	1750 *S. sclerotiorum* >1750 *S. trifoliorum*
β-tubulin[c]	TU1/TU2	CCTGAAAAGCACCCCACTAT ACGGCACGAGGAACATACTT	494 *S. sclerotiorum* *S. trifoliorum*
	TU2/TU3	ACGGCACGAGGAACATACTT AACTCAACTCGACCGATGCT	390 *S. trifoliorum*
β-tubulin[d]	Bt2a/Bt2b	GGTAACCAAATCGGTGCTGCTTTC ACCCTCAGTGTAGTGACCCTTGGC	452 *S. sclerotiorum* *S. trifoliorum* *S. minor*
Calmodulin[e]	STCadF/STCadR	TCCTAGATCGACTCT CCTCCTTT TCAAACGCCAAAGCT GTATG	97 *S. trifoliorum*
Aspartyl Protease[e]	SSasprF/ SSasprR	CATTGGAAGTCTCGTCGTCA TCAAACGCCAAAGCTGTATG	171 *S. sclerotiorum*
Laccase2[e]	SMLcc2F/SMLcc2R	CCCTCCTATCTCTCTTCCAAACA TGACCAATACCAATGAGGAGAG	264 *S. minor*
Glyceraldehyde 3-phosphate dehydrogenase[f]	G3PDHfor/G3PDHrev	ATTGACATCGTCGCTGTCAACGA ACCCCACTCGTTGTCGTACCA	985 *S. sclerotiorum* *S. trifoliorum* S. minor

[a]Njambere et al. (2008) and White et al. (1990)
[b]Powers et al. (2001) and White et al. (1990)
[c]Vleugels et al. (2012)
[d]Cho et al.(2013)
[e]Abd-Elmagid et al. (2013)
[f]Staat et al. (2005)

DISEASE MANAGEMENT

Diseases caused by *Sclerotinia* spp. are difficult to control due to the long-term survival of sclerotia in soil and development of airborne ascospores (Bolton et al., 2006). As with many other diseases, there is no single treatment that can completely control these pathogens. Growers therefore need to consider environmental variables, disease pressure, and risks when planning their management strategy. Preventing the spread of Sclerotinia diseases to other crops and regions requires early detection and diagnosis. Regular field inspections and monitoring for symptoms can aid in early detection, whereas laboratory examination may verify the pathogen's presence (Saharan & Mehta, 2008; Mihajlović, 2014; Mazumdar, 2021).

Implementation of multiple strategies, such as cultural practices (sanitation, crop rotation, and tillage), physical, chemical and biological protection, and deployment of resistant cultivars, would likely be necessary for effective disease management (Peltier et al., 2012; Mihajlović et al., 2015, 2017a).

Cultural Practices

Cultural and agronomic practices are an important factor in disease management because they reduce the amount of sclerotia in soil and mitigate disease severity. However, they are not sufficient by themselves to control the disease effectively. Cultural practices include crop rotation, plant density reduction, and practices to reduce ascospore production and release (Saharan & Mehta, 2008; et al., 2016c).

Crop rotation is not always an effective control practice against soilborne pathogens such as *Sclerotinia,* which has a wide host range and produces overwintering sclerotia that survive in soil for a long time (Rothmann & McLaren, 2018). Crop rotation with less susceptible crops will help reduce *S. minor*, but may not reduce *S. sclerotiorum* population. Small grain cereals, such as corn, wheat, barley, oats and sorghum, are not susceptible to infection with *Sclerotinia* spp. and are therefore acceptable for rotation. However, a break of two to three years may be required to decrease the amount of sclerotia in the soil (Peltier et al., 2012). Even though these small grain crops are still susceptible to *Sclerotinia* infection (Tian et al., 2020), avoiding infected or nearby fields for 1-4 years may be the best management strategy, provided that it is economically feasible. Lettuce drop, caused by *S. minor* and *S. sclerotiorum*, is an important disease of lettuce. It has already been demonstrated that rotating broccoli and lettuce reduces the amount of sclerotia in the field in the instance of *S. minor* infection of lettuce. Crop rotation with broccoli could be an effective strategy for reducing lettuce drop incidence caused by *S. minor*, particularly on farms with higher inoculum levels (Hao & Subbarao, 2005).

Resistant varieties - The use of Sclerotinia-resistant varieties may be the most effective way to reduce pesticide use. However, due to the specific nature of diseases caused by this pathogen, breeding programs have so far had limited success (Uloth et al., 2014; Lin et al., 2022). Resistant varieties are not yet available, although less severe lettuce drop may occur in varieties with upright growth, where leaves are more or less off the ground (Barbetti et al., 2014).

Sanitary measures - Sclerotia and mycelium can also be spread from infested to clean fields by contaminated agricultural equipment and footwear, contaminated and diseased seedlings and contaminated soil. Infected plants need to be found and destroyed as soon as possible prior to sclerotia development. To the greatest extent feasible, all debris and contaminated plant materials should be collected and destroyed (Saharan & Mehta, 2008).

Tillage operations have both positive and negative effects in reducing the sclerotia population in soil. While sclerotia can persist for several years in the plow layer, only those near the soil surface germinate and generate apothecia and ascospores. As a result, burying infected residues with a moldboard plow can prevent sclerotia from germinating. However, repeated plowing in another season might bring those sclerotia back to the surface, and any tillage activity can therefore contribute to sclerotia dissemination (Purdy, 1979).

Reducing fertilizers and delaying planting to control vegetative growth should also be taken into consideration, as overfertilization and early planting can produce tall, bulky plants during flowering that form a denser plant mass and increase the chance of disease incidence during periods of heavy rain (Webster et al., 2023).

Managing irrigation and moisture level might prevent soils from becoming excessively moist, and so promoting the development of *Sclerotinia* spp. The beds should be as high as possible to provide adequate drainage. Watering the soil while it is not actively in production might also help to decrease the survival of sclerotia, especially *S. minor*. Flooding soils with irrigation for 2-3 weeks during summer, drastically minimizes sclerotia viability. Nevertheless, this approach may not be relevant to all crops and producing regions (Matheron & Porchas, 2018). Keeping the optimum plant row spacing will help to produce a microclimate that will make it challenging for the fungus to survive. In addition, varietal selection is crucial to minimize excessive overlap of the leaves of nearby plants. By providing adequate ventilation, excessive moisture during vegetable cultivation will be reduced. *S. sclerotiorum* spreads primarily during the flowering or early stages of plant development, when ascospores can easily colonize the plant due to high amounts of water on leaves. White mold development is strongly induced by moisture content on leaves, and using sprinkler irrigation systems in vegetable production should therefore be minimized. Subsurface drip irrigation is less favourable for disease development than furrow irrigation, and high temperatures, soil moisture, and low oxygen levels can reduce the development of lettuce drop caused by *S. minor*. This can be done by starting an early-morning irrigation schedule that allows leaves enough time to dry during the day (Webster et al., 2023).

Weed control

Many broadleaf weeds serve as hosts for the pathogens and aid in their transmission between crops. When rotating with a non-host crop, the effect of tillage (or lack of it) on weed control is an additional consideration, as poor suppression of broadleaf weeds may lessen the benefit of crop rotation. If these weeds occur in a field, they may provide inoculum for a host crop (O'Sullivan et al., 2021).

Biological control

Environmentally friendly methods for eradicating microorganisms from soils by using bacteria, fungi or actinomycetes have become a replacement for chemical control of Sclerotinia disease (Mihajlović et al., 2012; Mihajlović et al., 2016b). Pathogen suppression biocontrol strategies include mycoparasitism, antagonism, competition for resources and space to enhance plant resistance and antibiosis (Ahemad & Kibret, 2014; Mihajlović et al., 2023b).

Bacterial biological control agents, including *Streptomyce*s spp. (Chen et al., 2016), *Bacillus* spp. (Hu et al., 2014; Mihajlović et al., 2017b; Mihajlović et al., 2023b), and *Pseudomonas* spp. (Lee et al., 2012) have been used successfully against *S. sclerotiorum*. Several species of fungi, such as *Coniothyrium minitans* or *Trichoderma* spp. are known to be antagonistic to *Sclerotinia* spp. (Vinale et al., 2008; Druzhinina et al., 2011; Hermosa et al., 2012; Mihajlović et al., 2022b). Some insects and nematodes have also been documented to have negative effects on *S. sclerotiorum* development (Coley-Smith & Cooke, 1971). According to Anas and Reeleder (1988), dark-winged fungus gnat larvae (*Bradysiu coprophila*) damage sclerotia during feeding, which affects sclerotia survival and increases their susceptibility to mycoparasitic species. Due to the action of the enzyme chitinase in salivary secretion, larval saliva inhibits the capacity of sclerotia to germinate. Moreover, *Trichoderma viride* parasitize sclerotia injured by *B. coprophila* more severely than healthy sclerotia (Anas & Reeleder, 1988).

Mycoviruses, which are viruses that infect fungi, have the potential to be used as novel biocontrol agents for fungal diseases (Zhang et al., 2022). *Sclerotinia* spp. are capable of hosting a variety of mycoviruses, including single-stranded circular DNA viruses, double-stranded RNA viruses, and single-stranded RNA viruses (Xie & Jiang, 2014). According to Yu et al. (2013), the DNA mycovirus *S. sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1) has the ability to infect and confer hypovirulence on *S. sclerotorium*.

In Serbia, only one bioproduct based on *Bacillus amyloliquefaciens* has been registered for use against *Sclerotinia* spp. in different crops (Team of Editors, 2022).

Chemical control

Fungicide application is necessary for efficient management of Sclerotinia diseases. The number of applications depends on weather conditions, the duration of crop vegetation and sensitive plant phenophases, such as flowers or petals availability for infection by ascospores (Mihajlović, 2014). More treatments are required for plants with longer flowering periods. A range of chemical substances with registration against *Sclerotinia* spp. are available in the USA, Canada, Australia, China and Europe, including: boscalid, fluazinam, fluxapyroxad, pyraclostrobin, penthiopyrad, picoxystrobin, prothioconazole, pyraziflumid and trifloxystrobin (Wang et al., 2015; Derbyshire & Denton-Giles, 2016; Kikutake et al., 2020; Team of Editors, 2022).

In Serbia, the majority of active ingredients registered for *Sclerotinia* spp. suppression are intended for application in field crops, primarily oilseed rape and sunflower. Thus, azoxystrobin is registered for sunflower and oilseed rape, while the triazole fugicides metconazole, prothioconazole and difenoconazole are permitted in oilseed rape. Boscalid, alone or in combination with pyraclostrobin, is registered for the suppression of white mold in rapeseed, sunflower, flax, as well as soya, mustard, hazelnut and walnut. To manage Sclerotinia disease in vegetable crops only a few registered products are available in Serbia, mostly for leaf crops such as lettuce, rocket, endive, and spinach (Table 2). New active ingredients from the pyrazole-4- carboxamide group, fluxapyroxad (+ difenoconazole) and penthiopyrad, are allowed for use in leaf vegetables, while fluxapyroxad (+ difenoconazole) can be used in carrots and parsley, besides leaf vegetables. Cyprodinil in combination with fludioxonil is registered for a wide range of vegetable crops, including leaf crops, melons, zucchini, squash, and beans. The only active ingredients with low resistance risk that are available for use in vegetable crops are copper oxychloride and *B. amyloliquefaciens*-based biofungicide. All other mentioned groups of fungicides have been listed by the Fungicide Resistance Action Committee (FRAC) as medium- to high-risk for resistance development, and should be used in accordance with the resistance management strategy (Team of Editors, 2022).

Table 2. List of fungicides with FRAC risk registered against Sclerotinia diseases in Serbia

Active ingredient	Chemical group	FRAC risk[1]	Crop
Azoxystrobin	Strobilurine	High	Sunflower, oilseed rape
Bacillus amyloliquefaciens	Biological control agent	Low	Lettuce, strawberries, cucumber, zucchini, melon, watermelon, beans, oilseed rape, spinach, rocket, radish, endive, common corn salad
Boscalid	Carboximide	Medium to high	Sunflower, oilseed rape
Boscalid + piracloxystrobin	Carboximide + strobilurine	High	Sunflower, oilseed rape, sunflower, soya, flax, mustard, hazelnut, walnut
Copper oxychloride	Inorganic	Low	Lettuce
Cyprodinil + fludioxonil	Anilino-pyrimidines + phenylpyrrole	Medium	Lettuce, rocket salad, endive, radicchio, common corn salad, chard, chives, leek, melon, watermelon, bean
Difenoconazole	Triazole	Medium	Oilseed rape, sugar beet
Fludioxonil	Phenylpyrroles	Low to medium	Sunflower, soya, oilseed rape
Fludioxonil + difenoconazole	Phenylpyrroles + triazole	Medium to high	Lettuce, rocket salad, endive, radicchio, common corn salad, carrot, parsley
Fludioxonil + prothioconazole	Phenylpyrroles + triazole	Medium	Soya, oilseed rape, sunflower
Fluopyram + prothioconazole	Pyridinyl-ethylbenzamides + triazole	Medium to high	Soya, oilseed rape, sunflower
Fluxapyrad + difenoconazole	Pyrazole-4- carboxamides + triazole	Medium to high	Lettuce, rocket salad, endive, radicchio, common corn salad, carrot, parsley
Metconazole	Triazole	Medium	Oilseed rape
Penthiopyrad	Pyrazole-4-carboxamides	Medium to high	Lettuce, rocket salad, spinach,
Prothioconazole	Triazole	Medium	Oilseed rape

[1]FRAC Code List ©*2022: Fungal control agents sorted by cross-resistance pattern and mode of action

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Vrste roda *Sclerotinia* u Srbiji i mogućnosti njihovog suzbijanja

REZIME

Vrste roda *Sclerotinia* su polifagne, nekrotrofne fitopatogene gljive koje izazivaju ekonomski značajne štete u poljoprivrednoj proizvodnji širom sveta. Poseduju sposobnost širenja vazdušnim putem i putem zemljišta, a mogu se prenositi i semenskim materijalom. Vrste ovog roda ugrožavaju biljnu proizvodnju tokom perioda vegetacije, ali i nakon skladištenja, a gubici koji nastaju mogu dostići i 100%. Najzastupljenija vrsta roda *Sclerotinia* u Srbiji je *Sclerotinia sclerotiorum*. Redovno se javlja na suncokretu i u pojedinim godinama može izazvati štete i preko 50%. Nedavno su u Srbiji opisane dve nove vrste ovog roda: *Sclerotinia trifolium* na lucerki i *Sclerotinia minor* na biljkama zelene salate. Suzbijanje vrsta roda *Sclerotinia* je vrlo izazovno, zbog sklerocija, tvorevina za preživljavanje, koje se mogu održati dugi niz godina u zemljištu, ali i zbog prisustva askospora u vazduhu. Kao i kod mnogih drugih prouzrokovača bolesti, ne postoji jedinstven tretman koji može u potpunosti suzbiti ili eliminisati patogene ovog roda. Implementacijom različitih strategija, kao što su primena agrotehničkih mera, hemijska i biološka zaštita, moguće je uticati na širenje bolesti uzrokovanih *Sclerotinia* spp.

Ključne reči: patogene gljive, *Sclerotinia sclerotiorum*, *S. trifoliorum*, *S. minor*, identifikacija, suzbijanje bolesti