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Review

Research Progress on Diseases Caused by the Soil-Borne Fungal Pathogen *Rhizoctonia solani* in Alfalfa

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Abstract: *Rhizoctonia solani* is a soil-borne fungal species with worldwide distribution and poses serious threats to a wide range of economically important crops such as grain and forage crops. This pathogen has survival capabilities within plants and soil, giving rise to sclerotia and persisting for several months or years. Alfalfa (*Medicago sativa*) is the most widely grown and important forage crop in China and worldwide. The unique characteristics of alfalfa, such as excellent forage quality, ruminant desirability, and substantial biomass output, distinguish it from other fodder crops, and it is also known as the "Queen of Forages". However, the production of alfalfa is seriously affected by *R. solani*, with yield losses of 20% to 60% globally. This review firstly summarizes diseases such as seedling damping-off, root rot, crown rot, root cankers, stem cankers, blight, and stem rot caused by *R. solani* in alfalfa and the survival mechanism of this pathogen. The techniques for *R. solani* detection and quantification from plants and soils, as well as management through host resistance, cultural practices, fungicides, and biological control, were then overviewed. This review provides scientific knowledge to enable researchers to efficiently manage *R. solani* in alfalfa production.

Keywords: *Medicago sativa*; forage legume; *Rhizoctonia solani*; pathogen detection; pathogen quantification; disease management

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1. Introduction

As we approach 2050, meeting the increasing demand for animal feed is expected to become challenging [1]. However, incorporating forage legume crops into feed production can significantly enhance the quality and availability of fodder [2]. Alfalfa, a perennial forage legume, is one of the most ancient crops, cultivated for over 2000 years and grown in more than 80 countries [3,4]. Currently, it is extensively cultivated and considered the primary source of commercial forage and feed in many countries [1]. Alfalfa is also known as the "Queen of Forage" because of numerous characteristics such as excellent forage quality, palatability for ruminant feed, and high biomass yield [5,6]. The deep root system of alfalfa enables it to absorb water from deep soil layers, and it can be grown in drought areas with only 200 mm of precipitation annually [7,8]. In addition, this crop provides a range of ecosystem services, including enhanced biodiversity and improved soil structure and fertility, as well as benefits such as increased infiltration and flood protection [3,9,10]. Due to all these benefits, alfalfa is being cultivated throughout the dry tropical and temperate regions of the world on approximately 32 million hectares. Its production area in China is 4 million hectares, which ranks first among cultivated grasslands in China [3,9,11]. China is currently the second-largest country for alfalfa cultivation [12]. Moreover, alfalfa plays a crucial role in improving China's agricultural economic structure by contributing to the expansion of grassland animal husbandry and the sustainability of farming ecosystems [9].

The genus *Rhizoctonia* was first introduced by de Candole (1815) while identifying an unknown fungus that attacked crops, namely alfalfa (*Medicago sativa*) and saffron

(*Crocus sativus*). However, *Rhizoctonia solani* was first described by Kühn in 1858. It is a necrotrophic/saprophytic fungal pathogen that may persist longer in soil and plant parts without a host [5,13,14]. *R. solani* is pathogenic against approximately 250 host plant species belonging to *Moraceae, Poaceae, Solanaceae, Linaceae, Brassicaceae, Fabaceae, Malvaceae, Amaranhtaceae, Rubiaceae, Asteraceae*, and *Araceae* [15]. It is a destructive fungal pathogen and has been reported to cause severe damage and significant yield losses in many economically important crops such as soybean, rice, potato, sugar beet, wheat, and chickpea [14,16,17]. *R. solani* is a species complex with several related but genetically different isolated groups and has been divided into 14 anastomosis groups (AGs) [18]. AG-2 has been reported as diverse and includes subgroups such as AG-2-1, 2-2 IIIB, 2-2 IV, 2-2 LP, 2-2 WB, 2-3, 2-4, and 2-B1 [19]. However, AG-1, AG-2, AG-3, AG-4, and AG-5 are frequently reported from legume crops [16]. The important diseases caused by *R. solani* and its subgroups include pre-emergence and post-emergence damping off, root rot, seed rot, hypocotyl rot, cankers, blights, pod rot, limb rot, black scurf, and stem rot [17].

R. solani is associated with serious diseases in alfalfa, including seed rot, seedling damping-off, stem-rot, root rot, root canker, stem canker, crown rots, and blight [20]. In alfalfa, diseases caused by *R. solani* tend to be more prevalent under elevated temperature and high moisture conditions [21,22]. Indeed, temperature is pivotal in developing *R. solani* diseases in alfalfa. The literature highlights that the optimum temperature range for these diseases is 21–30 °C [23–26]. It exhibits a destructive lifestyle that impacts various plant parts, including above-ground (pods, stems, and fruits) and below-ground (roots, hypocotyls, seeds) parts of the alfalfa plant [5,26–29]. Many studies have been conducted on different aspects of *R. solani* in other host crops under greenhouse and field conditions. However, limited studies have focused on *R. solani* in alfalfa (Figure 1), highlighting a major gap in our understanding of this pathogen's impact on alfalfa yields. Addressing this knowledge gap is essential for developing effective strategies to mitigate yield losses and safeguard alfalfa production. Therefore, this study discusses different alfalfa diseases caused by *R. solani* and their management approaches, including host resistance, cultural control, biological control, and chemical control. Further, it summarizes the survival structure of *R. solani* (sclerotia) and its detection and quantification from the rhizosphere and plant.

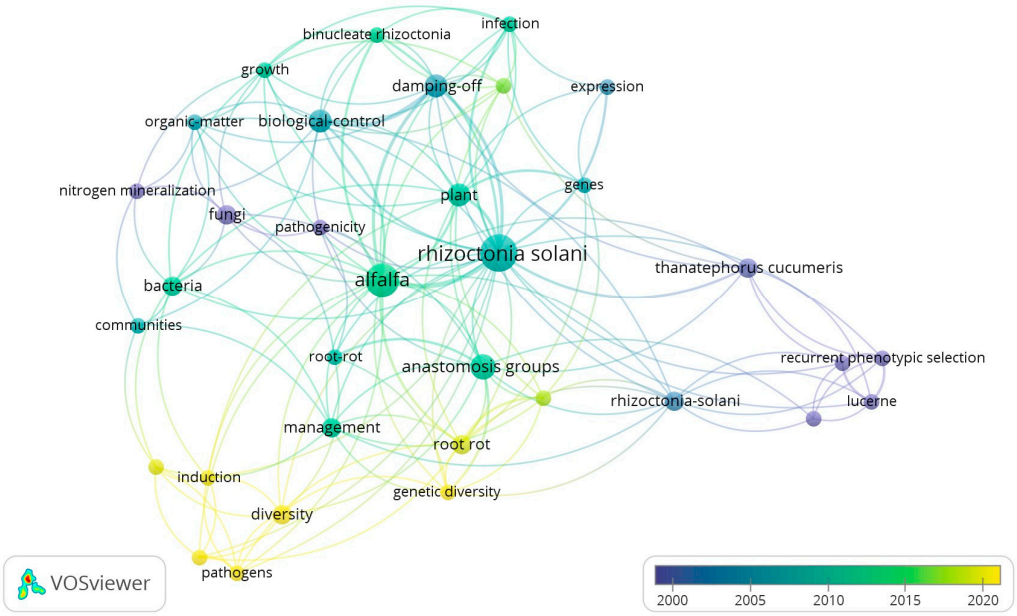


Figure 1. Keyword co-occurrence bibliometric analysis (Alfalfa and *Rhizoctonia solani*). The data were extracted from the Web of Science database and indexed until 31 December 2023. A total of 34 items were clustered into 6 clusters (shown in various colors).

2. Diseases Caused by *R. solani* and Worldwide Distribution

2.1. Damping-off

Damping-off disease caused by soil-borne fungal pathogens such as *R. solani* causes severe yield loss in alfalfa worldwide [29–33]. *R. solani* is associated with both pre- and post-emergence damping-off diseases of alfalfa; however, post-disease is common [21,34,35]. Addoh [36] described the symptoms associated with the sudden or severe damping-off of alfalfa, such as rapid wilting of plants, tissues becoming water-soaked, and plant death, although the leaves remain attached. However, symptoms in the gradual progression of the disease include brownish stems, gradual stunting of affected parts, and oval-shaped dark brown lesions appearing on the stem. These lesions merge rapidly around the stem and kill the plants. A total of 10 alfalfa genotypes were used against three types of fungus species, including *R. solani*, *Fusarium oxysporum*, and *Macrophomina*. The results showed that *R. solani* was the most pathogenic, followed by *Macrophomina phaseolina* and *F. oxysporum*. In addition, *R. solani* significantly reduced fresh and dry shoot yield compared to the other two fungus species [34]. In a study conducted in Ismailia Governorate, observations were made regarding fungal diseases affecting alfalfa crops. The collected data revealed that damping-off caused by *R. solani* was a predominant disease. The percentages of different fungus species for causing pre- and post-emergence damping-off were as follows: *R. solani* (70.0%, highest), *Sclerotium rolfsii* (31.25%), *F. solani* (28.75), and *M. phaseolina* (17.5%, lowest) [28].

2.2. Root Rot

Root rot disease accounts for 20–40% of the global annual yield loss of alfalfa [37]. Root rot caused by soil-borne fungal pathogens has been frequently reported in major alfalfa-growing regions of China such as northeast China (Jilin, Heilongjiang, and Liaoning), northwest China (Shaanxi, Ningxia Huizu, Gansu, Xinjiang, and Qinghai), and north China (Hebei, Inner Mongolia, and Shanxi). Moreover, in northwest China, alfalfa root rot is severe and has a disease incidence of 30–80% [5]. *R. solani* has been reported as one of the primary causative agents for root rot disease in alfalfa [5,6,38,39]. McKenzie and Davidson [40] surveyed 2-year-old alfalfa plants from 40 fields for root diseases. *R. solani* was identified as a causal organism of root rot. They also described the symptoms of alfalfa root rot caused by *R. solani* as brown to black lesions on root surfaces near the place of lateral root emergence from a primary root. In 1972, Fatemi first reported alfalfa root rot diseases caused by *R. solani* in the Fars province of Iran and reported the stunted growth of alfalfa plants during the early stages of root disease [41]. Different *R. solani* AGs are associated with severe root rot diseases of alfalfa in countries, such as AG-1, AG-2, AG-3, AG-4, AG-5, and AG-10 reported in Turkey [42], AG-1 and AG-4 in America [43], AG-1–AG-10 in Saudi Arabia, AG-11, AG-8, and AG-6 in Australia [44], and AG-4 in Iran [45].

2.3. Root Canker

Root canker in alfalfa caused severe losses in southwestern Queensland, Australia, and the Imperial and Palo Verdi Valleys of California [46,47]. Moreover, *R. solani* AG-6 was reported to cause root canker in all Australian commercial cultivars of alfalfa [48]. In another study, fourteen isolates of *R. solani* showing black canker symptoms were collected from alfalfa roots. It was concluded from the results that disease-causing strains were related to *R. solani* AG-4 [49]. Smith [23] identified the root canker disease of alfalfa caused by *R. solani* in the USA for the first time. The root canker of alfalfa is characterized by elliptical, sunken, and necrotic canker development at the junction of taproots and lateral roots. Hence, plant roots are girdled by these cankers, and plants can die from this disease [47]. Samac et al. [26] indicated that plant death occurs upon the complete invasion of roots by lesions; otherwise, roots can emerge again during fall and support the plant for the following year. Moreover, lesions usually heal in the winter season and turn blackish. The occurrence of root canker in *Rhizoctonia* is seasonal and strongly influenced by elevated temperatures. The disease's typical lesions mostly appeared in June, July, August, and

September, when temperatures in the soil varied between 21 to 35 °C. In winter, when soil temperatures were between 5 to 10 °C, diseases did not appear [23].

2.4. Crown Rot

The causal organisms of alfalfa crown rot are complex soil-borne fungi and can vary according to geographic location. *R. solani* is the frequently reported causal organism of crown rot disease in alfalfa [50]. *R. solani* enters the crown through cut stems and mechanical damage to the crown and root during multiple foliage cuttings [51]. Graham et al. [52] reported that *R. solani* crown rot is more severe in alfalfa after the second year of growth. In northern Mexico, plants showing crown rot symptoms were collected from 30 fields of alfalfa crops. The results identified nematodes, fungi, and oomycetes as causal organisms, and *R. solani* was the primary fungus causing crown rot in alfalfa [53]. Another study by Samac et al. [50] revealed that alfalfa plants affected by crown rot become stunted, wilt, and finally die. *R. solani* damages new shoots, and plants become weaker and more susceptible to attack by saprophytic fungi or worthless soil parasites [54].

2.5. Crown Bud Rot

R. solani has been associated with crown bud rot in forage legumes, including alfalfa [27]. Hawn and Cormack [55] found that *R. solani*, *Fusarium acuminatum*, and *Fusarium avenaceum* are involved in the alfalfa crown bud rot disease complex; however, *R. solani* was highly pathogenic. In addition, Hwang et al. [56] reported that plant age is a critical factor for this disease. Alfalfa crown bud rot resulted in dark brown to blackish lesions in tissues [52]. *R. solani* damages crown buds, causing bud killing and moving to the crown, stopping the new buds' vegetative production. It was also reported that *R. solani* ramified in alfalfa crown buds inter- and intracellularly [57]. McDonald [58] revealed that *R. solani* is a vital pathogen that produces crown bud rot problems in alfalfa during summer. Moreover, crown bud rot caused by *R. solani* is severe during the first month of growth, and plants that are 2 or 3 years old are more susceptible.

2.6. Stem Canker

Stem canker in alfalfa, caused by specific strains of *R. solani*, is a notable plant disease. Houston [59] showed that stem canker predominantly affects 1–2-year-old alfalfa fields, with higher incidence rates observed during spring, early summer, and fall. Stem canker symptoms include brown-edged, tan cankers that occasionally produce circular shapes resembling growth rings. Brown mycelial strands are usually visible on the canker's outer layer upon enlargement. Cankers girdle stems, which eventually die and resemble anthracnose-killed stems. The lack of setae in the acervuli of *Rhizoctonia* stem canker growths separates them from anthracnose stem cankers [26]. *R. solani* affects alfalfa stem tissues below the soil surface, with cankers extending up to 2.54 cm above the soil line post-infection [59].

2.7. Blights

R. solani-induced blight diseases in alfalfa, extensively documented in the literature [17,27,43,52,60], are particularly prevalent in the southeastern United States [56]. This pathogen persistently targets alfalfa foliage and stems, resulting in rapid tissue collapse and the formation of water-soaked masses, often mistaken for scalding water damage. Diseased plants can rapidly infect adjacent healthy plants. Large and irregularly shaped lesions are observed on less damaged stems and leaves [52,56]. Table 1 summarizes the disease severity induced by *R. solani* and associated yield reduction (%) in alfalfa, as documented in previous studies.

Table 1. Diseases caused by *Rhizoctonia solani* in alfalfa reported worldwide and yield losses.

Disease Name	Country/Region	Disease Everity (%)	Yield Losses (%)	References
Damping-off		95.0	90.0	[61]
	USA	62.2	—	[27]
	Turkey	87.2	—	[62]
	Canada	26.1	—	[28]
	Riyadh	—	21.1	[34]
	Egypt	70.0	—	[28]
		85.0	—	[29]
Root rot	Canada	68.0	—	[40]
		97.0	85.1	[62]
	Ryadh	19.7	—	[28]
	Al-Qasim	17.0	51.5	[63]
	China	≥60.0	≥60.0	[6]
		68.3	56.7	[5]
Seed rot	USA	—	—	[64]
	Riyadh	31.6	—	[65]
	Egypt	27.0	—	[28]
Crown rot	Mexico	82.5	24.3	[66]
Crown bud rot	Canada	80.6	—	[67]
Stem canker	USA	—	—	[43]
Blights	China	≥60.0	≥60.0	[6]
		80.0	55.5	[5]

2.8. Global Distribution

R. solani has affected alfalfa crops across the globe. Alfalfa diseases caused by *R. solani* have been reported in many countries, including China, Saudi Arabia, Japan, Egypt, Turkey, and Italy. Moreover, in some countries, significant yield losses have been reported by *R. solani* diseases. For instance, alfalfa root rot and damping-off are the most prevalent in Egypt, root rot is the most common in China, and damping-off is the most prevalent in KSA (Saudi Arabia). Yield losses because of these diseases are reported to be up to 60%, 20–40%, and 26.45% in Egypt [28], China [68], and KSA [69], respectively (Figure 2).

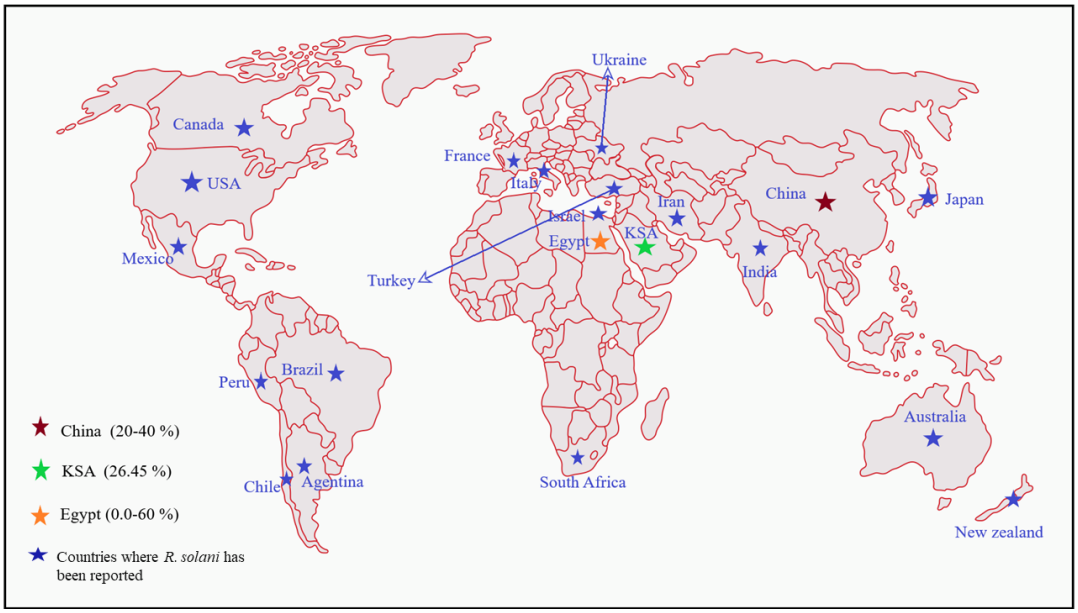


Figure 2. Countries where the alfalfa crop has been affected by *Rhizoctonia solani* and major yield losses.

3. Survival Structure of *R. solani*

R. solani develops sclerotia to survive in soil and plant debris. Sclerotia, known as "nutrient-independent propagules", persist in soil and crop residues [70]. Sclerotia formation is initiated by undifferentiated hyphae or monilioid cells. These small and ovular cells make chains or clusters. Monilioid cells are hyaline or brown with 30–35 µm and 20–22 µm average length and width, respectively. The chain formation of monilioid cells has been observed on the substrate surfaces and host tissues [71]. Upon germination, sclerotia form mycelia and increase the pathogen's inoculum density, resulting in disease spread. In most cases, *R. solani* infection occurs with sclerotia germination to form mycelia that grow toward the host plant [17]. Sclerotia have excellent resistance against unfavorable conditions such as extreme cold or heat and specific chemicals [70]. Sclerotia have three developmental stages: initiation, development, and maturation. Moreover, sclerotia change color (typically light and deep brown) during each developmental stage. Distinct color formation in sclerotia is due to the chemical composition of sclerotia exudates [72]. Aeration and humidity levels are essential factors for sclerotia formation. Light significantly influences sclerotia morphology and formation, and a few isolates of *R. solani* could not produce sclerotia in dark conditions [73]. Sclerotia can be distinguished into four types: (i) loose, (ii) terminal, (iii) lateral-chained, and (iv) lateral-simple. However, the "loose" type is the most abundant form of *R. solani* sclerotia [70].

Different studies have reported different periods of *R. solani* survival, as described below. Pots containing infested soil with *R. solani* isolates were buried in a field for 40 weeks. Results showed that isolates of AG-1 were recoverable after 86, 211, and 283 days. Isolates of AG-2-1 and AG-2-2 were recovered after 283 days [74]. Similarly, a recent study has reported that *R. solani* can survive in soil or plant debris as sclerotia for many years [75]. In another study, *R. solani* sclerotia were buried at a 5 cm depth in field soil and found to be viable after two years of burial with 25–50% germination [76]. The sclerotia of other *Rhizoctonia* species have different survival capabilities than *R. solani*. For instance, sclerotia produced by *Rhizoctonia cerealis* can survive for six months [77]. Sclerotia produced by *Rhizoctonia tuliparum* survived for ten years; however, the germination percentage after retrieval was <10% [78]. Environmental factors such as water potential, pH, temperature, and fungus nutrition significantly impact the growth and disease development of *R. solani* [79].

Proteins related to cell defense, amino acid metabolism, genetic information processing, and carbohydrate metabolism may play a role in sclerotia formation and maturation [80]. Regardless of earlier genomic and metabolomics research, the mechanisms regulating *R. solani*'s transformation from mycelia to sclerotia remain unexplained. The shift from mycelia to monilioid cells and the subsequent development of sclerotia may be associated with several signals, including oxidative stress, nutritional shortage, and other factors that have not yet been studied, such as QS mechanisms [81]. Previous research investigating the survival rates of *R. solani* sclerotia in alfalfa fields is lacking. As discussed earlier, sclerotia have a crucial role in *R. solani* disease dissemination; future studies should explore the formation process of sclerotia in alfalfa fields affected by different *R. solani* diseases. These investigations should focus on unraveling the longevity of sclerotia and understanding how the age of sclerotia influences the severity of alfalfa diseases. Additionally, it is essential to assess the impact of environmental factors on sclerotia survival rates (Figure 3).

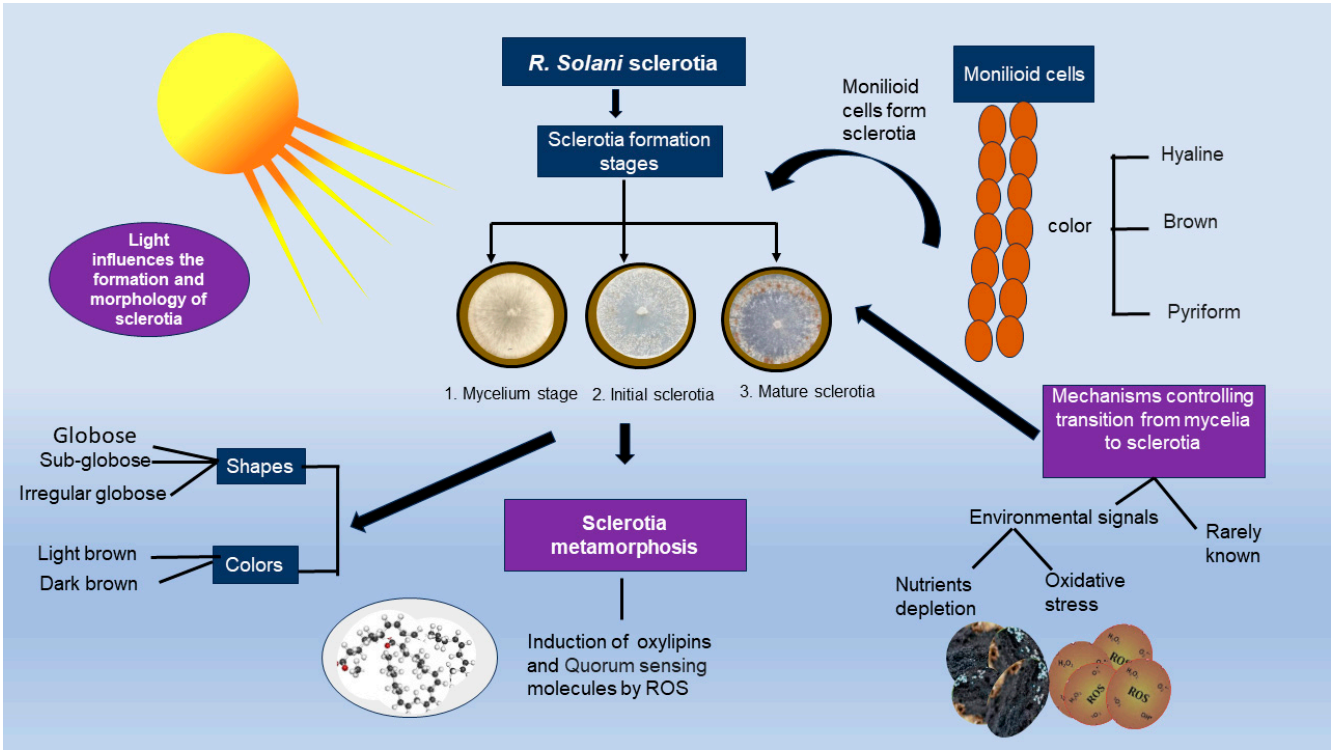


Figure 3. The structure and formation of *Rhizoctonia solani* sclerotia.

Disease Cycle of R. solani in Alfalfa

Under optimal conditions, such as warm temperatures of around 30 °C and high humidity, dormant sclerotia of *R. solani* become activated, initiating mycelium growth. This mycelium forms infection cushions, facilitating the penetration of alfalfa taproots through wounds created by young lateral roots. Mycelium then progresses into the cortex region, which induces decay, leading to the sloughing off of cortex tissues [82]. As the infection advances, visible symptoms begin to appear in the alfalfa plants, both above and below ground. Above-ground symptoms may include wilting, stunting, and yellowing of foliage, while below-ground symptoms involve root decay and discoloration. In severe cases, the extent of fungal attack can lead to the death of the alfalfa plants. Following plant death, *R. solani* can produce new sclerotia on infected alfalfa plant debris, completing the disease cycle. The sclerotia can persist in the soil, serving as a source of inoculum for future infections in subsequent growing seasons (Figure 4).

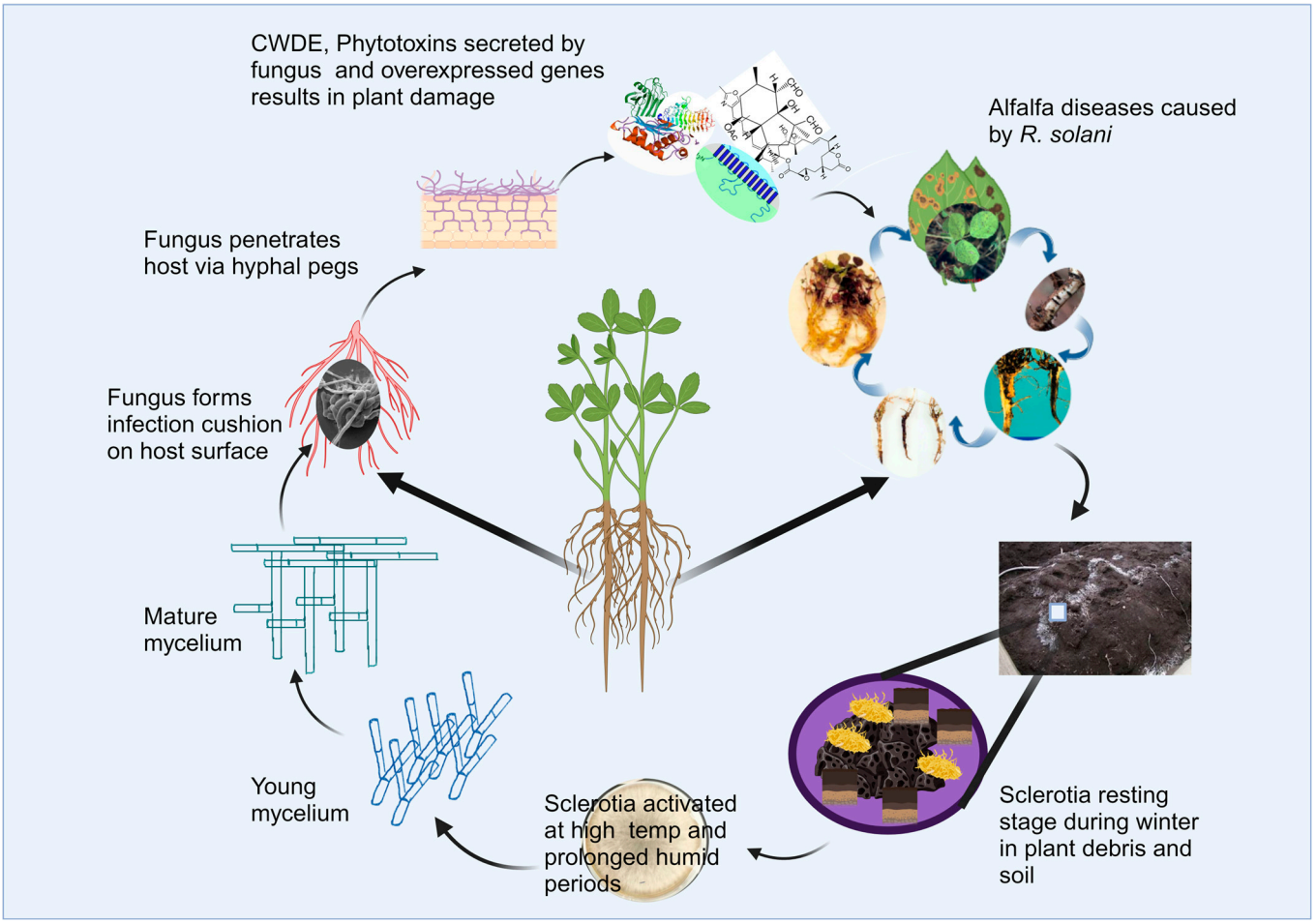


Figure 4. Disease cycle of *Rhizoctonia solani* in alfalfa.

4. Genetic Diversity and Pathogenic Mechanism of *R. solani*

4.1. Genetic Diversity

The genetic variability among populations of *R. solani* is an essential aspect that enhances its potential to cause disease and adapt to various environmental stress conditions and hosts. Researchers have examined the genetic makeup of *R. solani* populations using multiple molecular markers, including randomly amplified polymorphic DNA (RAPD) markers, restriction fragment length polymorphisms, analysis of sequence variations in ribosomal DNA (rDNA), β-tubulin genes, inter-simple sequence repeats, amplified fragment length polymorphisms, single-nucleotide polymorphisms, and simple sequence repeats [83–91]. Significant pathogenicity and variations in genetic composition have been seen in *R. solani* isolates collected worldwide [92]. Taheri et al. [87] used amplified fragment length polymorphism markers to categorize 150 Indian isolates of *R. solani* into 33 groups, with a similarity of 80%. Ali et al. [93] classified 29 isolates from Bangladesh into two separate clusters, while Moni et al. [94] grouped 18 isolates into four clusters. However, there was no substantial correlation between the difference in pathogenicity and the genetic groupings found using random amplified polymorphic DNA (RAPD) markers [95]. The internal transcribed spacer (ITS) regions of rDNA have also been demonstrated to be effective for identifying the genetic diversity of AG-1-IA isolates from soybean, AG-1-IB from lettuce, AG-2-1 from cauliflower, and AG-3 from potato [96].

The genome size of *R. solani* was estimated to range from 36.9 to 42.5 Mb, with 11 chromosomes varying in size from 0.6 to 6 Mb [97]. Later research included the release of a draft genome sequence for the *R. solani* AG-1-IA strain, which was determined to be 36.94 Mb using advanced next-generation sequencing technologies [98]. Further studies

led to the generation of another draft genome sequence for the *R. solani* AG-1-IA strain, 1802/KB, which was isolated from a widely cultivated rice variety in Malaysia and had a genome size of 28.92 Mb [99]. A detailed study conducted on 38 *R. solani* isolates from sugar beet and dry bean fields in western Nebraska showed greater morpho-genetic variation. The present study discovered no connection between the isolates of *R. solani* and their geographical location. It indicates that variables other than geographic distribution have a role in the genetic variation of *R. solani* in host crops [100]. The isolates from red cabbage were distinguished based on their anastomosis groups by utilizing the retrotransposon-based iPBS (inter priming binding site) amplification DNA profiling method. Pathogenicity tests demonstrated significant variation in disease severity index among the isolates [101]. Another study conducted on 175 isolates of AG-1-IA, which causes rice sheath blight in China, reported a higher degree of haplotype diversity and nucleotide diversity within AG-1-IA. The majority (97.8%) of genetic diversity was found within isolates of populations, while only a tiny portion (2.2%) of the genetic diversity was due to differences between populations. Pathogenicity experiments showed that all isolates caused disease; however, considerable differences were observed in the aggressiveness [96]. Research conducted on different crops worldwide has shown the complex genetics of *R. solani*. Therefore, understanding the genetic diversity of *R. solani* populations associated with alfalfa is crucial to facilitate breeding programs for successful disease management.

4.2. Pathogenic Mechanism of R. solani

Bioactive compounds such as toxins, enzymes, and secreted proteins play a major part in the infection process of this pathogen [102]. Notably, toxic substances such as succinic acid, PAA (phenylacetic acid), and furancarboxylic acid have been isolated from *R. solani* [103]. Substances such as PAA and 3-Methylthiopropionic Acid (MTPA) produced by *R. solani* AG-3 PT induce cellular destruction, including the breakdown of membranes, the alteration of chloroplast shape, and the swelling of the endoplasmic reticulum [102,104]. In addition, secondary metabolite formation enzymes, such as non-ribosomal peptide synthases (NRPSs), polyketide synthases (PKSs), hybrid NRPS-PKS enzymes, prenyltransferases (DMATSs), and terpene cyclases (TCs), enhance the fungus' ability to cause disease [105]. *R. solani* synthesizes a diverse range of carbohydrate-active enzymes (CAZymes) that degrade the cell walls of plants and exhibit considerable activity during the progression of diseases [106]. *R. solani* AG-1-IA produces 223 carbohydrate-active enzymes (CAZymes), along with an array of cell wall degrading enzymes (CWDEs) such as pectinase, xylanase, and laccase. These enzymes are linked to the pathogenic and saprophytic characteristics of the fungus [98]. In addition, numerous disease-causing fungi secrete proteins that have various functions in triggering disease [107]. These proteins, commonly referred to as effectors, have the dual function of promoting infection and inhibiting the defense mechanisms of the host [108]. The ratio of these effectors differs among fungi, and *R. solani* AG-1-IA was reported to produce 965 secretory proteins, many of which are still poorly characterized regarding their roles [98]. The effector AGLIP1, present in *R. solani* AG-1-IA, has been observed to disrupt plants' defense mechanisms and facilitate disease progression [109]. The primary processes by which *R. solani* causes disease in alfalfa plants are not well known, particularly in terms of whether specific toxins or enzymes cause the damage. Future studies on the pathogenic mechanism at the molecular level would address a knowledge gap and contribute to the development of appropriate disease management strategies.

5. Detection and Quantification of *R. solani*

Several methods have been used to quantify *R. solani* populations. These methods include baiting, screening, plating soil, and direct observation [110,111]. In the baiting method, seeds, plants, and toothpicks are used for fungal colonization. The toothpick-baiting method is widely acknowledged because it is cost-effective and rapid [111]. Moreover, other methods, including dry sieving, elutriation, and wet sieving, have been used to isolate *R. solani* from concentrated organic fractions of the soil [112]. *R. solani* produces

several diseases in different alfalfa-growing countries. However, we found a significant research gap in the detection and quantification of *R. solani* from alfalfa. AGs of *R. solani* have distinct host ranges and different virulence capabilities. The ITS (internal transcribed spacer region) is a crucial barcode for identifying each *R. solani* AG. We encourage researchers to isolate *R. solani* from alfalfa-infected plants and soil, confirm pathogenicity using Koch's Postulates, and identify specific AGs responsible for disease using PCR techniques. We have discussed PCR, qPCR, and selective mediums for detecting *R. solani* from other hosts to provide valuable insights for future studies on alfalfa.

5.1. Selective Mediums

Due to its low population levels, *R. solani* is often hard to isolate from soil with the dilution plating method [111]. Recovery of *R. solani* is also limited to symptomatic plant parts such as roots [112]. A selective medium for isolating *R. solani* is crucial for research and diagnostic purposes [113]. Some studies have developed effective selective media for this purpose. For example, a study found that a selective medium consisting of Malt Extract Agar (MA) supplemented with gallic acid (400 μg mL^{-1}) and fosetyl-Al (250 μg mL^{-1}) successfully isolated *R. solani* from soil samples, even when competing fungi such as *Macrophomina phaseolina* and other soil-borne fungal species were present [114]. Another agar medium containing Dexon, chloramphenicol, streptomycin, gallic acid, and sodium nitrate recovered 90 to 100% of *R. solani* from the soil and suppressed the growth of other microbes [115]. Recently, six different growth media, including cornmeal agar (CMA), 10% potato dextrose agar (PDA), 50% PDA, water agar (WA), amended clarified V8 (ACV8), and methylene-benomyl-vancomycin (MBV), were evaluated for promoting *R. solani* growth. However, V8 (ACV8) was the most effective medium for culturing *R. solani* [116]. In conclusion, it is crucial to prioritize research aimed at refining selective media formulations for improved detection and isolation of *R. solani* from host plant alfalfa.

5.2. Conventional PCR

R. solani is a destructive soil-borne pathogen with a vast host range and AG groups. There are a lot of similarities among different AG groups of *R. solani*, making its detection by morphology and isolation challenging [117]. However, molecular techniques such as PCR have been successfully used for *R. solani* detection and quantification [118]. Protocols are available for detecting specific AGs, including AG-1, AG-2, and AG-3, providing insights into the losses caused by each AG and management options for respective diseases [119]. This method involves the amplification of 5.8S ribosomal DNA and part of the ITS regions using the designed primers in combination with the general fungal primers ITS1F and ITS4B [120]. This method has been used to detect *R. solani* in various crops, including legumes such as common beans, cowpeas, and chickpeas. A study used and validated a conventional PCR-based assay to detect *R. solani* AG-1-IA from rice plants. Four sets of reverse and forward primers were used, and the specificity of the primer sets was checked using the primer BLAST tool. Conventional PCR was compared with a LAMP-based assay to develop a simple strategy for on-field pathogen detection. Results showed that traditional PCR was highly sensitive. However, instrumentation and lack of visual confirmation make conventional PCR unsuitable for on-field pathogen detection [121]. To find a reliable detection method for *R. solani* AG-3 from the soil, PCR assays were combined with the baiting method given by Thornton. The conventional primer set (Rs1F2 and Rs2R1) used in the study was designed from the ITS1 and ITS2 regions of *R. solani*. The conventional PCR sensitivity was proved suitable for detecting *R. solani* AG-3 from tuber tissues and plants. The study's findings also reported that conventional PCR sensitivity could be improved using a second-round nested PCR approach [122]. In another recent work, conventional PCR assays were developed to detect and quantify *R. solani* responsible for wet rot in pulse crops [123].

5.3. Real-Time Quantitative PCR (qPCR)

qPCR is a sensitive and rapid method with several advantages over traditional diagnostic methods, including being more time-efficient. Unlike conventional PCR, this advanced method can monitor the amplification of DNA molecules during PCR in real-time [124]. Moreover, qPCR has been reported to detect and quantify pathogens isolates from soil and plant parts in small quantities [125]. Fluorescent dyes such as SYBR Green I or sequence-specific DNA probes such as the TaqMan probe monitor reactions throughout the amplification steps [126]. SYBR Green dye is a cheaper monitoring agent; however, its drawback is that it is non-specific. Therefore, during the PCR cycles, it can bind with any DNA, leading to false positive results in pathogen quantification [127]. TaqMan is the most widely used probe out of all probe-based detection methods. Unlike SYBR Green dyes, probes have high specificity, making them more popular for qPCR methods [128].

qPCR has been implemented to detect and quantify many fungal species, such as *R. solani* [129,130]. For example, conventional and real-time PCR assays were designed to quantify and diagnose *R. solani* in pulses. Five *R. solani*-specific primers (ARSF1&R1, ARSF2&R2, ARSF3&R3, ARSF4&R4, and ARSF5&R5) were designed based on the ITS sequences of *R. solani* isolates from pulse crops. Results revealed that Rt-PCR was specific and sensitive for rapidly detecting and quantifying pathogens in mung beans. Moreover, Rt-PCR detected low levels of genomic DNA from infected plants and did not amplify other soil-borne pathogens [131]. Rt-qPCR assays were developed to isolate the *R. solani* AGs in soil collected from the *Brassica oleracea* field. PCR assays were designed by targeting the β-tubulin and ITS regions for different AGs, including AG-1-IA, AG-1-IC, AG-2-1, AG-2-2, AG-4HGI+II, AG-4HGIII, AG-8, AG-3, AG-4HGII, AG-5, and AG-9. Results reported all assays as target group-specific except AG-2-2 [132]. Rt-PCR has also been reported to increase the possibility of a false negative result when detecting pathogens (especially when examining emerging or extremely variable pathogens) [133]. Despite the successful application of qPCR in diagnosing *R. solani* across various economically important crops (Table 2), a notable gap exists regarding its implementation for detecting and quantifying *R. solani* in alfalfa. We propose the utilization of qPCR assays to diagnose *R. solani* in alfalfa plant tissues and field soil to enhance the management of this pathogen.

Although DNA-based methods enable quick pathogen detection, they have some limitations. It has been reported that PCR methods, including conventional and real-time qPCR methods, cannot differentiate between living and dead material. These assays work based on the detection of nucleic acids rather than living cells [134]. Hence, pathogen detection based on DNA can give false results because DNA can exist for a long time, even after the death of the fungus propagules [135]. Therefore, PCR amplification can overestimate the actual number of viable cells. However, consideration of some other practices can help to overcome these issues. For instance, for the detection of fungal species, the use of non-membrane-permeating dyes such as Propidium monoazide (PMA) with PCR-based tools has been reported to reduce the overestimation of cell counts due to the DNA of dead cells [136]. A study reported that including the baiting step before PCR proved helpful for the sensitive and specific detection of living propagules of *R. solani* AG-3 [137]. Other approaches to minimize the detection of dead cells in pathogen detection include the combination of qPCR with cultures (nutritive media) and using mRNA to indicate viable cells [134].

Table 2. qPCR assays used for *Rhizoctonia solani* detection and quantification in different crops.

Crop	AG	Primer and Sequence	Targeted Region	Annealing T (°C)	Purpose	Ref.
Wheat and barley	AG-2-1 and AG-8	Rs2.1/8F(GTTGTAGCTGGCCCATTCATTTG) Rs2.1/8R (AGCAGGTGTGAAGCTGCAAAAG)	ITS1 and ITS2	63	Detection and quantification of *Rhizoctonia* species from plant and soil	[138]
	AG-8	Rs8F(GGGGGAATTTATTCATTTATTGGAC) Rs8R (GGTGTGAAGCTGCAAAAG)		58		
	AG-10	Rs10F (GTAGCTGGCCTCTTAATTTG) Rs10R (CAAGTGTGAACCTGCAAGAC)		60		
Rice	AG-1-IA	Rs1F(GCCTTTTCTACCTTAATTTGGCAG) Rs2R(GTGTGTAAATTAAGTAGACAGCAAATG)	ITS	60	Detection and quantification of *R. solani* AG-1 IA from plant	[139]
Mung bean	AG-1, AG-2-2, AG-2-2LP, AG-2-3, AG-3, AG-4, AG-5	ARS (F1: GAGTTGTTGCTGGCCTTTTC) ARS (R1: TTTTTACGGGTGTCCTCAGC)	ITS	71	Detection and quantification of *R. solani* from plant	[131]
		ARS (F4: CAACGGATCTCTTGGCTCTC) ARS (R4: GGTGTCCTCGGCGATAGATA		67		
		ARS (F5: ACTAAGTTTCAACAACGGAT) ARS (R5: TTACTTTGAAGATTTCATGA)		52		
Soy bean	unknown	Rso1: RsolF-(GTGAACCAAATCAGACAGA) Rso1R-(CTACTCTACTGCTTACAG)	IGS	45.2	Detection and quantification of *R. solani* from soil and plant	[140]
Tomato	unknown	ST-RS1-F: (AGTGTTATGCTTGGTTCCACT) ST-RS1-R: (TCCTCCGCTTATTGATATGC)	ITS2	59.5	Detection and quantification of *R. solani* from soil	[141]
Potato	AG-1-IA	AG-1-1A_F(TTGTTGCTGGCCTTTTCTACCT) AG-1-A_R (ATGGAATTAAATCCACCAACTATTGC)	ITS1	50	Detection of pathogenic AGs and spatial distribution of *R. solani* in fields	[132]
	AG-3	AG-3_F (TCTACAGGGATTCCAGATTACGC) AG-3_R (TCACGGATCTTGGAAATCAACA)	β-tubulin			
Lettuce	AG-1-IB	AG1-IB-F3(TGGCCTTTTAACATTGGCATGT) AG1-IB-R(CCAACCCCAAAGGACCTTGA)	ITS	62	Detection and quantification of *R. solani* and *R. solani* AG1-IB from soil and plant	[142]
Sugar beet	AG-2-2 IIIB	AG22sp2-F(TAGCTGGATCCATTAGTTTG) 5.8SKhot-R(GTTCAAAGATCGATGATTCAC)	ITS	55	Detection and quantification of *R. solani* AG2-2IIIB from soil and plant	[143]
Tobacco	AG-3	RsTqF1(AGAGTTTGGTTGTAGCTGGTCTATTT) RsTqR4(AGACAGAAGGGTTCAATGACTTATTATA)	ITS	60	Detection and quantification of *R. solani* AG-3 from plant	[144]

6. Management Approaches

6.1. Host Resistance

Cultivating varieties with disease resistance is the most sustainable and effective way to control alfalfa diseases [145]. Zhang et al. [6] investigated the resistance potential of 68 alfalfa varieties with different worldwide geographic origins against *R. solani* infection. Only three varieties, including Gannong 9, Trifecta, and Common, displayed a high level of resistance, with the disease indices of shoots and roots, as well as reductions in the dry weight of shoots and roots, all being less than 40%. Some resistant varieties cannot perform well in soils with more than one soil-borne pathogen [14,146]. For example, a recent study revealed that the co-infection of *R. solani* and *F. oxysporum* is more destructive to plant growth than a single infection. Moreover, the varieties that are resistant to a single pathogen become susceptible to co-infection, emphasizing the importance of developing alfalfa varieties with resistance against multiple soil-borne pathogens [5]. Three varieties,

namely Siwa, Salt America, and New Salt, have been reported as highly resistant to damping-off disease caused by *R. solani* [34]. Alfalfa genotypes have been checked for resistance against damping-off caused by *R. solani*. Saponin treatment significantly reduced the mycelial growth of the pathogen. Roots of all genotypes had different saponin and lignin contents. Moreover, it was concluded that breeding for saponin concentration in the alfalfa crop could increase resistance against *R. solani* diseases such as root rot and damping-off [29]. Several molecular techniques, including CRISPR technology, pyramiding major R genes/QTLs, and host-induced gene silencing, have been used to increase plant resistance to many fungal diseases [147–150]. Bulked Segregation Analysis (BSA) and Random Amplified Polymorphism (RAPD) were used to examine molecular markers associated with genes that confer resistance toward alfalfa brown spots [151]. As reported from other host–pathogen interactions, implementing the above modern-day technologies might reduce *R. solani* diseases in alfalfa.

Although *R. solani* caused significant damage to crop plants, little is known about the host defense responses upon fungus attack. Due to increasing economic losses of legume crops such as alfalfa, chickpeas, and soybeans caused by soil-borne diseases, *Medicago truncatula*, a model legume plant, has been widely studied for its interactions with legume-infecting strains of *R. solani* [152]. *M. truncatula* provides a wide range of genetic and genomic resources, such as a sequenced genome, transformation methods, recombinant inbred lines, and reverse genetics populations, making it an ideal host for studying plant–microbe interactions [153]. It has been reported that different regions of the *M. truncatula* genome are responsible for its resistance or susceptibility against *R. solani* strains [152]. Multiple germplasm lines of *M. truncatula* were screened against *R. solani* isolates collected from legume fields. The results declared greater differences in susceptibility and resistance, with a majority of genotypes exhibiting susceptibility to *R. solani*. A17, a reference genotype of *M. truncatula,* exhibited moderate resistance against the root canker-causing isolate AG6; however, recombinant inbred line population analysis found a single locus producing resistance to *R. solani* [44]. A study reported that overexpression of IX *ERF* genes in the roots of a model legume increased resistance against *R. solani* [154]. Ethylene signaling was a key factor contributing to *M. truncatula* resistance to *R. solani* isolates, including AG-8 and legume-specific AG-11 [155]. RNA sequencing of moderately resistant (A17) and highly susceptible (*skl)* genotypes of a model legume was carried out to unravel the ethylene mechanisms for mediating resistance against *R. solani* AG-8. Several transcriptional changes were observed in A17, such as ethylene signaling, ROS metabolism, and isoflavonoid biosynthesis. Mass spectrometry detected increased levels of isoflavonoid-related compounds such as liquiritigenin, formononetin, medicarpin, and biochanin A in A17. Overexpressing an isoflavone synthase in *M. truncatula* roots led to higher isoflavonoid accumulation and resistance against *R. solani*. Additionally, adding exogenous medicarpin indicated that this phytoalexin might be essential among various isoflavonoids for resistance to *R. solani* [152]. Given the genomic similarities between alfalfa and *M. truncatula*, insights gained from studying *R. solani* interactions in this model legume should be extended to alfalfa to explore resistance mechanisms.

6.2. Cultural Control

Cultural control can reduce soil-borne diseases by creating unfavorable conditions for pathogen growth and survival [73]. Many cultural methods such as crop rotation, mixed cropping, cover cropping, green manuring, bio-fumigation, and pH and nutrient imbalance have been used to manage *R. solani* diseases in forage legumes [56,156–158]. Crop rotations contribute to disease prevention by increasing overall microbial populations that compete with pathogens, reducing inoculum levels when the host is absent, producing toxic compounds that directly inhibit pathogens, and enhancing specific antagonists that combat pathogens [159,160]. Implementing rotation practices has been reported to reduce the severity of *R. solani* diseases in many important crops [161–165]. The efficacy of crop rotation and green manure treatments in reducing *R. solani* diseases in alfalfa has

been demonstrated. These cultural practices were employed to examine their effect on *streptomycete* activity and suppress the alfalfa damping-off caused by pathogen complexes including *R. solani*. Green manuring treatments consisted of canola, sorghum-Sudan grass, buckwheat, and a fallow control. Crop sequences were potato-alfalfa, alfalfa-alfalfa, and corn-alfalfa. Results indicated that crop rotations increased alfalfa yield, and incorporation of green manures resulted in enhanced *streptomycete* activities and suppressed damping-off. Finally, the data suggested that green manures and crop rotation together can improve crop yield and suppress multiple pathogen complexes by increasing the antagonistic activity within *streptomycete* [166]. Perennial crops such as alfalfa are reported to increase soil-borne pathogenic populations such as *R. solani* and allow pathogen multiplication. Therefore, it is recommended that the alfalfa field be rotated with other crops that are not susceptible to *R. solani*.

Soil pH is an essential factor associated with plant pathogens, and altering the pH levels can reduce pathogenic diseases [156]. It has been proposed that soil pH directly influences the infection and development of plant diseases by affecting soil-borne pathogens [167,168]. Increasing the pH level is recommended for managing soil-borne diseases because a direct correlation has been observed between higher pH levels and lower disease severity caused by fungal pathogens [168,169]. The literature demonstrates that soil pH significantly influences crop diseases caused by *R. solani*. For instance, a study on sugar beet seedlings affected by *R. solani* damping-off showed that maximum biomass was produced at a pH near 6.0. In contrast, the minimum biomass was observed at a pH below 5.0 or above 7.0 [170]. Barbetti [171] reported that manipulating pH through the addition of lime influenced the alfalfa root rot complex caused by *Fusarium* sp., *R. solani*, and *Pythium* sp. However, lime application in alfalfa should be further investigated for its influence on various *R. solani* diseases. A combination of sanitation and bio-fumigation can reduce the survival capabilities of fungal pathogens' survival structures. However, it may be challenging for farmers to bear production losses from bio-fumigation [172]. Placing seeds deeper in the soil should be avoided because it causes late emergence, which increases the chances of pathogen infection. In addition, avoiding dense planting helps reduce disease spread [173]. Inorganic fertilizer application timings and quantity are critical factors for disease establishment. Suitable nutrition and excellent plant vigor have been reported to reduce crown bud rot caused by *R. solani* in alfalfa [174]. Over-seeding has been recommended to compensate for the seedling stand losses of alfalfa caused by *R. solani* seedling diseases [175]. Early cutting is suggested to manage alfalfa foliar blight caused by *R. solani* [56].

6.3. Biological Control

Beneficial microbes, including bacteria and fungi, have been widely used to improve growth, manage diseases, and enhance plant yield [176]. These microbes release antifungal compounds to suppress pathogens, which disrupt the pathogen's DNA, cellulose, hemicelluloses, and proteins [177]. Previous studies have investigated the biological control of *R. solani* in alfalfa. For example, streptomyces reduced disease severity through seed inoculation and/or soil infestation [178]. Similarly, microorganisms belonging to *Trichoderma* sp., *Penicillium* sp., and *Aspergillus* sp. have been suggested for managing *Rhizoctonia* diseases in alfalfa [179]. In a study conducted by Alsohim [180], it was determined that *Pseudomonas fluorescens* and its mutants 52-M12 and 45-M19 are effective against *R. solani* and improved various plant growth parameters, including root weight, root length, and shoot weight, as well as plant fresh and dry biomass. The observed disease suppression effect of these bioagents was attributed to their ability to produce antimicrobial substances. Furthermore, the study highlighted the substantial reduction in *R. solani*-induced damages in alfalfa fields upon application of these bioagents. Seed treatment *with Bacillus amiloliquefacaciens* and *Paenibacillus polymyxa* improved seed germination and increased the fresh and dry weights to 21% [63].

In another study, PGPRs, including *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas fluorescens, Paenibacillus polymyxa,* and *Sinorhizobium meliloti,* were checked against pre- and post-damping-off disease complexes. All four PGPR species produced HCN, siderophore, IAA, solubilized insoluble phosphate, and β-1,3-glucanase activities; however, *S. meliloti* only produced solubilized insoluble phosphate and IAA. Seed treatment with PGPR significantly reduced damping-off disease in field and greenhouse experiments. Seed treatment with a mixture of PGPR strains and *S. meliloti* resulted in increased nodules, plant height, dry weight, protein content, and tillers/m^2 under field conditions [181]. Additionally, the present study proved that biocontrol agents not only protect alfalfa from *R. solani* attack but can also help alfalfa plants produce resistance against disease complexes caused by *R. solani* and other soil-borne fungal pathogens. Moreover, unlike identifying bacterial species based on previous time-consuming and tedious phenotyping approaches, 16S rRNA is an accurate and reliable method for identifying several bacterial strains nowadays [182]. 16S rRNA can identify different types of bacteria, including slow- and fast-growth bacteria and rare and novel species [183]. Therefore, researchers are encouraged to isolate and identify the maximum number of bacterial species to investigate their potential in controlling *R. solani* alfalfa disease. Antimicrobial peptides (AMPs) are small, soluble molecules involved in plant defense mechanisms. They can inhibit the growth of pathogenic microorganisms by permeabilizing their cell membranes and inactivating intracellular targets within the cytoplasm [184]. The potential of AMPs has been explored against *R. solani* diseases in economically important crops. For instance, an antimicrobial peptide, AsR416, effectively inhibited the growth of AG1-IA sclerotia, successfully delayed the infection process, and reduced the disease severity of rice sheath blight in vitro and in vivo [185]. Some other studies [186,187] on soybeans and rice have also added similar findings for the effectiveness of different AMPs, including Purothionin, cecropin B, D4E1, Potato snakin-1, and phor21, against *R. solani*. Considering the need for sustainable farming, AMPs are better alternatives for managing R. solani diseases and improving alfalfa production.

6.4. Fungicides

Chemical control is the predominant method for managing pathogenic diseases in alfalfa and other crops [188]. Alternative options, such as resistant cultivars, often exhibit only partial resistance against pathogens, making them less practical within farming systems [189]. Seed treatment with fungicides containing metalaxyl and fludioxonil has been evaluated against seedling blight and root rot diseases caused by *R. solani*. Fludioxonil, alone or in combination with metalaxyl, improves seedling survival and reduces root rot under controlled conditions. However, the impact of seed treatment on forage yield under field conditions is limited. Additionally, metalaxyl alone proves ineffective under field and controlled conditions [62]. Berg et al. [190] documented the effectiveness of Stamina, containing the active ingredient pyraclostrobin, in combatting *R. solani* seedling diseases in alfalfa. Pyraclostrobin stops mitochondrial respiration in fungal cells, preventing energy synthesis for fungal growth and development [191]. Earlier research has also explored fungicide applications in alfalfa cultivation; for example, Hancock [192] observed increased alfalfa yield following the application of benomyl. Ahmad [193] found that seed dressing with Benlate T (a combination of benomyl and Thiram) improved germination rates and plant survival in alfalfa grown in *R. solani*-infested soil. Furthermore, Rhizolex (Tolclofos-methyl) is effective for controlling alfalfa disease complexes caused by pathogens such as *R. solani, Colletotrichum trifolii, Fusarium incarnatum*, and *Fusarium equiseti* [63,65].

Recently, fungicides with different modes of action have been used collectively to control alfalfa soil-borne diseases. For instance, seed treatment with fludioxonil (phenylpyrrole group) and difenoconazole (trizol group) in a 1:4 ratio prevented seedling and rot root diseases caused by *Bipolaris sorokiniana*. Difenoconazole reduces the participation of the enzyme C14-demethylase in ergosterol production by soil-borne pathogens, whereas fludioxonil stops glucose phosphorylation [194]. We suggest that more trials should be conducted to evaluate the fungicides mentioned above, and others have reported sound

results against *R. solani* diseases. However, high dependency on fungicides has some limitations. Seed treatment with fungicides can suppress endophytes, which protect plants from several pathogens and play an essential role in plant growth. Overdoses of fungicides have also been reported to affect plant metabolism [195]. Moreover, some fungicides are very effective against a single pathogen; however, sometimes, many pathogens are involved in a particular disease. Using a mixture of fungicides could produce better effects on pathogen complexes, but the cost may be difficult for some farmers [196]. Generally, due to its lack of spore production, *R. solani* is supposed to develop less resistance against different fungicides. However, repeated use of chemicals and remarkable genetic diversity among *R. solani* AGs resulted in the development of resistance [197]. In a study, RNA-sequencing qPCR analysis revealed that increased expression of ATP-binding cassette and major facilitator superfamily transporter genes produced resistance in *R. solani* against the fungicide thifluzamide (a succinate dehydrogenase inhibitor) [197]. Furthermore, a cross-resistance assay showed that fungicides such as SYP-14288 induced multidrug resistance (MDR) in *R. solani* against several fungicides with the same or different MOAs [198]. One potential approach for tackling multidrug resistance (MDR) in *R. solani* is to utilize metabolic enzyme inhibitors. The inhibitors can target the enzymes responsible for detoxifying and metabolizing fungicides [199]. In conclusion, adopting integrated disease management practices, such as using fungicides with biocontrol agents and cultural practices, could be a good option for managing *R. solani* in alfalfa (Figure 5).

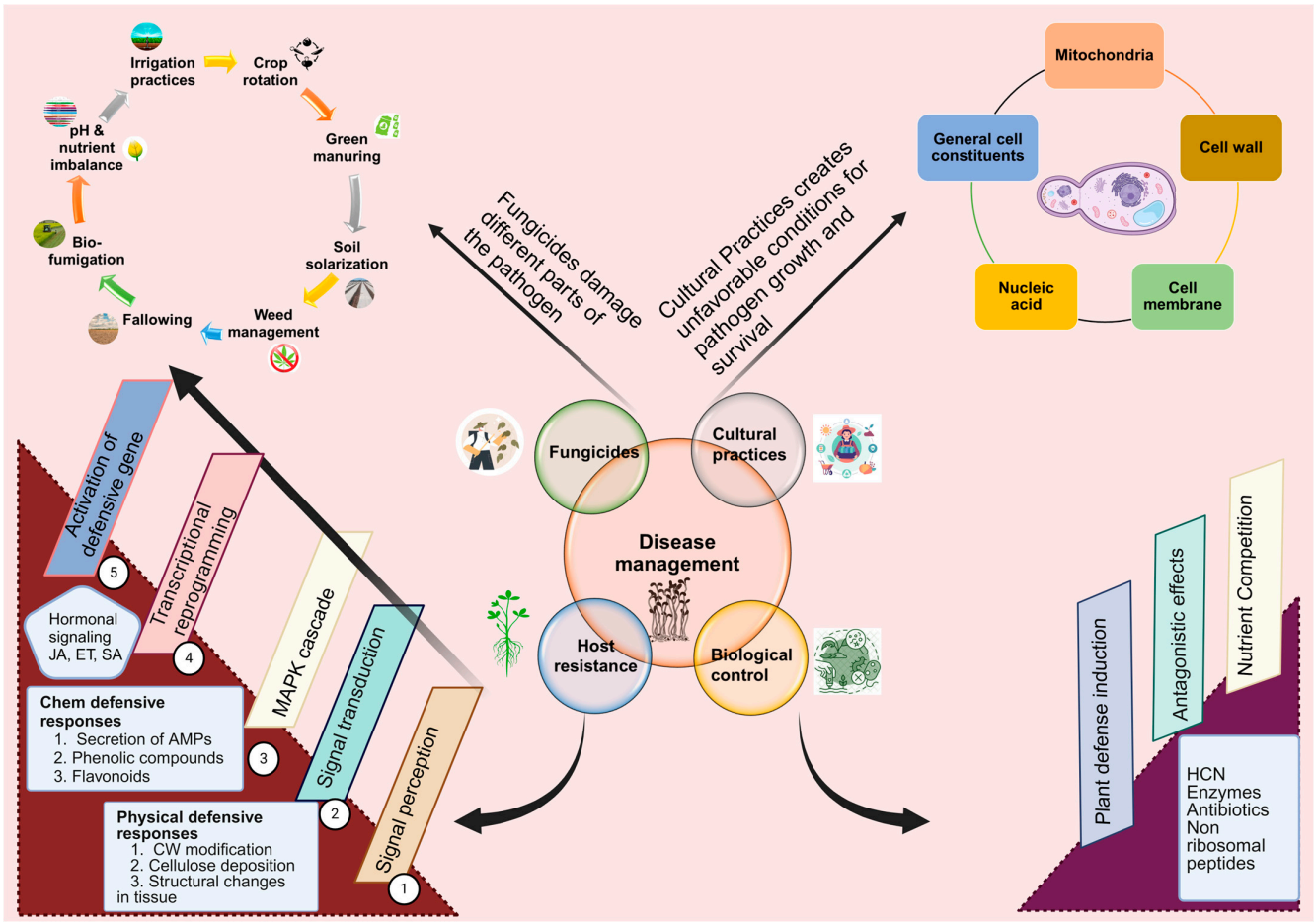


Figure 5. Disease control mechanisms through host resistance, fungicides, cultural practices, and biological control.

7. Future Directions in Alfalfa Resistance Research

The management of *R. solani* diseases in alfalfa is critical and future investigation areas could significantly advance our knowledge towards controlling this pathogen. Several recent developments and trends can potentially enhance alfalfa resistance and crop health [200]. Integrated use of genome-wide association studies (GWAS) with gene editing and synthetic biology could accelerate the molecular breeding of alfalfa to produce resistance against pathogens. For example, GWAS efficiently found the ZmFBL41 gene, which confers resistance to maize-banded leaf and sheath blight [201]. The genes facilitating pathogens to cause infection in host plants are known as susceptible (S) genes. Disruption of these S genes may hinder the compatibility of pathogens and hosts, leading to the development of broad-spectrum disease resistance [202]. Based on similar research studies [203–205], it is suggested that modifying S genes could increase alfalfa resistance against *R. solani* diseases. Plant pathogens are observed to secrete various effector proteins to weaken host immunity during the infection process. For instance, *Phytophthora sojae* secretes PsXEG1 in soybeans, which advances pathogenicity through its glycoside hydrolase activity [206]. Identifying *R. solani*-secreted effector proteins and studying their molecular mechanism in triggering the alfalfa immunity system will enhance theoretical understanding and allow the application of resistant genes. Studies on various plant–microbiome interactions have proven that the plant immune system has a strong relationship with its rhizosphere microbiome [207]. In China, artificial inoculation of the endophytic fungus *Epichloë bromicola*, belonging to wild barley, has increased the seed yield and biomass ratio in cultivated barley [208]. Therefore, researching the alfalfa microbiome would significantly contribute to future research on resistance against soil-borne diseases caused by *R. solani* in alfalfa. New pest and disease control technologies could improve alfalfa resistance, such as developing immune inducers, small RNAs, and host-induced gene silencing (HIGS) technology [209]. For example, silencing of the virulence gene VdH1 of *Verticillium dahliae* through HIGS technology improved cotton resistance against *V. dahliae* diseases [210].

8. Conclusions and Future Perspectives

The review highlights significant research gaps in understanding host–pathogen interactions between alfalfa and *R. solani*. Future studies should prioritize detecting and diagnosing *R. solani* in alfalfa fields, exploring management strategies, and conducting genomic comparisons between resistant and susceptible varieties. The rhizosphere is a reservoir of microorganisms, such as bacteria and fungi, that could harm or benefit the host plant. Root exudates alter the rhizosphere microbiome; however, several factors such as crop genotypes, growth stage, and biotic and abiotic factors such as soil types and pathogens influence the quality and quantity of root exudates. It has also been reported that host plants recruit disease-suppressive microbes during pathogen infection. Therefore, it would be interesting and crucial to investigate how the alfalfa rhizosphere microbiome behaves during *R. solani* infection. Alfalfa-resistant cultivars that can survive against *R. solani* and potentially persist against multiple pathogens should be introduced, as *R. solani* has also been reported to cause alfalfa diseases in the form of pathogen complexes. However, we suggest isolating PGPRs from alfalfa fields, testing their efficacy against *R. solani* in labs, and then applying them in greenhouse and field trials for disease control. These integrated management strategies can reduce *R. solani* impact, safeguard alfalfa yields, and ensure high-quality fodder for livestock.

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