We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter 2

Infection Mechanisms and Colonization Patterns of Fungi Associated with Soybean

Michelle L. Pawlowski and Glen L. Hartman

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62305

Abstract

Fungi have many kinds of unique associations with plants. These associations can benefit both the fungus and the plant, or can be detrimental to the plants and cause disease and even plant death. Land plants evolved over 425 million years ago, and fungi have been associated with their evolutionary development over the millennia. In reference to nutrient sequestration, fungal associations with plants are characterized as biotrophic, necrotrophic, or a mixture of these types. Biotrophs usually grow only on living plant tissue extracting nutrients from living plant cells. They can be pathogenic or symbiotic. In a symbiotic relationship, fungi gain carbon from the plant in exchange for nutrients and water unattainable by the plant. Necrotrophs promote host cell death to acquire nutrients for growth and reproduction. Each type of association is equipped with its own unique collection of biochemical and mechanical infection and colonization mechanisms. In turn, plants have evolved to have a complex network of genes to interact with a broad range of fungi. This chapter will provide an overview of three different types of fungal infection and colonization patterns with examples relevant to soybean as well as define defense mechanisms that the plant uses to interact with these microbes.

Keywords: Arbuscular mycorrhizal fungi, biotroph, *Glycine max*, hemibiotroph, necrotroph

1. Introduction

There is evidence based on molecular predictions to suggest that fungi evolved over one billion years ago [1]. Their associations with plants most likely began over 425 million years ago when plants began to colonize land [2]. There are well over 10,000 fungal species known to be associated with living plants and they range from beneficial to pathogenic. Fungal associations with plants can be characterized based on the different ways fungi infect and colonize host

INTECH
open science | open minds

© 2016 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

plants as well as how fungi use plants as a food source. Some fungal species cannot live without a host plant and are referred to as obligate biotrophs. Obligate biotrophs do not have a saprophytic independent life stage , normally only grow on living plants, and cannot be cultured. Fungi that derive their nutrition from dead tissue are referred to as necrotrophs. These have mechanisms to kill the host tissue prior to infection and colonization and tend to be easily cultured. Other fungal species that may have both biotrophic and necrotrophic phases in their life cycle are referred to as hemibiotrophs. We have chosen soybean [*Glycine max* (L.) Merr.] as the host to demonstrate these different associations that fungi have with plants.

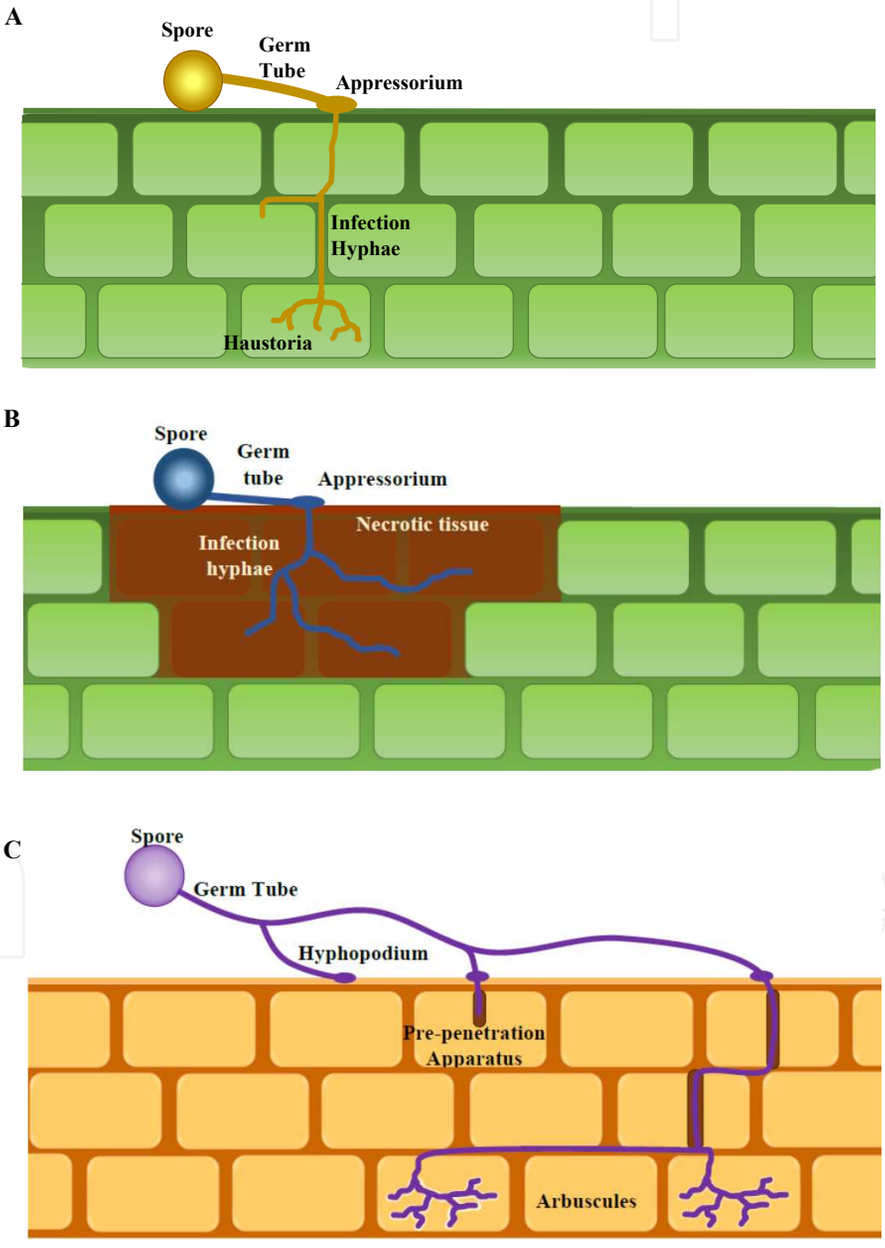


Figure 1. Generalized diagram of infection and colonization patterns of a) biotrophic pathogens; b) necrotrophic pathogens; and c) biotrophic mutualists.

http://dx.doi.org/10.5772/62305

Worldwide, soybean is the most important legume crop and the fourth leading crop in area of production [3]. There are many fungi associated with soybean plants and there are more than 40 named diseases [3]. Since soybean is planted in most cropping areas of the world, it has been exposed to many different fungi. We describe fungal interactions with soybean as biotrophs, necrotrophs, and hemibiotrophs and provide examples of these interactions as well as discuss the soybean response to both beneficial and pathogenic fungi.

2. Biotrophic pathogens

Biotrophs form infection structures to establish compatibility with the host and feed from living plant cells. The stages of interaction with the host may include a number/variety of infection structures as well as complex regulatory pathways established with the host. These regulatory pathways are not completely known for all infection structures due to the numerous types of biotrophic associations, but some generalizations can be made. Biotrophic fungi generally have (i) highly developed infection structures, (ii) limited lytic enzyme activity, (iii) interfacial layers made of carbohydrates and proteins that separate fungal and plant plasma membranes, (iv) long-term suppression of host defense mechanisms and (v) often develop specialized structures called haustoria for nutrient absorption and metabolism (Figure 1A) [4].

There are a number of diseases of soybean that are caused by biotrophic pathogens, including powdery mildew (*Erysiphe diffusa* (Cooke & Peck) U. Braun & S. Takam and soybean rust (*Phakopsora pachyrhizi* Syd.). In general, these pathogens have greater host specificity than pathogens classified as necrotrophs and often have developed more strain specificity with specific soybean genotypes, leading to gene-for-gene interactions and race development in the pathogen [3]. The example we are using as an obligate biotroph is *P. pachyrhizi*.

Like most rust fungi, *P. pachyrhizi* produces copious amounts of wind-blown spores called urediniospores. When these urediniospores land on a soybean leaf, they germinate and each forms a single germ tube of about 3 μm in width and of varying length from 100 μm or more before terminating into an appressorium that is approximately the same size as a urediniospore. The appressorium serves as an attachment structure at the site of penetration (Figure 2) [5, 6]. The appressorium develops an appressorial cone that initiates the penetration into the epidermal cell by turgor pressure independent of melanin accumulation [7]. The penetration hyphae grow through the epidermal cell and intercellular space, first forming primary invading hyphae, and then secondary hyphae populating intercellular spaces. In compatible interactions, primary haustoria form in the mesophyll cells, and within 12 days the formation of secondary haustoria occurs. A domed-shaped eruption occurs in the epidermis sometimes as early as 8 days after infection to form uredinia that produce urediniospores, causing the leaf to have a rusty-tan appearance. These urediniospores are wind-blown and serve as the source of inoculum for new infections.

When the soybean defense system is activated, an incompatible interaction occurs. In this case, fungal hyphae in the mesophyll cells become less common as mesophyll cells become necrotic (Figure 3). In addition, organized haustoria and uredinia do not develop, resulting in no

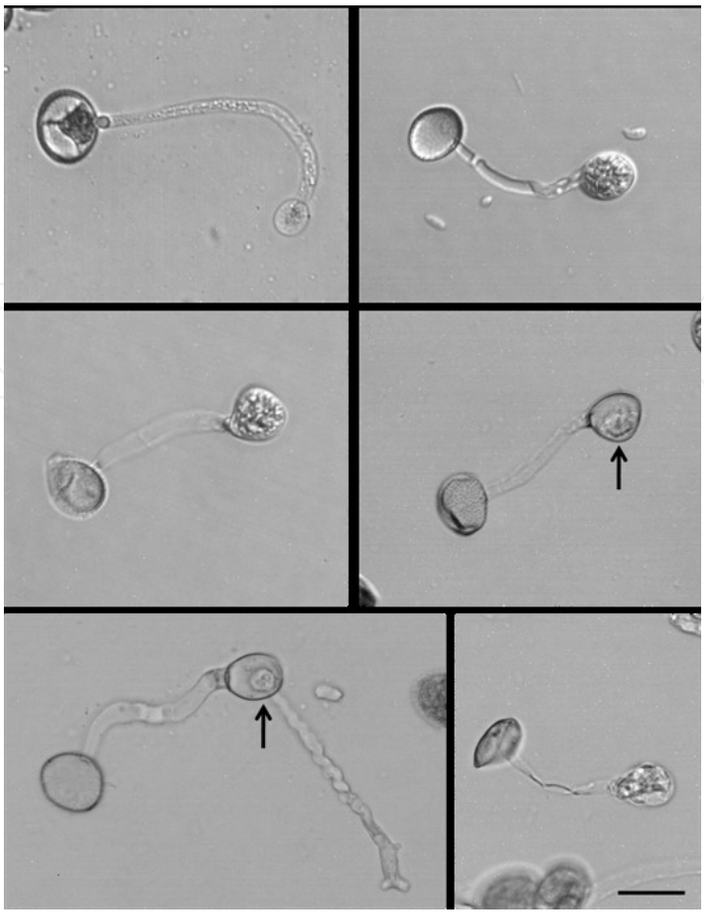


Figure 2. Development of *Phakopsorapachyrhizi* appressoria from 6, 8, 12, 24 to 48, respectively (top left to bottom left), and collapse of the spore and appressorium due to plasmolysis appressorium (bottom right). Arrows point to appressoria. Bar on bottom right represents 25 μm (Chang et al. 2014).

sporulation [5, 8]. The genetics of the incompatible interaction have been studied and six loci (*Rpp1* to *Rpp6*) are known to have dominant genes that segregate independently [9]. Some of these genes provide a strong or absolute resistance to certain isolates of the fungus while other genes show an incomplete resistance to certain isolates, which allows for sporulation although usually reduced compared to a compatible interaction [10].

A study that combined both histological development and fungal DNA quantification during infection and colonization [5] showed that early events of spore germination, appressorium formation, and fungal penetration of the epidermis occurred within 24 hours post-inoculation among the all tested soybean genotypes; differences in infection among genotypes were evident once the hyphae penetrated into the intercellular spaces between the mesophyll cells. For example, at 2 days after inoculation, the compatible soybean genotype Williams 82 had a higher percentage of hyphae in the mesophyll tissue than the incompatible soybean genotype, and the percentage of interaction sites with mesophyll cell death was higher in the most incompatible genotype starting at 3 days after inoculation. This study also reported a positive correlation between quantities of hyphae in the mesophyll cells and fungal DNA and demon-

http://dx.doi.org/10.5772/62305

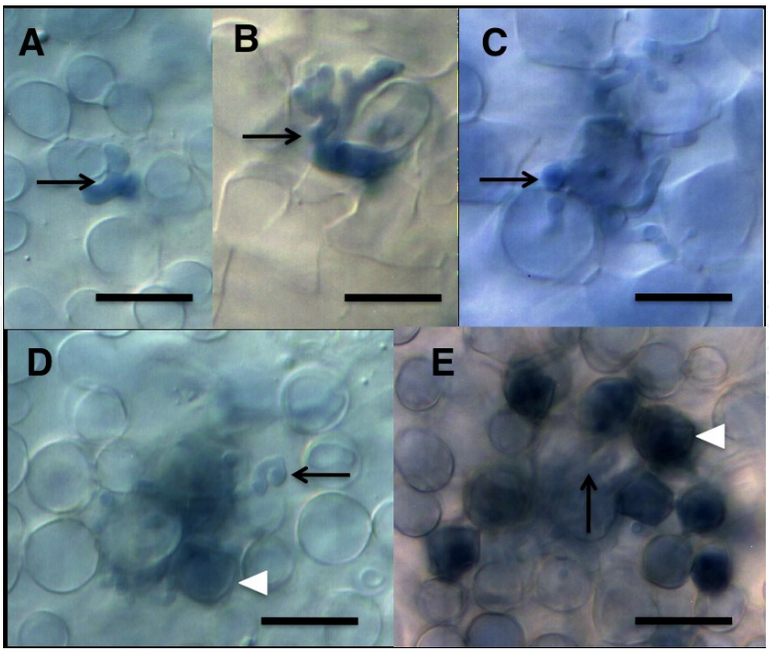


Figure 3. Infection of *Phakospora pachyrhizi* isolate FL07-1 on resistant soybean genotype PI 224268 observed between one to five days after inoculation (dai). The fungus entered the intercellular spaces of the mesophyll, gradually grew (see arrows) until 3 dai after which the growth was arrested by dying mesophyll cells, visualized by their retention of trypan blue stain (see arrow-heads). **A, B, and C,** Hyphae in the intercellular space of the mesophyll at 1, 2, and 3 dai, respectively. **D,** Slight discoloration of the mesophyll cells at 4 dai indicating the initiation of defense to arrest the spread of the hyphae. **E,** Increased discoloration of several mesophyll cells around and near the fungus at 5 dai. Leaves were stained with trypan blue and observed with differential interference contrast microscopy using an Olympus BX 51 microscope. Scale bar represents 20 μm (Vittal et al 2014).

strates that an incompatible soybean-*P. pachyrhizi* interaction results in restricted hyphal development in mesophyll cell tissue, likely due to hypersensitive apoptosis [5].

In another study, soybean transcriptome changes during soybean rust infection showed an early burst of gene expression at 12 h in both compatible and incompatible interactions [11]. This corresponds to the progression of urediniospore germination and appressorial formation, showing that these infection events induce dramatic changes in plant gene expression. Gene expression was much less from 1 to 2 days after inoculation, the period when hyphae entered into the mesophyll cells, with major differences in gene expression between compatible and incompatible genotypes noted at 3 days after inoculation, corresponding to haustoria formation and suggesting that genes expressed at 3 days after inoculation were involved in resistance gene-mediated defense responses [11]. In another study, the inoculation with a *P. pachyrhizi* isolate and an incompatible soybean genotype showed an up-regulation of peroxidases with an oxidative burst in infected cells that triggered programmed cell death or apoptosis [12].

The soybean rust pathogen has a wide host range of legumes specifically those in the subfamily Papilionoideae [13]. Although it is not known if these hosts have similar infection and colonization processes to soybean, some studies have focused on the interaction of this fungus with non-hosts. For example, it was shown that plants of *Arabidopsis thaliana* (L.) Heynh. inoculated with *P. pachyrhizi* urediniospores germinated and penetrated the epidermal cells,

but did not grow either inter- or intracellularly into the mesophyll tissue layer [14]. In *Hordeum vulgare* L., it was shown that fungal urediniospores germinated, but mostly failed to penetrate through the cuticle and the epidermal cells; however, when the fungus successfully penetrated, the epidermal cells died and colonization was arrested by the hypersensitive collapse of the mesophyll cells [15]. In a study using *Medicago truncatula* Gärtner as a non-host, urediniospores germinated to form appressoria and the fungus penetrated into the epidermal cells, but the fungus did not sporulate [16]. Furthermore, this study showed that genes that produced mecicarpan and chlorophyll catabolism were induced by *M. truncatula* soon after infection.

3. Necrotrophs

Necrotrophic pathogens survive by killing plant tissue to obtain nutrients and advance through colonization of dead tissue. Historically, necrotrophic infection was not considered a specific host-pathogen interaction because these fungi often excrete phytotoxins and cell wall degrading enzymes to kill host tissue with no reciprocating response from the host. Some have characteristics of both biotrophs and necrotrophs and are classified as hemibiotrophs, such as in the genus *Colletotrichum*. Hemibiotrophs will be covered in the next section.

Initial stages of infection by necrotrophs often start when conidia germinate and form infection hyphae that directly penetrate or the fungus develops appressoria that form penetration pegs to penetrate the epidermis (Figure 1B) [17]. Appressoria excrete a number of lytic enzymes including oxidases, cutinases, and lipases to degrade the plant cuticle and wax layers. The penetration pegs breach through the compromised epidermal cells and secrete various cell wall degrading enzymes (CWDEs) to kill cells and progress through the plant causing necrotic lesions and, in some cases, plant death. These enzymes are used by necrotrophs to break down cells and gain access to host nutrients and carbon. *Macrophomina phaseolina* (Tassi) Goid. isolates are known to produce an abundance of CWDEs including hydrolytic and lignin degradation enzymes [18]. Necrotrophs will also commonly secrete phytotoxins to facilitate colonization. These can be broad spectrum, such as (-)-botryodiplodin and phaseolinone produced by *M. phaseolina* (Radwan et al. 2014), or host-specific, such as tomato-specific AAL-toxins produced by *Alternaria alternate* f. sp. *lycopersici* Keissl. [19]. Botrydial, a toxin produced by *Botrytis cinerea* Pers., will cause chlorosis, cell collapse, and aids in fungal penetration of tissues [20]. Botrydial, similar to other necrotrophic phytotoxins, may contribute to the host unspecificity of the fungus [20].

There are several necrotrophs that infect and colonize soybean. Root pathogens *Cadophora gregata* (Allington & D.W. Chamb.) T.C. Harr. & McNew (brown stem rot), *Fusarium virguliforme* O'Donnell & T. Aoki (sudden death syndrome), *M. phaseolina*, and *Rhizoctonia solani* J.G. Kühn (Rhizoctonia damping off and root rot) among others are common to soybean are ubiquitous and have a broad host range [3]. Necrotrophs can also be above ground pathogens like *Cercospora kikuchii* T. Matsumoto & Tomoy. (purple seed stain), *Cercospora sojina* K. Hara (frogeye leaf spot), and *Septoria glycines* Hemmi (brown spot) that are also ubiquitous [3].

In some necrotrophs, there is a sophisticated microbe-host interaction where the pathogen takes over host defenses. *Sclerotinia sclerotiorum* (Lib.) de Bary has previously been considered

http://dx.doi.org/10.5772/62305

a primitive fungal pathogen, but actually has a sophisticated interaction with the host plant. The pathogen survives as sclerotia in the soil that germinate to produce mycelia or produce apothecia that contain ascospores. Apothecia are sexual fruiting bodies produced on a stipe that emerge from sclerotia [21]. When there is a relative change in humidity or a physical disturbance, mature apothecia forcibly eject ascospores in a puff. The majority of ascospores will remain in the field where they were produced; however, they can also be carried several kilometers in air currents [21].

The infection process begins when ascospores germinate on host tissue. To germinate, ascospores need free water and an exogenous nutrient source to grow like that of senescing flower petals. As the fungus colonizes these petals, it excretes oxalic acid and kills cells for further growth on the peduncle or onto a petiole or a leaf [21]. A study compared the infection patterns of *S. sclerotiorum* on cotyledons of a susceptible and a resistance cultivar of *Brassica napus* L. and showed that fungal hyphae grew along the surface of the plant and directly penetrated the cuticle by forming an appressorium or indirectly through stomata [22]. This process differed between susceptible and resistant genotypes after 24 hours post-inoculation when it was observed that there were longer hyphal strands on the susceptible genotype followed by dichotomous branching of terminal hyphae and appressorium development. In the resistant genotype, the fungus produced swollen hyphal apices and hyphal progression was restricted to the epidermal layer while the fungus continued to grow in the susceptible genotype and was able to colonize the mesophyll cells causing cell death. Hyphal cell collapse was also observed only on the resistant genotype. These contrasting interactions during infection and colonization between the susceptible and resistant genotypes indicates signaling between fungal growth and the host occurred at first contact or soon after and caused the fungus to be suppressed in the resistant genotype and not suppressed in the susceptible genotype as appressoria formed and the plant became colonized.

Gene expression profiling was used to characterize gene expression differences of a highly susceptible soybean cultivar and a partially resistant cultivar during the early stages of *S. sclerotiorum* infection [23]. Over 100 genes of interest including genes regulating cell wall composition, signaling pathways, and anthocyanin and anthocyanidin synthesis were reported. The fungus also has over 100 genes that encode for CWDEs and genes for the production of the phytotoxin sclerin [17]. In addition, the fungus produces and secretes oxalic acid [24, 25], which in itself is toxic to the plant, but also modulates a number of plant physiological processes that allow the fungus to invade unrecognized by the host. This phenomenon is similar to biotrophic pathogens, and newer information has stated that *S. sclerotiorum* is a hemibiotroph [26]. The main role of oxalic acid, or oxalate, in pathogenicity is the manipulation of host defense mechanisms. Initially, oxalate renders the reactive oxygen species (ROS) response ineffective. The oxidative burst and callose deposition is an initial defense mechanism to inhibit further colonization. In later stages, the fungus triggers the ROS mechanisms to initiate programmed cell death [25]. The fungal hyphae are then able to colonize the dead tissue and continue this process down the petiole and through the stem of the soybean eventually killing the entire plant (Figure 4). Oxalic acid is also involved in pH reduction, guard cell regulation, and chelation of calcium [25].

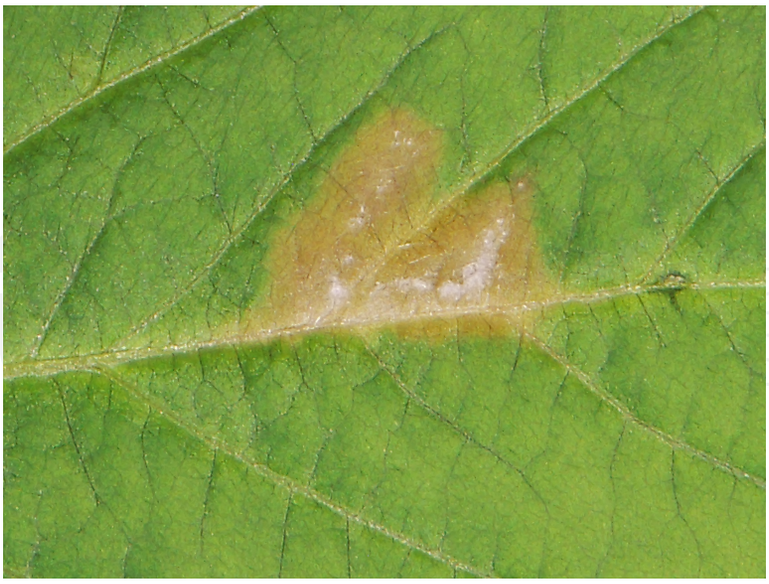


Figure 4. Necrotic tissue and water-soaking on a soybean leaf infected with *Sclerotinia sclerotiorum*.

4. Hemibiotrophs

There are some pathogens that deploy both biotrophic and necrotrophic mechanisms for infection and colonization and are considered by some to be the best-armed phytopathogens [27]. Commonly, a hemibiotrophic fungus has a biotrophic phase where it secures a relationship with the host plant before switching to a necrotrophic phase to obtain nutrients and colonize the tissue. Some pathogens that have traditionally been classified as necrotrophs exhibit some biotrophic characteristics.

Genomic and transcriptomic analyses of species in the hemibiotrophic *Colletotrichum* genus indicates that they use a wide range of biochemical processes to combat host defenses [27-29]. Although differences were observed between species, hundreds of genes that encode small, secreted proteins were found and proposed to be effector molecule homologues to other biotrophic fungi [27]. Interestingly, species in the genus *Colletotrichum* contain the largest collection of proteases and carbohydrate-active enzymes of any fungus. They also produce secondary metabolites, which are commonly phytotoxic, in both their biotrophic and necrotrophic phases [27].

There are a number of species of *Colletotrichum* that infect soybean [30, 31]. *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore is one of the species that causes anthracnose of soybean. The fungus can infect the soybean plant at any growth stage, but symptoms are most commonly observed as the plant matures. The disease symptoms are more prevalent in warm and humid conditions, and it causes poor stand and seed quality issues with reduction in yields by up to 20% [32]. Symptoms of anthracnose include irregularly shaped, brown to black blotches, and sunken cankers on stems, petioles, and pods. Infected leaves will be shrunken, rolled, or wilted with necrotic laminar veins [31].

http://dx.doi.org/10.5772/62305

The pathogen is commonly seed-borne but can persist in plant debris as mycelia or acervuli, an asexual fruiting body. In conducive conditions, the acervuli will produce colorless conidiophores and abundant conidia (Figure 5A). Conidia are frequently dispersed onto plant tissues by splashing rain. Infection begins when conidia germinate on plant tissue, forming a melanized appressorium to directly infect the cuticle layer of the host. Inside the sub-cuticular layer, the appressoria (Figure 5B) form primary infection hyphae, unrecognizable to the host, that grow along the cell walls and branch profusely to colonize the intercellular space [33]. The necrotrophic phase of the pathogen starts when the secondary hyphae emerge from the primary hyphae and quickly penetrate the epidermal and mesophyll cells. The cells collapse causing necrotic lesions. As early as 36 hours after infection, acervuli will develop [32]. These structures overwinter on plant debris until conditions in the spring allow for the cycle to repeat.

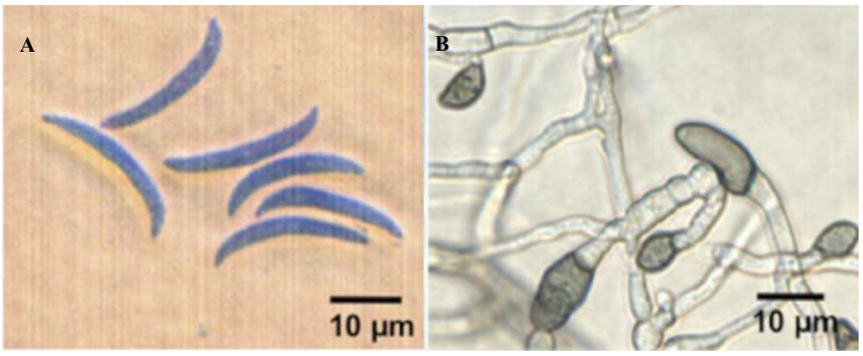


Figure 5. A) *Colletotrichum truncatum* conidia stained with trypan blue and B) appressoria (Yang et al. 2014).

5. Beneficial fungi

The phytobiome is the area surrounding a plant that associates or influences plant growth; it includes microbes, insects, nematodes, and abiotic factors, such as temperature and moisture [34]. A major part of the phytobiome is the rhizosphere. The rhizosphere is the soil surrounding the root tissues, a few millimeters thick, and potentially support 10^{11} microbial cells/g of soil. In grasslands, fungi made up 20-66% of the microbial biomass in the rhizosphere [35]. Some of these microbes can be pathogens, but many of these microbes have beneficial attributes to offer the plant. It has been well known that beneficial microbes in the rhizosphere have a crucial impact on host health, including uptake of nutrients, disease suppression, and host immunity [36]. These include mycorrhizal fungi and fungal endophytes.

Mycorrhizal fungi are one group of fungi that often benefit plants and have a mutualistic, biotrophic relationship. Mycorrhizal fungi are categorized into arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi, and orchid mycorrhizal fungi. Many of these fungal species are credited with helping plants first move onto land [37]. With over 200 species, AMF are ubiquitous and survive in a broad range of environments and form mutualistic associations with approximately 80% of all land plants. AMF are found in the rhizosphere and have a positive impact on the host by moving nutrients and water from outside the rhizosphere to

give to the host in return for carbon. In order for these nutrients to move efficiently, these fungi infect and colonize the cortex of plants. This sets off innate defense responses, and mycorrhizal fungi have a sophisticated repertoire of mechanisms to maintain a mutualistic relationship with the host.

Soybean plants colonized by AMF have shown greater drought tolerance and an increase capacity to uptake nutrients [38-40]. All AMF have a broad host range, but there is some host preference among crops. *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler and *Glomus* spp. Tul. & C. Tul. are AMF commonly associated with soybean [41, 42]. Much of the basic research of AMF with legumes has been on *M. truncatula* and not soybeans, although the findings may be applicable to soybean plants as well.

AMF interact with their host well before any physical contact occurs. Since AMF are obligate biotrophs, presymbiotic signaling between the plant and the fungus is required for spore germination [43]. The first step to initiate this symbiotic relationship originates from the plant when grown under low orthophosphate conditions. The plant releases strigolactones through root exudates that stimulate AMF spore germination [43-45]. Flavonoids from the plant have also been associated with AMF spore germination, but their role in pre-symbiosis is not known. The fungus recognizes strigolactones and/or other potential signals that activate mobilization of triacylglycerides and glycogen within the spores to produce germ tubes and extra radical hyphae. This carbon storage can maintain growth for a couple days while the fungus is finding and establishing a relationship with the plant. In absence of plant signals, a germinated spore is able to retract fungal cytoplasm for a later attempt [43].

Reciprocating fungal diffusible signals following host recognition stimulate a counter response from the plant. Two fungal diffusible signals have been identified as short- and long-chain chitin oligomers [46, 47]. These signals will stimulate expression of the early nodulation gene, ENOD11, and activate the common symbiosis signaling pathway [43, 45]. This pathway is comprised of cation channels, a leucine-rich repeat receptor kinase, and nuclear porins all of which are required to induce calcium spiking along with a calcium/calmodulin-dependent kinase [45]. Lateral root formation is also stimulated by diffusible signals, potentially by a separate pathway.

Once in contact with the root tissues, AMF hyphae will move along the root surface of young, lateral roots to find an ideal infection location. The root surface provides cues to the fungus to adhere to the host and establish what is known as a hyphopodium (Figure 1C) [45, 47]. This was shown when *Gigaspora gigantea* (T.H. Nicolson & Gerd.) Gerd. & Trappe colonized cell wall fragments of a carrot host but did not colonize root fragments of a non-host, common beet [48]. Hyphopodia formation is severely reduced on plant mutants (*required for arbuscular mycorrhiza, ram1* and *ram2*). [45]. RAM1 encodes for a gibberellic acid-insensitive transcription factor upstream of RAM2, a glycerol-3-phosphate acyl transferase, and produces cutin monomers [49]. Cutin monomers trigger appressorium induction in a number of pathogenic fungi indicating it could be a conserved feature among all fungi that colonize plants [45].

Following hyphopodia formation, the plant epidermal cells prepare for colonization by restructuring the cytoplasm (Figure 1C) [47, 50]. The nucleus of the cell moves toward the contact site followed by reorganization of microtubules and actin bundles between the nucleus and the contact site. The nucleus then migrates towards the underlying cell causing more

http://dx.doi.org/10.5772/62305

reorganization of microtubules to eventually form a column through the plant cell. This structure is termed the pre-penetration apparatus. Once the pre-penetration apparatus is established, the fungus will penetrate the cell directly and follow through the pre-penetration apparatus to not destroy the integrity of the cell. This compartment is now part of the apoplast [47, 50]. The hyphae will branch out in the apoplast colonizing the cortex of the plant and arbuscules will become established (Figure 1C).

Arbuscules are ephemeral structures that are engulfed by plant cells to deliver nutrients to the host in exchange for carbon. Arbuscules are formed by repeated branching of intercellular hyphae that connects to the plant cell by a periarbuscular membrane [51]. The periarbuscular membrane is the center of nutrient transfer. To form this association, extreme re-programming of the inhabited cortical cell transcriptome is required. Over 500 protein-coding genes are upregulated in mycorrhizal plant cells [51]. Proliferation of endoplasmic reticulum, and Golgi apparatus, increase and deployment of plastids, and enlargement of the nucleus are some actions of the host cell needed to accommodate an arbuscule [51]. Individual arbuscules live up to five days, but are continuously being renewed in other cells so there is still a plethora of benefits to the plant including increased disease resistance, drought tolerance, and nutrient uptake. Roughly 20% of the carbon sequestered from photosynthesis is transferred to the fungus through these arbuscules.

Phosphate is an essential nutrient involved in photosynthesis and energy production. Orthophosphate is found in low concentrations in the soil and relatively immobile so it is depleted quickly from the rhizosphere [44, 45]. Using its extra-radial mycelia, AMF can reach beyond the rhizosphere to uptake orthophosphate. Within the extra-radial mycelia, orthophosphate is converted to polyphosphate until it reaches the inter-radial mycelia where it is converted back to orthophosphate and brought to the plant via phosphate transporters.

Though phosphate has been the main focus in AMF research, these fungi also can transfer other nutrients, such as nitrogen. Two nitrogen transporters are located in the extra and inter-radial mycelia of *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler [52]. Three ammonium transporter genes were discovered in *Geosiphon pyriformis* (Kütz.) F. Wettst. showing is also taken up in the form of ammonium. A nitrate reductase, two nitrite reductases, a glutamine synthetase (GS), and a putative glutamate synthase (GOGAT) transcripts have all been identified in *R. irregularis*, which points to a potential extension of the GS/GOGAT cycle within the extra-radial mycelia of the fungus [44].

Fungal endophytes are another group of organisms that form a symbiotic relationship with their host plant and colonize all parts of the plant. Endophytes emit beneficial compounds such as phytohormones like auxin and gibberellins and secondary metabolites that have shown antimicrobial activity [53]. Genera found to include endophytes associated with soybean are *Alternaria, Cladsporium, Diaporthe,* and *Epicoccum* [54]. Although their mechanisms for host-microbe interactions are not well-known, endophytic fungi have gained attention in recent years as potential amendments in agriculture because of their positive impacts on plant health by promoting nutrient uptake, water acquisition, and by providing protection from abiotic and biotic stresses [53].

6. Influences on infection and colonization

The fungi portrayed in this chapter show many examples of the complex and effective host mechanisms that fungi have to overcome or even manipulate. Most of the fungal-plant interactions have been studied under highly controlled laboratory environments where most factors can be controlled. In the field, the plant interacts with an array of microbes simultaneously along with abiotic stresses, which can alter interactions among organisms. The success of a pathogen is based on the disease triangle (virulent pathogen, susceptible host, and a conducive environment).

The benefits of mycorrhizal associations are influenced by the environment and are considered to have a mutualistic-parasitic continuum [55]. Mycorrhizal fungi do not always benefit the plant, and can negatively impact biomass depending on external factors, including microbial populations, nutrient composition, and soil pH. AMF provide nutrients to their hosts in return for carbon, but in high quality soils, this assistance may not have a substantial benefit to the plant and the fungus may reduce carbon thus reducing biomass as well as directly compete for host resources.

7. Plant defense pathways

How fungi infect plants depends on the type of fungus, the plant, and the association. The association includes how the plant defends itself or whether it allows fungal infection and colonization. In response to fungi, plants have evolved different ways to recognize fungi through a network of different plant processes. Several processes important in plant defense are activated through the salicylic acid (SA) and the jasmonic acid (JA) pathways [56]. The SA pathway uses SA as a signaling molecule that is active in defense against biotrophic pathogens. SA is synthesized through the phenylalanine ammonia-lyase (PAL) and the isochorismate synthase (ICS) pathways, and accumulation of SA will induce a systemic immune response throughout the plant termed systemic acquired resistance (SAR). This induces *pathogenesis-related* (*PR*) genes, that encode for enzymes such as chitinases and β-1,3-glucanases that affect fungal cell walls [57]. The JA pathway is SA-independent and is traditionally attributed to immunity against necrotrophic pathogens. JA induces transcripts *DAD1, LOX2, AOS,* and *OPR3* as well as PR genes [58]. JA also stimulates induced systemic resistance, a systemic resistance similar to SAR by defending the plant against a broad range of pathogens [59]. There are also immune response-independent mechanisms that are involved in plant defense. Some pathogens have evolved to manipulate or surpass defense responses; therefore, plants require immune response-independent factors for survival. Four transcription factors in *Arabidopsis* were found to be required for host resistance to necrotrophs [60]. Mutants without one or more of these transcription factors were susceptible to infection without compromising either the SA- or JA-defense pathways indicating there are other biological processes involved in host defense.

AMF have a sophisticated relationship with the host defense pathways to maintain a symbiotic relationship. Similar to most biotrophs, AMF stimulate SAR by the SA-dependent defense pathway and activate defense compound production such as ROS [61]. SA-induced defense is a negative regulator of AMF symbiosis and is quickly repressed by the fungus by Myc factors

http://dx.doi.org/10.5772/62305

and effectors. A superoxide dismutase has been characterized in *R. irregularis* and *Gigaspora margarita* W.N. Becker & I.R. Hall to reduce the amount of ROS [44]. In *R. intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler, the SP7 effector has shown defense-suppressive activity and interacts with the ethylene-responsive factor ERF19 [62]. JA and ET pathways are upregulated in well-established symbiosis and have a positive impact on colonization of pathogens related to these pathways, such as necrotrophs and chewing herbivores.

8. Summary

Associations of fungi and plants are a continuum from beneficial to mutualistic to pathogenic. Based on how fungi obtain nutrients from plants, they can be classified as biotrophs, necrotrophs, or hemibiotrophs. Biotrophic fungi, such as *P. pachyrhizi* and arbuscular mycorrhizal fungi, grow only on living plant tissue, extracting nutrients from living plant cells. On the other hand, necrotrophic fungi, such as *S. sclerotiorum*, acquire nutrients for growth and reproduction from dead plant tissue. Some of these necrotrophic fungi also have a biotrophic phase in the life cycle, making them hemibiotrophs, such as *C. truncatum*.

Plants have developed a complex network of biosynthetic pathways, some of which are known to respond to fungal infection and colonization. In turn, fungi have developed sophisticated infection and colonization mechanisms to interact with the host. Advances in technology have allowed for a better perspective on this association in terms of understanding the specific protein-protein interactions and genetic sequencing to find genes controlling these processes. These genes can be directly inserted into plants through genetic engineering. As an example, non–host-linked genes in *A. thaliana* have been transferred to soybean to enhance resistance to soybean rust [63]. In the future, the exploitation of interspecies non–host-linked genes could be used to genetically engineer crops to control diseases or enhance beneficial fungal associations with plants.

Acknowledgements

We would like to thank Drs. Chang, Vittal, and Yang for their willingness to provide images and Theresa Herman for editing assistance.

Author details

Michelle L. Pawlowski[1] and Glen L. Hartman[1,2*]

*Address all correspondence to: hartman@Illinois.edu

1 University of Illinois, Department of Crop Sciences, USA

2 USDA-ARS, National Soybean Research Center, Urbana, IL, USA

References

[1] Parfrey, LW, Lahr, DJ, Knoll, AH, Katz, LA. Estimating the timing of early eukaryotic diversification with multigene molecular clocks. Proceedings of the National Academy of Sciences. 2011; 108: 13624-13629.

[2] Redecker, D, Kodner, R, Graham, LE. Glomalean fungi from the Ordovician. Science. 2000; 289: 1920-1921.

[3] Hartman, GL, Rupe, JC, Sikora, EJ, Domier, LL, Steffey, KL, Davis, JA. Compendium of Soybean Diseases and Pests, fifth edition 2015.

[4] Mendgen, K, Hahn, M. Plant infection and the establishment of fungal biotrophy. Trends in Plant Science. 2002; 7: 352-356.

[5] Vittal, R, Paul, C, Hill, C, Hartman, G. Characterization and quantification of fungal colonization of *Phakopsora pachyrhizi* in soybean genotypes. Phytopathology. 2014; 104: 86-94.

[6] Koch, E, Ebrahim-Nesbat, F, Hoppe, H. Structural studies on the development of soybean rust (*Phakopsora pachyrhizi* syd.) in susceptible soybean leaves. Phytopathology Z. 1983;106:302-320. 1983.

[7] Chang, H-X, Miller, LA, Hartman, GL. Melanin-independent accumulation of turgor pressure in appressoria of *Phakopsora pachyrhizi* . Phytopathology. 2014; 104: 977-984.

[8] McLean, R. Histological studies of resistance to soybean rust, *Phakopsora pachyrhizi* syd. Crop and Pasture Science. 1979; 30: 77-84.

[9] Kelly, H, Dufault, NS, Walker, D, Isard, S, Schneider, RW, Giesler, L, Wright, DL, Marois, JJ, Hartman, G. From select agent to an established pathogen: The response to *Phakopsora pachyrhizi* in North america. Phytopathology. 2015; 105:905-916.

[10] Miles, M, Bonde, M, Nester, S, Berner, D, Frederick, R, Hartman, G. Characterizing resistance to *Phakopsora pachyrhizi* in soybean. Plant Disease. 2011; 95: 577-581.

[11] Schneider, KT, Van DE Mortel, M, Bancroft, TJ, Braun, E, Nettleton, D, Nelson, RT, Frederick, RD, Baum, TJ, Graham, MA, Whitham, SA. Biphasic gene expression changes elicited by *Phakopsora pachyrhizi* in soybean correlate with fungal penetration and haustoria formation. Plant Physiology. 2011; 157: 355-371.

[12] Choi, J, Alkharouf, N, Schneider, K, Matthews, B, Frederick, R. Expression patterns in soybean resistant to *Phakopsora pachyrhizi* reveal the importance of peroxidases and lipoxygenases. Functional & Integrative Genomics. 2008; 8: 341-359.

[13] Slaminko, T, Miles, M, Frederick, R, Bonde, M, Hartman, G. New legume hosts of *Phakopsora pachyrhizi* based on greenhouse evaluations. Plant Disease. 2008; 92: 767-771.

[14] Loehrer, M, Langenbach, C, Goellner, K, Conrath, U, Schaffrath, U. Characterization of nonhost resistance of Arabidopsis to the asian soybean rust. Molecular Plant-Microbe Interactions. 2008; 21: 1421-1430.

[15] Hoefle, C, Loehrer, M, Schaffrath, U, Frank, M, Schultheiss, H, Hückelhoven, R. Transgenic suppression of cell death limits penetration success of the soybean rust fungus *Phakopsora pachyrhizi* into epidermal cells of barley. Phytopathology. 2009; 99: 220-226.

[16] Ishiga, Y, Uppalapati, SR, Gill, US, Huhman, D, Tang, Y, Mysore, KS. Transcriptomic and metabolomic analyses identify a role for chlorophyll catabolism and phytoalexin during Medicago nonhost resistance against asian soybean rust. Scientific reports. 2015; 5:1-17.

[17] Laluk, K, Mengiste, T. Necrotroph attacks on plants: Wanton destruction or covert extortion? The Arabidopsis book / American Society of Plant Biologists. 2010; 8:1-34.

[18] Radwan, O, Rouhana, LV, Hartman, GL, Korban, SS. Genetic Mechanisms of Host–Pathogen Interactions for Charcoal Rot in Soybean. Plant molecular biology reporter. 2014; 32: 617-629.

[19] Wolpert, TJ, Dunkle, LD, Ciuffetti, LM. Host-selective toxins and avirulence determinants: What's in a name?*. Annual Review of Phytopathology. 2002; 40: 251-285.

[20] Colmenares, AJ, Aleu, J, Duran-Patron, R, Collado, IG, Hernandez-Galan, R. The Putative Role of Botrydial and Related Metabolites in the Infection Mechanism of *Botrytis cinerea*. Journal of Chemical Ecology. 2002; 28: 997-1005.

[21] Bolton, MD, Thomma, B, Nelson, BD. *Sclerotinia sclerotiorum* (lib.) de bary: Biology and molecular traits of a cosmopolitan pathogen. Molecular Plant Pathology. 2006; 7: 1-16.

[22] Garg, H, Li, H, Sivasithamparam, K, Kuo, J, Barbetti, MJ. The infection processes of *Sclerotinia sclerotiorum* in cotyledon tissue of a resistant and a susceptible genotype of *Brassica napus*. Annals of Botany. 2010; 106: 897-908.

[23] Calla, B, Vuong, T, Radwan, O, Hartman, GL, Clough, SJ. Gene expression profiling soybean stem tissue early response to *Sclerotinia sclerotiorum* and in silico mapping in relation to resistance markers. Plant Genome. 2009; 2: 149-166.

[24] Amselem, J, Cuomo, CA, van Kan, JAL, Viaud, M, Benito, EP, Couloux, A, Coutinho, PM, de Vries, RP, Dyer, PS, Fillinger, S, Fournier, E, Gout, L, Hahn, M, Kohn, LM, Lapalu, N, Plummer, KM, Pradier, JM, Quevillon, E, Sharon, A, Simon, A, ten Have, A, Tudzynski, B, Tudzynski, P, Wincker, P, Andrew, M, Anthouard, V, Beever, RE, Beffa, R, Benoit, I, Bouzid, O, Brault, B, Chen, ZH, Choquer, M, Collemare, J, Cotton, P, Danchin, EG, Da Silva, C, Gautier, A, Giraud, C, Giraud, T, Gonzalez, C, Grossetete, S, Guldener, U, Henrissat, B, Howlett, BJ, Kodira, C, Kretschmer, M, Lappartient, A, Leroch, M, Levis, C, Mauceli, E, Neuveglise, C, Oeser, B, Pearson, M, Poulain, J, Poussereau, N, Quesneville, H, Rascle, C, Schumacher, J, Segurens, B, Sex-

ton, A, Silva, E, Sirven, C, Soanes, DM, Talbot, NJ, Templeton, M, Yandava, C, Yarden, O, Zeng, QD, Rollins, JA, Lebrun, MH, Dickman, M. Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. Plos Genetics. 2011; 7. 1-27.

[25] Rollins, JA, Cuomo, CA, Dickman, MB, Kohn, LM. Genomics of *Sclerotinia Sclerotiorum*. In: Genomics of Plant-Associated Fungi and Oomycetes: Dicot pathogens, Springer; 2014. 1-17.

[26] Kabbage, M, Yarden, O, Dickman, MB. Pathogenic attributes of *Sclerotinia sclerotiorum*: Switching from a biotrophic to necrotrophic lifestyle. Plant Science. 2015; 233: 53-60.

[27] McDowell, JM. Genomic and transcriptomic insights into lifestyle transitions of a hemi-biotrophic fungal pathogen. New Phytologist. 2013; 197: 1032-1034.

[28] Gan, P, Ikeda, K, Irieda, H, Narusaka, M, O'Connell, RJ, Narusaka, Y, Takano, Y, Kubo, Y, Shirasu, K. Comparative genomic and transcriptomic analyses reveal the hemibiotrophic stage shift of *Colletotrichum* fungi. New Phytologist. 2013; 197: 1236-1249.

[29] O'Connell, RJ, Thon, MR, Hacquard, S, Amyotte, SG, Kleemann, J, Torres, MF, Damm, U, Buiate, EA, Epstein, L, Alkan, N, Altmuller, J, Alvarado-Balderrama, L, Bauser, CA, Becker, C, Birren, BW, Chen, ZH, Choi, J, Crouch, JA, Duvick, JP, Farman, MA, Gan, P, Heiman, D, Henrissat, B, Howard, RJ, Kabbage, M, Koch, C, Kracher, B, Kubo, Y, Law, AD, Lebrun, MH, Lee, YH, Miyara, I, Moore, N, Neumann, U, Nordstrom, K, Panaccione, DG, Panstruga, R, Place, M, Proctor, RH, Prusky, D, Rech, G, Reinhardt, R, Rollins, JA, Rounsley, S, Schardl, CL, Schwartz, DC, Shenoy, N, Shirasu, K, Sikhakolli, UR, Stuber, K, Sukno, SA, Sweigard, JA, Takano, Y, Takahara, H, Trail, F, van der Does, HC, Voll, LM, Will, I, Young, S, Zeng, QD, Zhang, JZ, Zhou, SG, Dickman, MB, Schulze-Lefert, P, van Themaat, EVL, Ma, LJ, Vaillancourt, LJ. Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. Nature Genetics. 2012; 44: 1060-1065.

[30] Yang, HC, Haudenshield, JS, Hartman, GL. *Colletotrichum incanum* sp nov., a curved-conidial species causing soybean anthracnose in USA. Mycologia. 2014; 106: 32-42.

[31] Yang, HC, Haudenshield, JS, Hartman, GL. Multiplex real-time pcr detection and differentiation of *Colletotrichum* species infecting soybean. Plant Disease. 2015; 99: 1559-1568.

[32] Manandhar, JB, Kunwar, IK, Singh, T, Hartman, GL, Sinclair, JB. Penetration and infection of soybean leaf tissues by *Colletotrichum truncatum* and *Glomerella glycines*. Phytopathology. 1985; 75: 704-708.

[33] Ranathunge, NP, Mongkolporn, O, Ford, R, Taylor, PWJ. *Colletotrichum truncatum* pathosystem on *Capsicum* spp: Infection, colonization and defence mechanisms. Australasian Plant Pathology. 2012; 41: 463-473.

[34] Beattie, G., and J. E. Leach. "The Phytobiomes Initiative." Phytopathology: 2014; 104:166-166.

[35] Joergensen, RG. Ergosterol and microbial biomass in the rhizosphere of grassland soils. Soil Biology & Biochemistry. 2000; 32: 647-652.

[36] Berendsen, RL, Pieterse, CMJ, Bakker, PAHM. The rhizosphere microbiome and plant health. Trends in Plant Science. 2012; 17: 478-486.

[37] Akhtar, MS, Siddiqui, ZA, Wiemken, A. Arbuscular mycorrhizal fungi and rhizobium to control plant fungal diseases. New York, Springer; 2011. 263-292.

[38] Liu, ZL, Li, YJ, Ma, LN, Wei, HC, Zhang, JF, He, XY, Tian, CJ. Coordinated regulation of arbuscular mycorrhizal fungi and soybean mapk pathway genes improved mycorrhizal soybean drought tolerance. Molecular Plant-Microbe Interactions. 2015; 28: 408-419.

[39] Meng, LB, Zhang, AY, Wang, F, Han, XG, Wang, DJ, Li, SM. Arbuscular mycorrhizal fungi and rhizobium facilitate nitrogen uptake and transfer in soybean/maize intercropping system. Frontiers in Plant Science. 2015; 6:1-10.

[40] Abdel-Fattah, GM, Asrar, AA, Al-Amri, SM, Abdel-Salam, EM. Influence of arbuscular mycorrhiza and phosphorus fertilization on the gas exchange, growth and phosphatase activity of soybean (glycine max l.) plants. Photosynthetica. 2014; 52: 581-588.

[41] Jie, WG, Liu, XR, Cai, BY. Diversity of rhizosphere soil arbuscular mycorrhizal fungi in various soybean cultivars under different continuous cropping regimes. Plos One. 2013; 8:1-9.

[42] Kojima, T, Oka, N, Karasawa, T, Okazaki, K, Ando, S, Takebe, M. Community of arbuscular mycorrhizal fungi in soybean roots after cultivation with different cropping systems. Jarq-Japan Agricultural Research Quarterly. 2014; 48: 279-290.

[43] Schmitz, AM, Harrison, MJ. Signaling events during initiation of arbuscular mycorrhizal symbiosis. Journal of Integrative Plant Biology. 2014; 56: 250-261.

[44] Malbreil, M, Tisserant, E, Martin, F, Roux, C. Genomics of arbuscular mycorrhizal fungi: Out of the shadows. In: Fungi. F. M. Martin; 2014. 70: 259-290.

[45] Gutjahr, C, Parniske, M. Cell and developmental biology of arbuscular mycorrhiza symbiosis. In: Annual Review of Cell and Developmental Biology. R. Schekman; 2013. 29: 593-617.

[46] Genre, A, Chabaud, M, Balzergue, C, Puech-Pages, V, Novero, M, Rey, T, Fournier, J, Rochange, S, Becard, G, Bonfante, P, Barker, DG. Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear ca^{2+} spiking in *Medicago truncatula* roots and their production is enhanced by strigolactone. New Phytologist. 2013; 198: 179-189.

[47] Maillet, F, Poinsot, V, Andre, O, Puech-Pages, V, Haouy, A, Gueunier, M, Cromer, L, Giraudet, D, Formey, D, Niebel, A, Martinez, EA, Driguez, H, Becard, G, Denarie, J.

Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature. 2011; 469: 58-63.

[48] Nagahashi, G, Douds Jr, DD. Appressorium formation by am fungi on isolated cell walls of carrot roots. New Phytologist. 1997: 299-304.

[49] Wang, E, Schornack, S, Marsh, JF, Gobbato, E, Schwessinger, B, Eastmond, P, Schultze, M, Kamoun, S, Oldroyd, GE. A common signaling process that promotes mycorrhizal and oomycete colonization of plants. Current Biology. 2012; 22: 2242-2246.

[50] Smith, SE, Read, D. Mycorrhizal symbiosis : third edition. San Diego, Elsevier Academic Press Inc; 2008.

[51] Bonfante, P, Genre, A. Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nature Communications. 2010; 1:1-48.

[52] Madala, NE, Steenkamp, PA, Piater, LA, Dubery, IA. Metabolomic analysis of isonitrosoacetophenone-induced perturbations in phenolic metabolism of *Nicotiana tabacum* cells. Phytochemistry. 2013; 94: 82-90.

[53] Rai, M, Rathod, D, Agarkar, G, Dar, M, Brestic, M, Pastore, GM, Marostica, MR. Fungal growth promotor endophytes: A pragmatic approach towards sustainable food and agriculture. Symbiosis. 2014; 62: 63-79.

[54] Impullitti, AE, Malvick, DK. Fungal endophyte diversity in soybean. Journal of Applied Microbiology. 2013; 114: 1500-1506.

[55] Smith, FA, Smith, SE. How useful is the mutualism-parasitism continuum of arbuscular mycorrhizal functioning? Plant and Soil. 2013; 363: 7-18.

[56] Kachroo, P, Kachroo, A. The roles of salicylic acid and jasmonic acid in plant immunity. Molecular Plant Immunity. 2012: 55-79.

[57] An, C, Mou, Z. Salicylic acid and defense responses in plants. In: Phytohormones: A Window to Metabolism, Signaling and Biotechnological Applications, Springer; 2014. 191-219.

[58] Wasternack, C. Jasmonates in plant growth and stress responses. In: Phytohormones: A Window to Metabolism, Signaling and Biotechnological Applications, Springer; 2014. 221-263.

[59] Pieterse, CM, Zamioudis, C, Does, DV, Van Wees, S. Signalling networks involved in induced resistance. Induced Resistance for Plant Defense: A Sustainable Approach to Crop Protection. 2014: 58-80.

[60] Dobón, A, Canet, JV, García-Andrade, J, Angulo, C, Neumetzler, L, Persson, S, Vera, P. Novel disease susceptibility factors for fungal necrotrophic pathogens in arabidopsis. Plos Pathogens. 2015; 11: e1004800-e1004800.

[61] Jung, SC, Martinez-Medina, A, Lopez-Raez, JA, Pozo, MJ. Mycorrhiza-induced resistance and priming of plant defenses. Journal of Chemical Ecology. 2012; 38: 651-664.

[62] Zamioudis, C, Pieterse, CMJ. Modulation of host immunity by beneficial microbes. Molecular Plant-Microbe Interactions. 2012; 25: 139-150.

[63] Langenbach, C, Schultheiss, H, Rosendahl, M, Tresch, N, Conrath, U, Goellner, K. Interspecies gene transfer provides soybean resistance to a fungal pathogen. Plant Biotechnology Journal. 2016; 14:699-708.

IntechOpen

IntechOpen

IntechOpen

IntechOpen