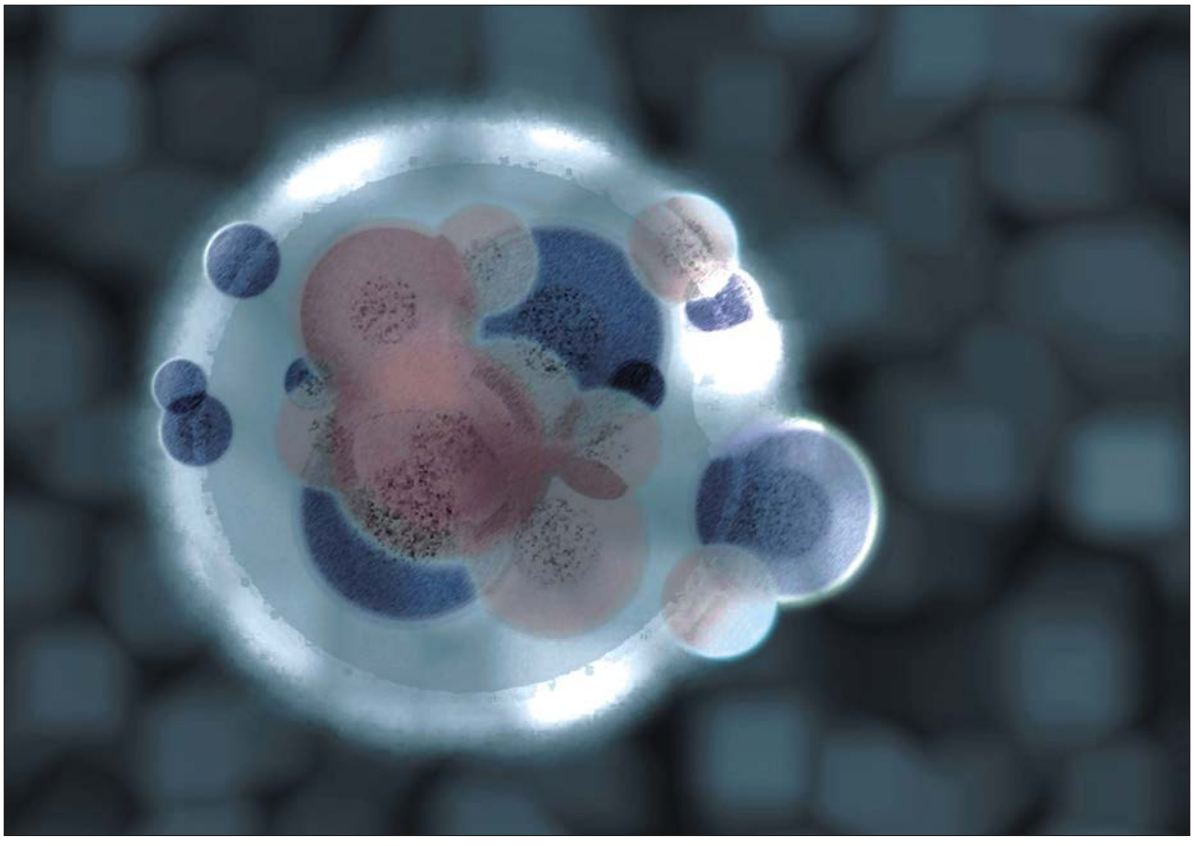

Journal of Biological Sciences

ISSN 1727-3048

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OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2019.300.313

Mini Review
How to Knock down a Plant: The Three Weapons of *Sclerotinia sclerotiorum*

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Abstract

The infection process of *Sclerotinia sclerotiorum* (Lib.) de Bary, a necrotrophic plant pathogen with more than 600 host plants, causing several disease symptoms such as cottony rot, watery soft rot, stem rot, white mould etc in a wide range of host plants remains sketchy. Specifically, virulence factors produced during host invasion require a special compilation to provide various researchers with this critical knowledge. This review discussed the virulence factors produced by *S. sclerotiorum* during plant invasion and colonization. The discussion was organized under the topics of *S. sclerotiorum* necrotrophic lifestyle, weaponry and the molecular aspect of its pathogenicity, zooming-in on the roles of its virulence factors (Cell wall degrading enzymes, effectors and oxalic acid) during pathogenicity.

Key words: Cell wall degrading enzymes, effectors, necrotrophy, oxalic acid, plant pathogen, *Sclerotinia sclerotiorum*

Citation: Sarah Otun and Khayalethu Ntushelo, 2019. How to knock down a plant: the three weapons of *Sclerotinia sclerotiorum*. J. Biol. Sci., 12: 300-313.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is a major phytopathogen which causes diseases to more than 600 plant species including oilseed rape, beans, cotton, sunflower, various vegetables and weeds[1]. *Sclerotinia sclerotiorum* is regarded as a model necrotroph[2], due to its vast host range and its vast arsenal of attack weapons, such as; cell wall degrading enzymes (CWDEs), effectors and oxalic acid for host cell apoptosis[3]. Upon successful infection, *S. sclerotiorum* obtains nutrients from the oozing plant sap which leaks from damaged tissues[2]. The major symptom associated with *S. sclerotiorum* is 'rot' which results from the maceration of tissue and among the common diseases are white mould on bean, rot in cotton, drop of lettuce, Sclerotinia rot of cabbage[4] etc. These diseases cause massive global economic loss of crops annually[1]. Outstanding research advancements have been made in recent years on the characterization of *S. sclerotiorum* virulence factors[5], resulting in the generation of a plethora of knowledge which has, unfortunately, not been perused and compiled in a single manuscript. This review which attempts to address this shortfall selectively reports on the production of CWDEs, effectors and oxalic acid produced during pathogenicity.

Proposed models of plant pathogen's infection cytology: Plant pathogens can be grouped based on their feeding lifestyles as; (a) Biotrophic: Those who feed through 'haustorium' and secrete minimally CWDEs and largely no toxins are produced, (b) Necrotrophic: They feed by attacking the living host cells with a myriad of CWDEs and toxic metabolites leading to the death of the host cell and (c) Hemibiotrophic: They utilize both biotrophs and necrotrophs characteristics[2].

However, this classification can further be categorised under two conceptual models based on the evolutionary and mechanistic plant-pathogen interactions[6]. In the first model, pathogens that rely on the production of effector proteins to suppress or evade host defense mechanisms or pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI). However, plant hosts have also evolved to detect these effector proteins and initiate a rapid counter-attack known as the effector-triggered immunity (ETI)[7]. Within this model are host specific necrotrophic pathogens, in which their effectors form an inverse gene-for-gene interaction with the host plant's toxin[8]. This inversed gene-for-gene model exceed genetic analogy, however, it functionally assists these necrotrophs to utilize the host resistance genes found in the gene-for-gene model by triggering all or specific components of the HR resistance pathway, susceptibility is achieved[9].

Whereas in the second model (two-phase model), which is mostly for broad host range necrotrophs, in which the gene-for-gene model of the host to pathogen compatibility is sadly partial and it the exact function of their effectors contribute toward virulence and host susceptibility is vague[6]. *Sclerotinia sclerotiorum*, a wide host range necrotroph (with over 600 host plants) exemplifies this model. The summary of *S. sclerotiorum* infection cytology, suggest that the pathogen first evades, counteracts and subverts host basal defense mechanisms, possibly in the absence of OA. Subsequently, the pathogen switches from biotrophic to necrotrophic lifestyle, initiating the death and host cell wall degradation through OA, OA-independent toxins and cell wall degrading enzymes. In the following sections, this two-phase model of *S. sclerotiorum* infection mechanisms will be discussed. The feeding lifestyle of a fungal pathogen and its host specificity can be important for various applications which include import of biological control agents. For an example, Retief *et al.*[10] performed a comprehensive host specificity testing of the rust fungus *Puccinia xanthii* var. *parthenii-hysterophorae* on various *Helianthii* plants and upon discovering that *P. xanthii* var. *parthenii-hysterophorae* is specific to the target weed *Parthenium hysterophorus* they recommended its import to South Africa to control *P. hysterophorus*.

Main characteristics of *Sclerotinia sclerotiorum* pathogenicity: The infection process of *S. sclerotiorum* is usually described by the following stages: attachment and penetration by the appressoria to the host surface, suppression of host defence mechanism and eventual killing of host tissues.

***Sclerotinia sclerotiorum* develops Appressoria; an infection structure for attachment and penetration of the host:** Formation of appressoria (infection cushions) from a dormant sclerotia is essential during host infection, with an exception of stomata infection[11]. *Sclerotinia sclerotiorum* forms appressoria in response to physical factors such as contact with the cuticle layer of host tissue or hydrophobic surfaces such as petri dishes, microscopic cover slides and parafilm[11]. This leads to the formation of the asci from the appressorium-like structures, which is depicted as swollen tips and it is necessary for the generation of an osmotic pressure

needed for penetration into the host cell wall[11]. The ascospores usually penetrate directly through the cuticle and not through stomata, hence sensing and recognition of the host surface characteristics, such as hydrophobicity and sugar sources, is essential for proper adhesion to the host surface. After inoculation with mycelium, a different form of penetration was observed, whereby hyphae growing on the plant surface heavily ramify into short bulbous cell aggregates as 'claw-like' structures. The importance of infection cushions in fungal penetration has not yet been molecularly investigated in *S. sclerotiorum*. Their germination and adhesion on plant surfaces represent crucial steps preceding host penetration and colonization[12].

With the availability of genomic data, seven genes involved appressorium development have been identified, these are; SMK3 [13], Ss-caf1 [14], Ss-ggt1 [15], Ss-odc2 [16], Ss-rhs1 [17], Ss-pth2 [18], Ss-nsd1 [19] and Ss-sac1 [20] (Table 1). SMK3, Ss-sac1, Ss-ggt1 and Ss-caf1 are major components of cellular signaling processes, in which their mutants display some defects in some specific developmental or penetration aspect of pathogenicity, hence virulence defects. This virulence defect can be salvaged by wound inoculation of the host. This shows the important role of compound appressorium in the infection process[13-16]. Also, *S. sclerotiorum* appressorium is melanized with dihydroxynaphthalene (DHN) derived compounds[21], in which polyketide synthase (Sspks13) regulates melanin accumulation in compound appressoria but does not affect melanin accumulation in sclerotia and Sspks13 mutants are still fully pathogenic[22].

***Sclerotinia sclerotiorum* cell wall degrading enzymes; the frontline virulence factor relies on multigenic families:** *Sclerotinia sclerotiorum* like other plant necrotrophic pathogens utilizes a myriad of CWDEs such as cellulases, pectinases and xylanases when infecting its hosts[23].

Cell wall degrading enzymes have different molecular weights, isoelectric points, transcriptional regulations, biochemical properties and pathogenicity potential on different plant hosts[24,25]. Cell wall degrading isozymes are largely responsible for the 'flexible penetration and colonization' characteristics of the pathogen during pathogenicity[26].

Sclerotinia sclerotiorum has been confirmed to secrete cellulolytic, hemicellulolytic and pectinolytic enzymes with varying levels that correlate with the disease progression[27].

Cellulose-degrading enzymes: The degradation of the host plant cell wall cellulose involves the action of endoglucanases and exoglucanases in a synergy, this is followed by the hydrolysis of the soluble cellodextrin oligomers to glucose by β-glucosidase. Although the exact function of each enzyme (Table 2) in cellulose degradation is largely unknown[28]. Analysis of the genome sequences of *S. sclerotiorum* show that these enzymes are confined to a relatively low number of glycoside hydrolases (GHs). Glycoside hydrolases are enzymes that are able to hydrolytically cleave glycosidic bonds in oligo or polysaccharides (including cellulose and hemicellulose) families[29].

Hemicellulose-degrading enzymes: Hemicellulose-degrading enzymes are involved in the cleavage and degradation of the non-cellulose polysaccharides of the plant cell wall that contain galactomannans xyloglucans and xylans. Although the linkage and sugars in the core chains of hemicellulose are different from major polysaccharides[28].

Pectin-degrading enzymes: *Sclerotinia sclerotiorum* produces many pectinolytic isoenzymes whose major role in pathogenicity is the degradation of the pectin component of the host's cell wall[26]. For instance, *S. sclerotiorum* pectinases are implicated in pectin (a major constituent of the plant cell wall) degradation. *Sclerotinia sclerotiorum* produces several forms of pectinase which weaken the cell wall to facilitate penetration and colonization of the host while also providing the pathogen carbon sources for growth[26].

Fraissinet-Tachet *et al.*[30] reported that he multiplicity of *S. sclerotiorum's* pectinolytic enzymes and polygalacturonase isozymes are coded by a multigene family of seven members and two subfamilies. It can be proposed that multiple copies of functionally related genes confer flexibility and adaptability to *S. sclerotiorum*, although this has to be proven experimentally.

Furthermore, endo-PGs are endo-acting enzymes that catalyse the hydrolysis of homogalacturonan while exo-PGs cleave monomeric or dimeric glycosyl groups from the pectic cell-wall polysaccharides, resulting in breakdown and release of potential nutrients from the substrate[25]. Several endo-PGs and exo-PGs (which are accommodated in family GH28) have been cloned and characterized in *S. sclerotiorum*[27]. During infection, *S. sclerotiorum* secretes a full complement of CWDEs that can facilitate penetration, macerate tissues and

Table 1: List of some characterized genes implicated in *Sclerotinia sclerotiorum* pathogenesis

Biological processes	Genes	Functions	References
Appressoria formation	Ss-odc2	Elevated inoculum nutrient level increases appressorium formation but not cuticle penetration on soybean leaflets	Liang *et al.*[16]
	Ss-Rhs1	Virulence, sclerotial and appressorium formation	Yu *et al.*[17]
	Ss-pth2	Sclerotial development and virulence	Liberti *et al.*[18]
	Ss-nsd1	The GATA-type IVb zinc-finger transcription factor	Li *et al.*[19]
	Ss-sac1	Adenylate cyclase and cAMP signalling	Jurick and Rollins[20]
	MAT1-1-1	Mating-type genes and apothecial development	Doughan and Rollins[84]
	MAT1-2-1	Mating-type genes and apothecial development	Doughan and Rollins[84]
	MAT1-1-5	Mating-type genes and apothecial development	Doughan and Rollins[84]
Sclerotia formation and development	SAC1	Adenylate cyclase, sclerotial development, virulence and cAMP-signaling pathway	Jurick and Rollins[20]
	pph1	Type 2A Ser/Thr phosphatase, catalytic subunit (PP2Ac), growth and sclerotial development	Erental *et al.*[85]
	rgb1	Type 2A Ser/Thr phosphatase, B subunit, sclerotial development, virulence and infection cushions	Erental *et al.*[85]
	CNA1	Catalytic subunit, calcineurin-encoding genes, sclerotial development and virulence	Harel *et al.*[86]
	SMK3	Slt2 ortholog (MAPK associated with the cell wall integrity pathway), sclerotia development and cuticle penetration	Bashi *et al.*[13]
	Ss-Ggt1	γ-Glutamyl transpeptidase, sclerotial and appressorium formation	Li *et al.*[15]
	Shk1	Histidine kinases, growth, sclerotial development and stress tolerance D	Zhu *et al.*[37]
	SSITL	Growth, sclerotial development, virulence, germination and suppress host defense	Zhu *et al.*[37]
	Ss-Ggt1	Sclerotia development defect (increased sclerotia initials, delayed sclerotia maturation, thickened and disorganized rind layer, failed carpogenic germination)	Li *et al.*[19]
	SsNox1	ROS generation	Kim *et al.*[87]
	Ss-sop1	Reduced radial growth, aberrant sclerotia formation, increased sensitivity to salt, osmotic and cell wall stresses and reduced sensitivity to oxidative stress	Lyu *et al.*[88]
	Ss-SOD1	Increased sensitivity to ROS stress and abolished sclerotia development in one but not another strain	Xu and Chen[43]
Signal transduction	pka1	Protein kinase A (PKA) activity	Jurick and Rollins[20]
	Smk1	ERK (extracellular signal-regulated kinase)-type mitogen-activated protein kinases (MAPKs), growth and sclerotial development via a pH-dependent signaling pathway	Chen *et al.*[89]
Host cell death	SsNep1	Necrosis and ethylene-inducing peptides, induce necrosis and cell death	Bashi *et al.*[75]
	SsNep2	Necrosis and ethylene-inducing peptides, induce necrosis and cell death and calcium and cAMP signaling	Bashi *et al.*[75]
	Ss-pth2	Increased transcripts accumulation of the glyoxylate cycle genes Ss-mls1 and Ss-icl1 and the oxalate biosynthetic gene, Ss-oah1 on MM medium with glucose as the carbon source	Liberti *et al.*[18]
Genes involved in fungal nutrition and responding to environment	CRE1	Putative glucose repressor and carbon catabolite repression	Veluchamy *et al.*[42]
	CRY1	Cryptochrome family, CRY-DASH (members of this branch exhibited no or trace levels of DNA repair activity) ortholog, sclerotial development and response to UV light	Veluchamy *et al.*[42]
	SOP1	Microbial opsin homolog gene, growth, sclerotial development and virulence	Lyu *et al.*[45]
	Ss-pac1	Reduced radial growth at higher pH and aberrant sclerotia development (lacking the melanized)	Rollins[63]

Table 1: Continue

Biological processes	Genes	Functions	References
Fungal development	ssp1	Development-specific protein and sclerotial development	Li *et al.*[15]
	ssp2	Development-specific protein and sclerotial development	Li *et al.*[15]
	MAT1-2-4	Mating-type gene, carpogenic germination and disc morphology	Doughan and Rollins[84]
Suppress reactive oxygen species (ROS)	Ssnox1	NADPH oxidase, sclerotial development, virulence and ROS regulation	Kim *et al.*[87]
	Ssnox2	NADPH oxidase, sclerotial development and ROS regulation	Kim *et al.*[87]
	Ss-SoD1	Cu/Zn superoxide dismutase, growth, sclerotial development, virulence and stress tolerance	Veluchamy *et al.*[42]
	Ss-oah1	Reduced radial growth at higher pH and increased fumaric acid accumulation in culture	Li *et al.*[19] and Liang *et al.*[66]
Secreted proteins	Ssv263	Virulence	Liang *et al.*[6]
	SsCVNH	Virulence, sclerotial development	Lyu *et al.*[88]
	Ss-Caf1	Secreted protein with a putative Ca^{2+}-binding, EF-hand motif, appressorium formation, sclerotial development and induces host cell death	Xiao *et al.*[14]
	Ss-Bi1	Bax inhibitor-1 protein, development, virulence and putative antiapoptosis	Yu *et al.*[90]
	SsPemG1	Elicitor-homologous protein, elicitor and negative virulence factor	Pan *et al.*[91]
	SsNACα	Nascent polypeptide-associated complex α-subunit, sclerotial development and virulence	Li *et al.*[92]
	SsSSVP1	Small secreted virulence-related protein, virulence and induces plant cell death	Lyu *et al.*[45]
	SsCP1	Cerato-platanin protein, virulence, induces plant cell death and interacts with PR1	Yang *et al.*[79]
	SsSm1	Cerato-platanin protein, virulence, growth, sclerotial formation and induces plant cell death	Pan *et al.*[93]
	Ss-Rhs1	Rearrangement hot spot repeat-containing protein, virulence, sclerotial and appressorium formation	Yu *et al.*[17]
	Ss-caf1	Appressorium formation, sclerotial development and induces host cell death	Xiao *et al.*[14]
	Ss-ITL	Suppresses host defense reactions	Zhu *et al.*[37]
	Ss-SSVP1	Reduced growth rate and reduced sclerotia formation	
	Ss-CVNH	Normal colony morphology, growth rate and sclerotial development	Lyu *et al.*[88]
	Ss-CP1	Higher growth rate, higher tolerance toward salt and SDS stresses, higher cellulase and pectinase activities	Yang *et al.*[79]
	Ss-PemG1a	Elicitor	Pan *et al.*[91]
	SS-ggt1	γ-Glutamyl transpeptidase, involved in oxidative stress responses	Li *et al.*[19]
	Ss-NSD1	A GATA-type IVb zinc-finger transcription factor	Li *et al.*[19]
	Ss-MADS	Transcription factor	Qu *et al.*[94]
	ScAT1	Type A catalase	Yarden *et al.*[95]
	Ss-FKH1	Forkhead transcription factor	Fan *et al.*[44]
	Ss-axp	Arabinofuranosidase/ B-xylosidase precursor	Yajima *et al.*[81]
	Ss-Xyl1	Endo-β-1,4-xylanase	Yu *et al.*[82]

Table 2: Cell wall degrading enzymes (CWDEs) produced by *Sclerotinia sclerotiorum*

Classification	CWDEs	Family	Number of enzymes
Cellulose degrading enzymes	Cellulases	GH6	2
		GH7	3
		GH5	14
		GH12	4
		GH45	2
		GH1	3
	β-Glycosidases	GH3	13
		GH61[b]	9
		CBM	19
	Accessory enzymes	GH10	2
		GH11	3
		GH74	3
Hemicellulose-degrading enzymes	Xylanases	GH27	3
		GH43	5
	Xyloglucanases	GH51	2
	α-Galactosidases	GH54	1
	α-Arabinosidases	GH35	4
		GH115	1
Pectin-degrading enzymes endo-PG	β-Galactosidases	GH28	17
		GH78	4
	β-Glucuronidases	GH115	1
	Polygalacturonases (PG)	GH28	17
		GH78	4
	Polygalacturonate lyases	PL11	4

GH: Glycoside hydrolase, PL: Polysaccharide lyase, CE: Carbohydrate esterase, CBM: Carbohydrate-binding module, Source: Riou *et al.*[27] and Kubicek *et al.*[28]

degrade plant cell-wall components (Table 2). Table 2 showed the classification of CWDEs based on their function, their family and the number of the enzymes in each family that is implicated in each category.

Lastly, it has been established that *S. sclerotiorum* releases large amounts as well as numerous CWDEs to aid it in its attempt to colonize a host. Although this seems like an abundance of weapons, apparently it is only a fraction of the possible arsenal released from this pathogen[31]. In addition to CWDEs, *S. sclerotiorum* releases many other protein-effectors that can influence disease.

Most of the degrading enzymes are encoded by multigenic families and some may have partially redundant functions. This would explain why inactivation of several genes encoding CWDEs[25] or cutinolytic enzymes, i.e., cutinase[32] A and lipase 1, did not affect fungal virulence. Taken together, the multiplicity of the reported degrading activities and the reduction of virulence observed for several mutants impaired in degrading enzymes strongly support a major role in pathogenicity for the enzymatic degradation arsenal of *S. sclerotiorum.* When genes for CWDEs are expressed it would interesting to understand the role of microRNAs. Djami-Tchatchou *et al.*[33] have reviewed the functional roles of microRNAs in agronomically important crops and found that they can be exploited for crop improvement. Similarly, microRNAs can be exploited in *S. sclerotiorum* but this time not to improve the fungal pathogen but to weaken its virulence machinery.

Host defense suppression; activities of effectors and oxalic acid at the early infection phase: All pathogens colonise host differently depending on their feeding lifestyle but they all encounter different defense responses from the host plants, however, the innate immunity pathogen-associated molecular pattern (PAMP) suppression is a common theme for all types of pathogens[34,35].

Virulence factors such as effectors (Small secretory proteins) play serious roles in host PTI suppression for both biotrophic, hemibiotrophic pathogens and necrotrophic pathogens such as; *S. sclerotiorum* based on recent molecular evidence. For example, secreted chorismate mutase enzyme Ss-Cmu1 in Ustilago maydis apparently translocates inside host cells and inhibits the synthesis of salicylic acid (SA) by shifting chorismate into the phenylpropanoid pathway to stimulate infection[36]. Amazingly, Ss-Cmu1 is among the predicted effectors secreted during the biotrophic phase, associated with *S. sclerotiorum* infection[2]. Other putative effectors include the Ss-ITL gene which encodes a secreted

integrin-like protein and is highly up-regulated during early infection[37]. Other *S. sclerotiorum* predicted effectors (Table 1) require further functional studies[38,39].

For *S. sclerotiorum* to achieve full virulence, it requires the detoxification of ROS and host-derived secondary metabolites, which are vital components of PTI defense reactions[40]. For instance, the disruption of a Cu/Zn superoxide dismutase Ss-Sod1 largely weakens virulence[41-43]. But, the disruption of *S. sclerotiorum* redox status, negatively affects the OA accumulation level[41-43], signifying a strong connection between ROS signaling and OA accumulation. Hence, virulence genes that are functional in responses to osmotic, high salt and cell wall stresses are upregulated[44,45].

Role of effectors in *Sclerotinia sclerotiorum* pathogenicity: Effectors are small secreted proteins that have been linked to many of the virulence-associated genes of plant pathogens such as *S. sclerotiorum*[46]. The major role of effectors is to manipulate plant defence mechanisms, in order to promote fungal infection and establishment of disease[47]. In many fungal phytopathogens, effectors were discovered with varying functions based on individual fungal lifestyle[45].

Effector proteins, termed "effectors," have been discovered in multiple plant pathogenic fungi and exhibit numerous different functions depending on fungal lifestyle. For example, necrotrophic fungi, which require dead tissue on which to feed, often produce effectors that promote cell death, whereas biotrophic fungi, which require living tissue, produce effectors that prevent cell death[48-51]. Hemibiotrophic fungi, which require both living and dead tissue at different life cycle stages may produce different effectors at different time points during infection[52-54].

Since the release of the *S. sclerotiorum* genome sequence, bioinformatics analysis aimed at systematically identifying candidate proteins associated with virulence have been and continue to be conducted[55]. Genomic analysis of *S. sclerotiorum* and *B. cinerea* secretomes highlighted over 400 secreted proteins including nearly 80 virulence factor candidates[38,39].

Thus far, in *S. sclerotiorum*, several proteins (Table 1) have been identified and classified based on their effector-like properties and functions in plants[47]. Several studies on *S. sclerotiorum* have attempted to classify effector-like or secreted small proteins implicated in infection pathways[38,39] and these studies have used several benchmarks to distinguish effector-like from none effector-like genes.

Oxalic acid is another virulence factor that has associated with host defence suppression for so long until the emergence of recent molecular findings.

Role of oxalic acid in *Sclerotinia sclerotiorum*'s pathogenicity: The role of oxalic acid in *S. sclerotiorum*'s pathogenicity has been a major research focus by many researchers, leading to the identification of its numerous roles, which includes; its unswerving toxicity to its host, probably due of its acidity, hence weaken the host and facilitating invasion and tissue colonization[56-57], enabling the pathogen to escape detection and recognition by PGIPs[58], affecting the proper functioning of the host guard cells, by activating stomata opening and preventing abscisic acid (plant hormone which activates leaf detachment) induced stomata closure hence leading to *S. sclerotiorum* foliar wilting during infection[59], chelation of cell wall Ca^{2+}, i.e., degeneration of the plant cell wall components[57], suppression of the host's oxidative burst[60], the creation of a low pH environment to facilitate hydrolytic enzyme activities[57,61-63], triggering apoptotic programmed cell death to permit necrotrophic colonization[64] and manipulation of host cell death fate from a resistance-related autophagy to a susceptibility-related apoptosis[2]. These findings suggest that oxalic acid is an important *S. sclerotiorum* virulence factor playing multifaceted roles and more broadly, high levels of OA accumulation have been implicated in the evolution of broad host-range necrotrophy within the family Sclerotiniaceae[65].

Despite this plethora of functions, yet experimentally it was discovered that *S. sclerotiorum* oxalic acid mutants still retain their pathogenic ability, although the severity varies from host to host[22,66,67]. This OA-mutant pathogenic capacity has been credited to the relative pH buffering capacity of host tissue[67]. Loss of function oah1 mutants created recently using CRISPR technology in three independent wild-type backgrounds and comparison with the oah1 knockout mutant previously created by Xu *et al.*[67] reconcile previously reported phenotypic incongruences among oah1 gene deletion mutants[66-67]. Multiple examined CRISPR-mediated mutants in all three wild-type backgrounds produced essentially identical phenotypes when compared with the Xu *et al.*[67] mutant[22]. All mutants fail to produce oxalic acid, over-produce compound appressoria on artificial surfaces and produce functional sclerotia in culture. In host tissues in which lesions can expand, symptom development is obviously different from wild-type with less water soaking maceration, a decreased breakdown of chlorophyll and in some interactions, a reduced rate of colonization[22,67]. Thus, many hosts produce limited lesions when infected by OA-minus mutants and although some host tissues are colonized in the absence of OA, the full range of disease symptoms is not observed. Thus, OA is an important virulence factor that

plays a primary role in host colonization rather than in establishing basal host-pathogen compatibility.

Also, Williams *et al.*[68] unveiled how oxalic acid suppresses host defence by manipulating the redox reaction after *S. sclerotiorum* attack on the host. It was reported that after few hours of inoculation with *S. sclerotiorum* there was a marked generation of a reducing environment which was followed by host oxidation, which eventually led to apoptotic cell death and disease but these reactions were absent in oxalate deficient *S. sclerotiorum* strains. Another difference that was noted during this experiment was that the wild-type caused typical disease symptoms while oxalic acid deficient strains were avirulent and had restricted growth on the host[68]. Therefore, it was concluded that in the absence of oxalate, the host was able to identify oxalate deficient mutants and immediately activated its defence system to stop them.

This hypothesis that OA is mainly responsible for the host colonization is buttressed by the experiment conducted by Heller and Witt-Geiges[69] in which they monitored the infection-related calcium oxalate depositions based on potassium pyroantimonate histological staining procedure. The result showed that at the early infection stage, calcium oxalate was not discovered on the surface hyphae, appressorium and subcuticular infectious hyphae, rather it was found in the vesicles of plant surface hyphae, suggesting that OA accumulation is at a low level. But the OA concentration that was detected at the colonization stage (late infection phase), where the host tissues become fully macerated, was higher. In another study, Davidson *et al.*[70] generated transgenic soybean plants overexpressing oxalate oxidase, these OA-degrading transgenic lines block lesion expansion but not primary lesion formation following *S. sclerotiorum* inoculation. On detached leaflets, primary lesions form similarly between the wild-type and the OA-degrading line 18-24 h postinoculation although the wild-type lesions accumulate significantly more OA. Histological observation shows that during this early period, *S. sclerotiorum* aggressively penetrates and infects both lines, producing subcuticular, intercellular and vascular hyphae with similar densities. At three days postinoculation, plant tissue damage is similar in appearance between the wild-type host and the transgenic oxalate oxidase over-expression host but infectious hyphae formed on the transgenic lines are highly vacuolized and degenerated. The authors suggested a two-phase model for lesion establishment and lesion expansion explain the lack of lesion expansion by wild-type *S. sclerotiorum* when inoculated on the oxalate oxidase over-expressing line[70]. The results of this study[70] are congruent with those in which OA accumulation is eliminated by mutation of the pathogen[22,66,67] in that both produce only limited lesions on soybean when OA is reduced or eliminated. These independent studies support a two-phase model of pathogenesis.

While the importance of OA in virulence appears to be colonization phase-specific, experimental evidence suggests its virulence functions encompass necrosis inducement as well as defense suppression[2,64,68]. Based on observations with a redox-regulated GFP reporter, Williams *et al.*[68] showed that OA induces an immediate lowered redox environment which suppresses host basal defense reactions. Moreover, Arabidopsis plants infected with UV mutagenesis-generated OA mutants show restricted colonization and undergo cytological changes consistent with host autophagy. Arabidopsis lines with known mutations in the general autophagic pathway are unable to mount an oxidative burst and exhibit increased colonization by these OA mutants[2]. At the same time, OA also induces ROS-dependent apoptosis in promoting necrotic lesion development[64]. Worthy of note, many studies concerning the virulence functions of OA have been mostly based on these UV-induced "OA-minus" A mutants. These mutants differ significantly from OA-minus mutants generated by gene-specific mutagenesis in terms of OA accumulation and morphological phenotypes. The UV-induced mutant strains still accumulate a low-level OA and the genetic basis for their pathogenicity defects has not been fully characterized. The availability of genetically-defined OA-minus mutants created through gene deletion[67] or gene disruption[22] should be utilized for the further study of the role and phase-specificity of OA during *S. sclerotium* colonization. *Sclerotinia sclerotiorum* then produces cell wall degrading enzymes, which facilitates the penetration process by breaking down the host cell wall and other barrier tissue, initiating an oxidative burst which ultimately leads to the death of the host cell[31].

The toxic effect of OA is wide-ranging and OA accumulation level variation has been related to host range evolution within the Sclerotiniaceae family[65]. Despite these demonstrated roles for OA, mutants which do not produce OA have the capacity to colonize some hosts under laboratory conditions[66,67], suggesting that fumaric acid[67] or other factors may at least partially compensate for the lack of OA during colonization. Chemical profiling of *S. sclerotiorum* needs to continue to delineate other chemical compounds which play various roles in the virulence or any phenomenon for that matter, of *S. sclerotiorum*. For an example, Ntushelo and Setshedi[71], Ntushelo[72], identified various benzene derivatives and chlorinated organic compounds in the plant pathogenic fungus *Fusarium graminearum*.

***Sclerotinia sclerotiorum* virulence factors involved in the death of host cells:** Symptomatically, *S. sclerotiorum* causes tissue maceration following infection indicating rapid cell death and host cell wall degradation during colonization. Toxins and host cell wall degrading enzymes (CWDEs) are thought to play critical roles in promoting these processes. The OA, the primary necrotrophic effector in *S. sclerotiorum*, is known to contribute toward both killing and host cell wall degradation in different ways including pH acidification, calcium chelation and wilt, induction of programed cell death and disruption of chloroplast function[56,59,73,74].

In addition to OA, the *S. sclerotiorum* genome encodes proteinaceous factors able to induce necrosis, such as necrosis and ethylene-inducing peptides[75], endo-polygalacturonase[76,77] and a cutinase[78]. Recently, two small secretory necrosis-inducing protein, Ss-SSVP1 and Ss-CP1 have been demonstrated to contribute toward full virulence and have been characterized in detail[51,79]. Ss-SSVP1 induces host cell death upon transient expression in *Nicotiana benthamiana*, either with or without a signal peptide, suggesting that the protein functions inside the host cell. Importantly, based on fluorescent protein tagging, Ss-SSVP1 is demonstrated to be internalized inside and translocated among host cells. Ss-SSVP1 interacts with the subunit 8 of cytochrome b-c1 complex (QCR8), a highly conserved mitochondrial protein in plants; silencing of QCR8 causes abnormal plant development and cell death. Likely, Ss-SVP1 promotes infection by inducing QCR8 mislocalization and thus necrosis. QCR8 is highly conserved in plant species, indicating the broad spectrum of Ss-SSVP1 virulence function. Ss-CP1 is a small secreted protein with 138 amino acids. It belongs to the cerato-platanin protein family and induces necrosis-like cell death when transiently expressed in *N. benthamiana*. Arabidopsis thaliana plants stably expressing Ss-CP1 exhibit hallmarks of an activated salicylic acid defense pathway and show enhanced disease resistance. Ss-CP1 localizes in the apoplastic space and interacts with plant PR1; however, the protein region required for this interaction is dispensable for plant immunity activation[79].

The rapid tissue maceration associated with *S. sclerotiorum* infection is a result of highly active plant cell wall degradation. This activity may be mediated by pectolytic activity and endo-polygalacturonase in particular. The *S. sclerotiorum* genome encodes five endo-polygalacturonase and their expression during infection and in response to pH and nutrient conditions have been well-characterized[61,80]. However, none of the endo-polygalacturonase has been functionally analyzed via gene mutation. The *S. sclerotiorum* and *B. cinerea* genomes encode a similar number of carbohydrate-active enzymes (CAZyme) as their hemibiotrophic and saprophytic relatives, suggesting that gene content variations are not key characteristics distinguishing different trophic lifestyles, on the other hand, gene expressional regulations may play a more important role[55]. Despite functional redundancy commonly observed with cell wall degrading enzymes, gene deletion of an arabinofuranosidase/β-xylosidase precursor gene and an endo-b-1, 4-xylanase encoding gene caused significant virulence reduction in *S. sclerotiorum*[81,82]. Various factors of importance have been left out in this review, firstly how *S. sclerotiorum* deals with toxic agents to thwart its infection processes. Recently, Mbovane *et al.*[83] proved that acetaldehyde reduces the growth of *Alternaria alternata* and decreases the quantity of adenosine 3',5'-cyclic monophosphate. It would be interesting to extract from existing literature similar issues about the *S. sclerotiorum*.

CONCLUSION

Sclerotinia sclerotiorum, a typical model of a necrotrophic plant pathogen and an economically devastating pathogen has evolved and developed a systematic and coordinated attack 'formation' against its plant host, despite the presence of the potent host plant's defence mechanisms against pathogenic invasion. These developments have provided important insights into the mechanisms of broad host range necrotrophic pathogenicity. Over the past several years, several *S. sclerotiorum* virulence genes have been identified and functionally characterized hence revealing the complexity of its infection mechanism. In this review, infection models and virulence factors involved in the infection process were discussed, zooming in on the virulence factors involved in each stage of infection. Hence, this review has enhanced the conventional knowledge of the host plant's defence mechanism and the necrotrophic lifestyle of *S. sclerotiorum*. There is, however, room for improvement, viz a viz, identification of; novel genes involved in pathogenicity, novel virulence factors and their specific functions, confirmatory test for all the proposed functions of known virulence genes and functional study of the interaction between *S. sclerotiorum* and its host plants.

SIGNIFICANCE STATEMENT

This article identified and collated all the infection models of the plant pathogenic fungus, *Sclerotinia sclerotiorum*, highlighting *S. sclerotiorum* cell wall

degrading enzymes, effectors and oxalic acid as the three weapons for plant attack. This review article is a unique combination of the three different modes of infection by this plant pathogen. Hence, this review has enhanced the conventional knowledge of the necrotrophic lifestyle of *S. sclerotiorum*.

ACKNOWLEDGMENTS

The authors appreciate the financial contribution of the University of South Africa (UNISA) and the National Research Fund (NRF) for funding this research program.

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