Mechanisms of Plant Signal Perception and Transduction in Fungal Pathogens

Prof. Dr. Mehmet Erhan GÖRE

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PREFACE

The interaction between plants and pathogenic fungi represents a dynamic domain where survival is contingent upon a silent yet intricate molecular exchange. This complex communication, initiated at the moment of contact, determines whether the host will mount a successful defense or succumb to disease. For many years, the study of phytopathology has strived to unravel this interaction, progressing from noting visible symptoms to comprehending the microscopic and molecular complexities of the host-pathogen struggle. This book focuses on the essence of this conflict: the signal transduction pathways that control the early stages of fungal infection.

The book systematically examines this critical interaction, meticulously mapping the sequence of events from the initial, brief contact to the establishment of a successful infection. The process starts by examining how a fungal spore, once it lands on a host surface, detects a variety of physical and chemical cues. The text elucidates how surface topography, hydrophobicity, and the chemical composition of plant waxes and cuticles function not merely as passive barriers but as active signaling environments. These signals start a conversation that sets off a sequence of predetermined developmental processes in the fungus, beginning with attachment and the release of an extracellular matrix, followed by germination and the development of highly specialized infection structures like the appressorium.

The text explores the inner workings of fungal cells by examining the fundamental signaling processes. It highlights the crucial functions of G-protein-coupled pathways, the complex interactions of Ca^{2+}/calmodulin signaling, and the broad cAMP/PKA and Mitogen-Activated Protein Kinase (MAPK) cascades. Each of these pathways is thoroughly analyzed, connecting the recognition of external cues to the subsequent activation of specific genes essential for morphogenesis, penetration, and pathogenicity. This study illustrates how the integration of classical genetics with contemporary molecular biology reveals the adaptation of essential signaling modules by different fungal pathogens to align with their specific infection methods.

This text demonstrates the application of universal principles by employing key model systems, such as the rice blast fungus *Magnaporthe*

grisea, the powdery mildew pathogen *Blumeria graminis*, and the anthracnose agent *Colletotrichum gloeosporioides*. It emphasizes both the remarkable conservation of signaling architecture across the fungal kingdom and the subtle variations that permit host specificity. We expect this thorough synthesis to be an invaluable asset for seasoned researchers, graduate students, and those new to the field, enhancing their comprehension of fungal pathogenesis and sparking innovative approaches to protect our vital agricultural and natural ecosystems.

Prof. Dr. Mehmet Erhan GÖRE
October 27, 2025

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1. INTRODUCTION

The interplay between the signal transduction pathways of plants and pathogens is a fundamental component in the development of disease. Communication between fungal pathogens and host plants is initiated upon their first encounter with the plant surface. Strong evidence suggests that interactions between hosts and pathogens are initiated within minutes, or possibly even seconds, of contact. The result of these interactions, whether it leads to susceptibility or resistance, is determined during these initial moments. When signals from plants are recognized as beneficial by the fungus, it triggers the germination of spores, leading to penetration through the cuticle, either directly or by developing specialized infection structures like the appressorium and penetration peg. The process of penetration can be influenced by both physical and chemical cues from plants. These signals are often utilized by the fungus to trigger the activation of genes necessary for host invasion.

Plants have developed intricate mechanisms for perceiving and responding to pathogen-derived signals, enabling them to initiate a cascade of defense responses. These reactions include the creation of antifungal substances and the strengthening of cell wall elements. For pathogens to establish a successful infection, they must circumvent these defense barriers by producing specific signals that disrupt host immunity. Upon detection of pathogen signals, plants activate a range of intracellular signaling pathways to orchestrate an effective defense response. The interaction of signals between the host and the pathogen influences plant immunity and ultimately decides whether the invading microorganism will be eliminated or manage to establish itself within the tissue. Susceptibility and resistance represent two aspects of the same interaction, reflecting the outcome of this molecular arms race. Notably, these outcomes are frequently determined at the single-cell level.

This book explores the complex signaling systems between plants and fungal pathogens under both susceptible and resistant conditions during the pathogenesis process. We will specifically address how fungal organisms perceive host-derived signals and the molecular events triggered in response to these cues.

2. SIGNAL TRANSDUCTION DURING INITIAL CONTACT AND ADHESION OF FUNGAL SPORES

2.1. Initial Contact as a Crucial Catalyst in the Pathogenesis Process

The moment a fungal spore first makes contact with the host surface, often referred to as the "initial touch," is widely acknowledged as a vital trigger for the activation of various fungal signaling pathways. This initial interaction is sufficient to initiate spore germination and the development of infection structures such as the appressorium. In *Colletotrichum* species, merely touching a hard surface can prompt spore germination. *Colletotrichum gloeosporioides* spores respond to various signals from plants to start germination and form appressoria; nonetheless, it appears that contact with a solid surface is essential for appressorium development to commence. For example, when *C. gloeosporioides* spores were exposed to plant cuticular waxes or ethylene, they formed appressoria on both hydrophilic glass coverslips and hydrophobic polystyrene surfaces. In contrast, on soft hydrophilic surfaces like 2% agar or soft hydrophobic environments such as petrolatum, only germination occurred without appressorium formation. All examined hard surfaces, encompassing both the hydrophobic and hydrophilic sides of GelBond film, successfully prompted appressorium formation, while soft surfaces such as water agar failed to initiate this developmental process (Fu et al., 2022; Uhm et al., 2003). These findings suggest that interaction with a solid surface triggers a series of molecular events that eventually result in the transformation of germ tubes into appressoria (Figure 1).

When fungal spores come into contact with a host surface, they quickly identify the substrate's specific features. During the initial recognition stage, an extracellular matrix (ECM) is rapidly released even before the spores begin to germinate. For the barley powdery mildew pathogen, *Blumeria graminis* f. sp. *hordei*, ECM secretion is associated with changes on the spore surface and takes place in two separate stages: an initial quick release around 2 minutes after contact, followed by another phase approximately 15 minutes later (Huth et al., 2023; Nicholson et al., 1988). Remarkably, ECM release by *B. graminis* f. sp. *hordei* conidia was detected just 20 seconds after contact with the surface (Carver et al., 1999; John et al., 2024). Additionally, Nielsen et al. (2000) observed the presence of hydrolytic enzyme activity in the ECM-associated areas within three minutes of contact. According to Carver et al. (1999), the extracellular matrix (ECM) continued to accumulate at the point where the spore and substrate meet for up to an hour after they first made contact.

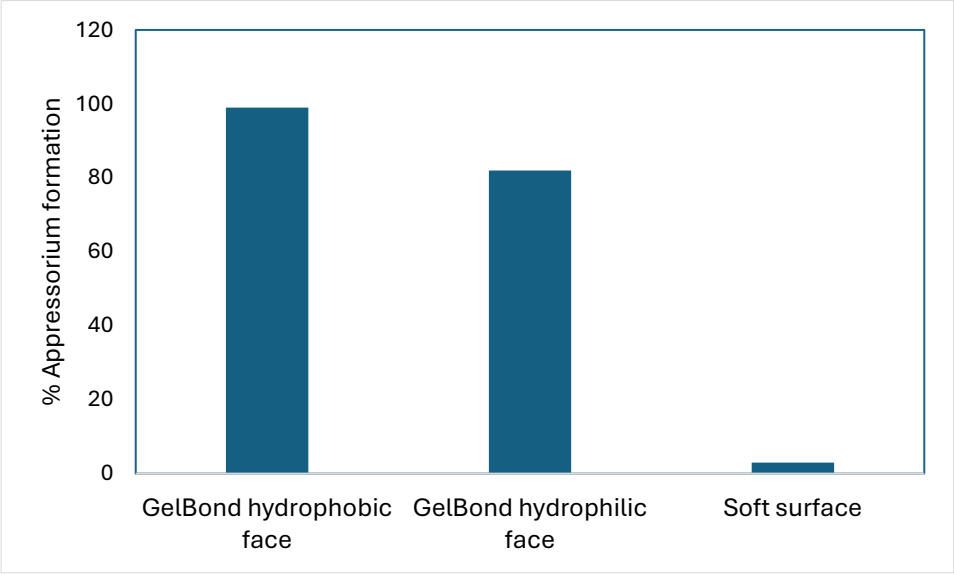

Figure 1. Evaluating the relative effectiveness of hard (either hydrophobic or hydrophilic) versus soft surfaces on the formation of appressoria in *C. gloeosporioides*.

2.2. The Initiation of Fungal Infection is Mediated by Adhesion or Close Contact

Upon reaching their destination, spores from a variety of pathogens promptly cling to the surfaces of host plants. Research has shown that conidial adhesion occurs in *Blumeria graminis* (Vigreux et al., 2025; Yamaoka & Takeuchi, 1999), *Uromyces appendiculatus* (Epstein et al., 1987), *U. viciae-fabae* (Beckett et al., 1990; Negussie & Pretorius, 2012), *Botrytis cinerea* (Doss et al., 1993; Plaza et al., 2025), *Colletotrichum musae* (Sela-Buurlage et al., 1991; Vasselli et al., 2022), *C. lindemuthianum* (Huang et al., 2020; Young & Kauss, 1984), *C. graminicola* (Mercure et al., 1994a; Vasselli & Shaw, 2022), *Magnaporthe grisea* (Hamer et al., 1988; Younas et al., 2025), *Haematonectria haematococca* (anamorph: *Fusarium solani*) (Coleman, 2016; Jones & Epstein, 1990), *Cochliobolus heterostrophus* (Arya & Cohen, 2022; Braun & Howard, 1994b), as well as in *Bipolaris maydis*, *B. zeicola*, and *B. turcicum* (Apoga et al., 2001; Evans et al., 1982). Studies have shown that foliar pathogens tend to cling to leaf surfaces (Jansson & Åkesson, 2003; Roy et al., 2023), while soilborne pathogens are typically found adhering to the surfaces of roots (Carlson et al., 1991; Han et al., 2010). This adhesion plays a vital role in the germination of spores, the extension of germ tubes, the formation of appressoria, and the creation of penetration pegs in various pathogens (Chethana et al., 2021; Jansson & Åkesson, 2003; Sugai et al., 2020; Yamaoka

& Takeuchi, 1999). The attachment of fungal spores to the surfaces of hosts is recognized as a crucial stage in the process of fungal infection. According to Jones & Epstein (1990), a mutant of *H. haematococca* lacking adhesion capabilities showed decreased virulence when compared to the wild type. In the grapevine pathogen *Phyllosticta ampelicida*, the adhesion of conidia is considered a crucial step for germination and the subsequent onset of infection (Kuo & Hoch, 1996; Tisch et al., 2024). The initial and crucial phase in achieving a successful infection is the adherence of spores to the surfaces of the host.

2.3. Hydrophobic Interactions in Spore Adhesion

The hydrophobic characteristics of a surface are a significant determinant in the adhesion of conidia from numerous plant pathogens. As demonstrated by Mercure et al. (1994b) and Vasselli & Shaw (2022), the conidia of *Colletotrichum graminicola* showed a greater tendency to adhere to hydrophobic polystyrene surfaces, whereas they did not attach at all to hydrophilic glass. Similarly, conidia of *Bipolaris sorokiniana* demonstrated selective adhesion to hydrophobic surfaces, adhering to polystyrene but not to hydrophilic glass. Conidial adhesion to polystyrene was hindered by the detergent Triton X-100, which is recognized for its ability to disrupt hydrophobic interactions (Apoga et al., 2001; Vasselli & Shaw, 2022). Conidial surfaces are naturally hydrophobic, and a number of studies have demonstrated a link between the hydrophobic nature of cell surfaces and their ability to adhere to polystyrene (Kuo & Hoch, 1996; Tisch et al., 2024). Research has shown that spores from *Colletotrichum musae* (Sela-Buurlage et al., 1991; Vasselli et al., 2022), *C. lindemuthianum* (Mercure et al., 1994b; Vasselli & Shaw, 2022), *B. cinerea* (Doss et al., 1993; Kou & Naqvi, 2016), and *Uromyces viciae-fabae* (Clement et al., 1994) tend to attach more readily to surfaces that are hydrophobic rather than hydrophilic. The requirement for hydrophobic surfaces in adhesion has also been observed *in U. appendiculatus* (Terhune & Hoch, 1993) and *P. ampelicida* (Kuo & Hoch, 1996). The role of hydrophobicity in adhesion was further validated by Doss et al. (1993), who found that oxidizing polyethylene surfaces, which lowers the water contact angle, greatly reduced the adhesion of *B. cinerea* conidia.

Hydrophobins, which are crucial elements of the fungal cell wall, significantly contribute to making the cell surface hydrophobic and are vital for forming the rodlet layer on spore surfaces (Lauter et al., 1992; Quarantin et al., 2019). These proteins are secreted, relatively small, and characterized by a high cysteine content (Wang et al., 2020; Wessels, 1993). They generally consist of 96 to 157 amino acids, include eight cysteine residues, and are characterized by

strong hydrophobic properties (Kirby et al., 2016; Stringer and Timberlake, 1993).A notable structural characteristic is the pairing of the second and third cysteines, which is generally succeeded by an asparagine residue (Lu & Edwards, 2016; Templeton et al., 1994). The hydrophobic characteristics of fungal pathogen surfaces play a crucial role in enabling them to adhere to host structures (Kirby et al., 2016; Stringer et al., 1991). The positioning of rodlets on the outside suggests they may play a role in facilitating initial contact during fungal interactions.

2.4. Spore Adhesion Is Accompanied by Extracellular Material Secretion

The adhesion of fungal spores may rely on both pre-existing and newly released adhesive materials, particularly mucilaginous extracellular matrices (ECMs) (Braun & Howard, 1994b; Chethana et al., 2021). Wright et al. (2002b) clarified the function of ECM as an adhesive in *Blumeria graminis* f. sp. *hordei*, the pathogen that causes powdery mildew in barley. Studies indicate that the adhesive strength of conidia is associated with both the rate at which ECM is secreted and the volume of ECM deposited on the substrate (Feng et al., 2009; Wright et al., 2002b). Before germination, the conidia of *B. graminis* f. sp. *hordei*, *B. graminis* f. sp. *tritici*, and *Erysiphe pisi* quickly secreted ECM when placed on barley coleoptiles. The secretion was significantly higher with full cell contact compared to partial contact (Fujita et al., 2004a; Mishra et al., 2012). At the tips of *M. grisea* conidia, preformed substances were observed to be released during hydration when the substrate was attached (Hamer et al., 1988; Ryder et al., 2022). Similarly, *H. haematococca* produced a tip-associated material linked to host adhesion (Coleman, 2016; Jones & Epstein, 1990). Numerous other fungal organisms also produced conidial mucilage following substrate contact (Fujita et al., 2004a; Mishra et al., 2012; Wright et al., 2002a). Studies using electron microscopy on *C. graminicola* conidia adhered to maize leaves have shown a unique layer of material at the point of contact, suggesting that substances are secreted during attachment (Mercure et al., 1994a; Vasselli & Shaw, 2022). The adhesion of *C. graminicola* conidia was markedly diminished when treated with cycloheximide, which inhibits protein synthesis, or brefeldin A, which blocks glycoprotein synthesis and transport (Mercure et al., 1994b; Vasselli & Shaw, 2022). This indicates that the new synthesis of proteins or glycoproteins is crucial for adhesion. Exposure to respiratory inhibitors also prevented the adhesion of conidia from *C. lindemuthianum* (Huang et al., 2020; Young & Kauss, 1984), *C. musae* (Sela-Buurlage et al., 1991; Vasselli et al., 2022), and *H. haematococca* (Jones & Epstein, 1989;

Vasselli & Shaw, 2022), suggesting that active metabolism is required for the production of adhesive substances necessary for spore adhesion.

Adhesive secretion from various pathogenic fungal spores has been demonstrated through techniques such as electron microscopy and the use of fluorescently labeled lectins or antibodies. In the early phases of infection, *C. graminicola* releases adhesives composed of glycoproteins from its conidia (Mercure et al., 1995; Vasselli et al., 2022). In a similar manner, *M. grisea* conidia emit mucilage from their tip during the adhesion process (Hamer et al., 1988; Younas et al., 2025). In *H. haematococca*, macronidia secrete extracellular material from one pole, contributing to adhesion (Coleman, 2016; Schuerger & Mitchell, 1993). Adhesive substances are also produced by the zoospores of *Phytophthora palmivora* (Singh & Bartnicki-Garcia, 1975) and *P. cinnamomi* (Gubler et al., 1989; Vasselli & Shaw, 2022), and similar materials have been noted in the urediniospores of *U. viciae-fabae* (Chethana et al., 2021; Clement et al., 1993).

Depending on the type of fungus, the substances that aid in spore adhesion have been identified as proteins, glycoproteins, or carbohydrates (Chethana et al., 2021; Mercure et al., 1995; Sugui et al., 1998; Vasselli et al., 2022). For *P. cinnamomi* zoospores, a protein larger than 200 kDa has been identified as facilitating attachment to host surfaces (Gubler & Hardham, 1988; Vasselli & Shaw, 2022). The extracellular matrix (ECM) secreted by adherent *H. haematococca* macronidia comprises a mannosylated protein (Coleman, 2016; Kwon & Epstein, 1997). In the process of conidial germination in *C. graminicola*, the extracellular matrix (ECM) that is secreted onto hydrophobic surfaces comprises both proteins and carbohydrates, with mannose being the primary sugar detected (Sugui et al., 1998). Notably, *C. graminicola* conidia produce two types of adhesive materials: a preformed proteinaceous adhesive and a newly secreted glycoprotein-based one (Mercure et al., 1994b; Vasselli & Shaw, 2022).

Some fungal adhesives exhibit lectin-like properties, capable of binding specific carbohydrate moieties. Zoospore-secreted adhesive substances from *Pythium aphanidermatum*, which facilitate attachment to the root surface of *Lepidium sativum*, and those from *Phytophthora* species involved in adherence to *Glycine max* and other plant cells, have been characterized as lectins (Longman & Callow, 1987; Hardham & Mitchell, 1998; Hohl & Balsiger, 1986; Van West et al., 2003).

2.5. The Role of Cutinases in Spore Adhesion

Esterases, including cutinases, are essential in the attachment processes of numerous fungal pathogens. Remarkably, the conidia of *B. graminis* f. sp. *hordei*, which are the cause of barley powdery mildew, emit a fluid when they make contact with the leaves of barley. This secretion exhibits esterase activity that is comparable to fungal cutinase (Huth et al., 2023; Nicholson et al., 1988). The fluid forms a thin film that disperses across the contact area, undermining the structural integrity of the host's cuticle (Chowdhury et al., 2019; Kunoh et al., 1990). The enzymatic activity found in this exudate is thought to aid in breaking down the cuticle, which may trigger recognition processes crucial for infection (Arya & Cohen, 2022; Pascholati et al., 1992; Apoga et al., 2001; Nicholson & Epstein, 1991). This degradation may provide critical signals for recognizing the host surface and ensuring successful spore adhesion.

Cutinase involvement in spore adhesion has been reported in multiple fungal species (Nicholson & Epstein, 1991). In urediniospores of the wheat rust fungus, both cutinase and non-specific esterases have been localized on the outer spore wall. Upon hydration, these enzymes are released and accumulate at the spore-substrate interface, contributing to spore adherence (Arya & Cohen, 2022; Deising et al., 1992). Even low levels of cutinase can enable fungal spores to detect host-specific cuticle monomers, thereby facilitating precise host recognition (Peng et al., 2024; Woloshuk & Kolattukudy, 1986).

2.6. The Potential Requirement of Plant-Derived Signals in Spore Adhesion

Evidence suggests that specific plant signals may be required to induce fungal spore adhesion. *H. haematococca* macronidia, for example, fail to adhere to polystyrene surfaces unless pre-exposed to host plant (squash) extracts. Exposure induces adhesive capability within minutes, coinciding with the production of fluorescein isothiocyanate-ConA-labeled mucilage at the conidial tip (Jones & Epstein, 1989; Vasselli & Shaw, 2022). Additionally, when spores are incubated in squash extract, a 90 kDa glycopeptide is secreted, a phenomenon not observed when spores are incubated in either Czapek-Dox medium or water, where adhesion is also absent (Coleman, 2016; Kwon & Epstein, 1993). These findings implicate the 90 kDa glycopeptide as a key factor in adhesion induction.

In the field of plant pathology, researchers have discovered a vitronectin-like protein called PVN1, originating from *Nicotiana tabacum*, which aids in the adhesion of tobacco cells to glass surfaces and is located within the plant cell wall (Pandey et al., 2010; Zhu et al., 1994). Although vitronectin-like

proteins have been linked to the attachment of bacterial pathogens to plant cells, their role in the adhesion of fungal spores has not been thoroughly investigated and warrants further academic research.

3. SIGNAL TRANSDUCTION IN FUNGAL SPORE GERMINATION

3.1. Host Signals Induce Pre-Germination Structural Changes In Spores

Upon landing on the plant surface, fungal spores initiate preparatory modifications for germination by detecting signals from the host. Within seconds to minutes of landing on plant leaves, the conidia of *B. graminis* and *E. pisi* quickly release extracellular matrix (ECM) substances, which facilitate their germination (Feng et al., 2009; Wright et al., 2002b; Fujita et al., 2004b; Meguro et al., 2001; Mishra et al., 2012; Pandey et al., 2010). Nielsen et al. (2000) state that when *B. graminis* f. sp. *hordei* conidia come into contact with a suitable surface, they start to take in anionic compounds of low molecular weight. Before this absorption occurs, the spores release a protein-rich ECM. Following the release of the matrix and its hydrolytic enzymes, host-derived molecules with low molecular weight are able to penetrate the conidia. These anionic molecules share physicochemical properties with cutin monomers (Kuska et al., 2018; Nielsen et al., 2000), which are liberated from the host cuticle due to the enzymatic actions of fungi (Arya & Cohen, 2022; Chowdhury et al., 2019; Huth et al., 2023; Nicholson et al., 1988; Kunoh et al., 1990; Pascholati et al., 1992). Signals originating from the host are essential for initiating spore germination and the subsequent development of infection structures.

Fungal spores, upon adhering to a host leaf, swiftly undergo structural transformations. In just 5 minutes, the conidia of *B. graminis* f. sp. *hordei* start to lose their unique reticulate surface pattern. By the 10-minute mark, only spiny projections are discernible, and by 30 minutes, spherical structures manifest on the conidial surface, all occurring prior to the initiation of germination (Chowdhury et al., 2019; Kunoh et al., 1988).It is proposed that these alterations are caused by the emission of an exudate with esterase activity from the spore. In a similar manner, the macroconidia of *H. haematococca* experience significant changes on their surface several hours prior to the emergence of the germ tube (Caesar-TonThat & Epstein, 1991; Kwon & Epstein, 1997). For *Hypoxylon fuscum*, a pathogen that targets beech trees (*Fagus sylvatica*), the germination of ascospores commences just moments after they come into contact with the host. The host cell wall molecules that

trigger this process bear structural resemblance to monolignol glycosides, such as z-isoconiferin and z-syringin, which are glycosylated forms of coniferyl and syringyl alcohols (Alam et al., 2021; Chapela et al., 1991).

3.2. Plant Surface Signals Promote Spore Germination

The initiation of conidia germination in many fungal pathogens is frequently prompted by their interaction with solid surfaces or by their subsequent adherence to these surfaces. For species like M. grisea (Zeilinger et al., 2016; Liu & Kolattukudy, 1999) and *Phyllosticta ampelicida* (Kuo & Hoch, 1996; Tisch et al., 2024), the ability to adhere is essential for the commencement of germination.In contrast, *Colletotrichum* spp. can initiate germination with mere surface contact (Kao et al., 2022; Kim et al., 1998). *B. sorokiniana*, however, does not depend on adhesion for germination, as it is capable of germinating in liquid media. Nonetheless, when placed in water, its germination is notably enhanced upon contact with a solid surface, indicating that both surface contact and adhesion can serve as effective stimuli for germination (Apoga et al., 2001; Vasselli & Shaw, 2022).

Microscopic examinations of *B. graminis* conidia reveal that germ tubes typically develop near the contact point with the host leaf, thus promoting interaction with the surface (Carver et al., 1995a,b, 1999; John et al., 2024). The development of appressoria in this species depends on the direct interaction between germ tubes and the host's surface. In *B. graminis* f. sp. *hordei*, *tritici*, and *avenae*, the initial germ tube usually forms close to the host interface, which facilitates the infection process (Chowdhury et al., 2019; Huth et al., 2023; Wright et al., 2000). Notably, minimal surface contact is sufficient to trigger germ tube emergence, with the site of emergence determined within a minute of substrate contact. As an example, when conidia are placed on spider silk, germ tubes often develop close to the point of contact with a microneedle tip, even after just two minutes of exposure, demonstrating the speed of this reaction (Wright et al., 2000). Moreover, merely touching spider silk can lead to the germination of a significant number of conidia. These observations suggest that in *B. graminis*, only a small contact area is necessary to initiate directed germ tube growth. On host leaves, even minimal interaction between the tips of conidial wall projections and the edges of epicuticular wax platelets is enough to trigger the development of infection structures.

Before germ tube formation begins, a series of molecular processes are likely activated. These processes involve signal transduction and cascades that control the specific sites of germ tube development, the targeted breakdown of the conidial wall, the reallocation of internal resources, and the new synthesis

of structural components (Huth et al., 2023; Wright et al., 2000). The extracellular matrix (ECM), rapidly released upon surface contact (Kuska et al., 2018; Nielsen et al., 2000), is thought to be involved in detecting the host surface and initiating intracellular signaling pathways. Dynamic alterations in cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) activity in germinating *B. graminis* f. sp. *hordei* conidia serve as evidence of intracellular signaling during the initial phases of germination (Chethana et al., 2021; Hall & Gurr, 2000). Interestingly, within 15 minutes of the interaction between powdery mildew conidia and a cellulose membrane, there is a noticeable rise in endogenous cAMP levels (Kinane et al., 2000; Liu et al., 2023). This indicates that the cAMP pathway is activated prior to the formation of germ tubes.

3.3. The Role of Flavonoids in Spore Germination

Fungal pathogens that reside in the soil can produce spores capable of remaining dormant for extended periods, waiting for a suitable host to appear (Bagga & Straney, 2000; Zhao et al., 2025). Flavonoids, which are secondary metabolites produced by plants and released from roots and seeds, have been identified as signaling molecules that trigger spore germination in certain fungi (Dakora et al., 1993; Bagga & Straney, 2000; Trush & Pal'ove-Balang, 2023; Zhao et al., 2025). In the species *Nectria haematococca* (anamorph: *Fusarium solani*), flavonoids play a pivotal role in the signaling cascade that controls spore germination. Inhibitors that target cyclic AMP (cAMP)-dependent protein kinase A (PKA) significantly reduce the ability of flavonoids to promote germination in *H. haematococca* spores (Pusztahelyi et al., 2015; Ruan et al., 1995). This evidence highlights the crucial role of the cAMP signaling pathway in the germination process triggered by flavonoids. Following exposure to flavonoids, transient increases in intracellular cAMP levels have been observed in the macroconidia of *H. haematococca* (Bagga & Straney, 2000). These cAMP levels are regulated by degradation through cAMP-specific phosphodiesterase enzymes (Miranda et al., 2015; Riley & Barclay, 1990). A notable correlation exists between the extent of cAMP elevation caused by flavonoids and the inhibition of phosphodiesterase activity (Bagga & Straney, 2000; Trush & Pal'ove-Balang, 2023). Furthermore, competitive phosphodiesterase inhibitors like IBMX and theophylline have been shown to promote spore germination in *H. haematococca*. This finding supports the theory that flavonoids might directly block phosphodiesterase activity, leading to increased cAMP levels and functioning as upstream signaling molecules in the initiation of fungal spore germination (Ruan et al., 1995; Bagga & Straney, 2000; Pusztahelyi et al., 2015).

4. SIGNAL TRANSDUCTION DURING GERM TUBE DIFFERENTIATION INTO INFECTION STRUCTURES

4.1. The Attachment of Germ Tubes and Infection Structures to the Host Surface

Fungal pathogens often undergo a defined series of morphological transformations, which are vital for their successful penetration and colonization of the host. The process begins with the development of a germ tube, which then evolves into an appressorium. A penetration peg is formed from this appressorium to penetrate the host's epidermal cell wall (Carver et al., 1995a; Huth et al., 2023). The systematic formation of these infection structures is controlled by recognizing signals originating from the host (Kuska et al., 2018; Nielsen et al., 2000). Germ tubes have the ability to sense different signals from the host surface, including the substrate's hydrophobic nature (Chethana et al., 2021; Kamakura et al., 2002), cutin monomers that are released from the host cuticle due to the activity of fungal cutinases (Arya & Cohen, 2022; Francis et al., 1996), and possibly products of cellulose breakdown released by fungal cellulases (de Oliveira Silva et al., 2024; Pryce-Jones et al., 1999). When they detect suitable signals, germ tubes continue to grow, develop into appressoria, and invade host tissues by either forming a penetration peg or directly breaking through the cuticle (Kolattukudy et al., 1995; Sun et al., 2019). The close interaction of germ tubes, appressoria, and penetration pegs with the host surface is essential for accurately detecting signals and activating intracellular signaling pathways. These processes are vital for developing infection structures and the subsequent onset of pathogenesis (Jansson & Åkesson, 2003; Roy et al., 2023).

During the process of appressorium differentiation, germ tubes can establish a firm connection with the plant surface (John et al., 2024; Pain et al., 1996; Mishra et al., 2012; Wright et al., 2002a). The successful formation of germination structures by many foliar pathogens largely relies on their ability to remain in contact with the host surface (Doehlemann et al., 2006; Staples & Hoch, 1997). Research has shown that disrupting hyphal adhesion can hinder the formation of appressoria in *M. grisea*, the organism responsible for rice blast (Ebbole, 2007; John et al., 2024; Xiao et al., 1994a). Once appressoria are differentiated, they must establish a secure attachment to the host tissue to initiate penetration. The appressoria of *M. grisea* adhere firmly to the plant surface, enabling either mechanical or enzymatic penetration into host tissues (Ebbole, 2007; Howard et al., 1991). Once the cuticle is penetrated, fungal hyphae come into contact with mesophyll cells, and it has been observed that the penetration hyphae adhere to the walls of these mesophyll cells. Unlike

cuticular adhesion, the interactions between fungal hyphae and the cell walls of the host are believed to mainly involve hydrophilic surface characteristics (Ebbole, 2007).

Fungal germination structures may necessitate a hydrophobic surface for effective adhesion (Chock, 2020; Lopatukhin et al., 2024). For *U. appendiculatus*, the attachment of germ tubes depends on how hydrophobic the surface is. In 1993, Terhune & Hoch used interference reflection microscopy to show that germ tubes create a much larger contact area on hydrophobic surfaces than on hydrophilic ones. Fungal hydrophobins, which are proteins associated with the cell wall and known for altering surface hydrophobicity, are believed to be essential in this adhesion process (Chock, 2020; Lopatukhin et al., 2024). Hydrophobins form the rodlet layer found on the surface of many fungal conidia (Wang et al., 2020; Wessels et al., 1991), and their ability to self-assemble at the boundary between hydrophilic and hydrophobic areas aids in the attachment of fungi to host surfaces. These proteins play a crucial role in the initial attachment of fungal infection structures during the early phases of pathogenesis (Lopatukhin et al., 2024; Wösten et al., 1994).

4.2. Role of ECM in Germling Adhesion

The secretion of an extracellular matrix (ECM) often aids in the attachment of germlings, playing a vital role in helping fungal germlings adhere to host surfaces. Studies have shown that in species like *B. sorokiniana* (Apoga et al., 2001; Vasselli & Shaw, 2022), *B. cinerea* (Doss et al., 1995; Kou & Naqvi, 2016; Plaza et al., 2025), *B. graminis* (Wright et al., 2002a), and *Cochliobolus heterostrophus* (Arya & Cohen, 2022; Braun & Howard, 1994a), the emission of ECM occurs in tandem with the adhesion of germlings. In a similar manner, in *U. viciae-fabae*, germ tubes attach to surfaces by releasing ECM or mucilage (Chethana et al., 2021; Clement et al., 1993). The extracellular matrix generated during the germination of fungi is regarded as crucial for facilitating this adhesion process (Nicholson & Epstein, 1991). For the tomato powdery mildew pathogen *Oidium neolycopersici*, ECM is observed beneath germ tubes and at the periphery of appressoria, but it is absent under spores that have not germinated (Jones et al., 2000; Lebeda et al., 2014).

Histological staining methods have demonstrated that in species such as *B. maydis*, *B. zeicola*, and *Setosphaeria turcica* (*Bipolaris turcicum*), the germ tubes are encased in a two-layered extracellular matrix (ECM) (Evans et al., 1982; Healy et al., 2004). In a similar manner, the germ tubes of *B. sorokiniana* are surrounded by a two-layered ECM, which plays a vital role in their adherence to host surfaces (Apoga & Jansson, 2000; Apoga et al., 2001; Healy

et al., 2004; John et al., 2024). In the same vein, *C. heterostrophus* germlings produce a two-layer ECM. Studies on ECM-deficient mutants of *C. heterostrophus* have demonstrated that each layer can independently remain and adhere to leaf surfaces, indicating that the inner layer might primarily facilitate adhesion (Pandey et al., 2010; Zhu et al., 1998).

The adherence of germlings to host surfaces may depend on extracellular matrices (ECMs) composed of proteins or glycoproteins. Pronase E treatment greatly reduces the adhesion of *U. appendiculatus* germ tubes and interferes with their development and nuclear division associated with appressorium formation (Epstein et al., 1987; Yan et al., 2025). While germ tubes exposed to Pronase E continue to grow at a normal pace, they develop into rounded forms and fail to produce a visible ECM, leading to weak adhesion. Additionally, Pronase E effectively detaches germ tubes that were previously adhered, whereas water or heat-inactivated Pronase E has minimal impact. These findings strongly indicate that extracellular proteins play a crucial role in the adhesion of fungi. The ECM of *U. appendiculatus* has been partially characterized, revealing six main extracellular peptides (Epstein et al., 1987; Klymiuk et al., 2021).

Research has consistently shown that fungal adhesives are largely made up of glycoproteins with high molecular weight (Apoga et al., 2001; Arya & Cohen, 2022). Specifically, in *P. palmivora*, surface glycoproteins play a crucial role in facilitating adhesion (Bircher & Hohl, 1997). These glycoproteins typically contain carbohydrate components such as α-mannosides and α-glucosides, which interact with the lectin concanavalin A (ConA). It has been observed that many fungi exhibit reduced adhesion of germ tubes when exposed to ConA (Shaw & Hoch, 1999; Apoga et al., 2001). For *P. megasperma* f. sp. *glycinea*, the suppression of glycoprotein function by ConA greatly reduces the attachment of germ tubes to soybean leaf discs and limits colonization (Figure 2) (Apoga et al., 2001; Ding et al., 1994).

Upon the deposition of a droplet containing *M. grisea* conidial suspension onto a polycarbonate substrate, the conidia adhere within a 30-minute timeframe. Once germinated, the conidia exhibit strong attachment to the surface, remaining affixed despite agitation in water for durations exceeding two minutes (Ebbole, 2007; Xiao et al., 1994a). According to Xiao et al. (1994a,b), scanning electron microscopy has revealed a significant amount of mucilage surrounding the germ tubes. The use of enzymes such as α-glucosidase, α-mannosidase, and protease has been demonstrated to effectively prevent the adhesion of spores. When these enzymes are present, germinated conidia fail to attach and remain suspended in water droplets. These

observations imply that the mucilage is predominantly glycoprotein-based, susceptible to disruption by proteolytic and glycolytic enzymes. Mucilage has also been observed encasing germ tubes on plant surfaces, supporting the theory that glycoprotein molecules aid in adhering to host surfaces (Xiao et al., 1994a).

In barley root infections caused by *B. sorokiniana*, ultrastructural studies have identified a fibrillar extracellular matrix (ECM) located at the junction between the fungal hyphae and the root surfaces (Carlson et al., 1991; Han et al., 2010). The use of the glycosylation inhibitor tunicamycin, in combination with the lectins ConA and GNA from Galanthus nivalis, greatly reduces the adhesion of *B. sorokiniana* germ tubes. This finding provides additional evidence for the role of surface glycoproteins in the fungus's attachment mechanism (Apoga et al., 2001; Vasselli & Shaw, 2022).

4.3. Role of the ECM in Appressorium Adhesion

The successful attachment of appressoria to host surfaces is vital for the initiation of infection structures. The extracellular matrix (ECM) is essential for ensuring that appressoria adhere effectively to plant surfaces (de Oliveira Silva et al., 2024; Jones et al., 2001). Numerous fungi produce ECM around their appressoria (Apoga & Jansson, 2000; Healy et al., 2004), and scanning electron microscopy has demonstrated a substantial presence of mucilage (Pandey et al., 2010; Xiao et al., 1994a). Glycoproteins in the ECM influence the adhesion and development of appressoria. In P. palmivora, the attachment of appressoria is facilitated by surface glycoproteins within the ECM (Bircher & Hohl, 1997). Similarly, in *M. grisea*, glycoproteins have been recognized as crucial elements for appressorium adhesion (Ebbole, 2007; Xiao et al., 1994a).

Cutinases located in the ECM could contribute to the attachment of appressoria. The presence of cutinases in the ECM of the maize pathogen *C. graminicola* is associated with the effective attachment of appressoria to host surfaces (Arya & Cohen, 2022; Pascholati et al., 1993). Conidia, which form within infected plant tissues or cultures, are surrounded by mucilage containing up to four distinct cutinases. The activity of cutinases is inhibited by diisopropyl fluorophosphate (DIPF). Even though conidia exposed to DIPF can develop into normal appressoria, they fail to induce disease because their adhesion is impaired. DIPF interferes with the firm attachment necessary for penetration, highlighting the critical role of cutinases in appressorial adhesion (Huth et al., 2023; Pascholati et al., 1993).

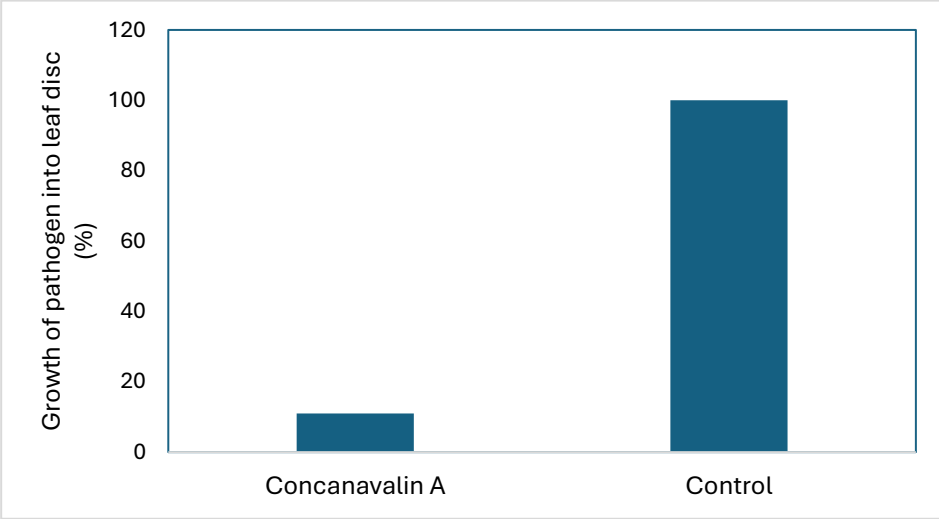

Figure 2. Effects of concanavalin A on how *Phytophthora megasperma* f. sp. *glycinea* colonizes soybean leaf discs.

4.4. Topographical Signals in Appressorium Formation

The development of appressoria is a crucial and active process necessary for numerous fungi to penetrate host plants (Apoga et al., 2001; Ding et al., 1994). Plant-derived signals can trigger the growth of germinated structures and result in the formation of appressoria. The formation of appressoria is triggered by both chemical and physical signals (Gilbert et al., 1996; Sugai et al., 2020; Younas et al., 2025).

Thigmotropic growth, characterized by directional development in reaction to contact with solid surfaces, has been observed in the germ tubes of several fungal pathogens on leaf replica surfaces (Doehlemann et al., 2006; Staples & Hoch, 1997). For example, the germ tubes of the bean rust fungus *U. appendiculatus* are capable of sensing the elevated regions of stomatal guard cells, which triggers the development of appressoria (Hoch et al., 1987; Panstruga & Moscou, 2020). In a similar manner, the thigmotropic development of hyphae has been noted in the indentations between epidermal cells on barley leaves infected by *B. sorokiniana* (Jansson & Åkesson, 2003; Roy et al., 2023). Despite this, hyphae frequently cross over epidermal protrusions and attach to them via ECM. For directed growth, it is essential for germ tubes and hyphae to maintain close contact with the cuticle surface.

The significant hyphal expansion of *B. sorokiniana* on leaf surfaces indicates that appressoria mainly form once the conidium's nutrient supplies are

exhausted. However, appressoria did not form on synthetic surfaces, indicating that their development is dependent on thigmotropic signaling (Jansson & Åkesson, 2003; Roy et al., 2023). Studies using leaf replica surfaces have demonstrated that appressoria often develop at the junctions of the anticlinal walls of epidermal cells, highlighting the importance of thigmotropic cues (Chethana et al., 2021; Clay et al., 1994). Thigmotropic growth similar to this has been noted in the germ tubes of other fungal pathogens as well (Staples & Hoch, 1997; Sugai et al., 2020; Ryder et al., 2022).

Topographical features on plant surfaces are recognized for their role in inducing appressoria formation. Studies have demonstrated that microstructured silicone surfaces with ridges measuring 0.5 mm are particularly effective in encouraging the development of appressoria in *U. appendiculatus*, as they mimic the ridge of stomatal lips present on leaf surfaces (Chethana et al., 2021; Hoch et al., 1987; Panstruga & Moscou, 2020). It is suggested that mechanosensitive chemicals could convert the membrane tension, resulting from the leaf surface's topography, into an influx of ions, especially Ca^{2+}, thereby triggering the appressorium development process (Chowdhury et al., 2019; John et al., 2024; Zhou et al., 1991). Research has shown that these topographical cues can initiate specific gene expression during the development of appressoria in U. appendiculatus (Han et al., 2010; Xuei et al., 1993). Among the genes that show increased expression, INF24 features a 450-bp open reading frame (Bhairi et al., 1989), whereas INF56 contains two overlapping open reading frames (Kolattukudy et al., 1995; Xuei et al., 1992). Although these genes are uniquely expressed during thigmotropic differentiation in *U. appendiculatus*, their specific functions remain unidentified.

In fungal pathogens like *M. grisea*, the hardness of a surface might play a significant role in how the substrate is recognized (Ebbole, 2007; John et al., 2024; Xiao et al., 1994b). When the conidia of this fungus are placed on soft, non-inductive surfaces that are unsuitable, they fail to develop appressoria. In *C. gloeosporioides*, eight genes that are upregulated during initial contact with hard surfaces have been cloned and are referred to as *chip* (*Colletotrichum* hard-surface induced protein) genes. Among these, chip1 encodes a ubiquitin-conjugating enzyme, and its expression is enhanced by both contact with a hard surface and the application of ethylene (Zeilinger et al., 2016; Liu & Kolattukudy, 1998). According to Kim et al. (2000), the proteins encoded by *chip2* and *chip3* have molecular weights of 65 kDa and 64 kDa, respectively. *Chip2* is characterized by the presence of a nuclear localization signal, a leucine zipper, and a heptad repeat region capable of forming a coiled-coil structure. *Chip3* encodes a protein with nine transmembrane domains. Both genes

become active two hours after coming into contact with a solid surface. However, their deletion did not affect appressorium development, indicating the existence of functionally redundant genes (Kao et al., 2022; Kim et al., 2000). Contact with a solid surface may initiate a molecular sequence that alters protein production, which is essential for the germination of conidia and the development of appressoria (Zeilinger et al., 2016; Liu & Kolattukudy, 1998).

Several studies (Huang et al., 2020; Lee & Dean, 1994; Lopatukhin et al., 2024) have highlighted the essential role of surface hydrophobicity in the development of appressoria in *M. grisea*. Scientists have discovered a gene in *M. grisea* that produces a chitin-binding protein called *CBP1*, which plays a crucial role in recognizing hydrophobic signals during the formation of appressoria (Kamakura et al., 2002; Kirby et al., 2016). The extracellular protein known as *CBP1* is distinguished by the presence of a signal peptide, two domains that bind to chitin, and a central region that is similar to fungal chitin deacetylases. Mutants that lack *CBP1* are unable to develop normal appressoria on synthetic surfaces, although they can do so on plant leaf surfaces, emphasizing the critical role of *CBP1* in recognizing physical cues on rigid surfaces.

Integrins, which are transmembrane proteins composed of two subunits, are crucial for cell adhesion, recognition, and signaling within cells (Hostetter, 2000; Kou & Naqvi, 2016). In relation to *Candida albicans*, the tripeptide composed of arginine, glycine, and aspartic acid (RGD) interferes with both adhesion and the recognition by the host (Bendel & Hostetter, 1993; Wu et al., 2023). The application of RGD externally to *U. appendiculatus* hinders the fungus's capacity to detect the host surface's topographical characteristics (Chethana et al., 2021; Hoch et al., 1987; Panstruga & Moscou, 2020). Research has shown that integrins are found in the same locations as Ca^{2+} channels (Huang et al., 2020; Kao et al., 2022; Levina et al., 1994). The role of appressoria has also been confirmed in *Oidium neolycopersici*, as shown by de Oliveira Silva et al. (2024). According to Jones et al. (2001), integrins are believed to play a role in host-specific interactions and intracellular signaling pathways in pathogenic fungi.

Numerous research efforts have highlighted the importance of substrate characteristics in the development of fungal appressoria. Appressorium development is typically initiated on hydrophobic surfaces, whereas hydrophilic surfaces do not elicit the same response (Beckerman & Ebbole, 1996; Ebbole, 2007). In *M. grisea*, there is a notable link between the rate of appressorium formation and the surface's hydrophobic nature (Lee & Dean, 1993b; Younas et al., 2025). Research indicates that the barley powdery mildew

pathogen, *B. graminis*, achieves more effective appressorium formation on surfaces that are hydrophobic (Vigreux et al., 2025; Yamaoka & Takeuchi, 1999). Conversely, *C. gloeosporioides* is capable of forming appressoria with equal efficiency on both hydrophobic and hydrophilic surfaces (Kao et al., 2022; Lee & Dean, 1993b; Younas et al., 2025). *Uromyces* species are incapable of forming appressoria on either smooth hydrophilic or hydrophobic surfaces, as they depend on specific topographical cues for the development of appressoria (Chethana et al., 2021; Hoch et al., 1987; Panstruga & Moscou, 2020). These observations indicate that various plant pathogens utilize different strategies to detect plant-derived signals for initiating appressorium formation.

4.5. Induction of Appressorium Formation by Plant Surface Waxes

Plant surfaces exposed to the air are covered with a complex blend of highly water-repellent substances known as "waxes," which include very long-chain aliphatic components. When fungal spores land on these surfaces, they first come into contact with these epicuticular waxes. Research has extensively shown that components of the plant cuticle can initiate spore germination and appressoria development in fungal pathogens (Feng et al., 2009; Huth et al., 2023; Kolattukudy et al., 1995). These waxes on plant surfaces can act as signals that promote the formation of infection structures such as appressoria and penetration pegs. Due to the variation in the chemical composition of waxes across different plant species, waxes specific to a host may selectively trigger spore germination and the differentiation of appressoria in fungi that are compatible. For example, *C. gloeosporioides* spores will only germinate and develop appressoria when exposed to waxes from their specific host. Waxes from non-host plants did not trigger these developmental processes (Arya et al., 2021; Podila et al., 1993). Even waxes from other *Colletotrichum* hosts failed to trigger the development of infection structures. The fatty alcohol component of avocado wax demonstrated the most significant biological activity. In particular, synthetic straight-chain aliphatic alcohols containing 24 or more carbon atoms were found to effectively promote spore germination and the formation of appressoria in *C. gloeosporioides* (Podila et al., 1993). These long-chain alcohols are found naturally in various plant waxes (Arya et al., 2021; Kolattukudy et al., 1995).

However, some plant waxes do not trigger these fungal reactions, possibly because they contain inhibitors that prevent the development of appressoria. In fact, when waxes from various plants were mixed with avocado wax, they hindered the formation of appressoria in *C. gloeosporioides*. The chemical characteristics of these inhibitory substances have not yet been

determined (Arya et al., 2021; Kolattukudy et al., 1995). In addition, polar compounds derived from terpenoids in avocado wax have been discovered to encourage the formation of appressoria. Ursolic acid and oleanolic acid, which are among the pentacyclic triterpenes typically present in fruit waxes, were notably successful in promoting spore germination and the development of appressoria in *C. gloeosporioides* (Feng et al., 2009; Huth et al., 2023; Kolattukudy et al., 1995). The results indicate that the lipids on plant surfaces include both elements that encourage and those that inhibit fungal growth, influencing it in a host-specific way through a complex network of signaling interactions.

In non-host plant species, the waxes present on their surfaces serve as a physical barrier, impeding the fungal pathogen from establishing close contact with the plant's epidermal cells, thereby preventing infection (Feng et al., 2009; Vidhyasekaran, 2004). For example, rust fungi like *P. hordei*, *P. triticina*, *P. recondita*, and *P. agropyrina* cannot infect *Hordeum chilense*, which is a species of wild barley. In this context, the germ tubes of the fungi elongate excessively and traverse the stomata without forming appressoria or penetrating the leaf tissue (Quarantin et al., 2019; Rubiales & Niks, 1996). This failure to infect is attributed to a substantial layer of epicuticular wax on the stomatal guard cells, which inhibits the development of appressoria in rust fungi (Huth et al., 2023; Vaz Patto et al., 2003).

In interactions where plants and pathogens are compatible, the breakdown of the wax layer often occurs, releasing cutin monomers that aid in adhesion and the formation of infection structures. In many plant-fungus interactions, areas lacking wax have been found beneath the fungal hyphae. These exposed regions might arise due to the breakdown or consumption of waxes when they come into contact with the fungus (Feng et al., 2009; Huth et al., 2023) or because wax plates become attached to fungal hyphae (Vasselli et al., 2022). The presence of a distinct area around the appressorium is often a sign of enzymatic activity, particularly involving hydrolytic enzymes like cutinases and esterases, which play a role in breaking down wax (Chethana et al., 2021; Jansson & Åkesson, 2003; Roy et al., 2023). Notably, the treatment of barley leaves with esterase extracts from *B. graminis* resulted in significant alterations in the wax layer (Kunoh et al., 1990). These enzymes are typically located in the fungal extracellular matrix (ECM), and their presence appears crucial during the initial stages of host penetration (Jansson & Åkesson, 2003; Sugai et al., 2020).

4.6. Cutin-Derived Monomers as Inducers of Pathogenic Development

Fungal spores produce cutinase both prior to and during the germination phase. This enzyme aids in breaking down the host's cuticle, which results in the release of cutin monomers that could function as signaling molecules (Arya & Cohen, 2022; Peng et al., 2024). Application of these monomers to microscope slides or plastic coverslips led to a notable rise in the percentage of germ tubes forming appressoria in *B. graminis* f. sp. *hordei* (Francis et al., 1996). The results lend credence to the theory that cutin monomers act as molecular signals triggering the formation of appressoria (Figure 3).

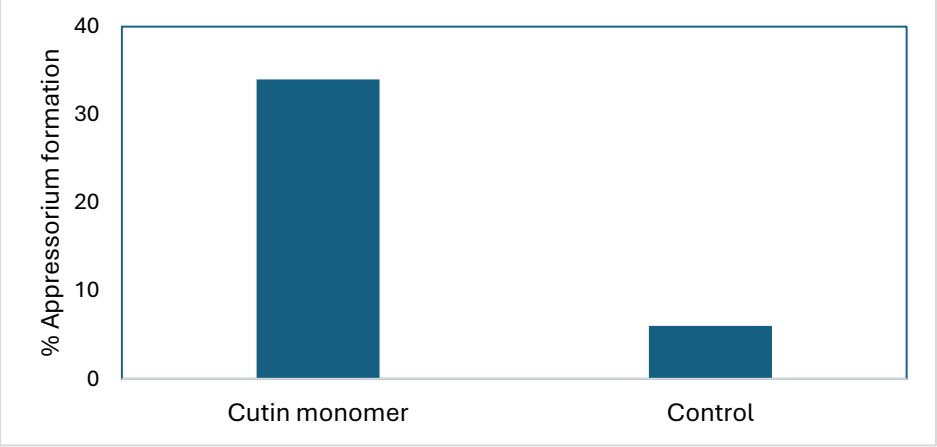

Figure 3. The role of cutin monomers in triggering appressorium formation in *Blumeria graminis* f. sp. *hordei*

4.7. Ethylene as a Trigger for Appressorium Development

Ethylene is a gaseous plant hormone primarily synthesized in climacteric fruits during postharvest ripening. It serves as a signaling molecule that initiates the germination of fungal spores and the formation of appressoria. In ethylene-emitting wild-type tomatoes, the spores of *C. gloeosporioides* developed numerous appressoria. In contrast, spores failed to germinate on transgenic tomatoes that were deficient in ethylene production. Nevertheless, the application of exogenous ethylene successfully reinstated spore germination, the formation of appressoria, and the emergence of visible infection symptoms (Arya et al., 2021; Sugai et al., 2020; Kirby et al., 2016; Kolattukudy et al., 1995). The results clearly demonstrate that ethylene functions as a signaling molecule that regulates fungal pathogenesis.

Studies have shown that ethylene can trigger both the germination and the formation of appressoria in *C. gloeosporioides* and *C. musae*, which are pathogens that impact climacteric fruits. In contrast, this phenomenon is absent in *Colletotrichum* species that target non-climacteric fruits (Kao et al., 2022; Flaishman & Kolattukudy, 1994). Norbornadiene, a substance recognized for mitigating the effects of ethylene in plants, also suppresses ethylene reactions in fungal spores. Nevertheless, the inhibitory effect can be counteracted by administering higher levels of ethylene (Feng et al., 2009; Huth et al., 2023; Kolattukudy et al., 1995). These results collectively provide strong evidence that ethylene acts as a signaling molecule derived from plants, playing a crucial role in the regulation of interactions between fungi and their hosts.

4.8. Signaling Dynamics in Appressorium Formation

Fungal growth and pathogenic capabilities are intricately regulated by a multitude of internal and external signals. Among these, signals originating from the fungi themselves are pivotal in initiating appressoria formation. Hydrophobins are small proteins rich in cysteine that are found in the cell walls of many fungi, as noted by Wang et al. (2020) and Wessels et al. (1991), and they are of particular importance. In the fungus *M. grisea*, which causes rice blast disease, a hydrophobin produced by the *MPG1* gene has been discovered to be an essential signaling molecule that triggers the formation of appressoria. The *MPG1* gene, responsible for coding a hydrophobic protein typical of the hydrophobin class, was extracted from *M. grisea* and shown to be actively expressed during the formation of appressoria (Huang et al., 2020; Talbot et al., 1996; Younas et al., 2025). Research involving the deliberate disruption of this gene demonstrated that mutants without mpg1 showed a markedly diminished ability to develop appressoria (Lopatukhin et al., 2024; Talbot et al., 1993).

The hydrophobin produced by *MPG1* is released into the extracellular matrix and is thought to aid in recognizing hydrophobic surfaces, which in turn triggers the development of appressoria (Healy et al., 2004; Pandey et al., 2010; Talbot et al., 1993, 1996). Empirical evidence indicates that *MPG1* is essential for the recognition of both natural and synthetic hydrophobic surfaces. In their 1996 study, Beckerman & Ebbole noted that *mpg1* mutants maintained the capacity to develop appressoria on certain man-made surfaces. Interestingly, when *mpg1* mutants were co-inoculated with wild-type conidia, the mutants regained their ability to form appressoria. This observation suggests that the *MPG1* protein secreted by wild-type strains can act as a diffusible signaling molecule, compensating for the functional deficiency in the mutants (Ebbole, 2007).

Proteins like *MPG1*, which are categorized as hydrophobins, are released into the extracellular space with the help of a distinctive N-terminal signal peptide that aids in their secretion (Beckerman & Ebbole, 1996; Ebbole, 2007). These proteins have the potential to gather at the junction where fungal hyphae meet the substrate, possibly acting as structural indicators of a "host" surface. This interaction might facilitate the attachment to hydrophobic surfaces and encourage the development of appressoria by conveying physical or chemical signals (Ebbole, 2007; Lopatukhin et al., 2024; Wösten et al., 1994; Kershaw & Talbot, 1998).

Beyond the role of hydrophobins, extracellular glycoproteins secreted by germinating conidia have been identified as potential signaling molecules crucial for appressorium formation. For *M. grisea*, a mucilaginous coating has been observed surrounding the germ tubes (Ebbole, 2007; John et al., 2024; Xiao et al., 1994a). The successful removal of this mucilage by using enzymes like protease, α-mannosidase, and α-glucosidase confirmed its nature as a glycoprotein. Moreover, Concanavalin A (ConA), a lectin known for its specific binding to α-D-mannose and α-D-glucose residues, was found to selectively impede the development of appressoria. Competitive inhibitors, including methyl-α-D-mannoside, methyl-α-D-glucoside, and D-mannose, effectively reversed this inhibition (Figure 4). This indicates that certain extracellular glycoproteins are essential for the recognition and transmission of signals necessary for appressorium formation (Ebbole, 2007; Pandey et al., 2010; Xiao et al., 1994a).

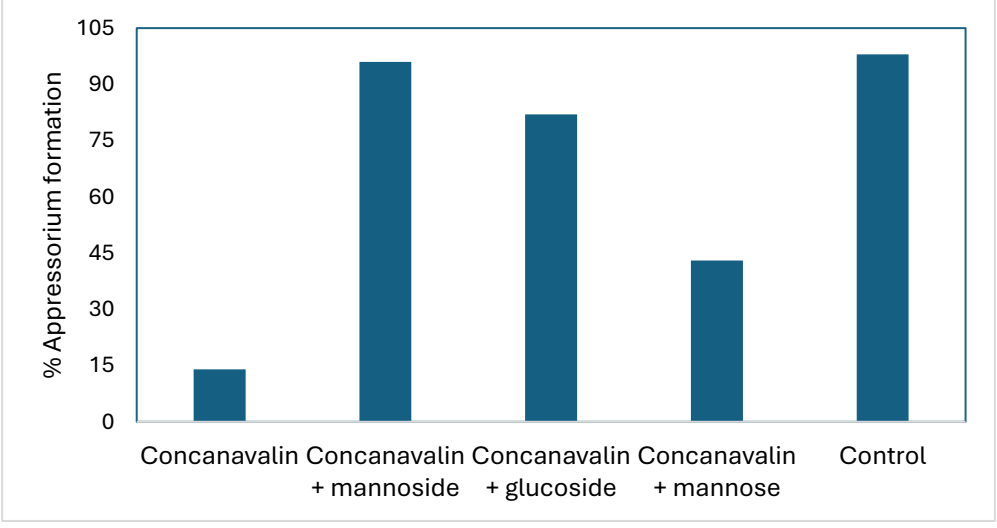

Figure 4. Impact of concanavalin A on the development of appressoria in *M. grisea*.

5. SIGNALING PATHWAYS IN FUNGAL PATHOGENICITY

5.1. Transmembrane Signal Perception Mechanisms in Fungal Pathogenesis

Fungal pathogens employ a complex set of external signals and internal communication pathways to regulate the development of infection structures. Signals originating from plants, such as surface topography (de Oliveira Silva et al., 2024; Jones et al., 2001), components of epicuticular wax (Feng et al., 2009; Huth et al., 2023; Kolattukudy et al., 1995), cutin monomers generated by cutinase activity (Arya & Cohen, 2022; Francis et al., 1996; Peng et al., 2024; Rumbolz et al., 2000), ethylene (Kao et al., 2022; Kolattukudy et al., 1995), and possibly byproducts from cellulose degradation (Suzuki et al., 1998; Carver et al., 1999), are recognized for triggering the formation of appressoria. Moreover, molecules derived from fungi, such as the hydrophobin Mpg1 produced by the *MPG1* gene, act as essential initial signals required for the differentiation of appressoria (Lopatukhin et al., 2024; Talbot et al., 1996). Transmembrane receptors located in the fungal plasma membrane detect these external signals (DeZwaan et al., 1999; Plaza et al., 2025; Wu et al., 2023).

The gene *PTH11*, which encodes a receptor in *M. grisea*, has been identified as crucial for detecting surface signals that induce responses. *PTH11* is responsible for encoding a transmembrane protein that is situated at the plasma membrane and plays a role early in the signaling pathway that regulates the formation of appressoria. Mutants lacking *PTH11* show impairments in advancing past the initial stage of appressorium formation. Conversely, introducing cyclic AMP (cAMP) and diacylglycerol (DAG) from outside sources resolved the developmental problem: cAMP successfully reinstated both appressoria formation and the organism's pathogenicity, while DAG was only successful in restoring appressorium formation (DeZwaan et al., 1999; Kao et al., 2022; Lu et al., 2016). The results indicate that Pth11p acts as a potential surface signal receptor, triggering internal signaling pathways crucial for developing infection structures.

Upon recognizing external cues, fungal cells trigger an intricate array of internal signaling pathways that oversee developmental activities, including the creation of appressoria (Fu et al., 2022; Uhm et al., 2003). Signal transduction, a process that involves secondary messengers, makes use of calcium ions (Ca^{2+}) (Huang et al., 2020; Lee & Lee, 1998; Li et al., 2024), cyclic nucleotides (Doehlemann et al., 2006; Fu et al., 2022; Lee & Lee, 1998; Li et al., 2024; Sun et al., 2019; Takano et al., 2001), inositol phosphates (Devecha & Irvine, 1995;

Oubohssaine et al., 2025), and lipid-derived molecules such as diacylglycerol, ceramides, and sphingomyelin (Ebbole, 2007; Thines et al., 1997). These molecules act as essential channels, conveying external signals to downstream effectors, thus enabling the morphogenetic transformations required for host penetration and the initiation of infection.

5.2. G-Proteins

Fungal pathogens have the ability to perceive signals from plants and relay these internally through heterotrimeric guanine nucleotide-binding proteins, also known as G-proteins (Doehlemann et al., 2006; Kao et al., 2022; Liu & Dean, 1997). These membrane-associated proteins are crucial elements in signal transduction pathways. G-proteins, as members of the GTPase superfamily, function by relaying signals from activated membrane receptors to a variety of intracellular effectors through a sequence of protein-protein interactions (Li et al., 2021; Neer, 1995). Furthermore, they function as both signal transducers and amplifiers (Kao et al., 2022; Mukhopadhyay & Kumar, 2020).

Heterotrimeric G-proteins consist of three unique subunits: α, β, and γ (Li et al., 2021; Neer, 1995). These proteins become active when seven-transmembrane domain receptors are stimulated, leading to interactions with effector molecules that trigger specific gene expression. When in its inactive state, the α subunit is attached to GDP, while the βγ dimer ensures the stability of the heterotrimeric configuration. Upon ligand binding to the receptor, GDP is replaced by GTP on the α subunit, prompting a structural alteration that results in the separation of the complex into GTP-bound α (GTP-Gα) and βγ dimers. Once separated, these components can interact with various downstream effectors, such as phosphodiesterases, protein kinases, adenylate cyclases, phospholipases, and calcium ion channels, thus triggering multiple signal transduction pathways (Mukhopadhyay & Kumar, 2020; Neer, 1995). When G-proteins are activated, they rapidly alter the levels of intracellular second messengers, including cAMP, inositol phosphates, diacylglycerol (DAG), and calcium ions in the cytosol (Gudermann et al., 1997; Mukhopadhyay & Kumar, 2020). Over time, the α subunit's inherent GTPase activity converts GTP back into GDP, which facilitates the reassembly of the signaling complex with the βγ dimer (Li et al., 2021; Kao et al., 2022; Neer, 1995; Fang & Dean, 2000).

Gα subunits are categorized into four main families based on sequence similarity: $G\alpha_i$, $G\alpha_s$, $G\alpha_q$, and $G\alpha_{12}$ (Mukhopadhyay & Kumar, 2020; Li et al., 2021; Simon et al., 1991). The $G\alpha_i$ family comprises the subunits $G\alpha_i$, $G\alpha_o$, $G\alpha_z$,

and $G\alpha_t$ (transducin). Researchers have successfully cloned genes responsible for encoding G-protein subunits from a variety of fungal species (Klymiuk et al., 2021; Fang & Dean, 2000; Nishimura et al., 2003). Research has demonstrated that genes responsible for encoding either the α (Liu & Dean, 1997; Mukhopadhyay & Kumar, 2020; Regenfelder et al., 1997) or β (Doehlemann et al., 2006; Nishimura et al., 2003) subunits are crucial for the formation of infection structures in fungi.

Studies have shown that the CGA1 gene, which encodes the Gα subunit in *C. heterostrophus*, is essential for the formation of appressoria (Horwitz et al., 1999; Kao et al., 2022; Lu et al., 2016). In *M. grisea*, researchers have identified three genes that encode Gα, with *MAGB* being crucial for appressoria formation (Doehlemann et al., 2006; Liu & Dean, 1997). Interference with *MAGB* leads to defective appressorium development (Liu & Dean, 1997; Fang & Dean, 2000). In a similar vein, the gene mgb1, responsible for encoding the β subunit, is crucial for the development of appressoria in *M. grisea* (Nishimura et al., 2003). Conidia with a mutation in *mgb1* are unable to develop appressoria, penetrate rice foliage, or commence invasive growth (Mukhopadhyay & Kumar, 2020; Kao et al., 2022; Nishimura et al., 2003). Additionally, the *cpg-1* gene, which encodes a Gα subunit in the chestnut blight fungus *Cryphonectria parasitica*, plays a role in the signal transduction pathways linked to infection (Choi et al., 1995).

The *ctg-1* gene, which is responsible for encoding a Gα homolog, was extracted from *Colletotrichum trifolii*, the pathogen that causes alfalfa anthracnose (Kao et al., 2022; Truesdell et al., 2000). The presence of *ctg-1* transcripts in germinating conidia indicates its regulatory function during this stage of development (Figure 5). Alteration of the *ctg-1* gene resulted in transformants that failed to germinate (Truesdell et al., 2000), suggesting that *ctg-1* is crucial for the initial pathogenic development of *C. trifolii*. In *B. cinerea*, the gene *bcg1*, which encodes Gα, has been demonstrated to play a role in virulence (Gronover et al., 2001; Kao et al., 2022; Li et al., 2021).

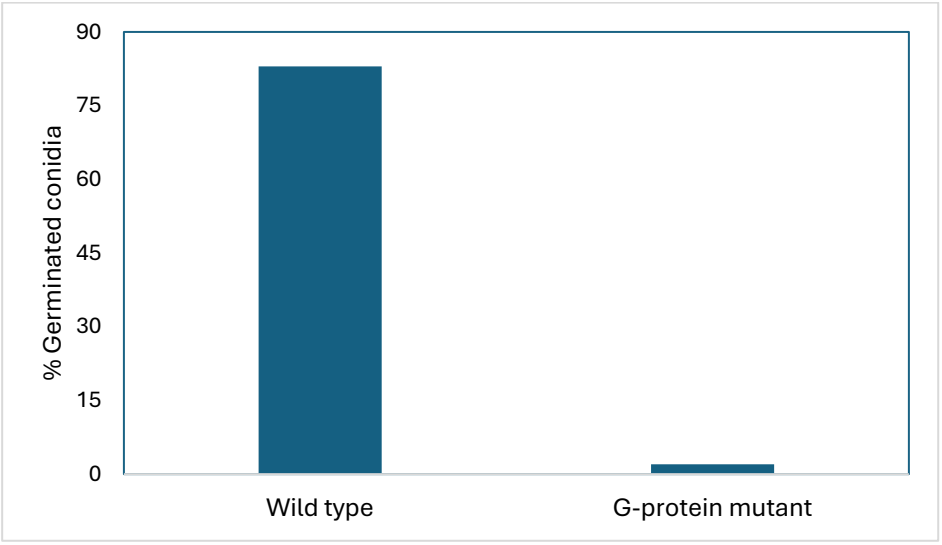

Figure 5. Germination characteristics of wild-type and *ctg-1* disrupted *Colletotrichum trifolii*

The collective findings emphasize the pivotal role of G-protein signaling in the progression of fungal diseases (Kao et al., 2022; Lu et al., 2016; Nishimura et al., 2003). Altering the genes responsible for Gα subunits leads to a notable decline in the virulence of a range of pathogens. For instance, *U. maydis* mutants deficient in Gα are incapable of inducing disease in maize (Mukhopadhyay & Kumar, 2020; Regenfelder et al., 1997). The removal of the *cpg-1* gene in *C. parasitica* results in the loss of both conidiation and the ability to infect chestnut trees (Mukhopadhyay & Kumar, 2020; Li et al., 2021; Gao & Nuss, 1996). In *Stagonospora nodorum*, a wheat pathogen, the disruption of the *Gna1* gene, which encodes a Gα subunit, results in compromised direct penetration (Solomon et al., 2004). In a similar manner, *M. grisea* strains with *magB* disruptions show decreased ability to form appressoria and reduced virulence (Doehlemann et al., 2006; Li et al., 2021; Kao et al., 2022; Liu & Dean, 1997; Fang & Dean, 2000). Mutants of *C. trifolii* that are missing *ctg-1* show reduced germination of conidia and impaired development of appressoria (Kao et al., 2022; Li et al., 2021; Truesdell et al., 2000). In *B. cinerea*, while *BCG1* mutants are capable of penetrating host leaves and initiating primary lesions, they are unable to further infiltrate plant tissue, which leads to the lack of secondary lesion development (Gronover et al., 2004; Oubohssaine et al., 2025).

The combined results underscore the essential function of G protein signaling in the signal transduction mechanisms linked to fungal infections.

Upon activation of G-proteins, pathways involving calcium-dependent messengers, cAMP/protein kinase cascades, and MAP kinase (MAPK) pathways are commonly initiated.

The enzyme adenylate cyclase, located in the cell membrane, facilitates the transformation of ATP into cAMP. The gene *MAC1*, which is responsible for the production of adenylate cyclase, has been successfully cloned from *M. grisea* (Choi & Dean, 1997; Fu et al., 2022). Mutants deficient in the *mac1* gene are incapable of forming appressoria on surfaces that typically induce their development and are unable to penetrate susceptible rice leaves. Nevertheless, introducing external cAMP can reestablish the formation of appressoria, indicating that *MAC1* acts prior to cAMP production (Choi & Dean, 1997; Miranda et al., 2015; Sun et al., 2019). Research has indicated that the β subunit of G-proteins engages directly with adenylate cyclase, thereby affecting the levels of intracellular cAMP (Chen et al., 1995; Kao et al., 2022; Li et al., 2021; Yan & Gautam, 1996; Nishimura et al., 2003). As a result, adenylate cyclase might serve as a downstream target for G-proteins that have been activated, and adding external cAMP can make up for certain deficiencies in Gα (Krüger et al., 1998; Loubradou et al., 1999). Encoded by *MAGB* in M. grisea, the Gα protein is responsible for the direct activation of adenylate cyclase, which in turn initiates cAMP-dependent signaling pathways (Liu & Dean, 1997; Oubohssaine et al., 2025). Introducing exogenous cAMP can reinstate the development of appressoria in *magB* mutants (Mukhopadhyay & Kumar, 2020; Liu & Dean, 1997). The results indicate that a signaling cascade reliant on cAMP, which follows adenylate cyclase, plays a crucial role in the signaling pathway mediated by G-proteins.

Additional proof of the interaction between fungal G-proteins and adenylate cyclase comes from research on *C. parasitica* strains, where the wild-type *cpg-1* gene, which codes for the Gα subunit, was substituted with a null allele (Mukhopadhyay & Kumar, 2020; Li et al., 2021; Gao & Nuss, 1996). These strains exhibited elevated intracellular cAMP levels, suggesting that *CPG-1* may typically inhibit adenylate cyclase or exert a negative effect on the Gβγ dimer, which otherwise activates the enzyme (Gao & Nuss, 1996). In contrast, the protein *MagB*, which is encoded by the *magB* Gα gene, has been demonstrated to increase the activity of adenylate cyclase (Doehlemann et al., 2006; Liu & Dean, 1997). Certain Gα subunits may also function through signaling pathways independent of cAMP. In B. cinerea, the expression of most genes controlled by the Gα subunit BCG1 continued to be observed in adenylate cyclase mutants, suggesting that BCG1 is likely involved in at least one other

signaling pathway beyond the cAMP-dependent mechanism (Fu et al., 2022; Gronover et al., 2004; Miranda et al., 2015; Sun et al., 2019).

In *M. grisea*, the introduction of external cAMP can initiate the formation of appressoria, even in the absence of conventional stimuli like hydrophobic surfaces or cutin monomers (Choi & Dean, 1997; Fu et al., 2022; Adachi & Hamer, 1998). Notably, *mac1* adenylate cyclase mutants, similar to *magB* mutants, exhibit defects in appressorium development. Despite the inability of *magB* mutants to differentiate on hydrophobic surfaces, they continue to react to soluble cutin monomers (Miranda et al., 2015; Liu & Dean, 1997; Sun et al., 2019). Conversely, *mac1* mutants fail to react to either of the inductive signals (Choi & Dean, 1997; Fu et al., 2022). The findings suggest that parallel sensory input pathways merge either at the adenylate cyclase protein (Mac1p) or before it, which is encoded by *MAC1*, in *M. grisea*.

5.3. Signal Modulation via Ca^{2+}/Calmodulin Complexes

Calcium ions (Ca^{2+}) in the cytosol are crucial cations that significantly influence the growth of hyphal tips and the development of appressoria in various organisms (Jackson & Heath, 1993; Huang et al., 2020). In fungal cells, the Ca^{2+} signaling cascade is generally initiated by conformational changes in membrane-associated GTP-binding proteins (G-proteins) following receptor activation (Larson et al., 1992; Li et al., 2021). Following their activation, these G-proteins stimulate phospholipase C, which then facilitates the breakdown of phosphatidylinositol-4,5-bisphosphate (PIP_2) into inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (Oubohssaine et al., 2025; Su et al., 2001). IP_3 subsequently triggers the release of endogenous Ca^{2+} from the body's internal calcium reserves (Belde et al., 1993; Li et al., 2024). The subsequent rise in cytosolic Ca^{2+} concentrations activates calmodulin, a receptor protein that binds calcium. This interaction causes changes in the structure of calmodulin, allowing it to activate a range of downstream enzymes, such as multifunctional calmodulin-dependent protein kinases and protein phosphatases (de Oliveira Silva et al., 2024; Li et al., 2024; Klymiuk et al., 2021; Kim et al., 1998).

The involvement of Ca^{2+} signaling pathways in the formation of appressoria has been thoroughly studied in *C. trifolii* (Huang et al., 2020; Kao et al., 2022; Warwar & Dickman, 1996) as well as in *C. gloeosporioides* (Kim et al., 1998; Klymiuk et al., 2021; Li et al., 2024). Research has shown that inhibitors affecting different phases of the calcium signaling pathway can hinder the formation of appressoria in *C. gloeosporioides* (Fu et al., 2022; Uhm et al., 2003). Intracellular Ca^{2+} levels can be regulated either by releasing calcium from internal stores or by allowing its entry through Ca^{2+} channels in

the cell membrane (Cornelius et al., 1989). TMB-8 [3,4,5-trimethoxybenzoic acid 8-(diethylamino)octyl ester], known for its ability to inhibit the release of Ca^{2+} from internal reserves, has been demonstrated to prevent the formation of appressoria in *C. gloeosporioides* (Figure 6). In a similar manner, methoxyverapamil, which inhibits calcium channels in the plasma membrane, also hinders the formation of appressoria. These findings suggest that calcium channels are crucial for initiating the signal transduction pathways that regulate appressorium development in fungal pathogens. In the context of appressorium formation in both *M. grisea* and *C. gloeosporioides*, the release of Ca^{2+} from intracellular stores is considered more crucial than the influx of calcium through the plasma membrane (Fu et al., 2022; Huang et al., 2020; Lee & Lee, 1998; Li et al., 2024; Sun et al., 2019; Uhm et al., 2003).

Calmodulin, a protein that binds calcium, primarily facilitates the intracellular actions of Ca^{2+}. Research has linked calmodulin to the development of appressoria in *C. gloeosporioides* (Kim et al., 1998; Klymiuk et al., 2021; Li et al., 2024) as well as in *C. trifolii* (Buhr & Dickman, 1997; de Oliveira Silva et al., 2024; Wang et al., 2020). Chlorpromazine acts as a calmodulin antagonist by competing with Ca^{2+} for binding to calmodulin, effectively inhibiting Ca^{2+}/calmodulin-dependent signaling and decreasing appressorium formation in *C. gloeosporioides* (Li et al., 2024; Sun et al., 2019; Figure 6; Uhm et al., 2003). Similar inhibitory effects have been noted with other calmodulin antagonists in this species (Uhm et al., 2003). Calmodulin antagonists have been shown to interfere with the development of appressoria in *C. trifolii* (Dickman et al., 1995; Huang et al., 2020; Li et al., 2024; Warwar & Dickman, 1996), *M. grisea* (Huang et al., 2020; Lee & Lee, 1998; Oubohssaine et al., 2025), and *Phyllosticta ampelicida* (Shaw & Hoch, 2000; Kao et al., 2022; Li et al., 2024).

Research has successfully cloned calmodulin (*cam*) genes from *C. gloeosporioides* (Kim et al., 1998; Li et al., 2024), *C. trifolii* (Kao et al., 2022; Warwar et al., 2000), and *M. grisea* (Liu & Kolattukudy, 1999), demonstrating an impressive level of conservation with nearly identical nucleotide sequences across these species. In *C. trifolii*, researchers cloned a calmodulin gene and used an antisense approach to suppress its expression, which resulted in a significant reduction in the frequency of appressorium formation (Doehlemann et al., 2006; Kao et al., 2022; Dean, 1997; Warwar et al., 2000). The mucilage secreted by *M. grisea* conidia is essential for attaching to host surfaces, a process that is critical for the formation of appressoria (Hamer et al., 1988). Concanavalin A (ConA), a type of lectin, was found to hinder surface adhesion, the expression of the cam gene, and the formation of appressoria, while leaving

conidial germination unaffected (Li et al., 2024; Liu & Kolattukudy, 1999). These findings highlight the crucial role of Ca^{2+}/calmodulin signaling in the formation of fungal appressoria.

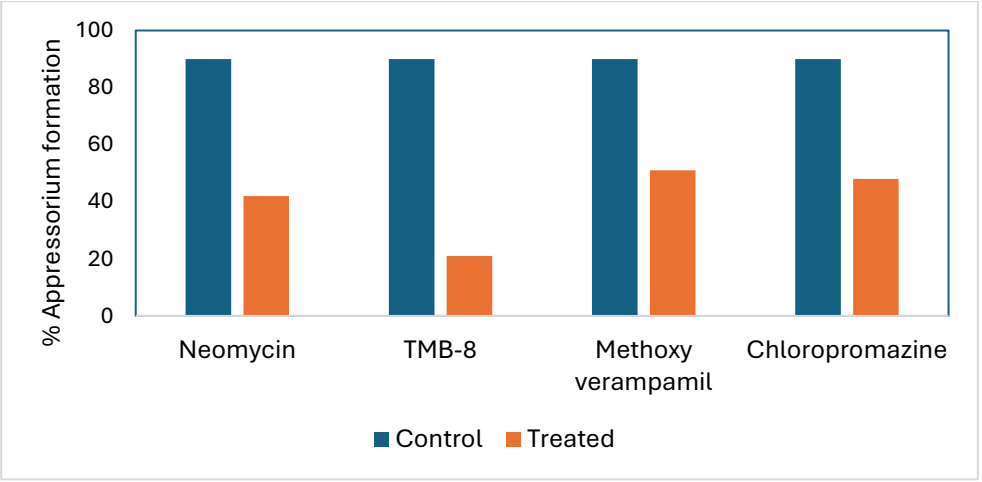

Figure 6. The effect of calcium/calmodulin signaling pathway inhibitors on the development of appressoria in *C. gloeosporioides*.

The phosphoinositide signaling pathway is closely linked to calcium signaling via inositol trisphosphate (IP_3), which acts as an intracellular trigger for calcium. IP_3 is produced from phosphatidylinositol 4,5-bisphosphate (PIP_2) through the enzymatic activity of activated phospholipase C and is then released into the cytosol (Chethana et al., 2021; Devecha & Irvine, 1995; Li et al., 2024; Oubohssaine et al., 2025; Ryder et al., 2022). Neomycin, recognized as an inhibitor of phospholipase C, has been shown to prevent the formation of appressoria in *C. gloeosporioides*, highlighting the crucial importance of the phosphoinositide signaling pathway in this developmental stage (Figure 6; Uhm et al., 2003).

5.4. cAMP/Protein Kinase Signaling Pathways

cAMP acts as an essential secondary messenger in signal transduction pathways. The cAMP signaling pathway operates downstream of signaling mediated by G-proteins (Fu et al., 2022; Miranda et al., 2015; Doehlemann et al., 2006; Nishimura et al., 2003). In eukaryotic cells, the regulatory subunit of protein kinase A (PKA) is one of the most extensively studied intracellular targets of cAMP (Kao et al., 2022; Taylor et al., 1990). PKA is a holoenzyme composed of four subunits: two regulatory and two catalytic. When cAMP attaches to the regulatory subunits, the catalytic subunits are released, leading

to the phosphorylation of target proteins that play a role in cellular processes regulated by cAMP (Adachi & Hamer, 1998; Li et al., 2022). It is widely recognized that cAMP signaling is crucial in the development of fungal diseases (Doehlemann et al., 2006; Kronstad, 1997).

In *M. grisea*, increased levels of intracellular cAMP have been linked to the early signaling processes that initiate the formation of appressoria (Fu et al., 2022; Miranda et al., 2015; Lee & Dean, 1993a; Sun et al., 2019). When *M. grisea* conidia begin to grow on hydrophobic surfaces, the germ tube's tip transforms into an appressorium. In contrast, on hydrophilic surfaces, the germ tube continues to grow vegetatively without forming an appressorium. Applying cAMP externally to either germinating conidia or vegetative hyphae triggers the formation of appressoria, even on surfaces that are hydrophilic (Lee & Dean, 1993a; Li et al., 2022). This observation suggests that cAMP can circumvent the necessity for specific surface signals that typically trigger appressorium formation (Figure 7). According to Lee & Dean (1993b), the interaction between the proteins in the germ tube's cell wall and the host's surface triggers the production of cAMP, which in turn initiates the developmental process for forming an appressorium.

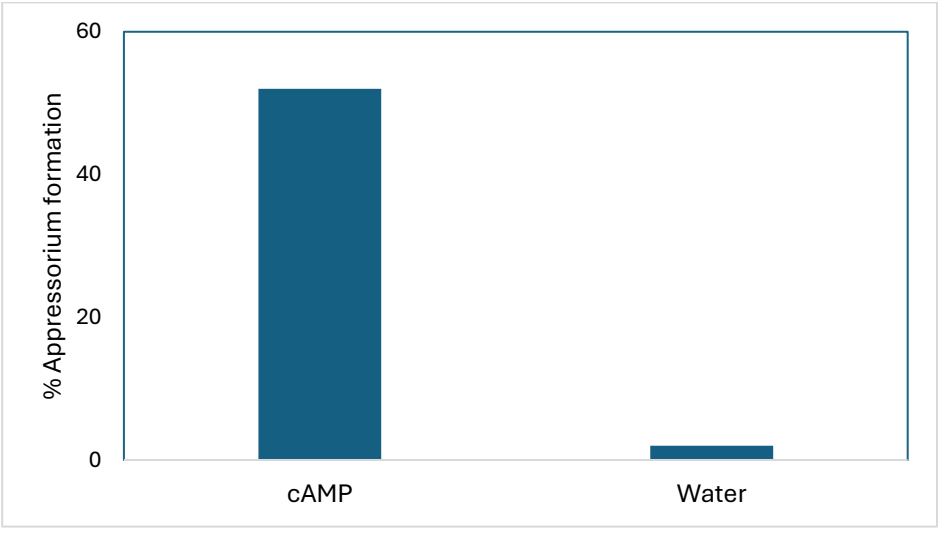

Figure 7. The impact of cyclic adenosine monophosphate (cAMP) on the initiation of appressorium formation in *M. grisea* on hydrophilic surfaces.

Yeast extract appears to prevent the development of appressoria in *M. grisea*, probably by disrupting the initial signaling required for differentiation

(Beckerman & Ebbole, 1996; Ebbole, 2007; Younas et al., 2025). However, the addition of cAMP alongside yeast extract restores appressorium development (Beckerman & Ebbole, 1996; Kao et al., 2022). Mutants lacking the *mac1* gene, responsible for coding adenylate cyclase, are unable to develop appressoria (Figure 8). However, the addition of external cAMP restores typical appressorium development in these mutants (Adachi & Hamer, 1998; Fu et al., 2022; Miranda et al., 2015; Younas et al., 2025). The collective evidence indicates that cAMP plays a vital role as a secondary messenger in the signal transduction pathway that triggers the formation of appressoria (Figure 9).

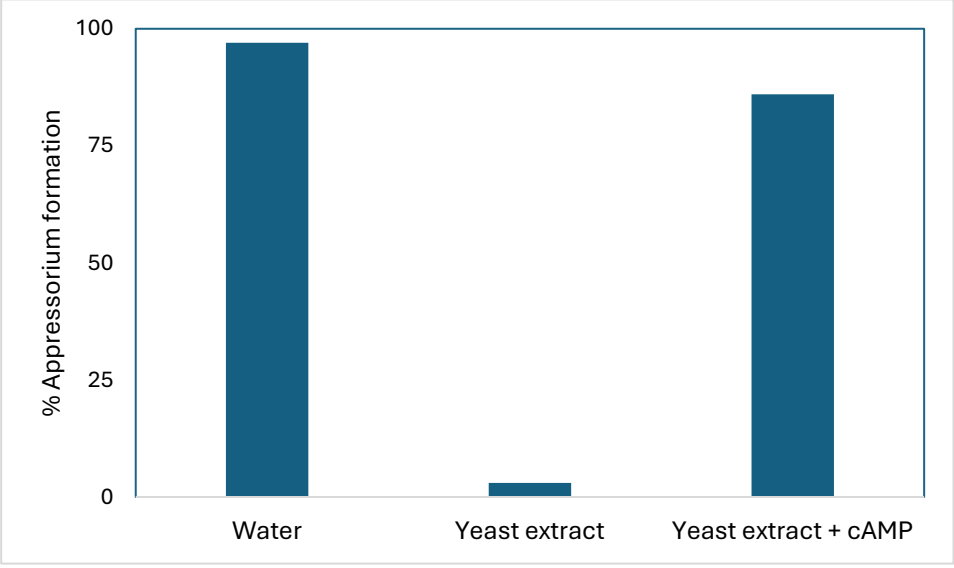

Figure 8. The involvement of cyclic adenosine monophosphate (cAMP) in initiating the formation of appressoria in *M. grisea* on hydrophobic surfaces.

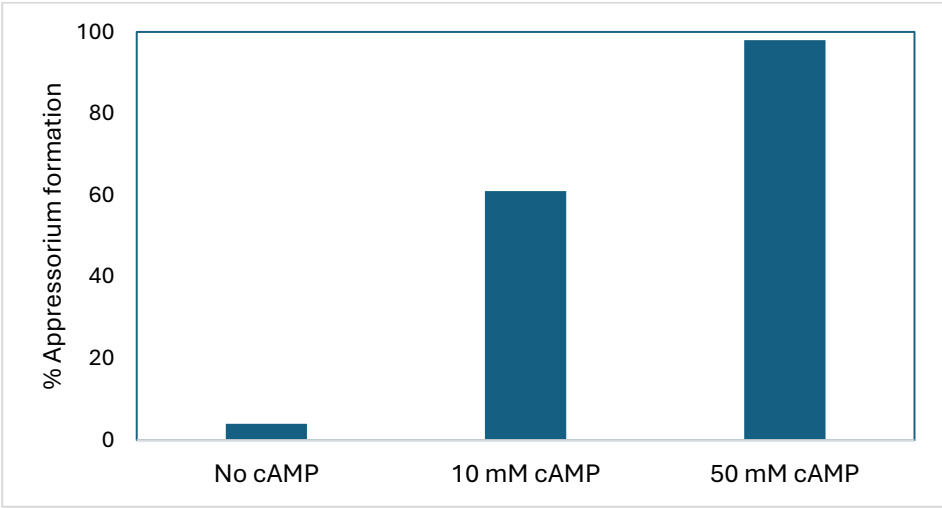

Figure 9. The addition of cAMP corrects the issues in appressorium development observed in mac1 mutants of *M. grisea*.

The cAMP signaling pathway serves as a crucial and conserved mechanism among a range of fungal pathogens, playing a role in the onset of diseases like rust (Chock, 2020; Gold et al., 1997; Negussie & Pretorius, 2012), blast (Mitchell & Dean, 1995; Choi et al., 1998), and powdery mildew (Hall & Gurr, 2000; Huth et al., 2023; Lee et al., 2003). This pathway plays a crucial role in the formation of appressoria in *B. graminis* f. sp. *hordei* (Feng et al., 2009; Hall & Gurr, 2000; Sugai et al., 2020). Similarly, in *Ustilago maydis*, the process of morphogenesis regulated by cAMP is essential, with the regulatory subunit of a cAMP-dependent protein kinase playing a critical role in the formation of galls on maize (Gold et al., 1994; Gold et al., 1997). The role of cAMP-dependent protein kinase in the formation of appressoria in *C. trifolii* is well-established (Fu et al., 2022; Yang & Dickman, 1997, 1999b).

In *U. maydis*, the union of haploid cells is crucial for developing infection structures. The pathogen's capacity to induce disease relies on its transformation from a non-infectious yeast-like haploid form into an infectious filamentous dikaryotic state. This change takes place when haploid cells with different alleles at both the a and b mating-type loci come together. The cAMP pathway plays a crucial role in controlling the genes responsible for mating and development (Fu et al., 2022; Miranda et al., 2015; Krüger et al., 1998; Müller et al., 1999). Essential elements of this pathway consist of the Gα subunit Gpa3 (Doehlemann et al., 2006; Regenfelder et al., 1997; Krüger et al., 1998), the adenylate cyclase Uac1, along with the regulatory (Ubc1) and catalytic (Adr1)

subunits of PKA (Gold et al., 1997). This signaling cascade is also critical for the fungus's subsequent development within plant tissues. According to Gold et al. (1997), the complete absence of function in the genes *gpa3*, *uac1*, *ubc1*, or *adr1* entirely prevents the development of tumors. Mutations in either the Gα or the PKA regulatory subunit lead to moderate cAMP activation, which subsequently changes the structure of maize tumors (Doehlemann et al., 2006; Krüger et al., 2000; Sun et al., 2019). The results collectively indicate that in *U. maydis*, the cAMP signaling pathway operates downstream of G-proteins.

It is thought that PKA mediates the influence of cAMP on the formation of appressoria in *M. grisea*. In 1998, Adachi & Hamer discovered *M. grisea* strains that displayed unstable *mac1* phenotypes. One of the bypass suppressor mutations was found in the PKA regulatory subunit, which enabled the formation of appressoria on non-inductive (hydrophilic) surfaces and when yeast extract was present. The results indicate that cAMP affects the formation of appressoria through PKA, most likely by means of its catalytic subunit. The catalytic subunit of cAMP-dependent PKA plays a vital role in the development of appressoria and the pathogenicity of *M. grisea* (Mitchell & Dean, 1995). The gene *CPKA*, responsible for encoding this catalytic subunit, is crucial for the correct formation of appressoria (Xu & Hamer, 1996; Xu et al., 1997). Mutants that lack *cpkA* show a delay in the formation of appressoria, but they eventually generate appressoria in quantities similar to those of wild-type strains. Despite being completely melanized, these mutant appressoria are unable to penetrate the host, suggesting that *CPKA* has a distinct function during the infection's penetration phase (Xu et al., 1997).

The gene known as *pka1*, which encodes a cAMP-dependent PKA catalytic subunit in *Sclerotinia sclerotiorum*, is crucial for the infection process (Jurick et al., 2004). According to Jurick et al. (2004), an increase in intracellular cAMP levels triggers the production of oxalate in this pathogen. There is a direct link between the rise in cAMP levels and the increased production of oxalic acid, with mutants that lack the ability to produce oxalate being non-pathogenic (Godoy et al., 1990; Rollins & Dickman, 1998). The results suggest that the cAMP signaling pathway plays a role in controlling oxalate production, which is essential for the pathogenicity of *S. sclerotiorum* (Jurick et al., 2004).

5.5. MAPK-Mediated Signal Transduction Pathways

Mitogen-activated protein kinases (MAPKs) in fungal cells are essential for interpreting a range of external signals, which subsequently influence key biological activities such as growth, differentiation, and pathogenesis (Chun et

al., 2019; Takano et al., 2000; Xu, 2000; Park et al., 2004; Zhang et al., 2021). MAPKs comparable to the yeast Fus3/Kss1, Slt2, and Hog1 have been found in a wide range of fungal pathogens, where they are vital for the development of appressoria, the expansion of invasive hyphae, and the pathogens' ability to cause disease (Doehlemann et al., 2006; Xu, 2000; Zhang et al., 2021). A standard MAPK cascade consists of three linked kinases: a MAPK, a MAPK kinase (MAPKK), and a MAPK kinase kinase (MAPKKK). When external signals are detected, MAPKKK phosphorylates MAPKK at serine/threonine sites within an SXXXS/T sequence, leading to its activation. MAPKK, which is a dual-specificity kinase, then activates MAPK by phosphorylating the conserved threonine and tyrosine residues in the TXY sequence found between kinase subdomains VII and VIII. Once activated, MAPK phosphorylates various downstream targets, including enzymes and transcription factors, thus influencing cellular responses and translating external signals into messages within the cell (Chun et al., 2019; Doehlemann et al., 2006; Herskowitz, 1995; Liu et al., 2000; Zhang et al., 2021).

MAPK cascades can operate either downstream or alongside the cAMP-PKA signaling pathway. Researchers have pinpointed two genes in *M. grisea* that encode MAPKs, specifically PMK1 and MPS1 (Chun et al., 2019; Doehlemann et al., 2006; Xu & Hamer, 1996; Xu et al., 1998; Zhang et al., 2021). Disruption of these genes impedes either the development of appressoria or the process of penetration. It is noteworthy that both mutants exhibit a partial reaction to external cAMP during the early phases of appressorium formation, suggesting a functional interplay between the cAMP-PKA and MAPK signaling pathways (Cruz-Mireles et al., 2024; Klymiuk et al., 2021; Kronstad et al., 1998). The MAPK gene *ubc3*, also known as *kpp2*, in *Ustilago maydis*, is a homolog of PMK1 from *M. grisea* and has been the subject of research (Fu et al., 2022; Mayorga & Gold, 1999; Miranda et al., 2015; Müller et al., 1999). Removing this gene does not affect vegetative growth or structure, but it greatly hinders mating, the development of filamentous dikaryons, and virulence. According to Müller et al. (1999), when plants are exposed to pathogenic haploid strains that lack *kpp2*, they only form small tumors on the leaves, each measuring less than 1 mm in diameter. Moreover, the genes *ubc4* (MAPKKK) and *ubc5* (MAPKK) within the MAPK pathway are crucial for the filamentous growth and pathogenicity of *U. maydis*, as reported by Andrews et al. (2000) and Yu et al. (2023). In this smut fungus, the MAPK module transmits signals to the transcription factor Prf1, which contains a high-mobility group (HMG) domain, subsequently initiating pathogenic development (Brefort et al., 2005; de Oliveira Silva et al., 2024; Doehlemann et al., 2006; Zhang et al., 2021).

The *kpp6* gene is responsible for producing another MAPK, which plays a crucial role in the infection process of *U. maydis* (Brachmann et al., 2003; Yu et al., 2023). This gene generates two transcripts of varying lengths, yet they produce the same protein. Mutants with a defective *kpp6* allele show reduced virulence. Although these mutants are capable of forming appressoria, they are unable to breach the plant cuticle. This suggests that *kpp6* plays a crucial role in controlling the expression of genes essential for the effective penetration of host tissue in response to host-derived signals (Brachmann et al., 2003). As noted earlier, cAMP signaling plays a crucial role in the pathogenesis of *U. maydis*, and the interaction between cAMP and MAPK signaling pathways is vital during the infection process (Yu et al., 2023).

The MAPKs Pmk1 and Mps1 are crucial for the pathogenic abilities of *M. grisea*. Pmk1 belongs to the ERK subfamily, which is a part of the larger MAPK superfamily, and plays a role in conveying environmental signals within eukaryotic cells. PMK1 plays a crucial role in the development of appressoria and the pathogenic abilities of the organism, much like the yeast Fus3 MAPK. Mutants that lack *pmk1* are unable to form appressoria and remain non-pathogenic, even when they are directly introduced into plant tissues. Although external cAMP cannot completely restore the phenotype, these Δpmk1 mutants are still capable of initiating the early stages of appressorium development, such as forming hooks, in the presence of cAMP (Cruz-Mireles et al., 2024; Xu & Hamer, 1996). Thus, Pmk1 plays a crucial role in the development of appressoria and the progression of invasive growth, although it is not necessarily needed for other developmental activities such as mating.

The Mps1 MAPK belongs to the yeast ERK (YERK2) subfamily and shares characteristics with the yeast Slt2 (Mpk1) kinase (Chun et al., 2019; Doehlemann et al., 2006; Lee et al., 1993; Xu et al., 1998; Zhang et al., 2021). It is essential for the penetration of host tissues (Cruz-Mireles et al., 2024; Klymiuk et al., 2021; Lee & Dean, 1993a; Xu et al., 1998). Moreover, MAPKs play a crucial role in the development of functional appressoria. The appressorium of *M. grisea* generates considerable turgor pressure to breach the rice cuticle via a slender penetration peg (Howard et al., 1991). The PMK1 MAPK cascade regulates the pressure increase, which is aided by the movement of stored carbohydrates and lipids (Cruz-Mireles et al., 2024; Thines et al., 2000).

Researchers have identified a MAPK gene in *B. cinerea* that shares a close relationship with the PMK1 gene found in *M. grisea*, and they have designated it as BMP1 (Botrytis MAPK required for pathogenesis 1) (Doehlemann et al., 2006; Zhang et al., 2021; Zheng et al., 2000). This MAPK

pathway enhances the pathogen's ability to penetrate. While conidia from *bmp1* mutants can germinate on the surfaces of plants, they are incapable of penetrating host tissues. Moreover, these *bmp1* mutants exhibit stunted growth, but the introduction of the wild-type *BMP1* allele reinstates normal growth rates (Zheng et al., 2000). The mutants do not lead to disease in either carnation flowers or tomato leaves, highlighting the essential function of MAPK signaling in the pathogenicity of *B. cinerea* (Figure 10).

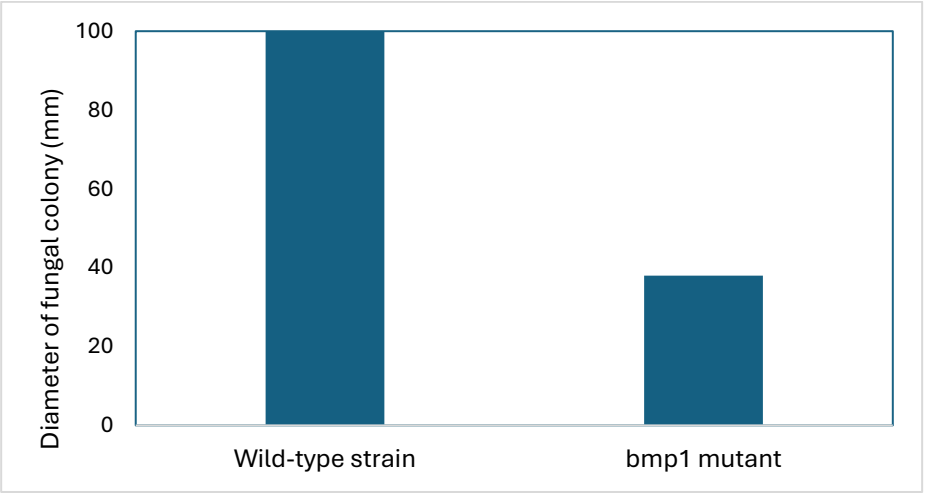

Figure 10. Role of the *BMP1* MAPK Gene in Regulating Growth of *Botrytis cinerea*

The infection process in *C. lagenarium*, which causes cucumber anthracnose, involves the germination of conidia, the formation of melanized appressoria, penetration, and subsequent invasive growth. The MAPK signaling pathway is essential at every stage of these processes (Chun et al., 2019; Takano et al., 2000; Zhang et al., 2021). Two MAPK genes, *CMK1* and *MAF1*, have been discovered in this pathogen by researchers (Takano et al., 2000; Kojima et al., 2002). In contrast to wild-type conidia, which can stick to solid surfaces, *cmk1* mutants show a marked decrease in adhesion (Table 1.1; Takano et al., 2000), indicating that signals from surface contact trigger infection through MAPK signaling. While wild-type conidia germinate effectively on hard surfaces, *cmk1* mutants fail to germinate under identical conditions (Table 1.1). Despite being exposed to yeast extract, which promotes germination, *cmk1* mutants fail to develop appressoria. They also fail to penetrate host surfaces, and their vegetative hyphal growth is markedly restricted when inoculated onto wounded leaves. The results highlight the

essential function of *CMK1* in facilitating invasive growth on cucumber leaves (Fu et al., 2022; Li et al., 2021; Takano et al., 2000).

MAPK signaling pathways are crucial in conidial germination, appressorium formation, and fungal pathogenicity. In *C. lagenarium*, the *CMK1* MAPK gene plays a role in melanin synthesis and the development of appressoria (Chun et al., 2019; Fu et al., 2022; Li et al., 2021; Takano et al., 2000). Interestingly, *cmk1* mutants are still capable of producing melanin during their vegetative phase. The genes *PKS1*, *SCD1*, and *THR1*, which play a role in the production of melanin, are expressed during the process of mycelial melanization in both wild-type and cmk1 mutant strains. In the wild-type strain, these gene transcripts are absent in conidia before inoculation but appear three hours post-inoculation under conditions that promote germination. Conversely, in the *cmk1* mutant, these transcripts are not present even after 16 hours of water incubation, during which germination does not occur. Nevertheless, when the *cmk1* mutant is placed in a yeast extract solution conducive to germination, there is a significant increase in the expression of all three melanin genes. This indicates that the transcription of genes involved in melanin production is intricately connected to the process of conidial germination. Despite the fact that *cmk1* mutants fail to undergo melanization during germination, the genes remain highly expressed, underscoring the essential function of *CMK1* in both the melanization process and the development of functional appressoria (Takano et al., 2000; Zhang et al., 2021).

Studies have pinpointed another MAPK gene in *C. lagenarium*, termed *MAF1*, as vital for the early stages of appressorium development. Mutants with a disrupted *maf1* gene fail to form this infection structure (Kojima et al., 2002; Zhang et al., 2021). The results suggest that *MAF1* is associated with the initial stages of morphogenesis, whereas *CMK1* is involved in the later stages of appressorium development. Collectively, these studies highlight the critical role of MAPK signaling in spore adhesion, germination, the development of a functional appressorium, and the invasive growth within host plants.

In *C. heterostrophus*, the pathogen responsible for southern corn leaf blight, a MAPK gene called *CHK1* has been identified and categorized under the ERK subfamily of the MAPK superfamily, which is known for enabling extracellular signal transduction in eukaryotic organisms (Chethana et al., 2021; Lev et al., 1999; Kültz, 1998; Ryder et al., 2022). The absence of *chk1* greatly impedes the formation of infection structures, highlighting its crucial role in the progression of pathogenicity.

In a similar vein, the MAPK gene *PTK1* has been identified and its function analyzed in *P. teres*, the pathogen responsible for net blotch in barley. Mutants with a disrupted *ptk1* allele are incapable of forming appressoria, cannot penetrate host tissue following artificial injury, and entirely lose their ability to infect barley leaves (Klymiuk et al., 2021; Ruiz-Roldan et al., 2001; Zhang et al., 2021).

The MAPK signaling pathway is crucial for the ability of fungi to cause disease in floral tissues. The MAPK gene known as *MAP1* is crucial for *F. graminearum*, the pathogen that causes Fusarium head blight in wheat, to achieve its full level of virulence. Mutants deficient in the *map1* gene are incapable of causing disease in the floral tissues of wheat, indicating that MAPK-mediated signaling is essential for the infection of not only leaves and roots but also reproductive organs (Doehlemann et al., 2006; Segorbe et al., 2017; Urban et al., 2003).

C. purpurea, a common pathogen affecting grasses and cereals with ergot disease, does not develop specialized structures like appressoria for penetration. Nevertheless, MAPK signaling is integral to its infection process within rye ovary tissue. The gene *cpmk1*, which encodes a MAPK analogous to *Fus3* in *S. cerevisiae* and *pmk1* in *M. grisea*, was cloned from *C. purpurea*. Disruption of *cpmk1* does not affect the fungus's growth rate or conidiation but completely abolishes its capacity to infect rye ovaries. Restoration of the wild-type *cpmk1* allele reinstates pathogenicity, highlighting its essential role in host infection (Fu et al., 2022; Mey et al., 2002a; Yin et al., 2016).

Additionally, the insertion of *cpmk1* into a *M. grisea pmk1* mutant completely restores its ability to produce appressoria and regain its pathogenicity on barley. Unlike *C. purpurea*, which invades host tissue by forming penetration hyphae without creating appressoria, *M. grisea* depends on appressoria to aid in penetration. Interestingly, *cpmk1* from *C. purpurea* plays a role in forming infection structures in these different fungi, indicating that the MAPK pathway involving *CPMK1* is evolutionarily preserved across various fungal pathogens (Fu et al., 2022; Mey et al., 2002a; Yin et al., 2016). *CPMK2*, another MAPK in *C. purpurea* and a counterpart of *SLT2*, has been recognized as crucial for its ability to infect rye (Mey et al., 2002b; Segorbe et al., 2017).

The *fmk1* gene, which encodes a MAPK from the *YERK1* subfamily, has been investigated for its role in the soilborne wilt pathogen *F. oxysporum*. Mutants lacking *fmk1* are non-pathogenic on tomato plants, show a diminished ability to attach to root surfaces, and exhibit reduced surface hydrophobicity, as indicated by their failure to move across liquid-air interfaces. Moreover, these

mutants are unable to develop penetration hyphae and exhibit reduced invasive growth on tomato plants, along with a marked decrease in the transcript levels of *pl1*, a gene that codes for a pectate lyase crucial for breaking down the cell wall (Di Pietro et al., 2001). The results underscore the crucial importance of MAPK signaling in the adhesion of spores, the development of penetration hyphae, invasive growth, and the enzymatic breakdown of host cell walls (Doehlemann et al., 2006; Segorbe et al., 2017).

The *czk3* gene, identified as a MAPKKK, has been successfully isolated from *Cercospora zeae-maydis*, the organism that causes gray leaf spot in corn. Research has shown that this gene plays a role in fungal development, the synthesis of cercosporin toxin, and disease advancement, suggesting that MAPK cascades have a wider function in controlling both virulence factors and infection structures (Segorbe et al., 2017; Shim & Dunkle, 2003).

MAPK pathways are a prevalent characteristic among a diverse array of fungal pathogens. The consistency is apparent in leaf pathogens like *C. heterostrophus* (Doehlemann et al., 2006; Segorbe et al., 2017; Lev et al., 1999), *C. lagenarium* (Takano et al., 2000; Kojima et al., 2002), and *P. teres* (Ruiz-Roldan et al., 2001); root pathogens such as *F. oxysporum* (Di Pietro et al., 2001) and *Gaeumannomyces graminis* (Dufresne & Osbourn, 2001); floral pathogens including *C. purpurea* (Mey et al., 2002a,b; Zhang et al., 2021) and *F. graminearum* (Segorbe et al., 2017; Urban et al., 2003); as well as fruit pathogens like *B. cinerea* (Zheng et al., 2000). The *FUZ7* gene in *U. maydis*, which codes for a MAPKK homolog, plays a vital role in the organism's ability to develop pathogenicity (Banuett & Herskowitz, 1994; Chun et al., 2019; Yin et al., 2016).

Genes within the MAPK family, such as fmk1 from *F. oxysporum* (Di Pietro et al., 2001; Segorbe et al., 2017), *cpmk1* and *cpmk2* from *C. purpurea* (Fu et al., 2022; Mey et al., 2002a,b), *MAP1* from *F. graminearum* (Segorbe et al., 2017; Urban et al., 2003), *BMP1* from *B. cinerea* (Zheng et al., 2000), a *pmk1*-like gene from *G. graminis* (Dufresne & Osbourn, 2001), *Cmk1* from *C. lagenarium* (Takano et al., 2000), and *CHK1* from *C. heterostrophus* (Lev et al., 1999), exhibit a significant similarity to pmk1 from *M. grisea*. Interestingly, the *fmk1* gene from *F. oxysporum* is capable of compensating for the shortcomings observed in *M. grisea pmk1* mutants, which include issues with adhesion, surface hydrophobicity, host penetration, and pathogenicity (Di Pietro et al., 2001; Doehlemann et al., 2006). Similarly, *Cmk1* from *C. lagenarium* can rectify issues in appressorium development in *M. grisea pmk1* mutants (Klymiuk et al., 2021; Ruiz-Roldan et al., 2001; Takano et al., 2000; Zhannon-pathogenic1), and the introduction of either *cpmk1* or *cpmk2* into *M.*

grisea fully reinstates appressorium formation and virulence on barley (Mey et al., 2002b). These findings provide strong evidence that the MAPK signaling pathway is highly conserved across various fungal species and plays a crucial role in both developmental and infection processes associated with pathogenesis.

5.6. Protein Kinase Signaling Pathways Mediated by Lipid Activation

A unique category of protein kinases, known as lipid-activated protein kinases (LIPKs), has been associated with the pathogenicity of fungi. Scientists have identified a LIPK gene from *C. trifolii*, the organism that causes alfalfa anthracnose (de Oliveira Silva et al., 2024; Dickman et al., 2003; Doehlemann et al., 2006). This gene is specifically activated by purified plant cutin or its monomeric constituents, such as long-chain fatty acids. Mutant strains, created by specifically disrupting the lipk gene, were incapable of forming appressoria and could not infect intact host tissues (Dickman et al., 2003; Klymiuk et al., 2021). The results indicate that the fungus detects the host's surface, leading to the release of cutin monomers, which then trigger the LIPK-mediated signaling pathway crucial for the development of the appressorium.

5.7. PAK-Driven Signal Transduction in Fungal Cells

p21-Activated kinases (PAKs), which belong to the STE20 subgroup of the "other protein kinase" (OPK) family, act as crucial effectors for Rho-type p21 GTPases and are essential in the process of intracellular signal transduction. In *S. cerevisiae*, the Ste20 PAK kinase is recognized for its role in regulating MAPK signaling. Research has identified two genes, *CHM1* and *MST20*, in *M. grisea* that are responsible for encoding PAKs (Ebbole, 2007; Klymiuk et al., 2021; Li et al., 2004; Younas et al., 2025). While *MST20* is not crucial for causing disease, mutants lacking *chm1* show a decrease in appressorium development, and the appressoria that do develop are unable to effectively penetrate the host. The *chm1* mutants exhibit a phenotype that is different from *pmk1* (MAPK pathway) mutants, which have issues with appressorium morphogenesis. The activation of the *PMK1* MAPK pathway does not require *CHM1* during the development of appressoria or the growth of infection hyphae. While *CHM1* is critical for appressorial penetration, its role in *M. grisea* is considered partially redundant with other pathways (Fu et al., 2022; Li et al., 2004; Yin et al., 2016).

5.8. Cascades of Protein Phosphorylation and Dephosphorylation

The process of protein phosphorylation, which is enabled by protein kinases, is essential in numerous biological signal transduction pathways (Flaishman et al., 1995; Fu et al., 2022; Vasselli et al., 2022). Genistein, a protein kinase inhibitor, has been demonstrated to decrease protein phosphorylation while also preventing the onset of appressorium formation in *C. gloeosporioides* (Flaishman et al., 1995; Kao et al., 2022). This finding implies that the activation of protein kinases and the resulting rise in phosphorylation might play a role in signaling pathways that encourage the differentiation of infection structures. Typically, nutrient-rich environments suppress the formation of appressoria in fungi. For example, while yeast extract encourages the germination and robust hyphal growth of *C. gloeosporioides*, it also suppresses the formation of appressoria (Flaishman et al., 1995; Li et al., 2021). In yeast extract, conidia do not exhibit phosphorylation of the 29- and 43-kDa proteins that are linked to the development of appressoria. Conversely, the application of calyculin A, a protein phosphatase inhibitor, restores the phosphorylation of these proteins—also stimulated by host waxes and ethylene—and initiates appressorium formation. The results suggest that certain phosphorylated elements temporarily build up soon after a signal is detected, with their stable levels being controlled by the interplay between kinase and phosphatase activities. As a result, the build-up of phosphorylated proteins and the subsequent formation of appressoria in *C. gloeosporioides* can occur through both the activation of kinases and the suppression of phosphatases (de Oliveira Silva et al., 2024; Flaishman et al., 1995).

5.9. Signaling Mechanisms Mediated by P-Type ATPases

P-type ATPases are known for their unique catalytic subunit, which functions through an enzyme intermediate that becomes phosphorylated during the catalytic process (Palmgren & Axelsen, 1998; Pei et al., 2022; Zhao et al., 2021). These enzymes, named for this "P-type" intermediate, facilitate the translocation of various charged substrates, including H^+, Ca^{2+}, K^+, Na^+, Mg^{2+}, Cu^{2+}, Cd^{2+}, and phospholipids. Plasma membrane H^+-ATPases are among the most thoroughly researched members (Balhadère & Talbot, 2001). P-type ATPases share common structural characteristics, including a site for phosphorylation, a domain for ATP binding, and three other consensus sequences. They participate in a variety of transport and signaling roles (Catty et al., 1997; Palmgren & Axelsen, 1998; Zhao et al., 2021).

P-type ATPases in fungal pathogens play a role in the signaling pathways that result in the formation of penetration hyphae. The PDE1 gene, responsible for encoding a P-type ATPase, was identified in *M. grisea* (Balhadère & Talbot, 2001; Pei et al., 2022). Mutants of *pde1* are nonpathogenic and show decreased ability to penetrate the cuticle, with their penetration structures limited to nonpolarized hyphae within the initial epidermal cell. *PDE1* is responsible for producing a protein that belongs to the DRS2 family of aminophospholipid translocases, which play a crucial role in preserving the asymmetrical distribution of membrane phospholipids. When this asymmetry is regulated, its disruption can serve as a signaling trigger (Bevers et al., 1999; Zhao et al., 2021). The ATPase activity associated with *PDE1* might play a vital role in the swift alterations of the plasma membrane's structure that take place during the development of penetration hyphae. The role of P-type ATPases in signaling during infection processes remains largely uninvestigated in other fungal pathogens.

6. GENES INVOLVED IN INFECTION STRUCTURE FORMATION

Various physical and/or chemical signals that pass through the fungal plasma membrane can trigger the transcription of specific fungal genes necessary for forming infection structures. The use of cyclic AMP (cAMP) in *M. grisea* stimulates the development of appressoria and boosts the expression of several genes associated with infection, including *MPG1*, which codes for the hydrophobin *MPG1* (Irie et al., 2003; Quarantin et al., 2019; Wang et al., 2020). It also affects *GAS1* (*MAS3*) and *GAS2* (*MAS1*), which are responsible for encoding *Magnaporthe*-specific appressorial proteins (Kirby et al., 2016; Xue et al., 2002; Irie et al., 2003), as well as the adenylate cyclase genes *PTH11* and *MAC1* (Irie et al., 2003). While *GAS1* and *GAS2* are not essential for the growth of mycelium, the formation of conidia, or sexual reproduction, they play a crucial role in the penetration of appressoria and the development of lesions (Sugai et al., 2020; Xue et al., 2002). According to Beckerman & Ebbole (1996), the disruption of *MPG1* leads to a marked decrease in the formation of appressoria and the infection of plants. The expression of *MPG1* is regulated by two genes, *NPR1* and *NPR2*, as identified by Lau & Hamer (1996) and Ryder et al. (2022). The complete expression of *MPG1* requires the *MAPK* gene *PMK1*, along with broad-domain regulatory genes like *NPR1* and *NUT1*, which are responsible for controlling nitrogen utilization (Lopatukhin et al., 2024; Soanes et al., 2002). The elevated expression of *MPG1* during the development of appressoria is also reliant on CPKA, which encodes the catalytic subunit of *PKA*, and *NPR1* (Soanes et al., 2002). While mutants deficient in *pth11* and

abc1 show diminished virulence, they still possess the capability to develop appressoria (Chethana et al., 2021; DeZwaan et al., 1999; Urban et al., 1999). Significantly, *PTH11* is capable of initiating the differentiation of appressoria in reaction to environmental signals that prompt this process (DeZwaan et al., 1999).

The *MST12* gene, sourced from *M. grisea*, is essential for the development of invasive hyphae (Huang et al., 2020; Park et al., 2002). While *mst12* mutants can develop standard melanized appressoria, these structures are incapable of breaching the tissues of host plants. This suggests that *MST12* operates downstream of the *PMK1* MAPK pathway, controlling genes crucial for the development of invasive hyphae. In 2003, Wang and colleagues discovered the *ICL1* gene in *M. grisea*, which is responsible for producing isocitrate lyase, an essential enzyme in the glyoxylate cycle. *ICL1* expression increases during the development of infection structures and the penetration of the cuticle. In *icl1* mutants, the initial stage before penetration is affected because the glyoxylate cycle is not functioning. Another gene, *APF1*, found in *M. grisea*, is crucial for the development of appressoria; mutants of *apf* exhibit shortcomings in this process (Silué et al., 1998; Younas et al., 2025). Research indicates that *APF1* functions either as a downstream component of the cAMP-dependent protein kinase within the cAMP signaling pathway or operates independently of it. The *PLS1* gene in *M. grisea* is responsible for producing *Pls1p*, a predicted integral membrane protein consisting of 225 amino acids. This protein is exclusively present in appressoria and is situated in plasma membranes and vacuoles (Clergeot et al., 2001; Ebbole, 2007; Yin et al., 2016). *Pls1p* shares a structural similarity with the tetraspanin protein family, which in animals are integral to membrane signaling complexes that control cell differentiation and adhesion (Gourgues et al., 2002). *Pls1p* is essential for host penetration, as *pls1* mutants form appressoria that cannot penetrate the leaf epidermis. The *ABC1* gene (ATP-binding cassette 1), another gene linked to pathogenicity, is thought to help shield the fungus from the defense mechanisms of host plants (Liu et al., 2023; Urban et al., 1999).

In *C. gloeosporioides*, the genes *cap3* and *cap5*, which have been cloned from spores that develop appressoria, are responsible for encoding cysteine-rich polypeptides made up of 26 and 27 amino acids, respectively (Fu et al., 2022; de Oliveira Silva et al., 2024; Kolattukudy et al., 1995; Lu et al., 2016). These genes are regulated by developmental processes and are specifically active during appressoria formation. Another gene, *cap22*, encodes a 22-kDa protein that contains two N-glycosylation consensus sequences and exhibits limited similarity to various surface glycoproteins (Hwang & Kolattukudy,

1995; Vasselli et al., 2022). Immunogold electron microscopy has revealed that *CAP22* is linked to the appressorial wall, suggesting its function as a glycoprotein that is secreted into this structure. The *cap20* gene, which is uniquely active during the formation of appressoria, produces a 20-kDa protein. The production of this protein begins roughly four hours after a differentiation signal is detected (Hwang et al., 1995; Li et al., 2021). *CAP20* is similarly localized in the appressorial wall. While spores with a *cap20* mutation germinate and develop appressoria that seem normal, they are unable to create lesions on avocado and tomato fruits, showing only minimal surface mycelial growth without significant penetration. These findings suggest that the expression of *cap20* is essential for effective infection mediated by appressoria.

The gene *ClaPEX6* is essential for the pathogenicity of *C. lagenarium*, the fungus that causes cucumber anthracnose (Fujihara et al., 2010; Kimura et al., 2001). *PEX* genes are responsible for encoding peroxins, which are proteins involved in the formation of peroxisomes. These organelles, encased in a single membrane, are responsible for a variety of metabolic processes, including the breakdown of fatty acids through β-oxidation, participation in the glyoxylate cycle, and the neutralization of reactive oxygen species. In *C. lagenarium*, the metabolic processes within peroxisomes are vital for infection via appressoria. Disruption of *ClaPEX6* leads to detrimental effects on peroxisomal metabolism, causing reduced efficiency in fatty acid β-oxidation and less than optimal growth on fatty acid substrates. *clapex6* mutants form small, inadequately melanized appressoria that are incapable of developing invasive hyphae.

According to Li et al. (2021) and Tsuji et al. (2003), the *CST1* gene plays a crucial role in the formation of invasive hyphae in *C. lagenarium*. This gene shares similarity with the *STE12* gene found in *S. cerevisiae*, which acts as a transcription factor following the MAPK pathway. In *cst1* mutants, while germination and the formation of melanized appressoria occur, the production of invasive hyphae is absent. A significant observation is that mature appressoria in these mutants exhibit a markedly reduced lipid droplet content. Initially, conidia are abundant in lipid droplets, but these are rapidly depleted during appressorium development. This rapid lipid depletion may contribute to the failure in penetration. The results indicate that *CST1* encodes a transcription factor that functions downstream of MAPK signaling and is essential in the signal transduction processes that control infection.

In the year 2000, Görnhardt and his colleagues achieved the successful cloning of three genes from *P. infestans*. These genes are distinctively expressed in germinating zoospores and appressoria, becoming active just before the

pathogen invades the host tissue. This group of genes belongs to a small, polymorphic family that produces proteins similar in structure to human mucins, referred to as Car (zoospore-germination-specific acidic repeat) proteins (Raffaele et al., 2010). During the germination of zoospores and the development of appressoria, car proteins are expressed temporarily and are found on the surface of the germinating spores. This positioning might facilitate attachment to the leaf surface, possibly indicating the initiation of infection structure formation.

The *CHK1* MAPK in the maize pathogen *C. heterostrophus* is integral to the infection process. During host colonization, the transcriptional activation of two cell wall–degrading enzyme genes—*CBH7*, encoding a cellobiohydrolase, and *EG6*, encoding an endoglucanase—occurs as a consequence of CHK1 signaling (Doehlemann et al., 2006; Klymiuk et al., 2021; Lev & Horwitz, 2003). The activation of these genes coincides with the beginning of invasive growth. Interfering with *CHK1* leads to a delay in hyphal penetration and a decrease in the expression of these cellulase genes, highlighting the essential function of cellulases in the penetration process (Vidhyasekaran, 2007; Zhang et al., 2021).

7. MOLECULAR SIGNALS INVOLVED IN THE FUNGAL INFECTION PROCESS

In the progression of a fungal infection, a variety of signal transduction pathways are actively involved, coordinating the detailed molecular events that lead to fungal pathogenesis. To illustrate these intricate signaling networks, several model fungal pathogens are presented here. Included among the chosen examples, each linked to distinct disease syndromes, are rice blast (*M. grisea*), anthracnose (*C. gloeosporioides*), powdery mildew (*B. graminis*), smut (*U. maydis*), and wilt (*F. oxysporum*).

7.1. Pathogenic Signaling in *Magnaporthe oryzae*

M. grisea is responsible for causing blast disease in both rice and barley. Upon making contact with the host plant, the conidia fasten themselves to the plant's surface, initiate germination, and produce a germ tube that later develops into an appressorium. This transformation unfolds in two distinct stages. At first, the tip of the germ tube develops into a hook-shaped form, with apical vesicles oriented towards the substrate, indicating the "recognition phase" (Bourett & Howard, 1990; Ebbole, 2007). During the subsequent phase, the germ tube tip continues to grow, resulting in the formation of a symmetrical appressorium. A septum then develops between the appressorium and the remaining part of the germ tube, and the appressorium undergoes the process

of melanization (Cruz-Mireles et al., 2024; DeZwaan et al., 1999; Yin et al., 2016). Upon reaching full development, the appressorium creates substantial hydrostatic pressure, enabling it to penetrate the host's cuticle and epidermis via a slender penetration peg (Howard et al., 1991). Following this penetration, the intracellular hyphae expand within the host's tissues, ultimately leading to the formation of conidiophores that penetrate the host's surface to disperse conidia into the surroundings (DeZwaan et al., 1999; Plaza et al., 2025; Wu et al., 2023).

The infection process involves several signaling pathways (Figure 11). Hydrophobins produced by the *MPG1* gene, in conjunction with the hydrophobic characteristics of plant surfaces, serve as signals for conidial adhesion. Conidia secrete pre-formed adhesive substances that facilitate attachment, thereby initiating signals that promote germination (Liu & Kolattukudy, 1999). The fungus releases cutinases that break down cutin into monomers (Choi & Dean, 1997; Fu et al., 2022). When conidia come into close physical and chemical contact with the hydrophobic host surface, they begin to germinate, leading to the development of short germ tubes that attach to the plant surface. This interaction is probably associated with hydrophobins (Wösten et al., 1994) and glycoprotein mucilage (Pandey et al., 2010; Xiao et al., 1994a,b). Being in such close proximity delivers essential positional cues necessary for the development of appressoria (Bourett & Howard, 1990; Xiao et al., 1994a). The *CBP1* gene is believed to code for an extracellular protein that binds to chitin, potentially playing a crucial role in detecting hydrophobic surfaces and initiating the formation of appressoria (Kamakura et al., 2002; Miranda et al., 2015; Sun et al., 2019). Hydrophobin encoded by *MPG1* (Ebbole, 2007; Kershaw & Talbot, 1998; Lopatukhin et al., 2024) and extracellular glycoproteins (Xiao et al., 1994a) are both involved in aiding the differentiation process of appressoria.

The expression of *MPG1* is influenced by several signal transduction pathways, such as the MAPK gene *PMK1*, the nitrogen utilization regulators *NPR1* and *NUT1*, and the PKA gene *CPKA* (Lopatukhin et al., 2024; Soanes et al., 2002). Signal detection is mediated by transmembrane G-protein-coupled receptors, like the receptor encoded by *pth11*, which promotes the formation of appressoria in reaction to certain stimuli (DeZwaan et al., 1999; Wu et al., 2023).

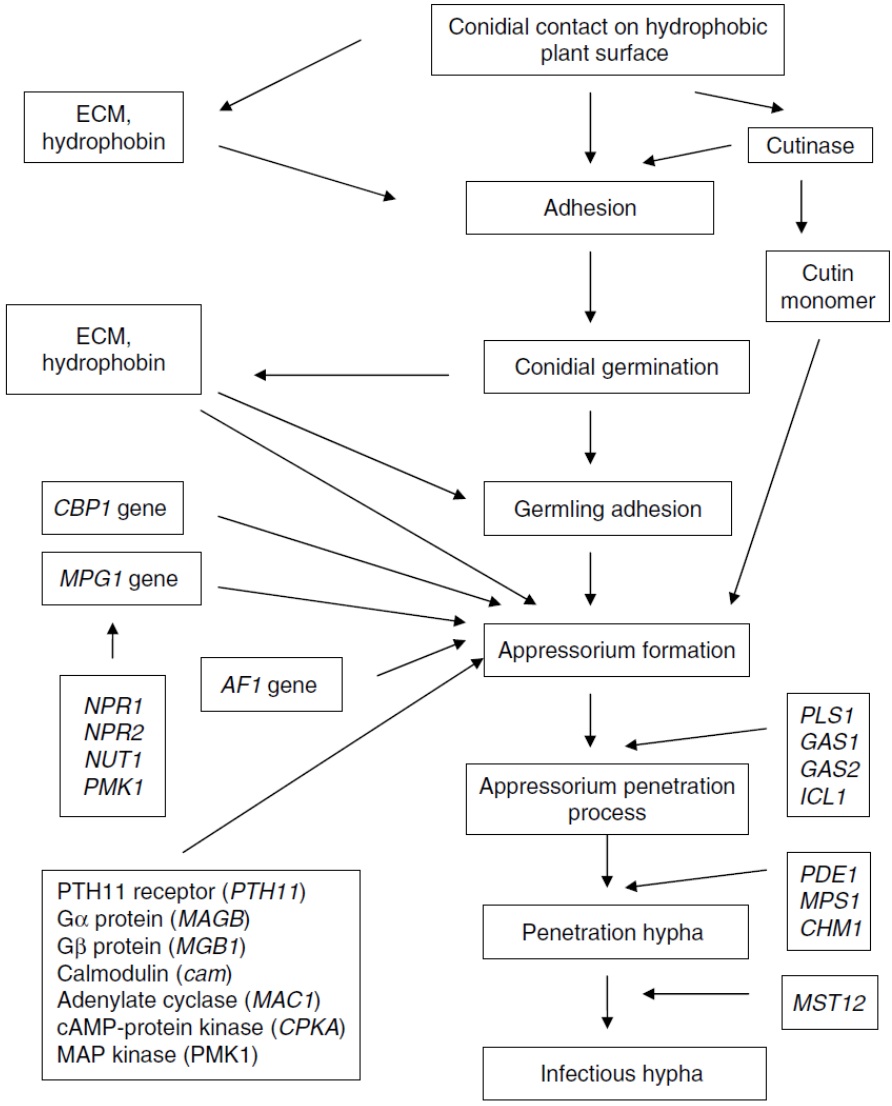

Figure 11. Molecular Signals Governing the Infection Process of *M. oryzae*.

In this signaling pathway, the Gα subunit, which is encoded by *MAGB* (Fang & Dean, 2000; Kao et al., 2022; Lu et al., 2016), along with the Gβ subunit encoded by *mgb1* (Li et al., 2021; Nishimura et al., 2003), are crucial elements of the G-protein signaling system. This pathway is probably linked to calcium/calmodulin-dependent signaling (Liu & Kolattukudy, 1999; Zeilinger et al., 2016) as well as the cAMP-mediated cascade (Kao et al., 2022; Li et al., 2021; Yan & Gautam, 1996). The adenylate cyclase produced by *MAC1* (Choi

& Dean, 1997; Miranda et al., 2015; Nishimura et al., 2003; Sun et al., 2019) plays a crucial role in interpreting sensory signals from hydrophobic surfaces and soluble cutin monomers, thereby influencing the levels of cAMP within the cell. The cAMP-dependent protein kinase A (PKA), which is encoded by the *CPKA* gene, plays a vital role in the formation of functional appressoria (Chun et al., 2019; Xu et al., 1997). Additionally, MAPK cascades might function as downstream components of the cAMP signaling pathway (Cruz-Mireles et al., 2024; Doehlemann et al., 2006; Xu & Hamer, 1996). The *APF1* gene is capable of triggering the formation of appressoria without relying on cAMP signaling pathways (Segorbe et al., 2017; Silué et al., 1998).

The *PMK1* MAPK is essential for appressoria development, aiding in the transformation of stored carbohydrates and lipids into the emerging infection structure (Cruz-Mireles et al., 2024; Thines et al., 2000).Once they are fully formed, appressoria attach securely to the host's surface (Ebbole, 2007; Howard et al., 1991) and facilitate penetration with the help of genes like *PLS1*, *GAS1*, *GAS2*, and *ICL1*. Encoded by *PDE1*, the P-type ATPase plays a crucial role in the signaling pathways that are necessary for the formation of the penetration peg (Balhadère & Talbot, 2001; Pei et al., 2022). The *CHM1* gene, associated with PAK kinase (Klymiuk et al., 2021; Li et al., 2004), along with the MAPK *MPS1* (Xu et al., 1998; Zhang et al., 2021), plays a role in the development of penetration hyphae. In contrast, *MST12* is essential for controlling the expansion of invasive hyphae (Park et al., 2002; Younas et al., 2025). In conclusion, *ABC1* plays a crucial role in pathogenicity by primarily serving to protect the pathogen from the host's immune responses (Urban et al., 1999).

7.2. Molecular Basis of Infection in *Blumeria graminis*

B. graminis is a fungus that exclusively relies on living hosts and is the cause of powdery mildew in cereal crops. Upon contact with a host leaf, its conidia rapidly secrete extracellular matrix (ECM) material (Carver et al., 1999; John et al., 2024; Fujita et al., 2004a) (Figure 12). According to Wright et al. (2002a), the ECM supports the fungus in adhering to the leaf surface and is vital for detecting the point of contact, as noted by Fujita et al. (2004a) and Mishra et al. (2012). Hydrophobic interactions play a role in the recognition process (Chowdhury et al., 2019; Huth et al., 2023; Wright et al., 2000), along with the function of structural cutinases that break down the plant cuticle, leading to the release of substances that are quickly taken up by the conidia (Nielsen et al., 2000). These interactions deliver cues that facilitate adhesion and the later germination of spores (Meguro et al., 2001; Mishra et al., 2012; Pandey et al., 2010).

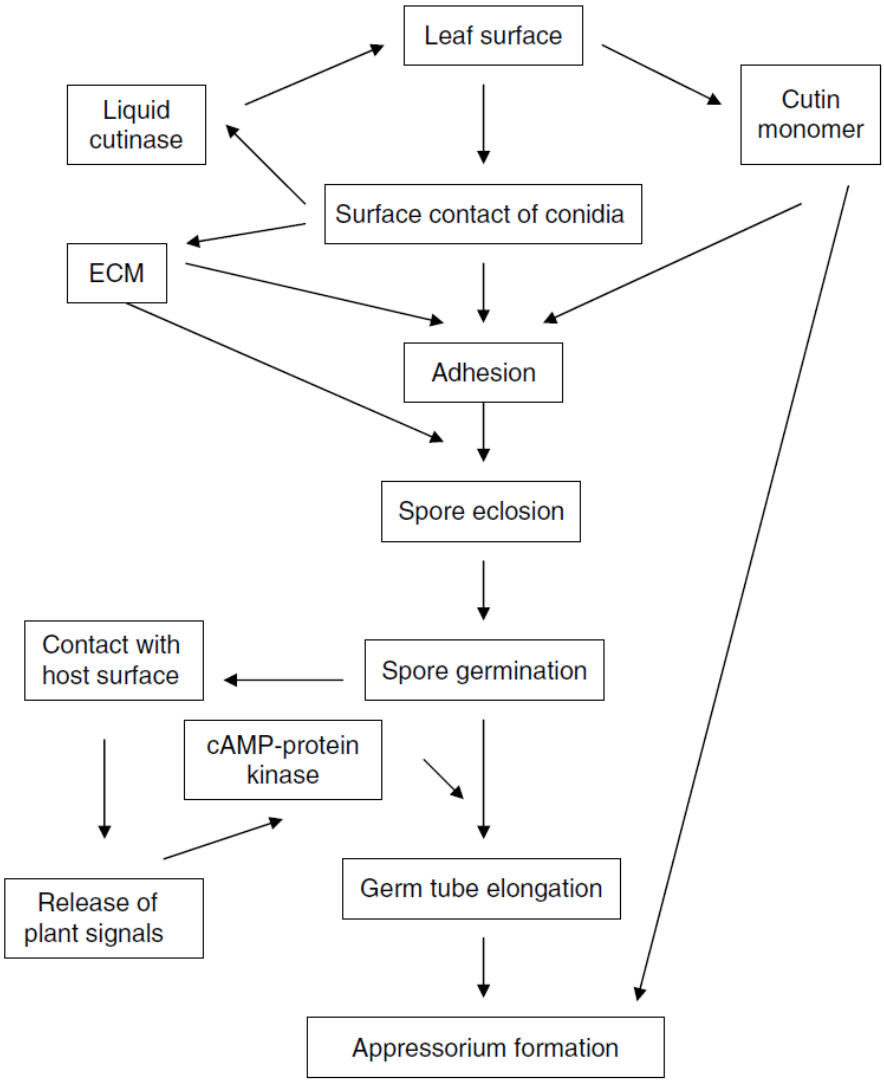

Figure 12. Signal-mediated regulation of pathogenesis in *B. graminis*

Within 5–10 minutes of attachment, spores undergo significant structural transformations as they commence germination (Chowdhury et al., 2019; Kunoh et al., 1988). At first, a brief primary germ tube sprouts from the spore that has germinated, and this is succeeded by the formation of a longer secondary germ tube, which eventually transforms into an appressorium. The initial germ tube releases sticky extracellular materials that secure the young germling to the host's surface (Wright et al., 2000). The elongation of the germ tube is initiated by the detection of particular characteristics on the host's

surface, likely facilitated by intracellular signaling through the cAMP–PKA pathway (Carver et al., 1996; Chowdhury et al., 2019; Hall & Gurr, 2000; Huth et al., 2023). Moreover, the enzymatic action of fungal cutinase produces cutin monomers, which act as additional signals to encourage the development of the appressorium (Arya & Cohen, 2022; Francis et al., 1996; Sun et al., 2019).

The initial stage of infection is marked by the formation of a hook-like extension at the far end of the appressorial germ tube (Figure 12). This extension aids the penetration peg in passing through the host's cuticle and epidermal cell wall, ultimately leading to the formation of a haustorium (Carver et al., 1995a; de Oliveira Silva et al., 2024; Pryce-Jones et al., 1999).

7.3. Signal Transduction Networks in *Colletotrichum gloeosporioides*

The primary pathogen responsible for anthracnose disease, *C. gloeosporioides*, affects over 197 different plant species. The process of infection is controlled by a network of complex signal transduction pathways. This sequence involves spores attaching to the host's surface, germinating, extending a germ tube, forming an appressorium, and penetrating via the penetration peg. When conidia land on the host surface, they detect hardness cues, which lead to the activation of the *chip1*, *chip2*, and *chip3* genes within two hours of contact (Figure 13). This recognition of surface hardness initiates the molecular events required for conidial germination and the formation of the appressorium.

Signal transduction cascades are started by conidial adhesion. Thigmosignals, such as the hydrophobic and firm characteristics of the contact surface, along with chemical cues like surface wax elements and ethylene, promote the formation of appressoria (Kolattukudy et al., 1995; Sun et al., 2019). The transmission of these signals occurs via a signaling pathway that relies on Ca^{2+} (Fu et al., 2022; Uhm et al., 2003). The cam gene, responsible for encoding calmodulin, initiates this signaling pathway (de Oliveira Silva et al., 2024; Kim et al., 1998; Klymiuk et al., 2021; Li et al., 2024), which ultimately leads to protein phosphorylation events carried out by protein kinases (Flaishman et al., 1995; Uhm et al., 2003). Moreover, a phosphoinositide signaling pathway, triggered by phospholipase C, is also a component of this fungus's signaling network (Uhm et al., 2003).

In response to these transmitted signals, several downstream genes become activated. The genes *cap3* and *cap5* are involved in the development of the appressorium (Feng et al., 2009; Huth et al., 2023; Kolattukudy et al.,

1995), whereas *cap22* is associated with the appressorial cell wall. The cap20 gene is likely crucial for the appressorium's ability to penetrate (Arya et al., 2021; Kolattukudy et al., 1995) (Figure 13).

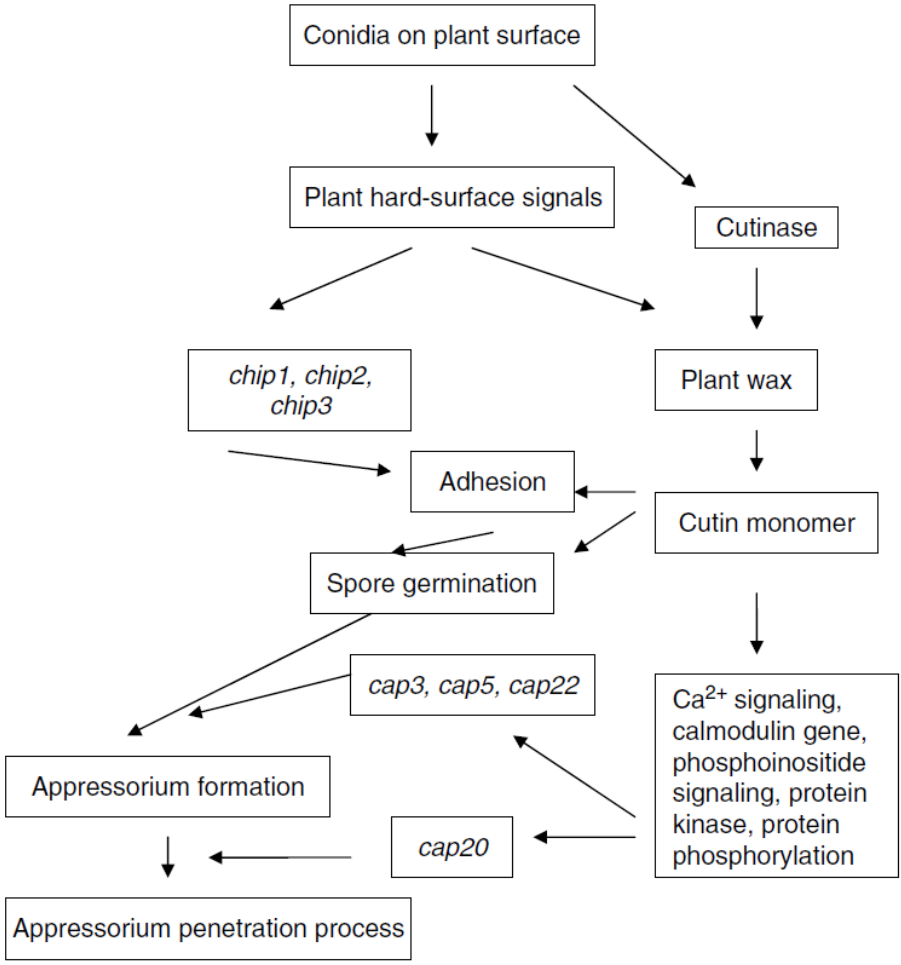

Figure 13. Signals at the molecular level that regulate the infection process of *C. gloeosporioides*.

7.4. Signaling Mechanisms in *Ustilago maydis* Pathogenesis

U. maydis is responsible for causing corn smut disease, which is marked by the appearance of tumor-like galls on maize. Galls may appear on the ears, tassels, leaves, and on the shoots that sprout from the nodes of the stem. In this fungus, the creation of infection structures necessitates the mating of compatible haploid sporidia. During the infection process, signals originating

from the plant initiate a series of intracellular signal transduction events (Figure 14).

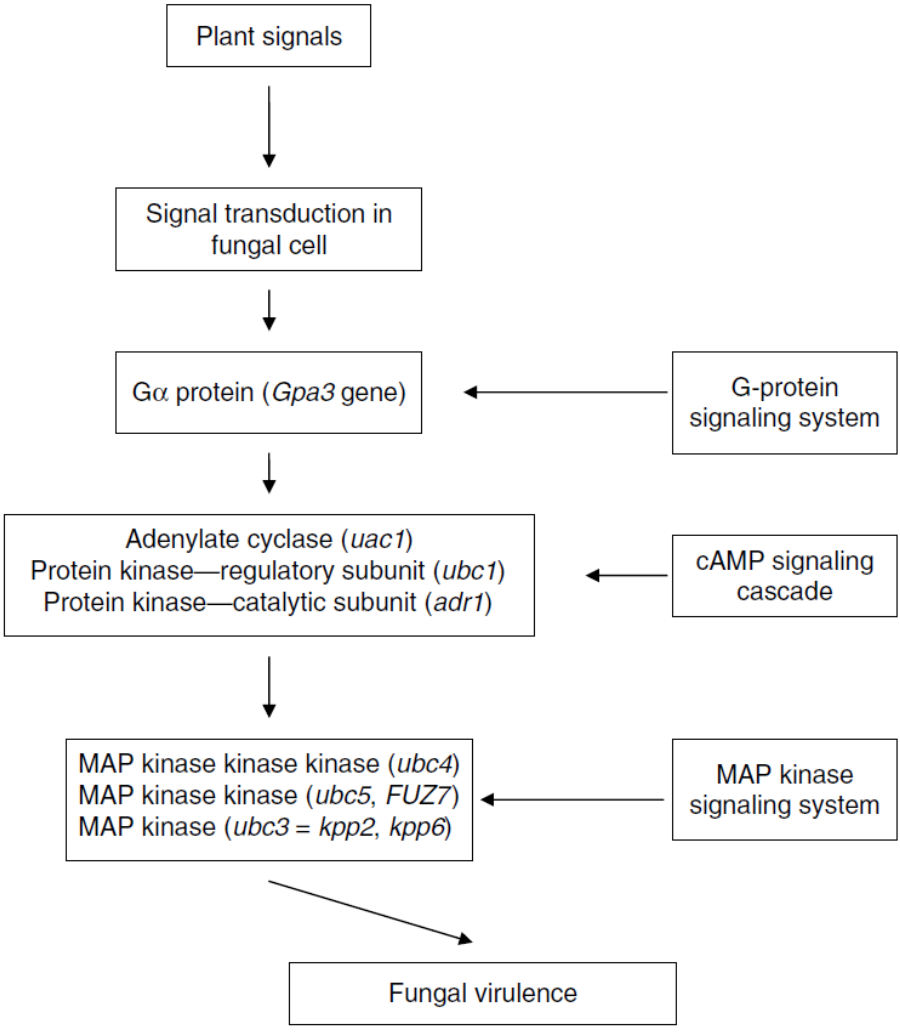

Figure 14. Molecular signals that regulate the infection process of *U. maydis*

The pathogenicity of *U. maydis* relies heavily on G-protein signaling, as highlighted by Doehlemann et al. (2006), Mukhopadhyay & Kumar (2020), and Regenfelder et al. (1997). The cAMP signaling cascade is crucial within the G-protein pathway, involving adenylate cyclase (*uac1*) and the regulatory (*ubc1*) and catalytic (*adr1*) subunits of protein kinase A, as noted by Gold et al. (1994, 1997) and Yu et al. (2023). Moreover, the MAPK pathway plays a vital role in

infection, with MAPKKK (*ubc4*), MAPKK (*ubc5*), and two MAPKs encoded by *ubc3* and *kpp6* serving as essential elements (Andrews et al., 2000; Brachmann et al., 2003; Yu et al., 2023).

7.5. Signaling Mechanisms Governing Pathogenicity in *Fusarium oxysporum*

Extensive research has been conducted on the roles of signaling and transduction systems in fungi that infect leaves, and it is assumed that comparable mechanisms exist in pathogens that reside in the soil. Unlike many pathogens that affect leaves, soilborne fungi often penetrate host roots directly, avoiding the need to form fully developed infection structures (Coleman, 2016; Mendgen et al., 1996; Quarantin et al., 2019). *F. oxysporum,* a facultative parasite found in soil, causes vascular wilt disease in numerous crops. It penetrates roots directly using penetration hyphae and establishes itself in the cortex, both intercellularly and intracellularly. Once the pathogen reaches the vascular tissue, it rapidly ascends through the xylem vessels, causing extensive wilting symptoms.

When *F. oxysporum* f. sp. *lycopersici* spores perceive signals from host roots, they start to germinate on the root surface. The germ tubes that develop subsequently attach to the surface of tomato roots (Di Pietro et al., 2001; Peng et al., 2024), in a manner akin to the way foliar pathogens adhere. The MAPK encoded by *fmk1* plays a crucial role in the signaling pathway that enables germ tubes to adhere to the host surface (Di Pietro et al., 2001; Doehlemann et al., 2006). These germ tubes subsequently develop into infection hyphae (Mendgen et al., 1996; Segorbe et al., 2017), which directly breach the walls of epidermal cells (Rodríguez-Gálvez & Mendgen, 1995). *FMK1* plays a vital role in invasive growth by controlling the development of infection hyphae, as well as facilitating root adhesion and penetration (Di Pietro et al., 2001). Therefore, the MAPK signaling pathway plays a crucial role in the infection process of *F. oxysporum*.

8. CONCLUSION

Fungal pathogens possess the capability to perceive signals emanating from plants, which are subsequently transmitted through internal pathways to initiate infection. The recognition happens almost immediately when the pathogen first encounters the host, with signaling possibly starting in as little as 20 seconds after contact. The presence or close contact of fungal spores with the host's surface is essential for recognizing plant signals. Pathogens have the ability to detect mechanosensitive thigmotropic signals that result from this interaction. Although it is well recognized that the surface topography of the

host affects the development of germ tubes and the formation of infection structures, the exact processes through which this physical information is converted into intracellular fungal signals are not yet fully understood (Heath, 2000; Kou et al., 2016; Vigreux et al., 2025).

The inhibition of appressorium formation triggered by contact through peptides with Arg–Gly–Asp (RGD) suggests a possible role for integrin-like proteins. These proteins, akin to those found in mammals, may aid in the interaction between extracellular matrices and cytoskeletal elements (Chethana et al., 2021; Ryder et al., 2022; Sugai et al., 2020). Nevertheless, simply coming into physical contact is not always enough to initiate infection structures; for example, the texture of the host's surface does not fully explain the high levels of appressorium formation seen in cereal rust fungi in vivo (Collins & Read, 1997; Fujihara et al., 2010). Numerous genes, including *INF24*, *INF56*, *CBP1*, *MPG1*, *chip1*, *chip2*, and *chip3*, have been recognized as playing a specific role in the transduction of signals on hard surfaces, though their precise functions remain unknown.

Components within plant cuticular waxes might initiate fungal signal transduction. Pentacyclic triterpenes such as ursolic acid and oleanolic acid, found in the waxes of fruits, are capable of triggering signaling pathways. Monomers of cutin, which result from the decomposition of cuticular waxes, act as potent triggers for fungal pathogenesis. Ethylene, another signal derived from plants, facilitates spore germination and the formation of appressoria. Moreover, flavonoids emitted from roots and seeds act as signals that can initiate the germination of fungal spores, potentially via the cAMP–protein kinase pathway. Upon landing on a host surface, fungal spores quickly release an extracellular matrix (ECM) that is abundant in cutinases and other esterases. These enzymes are crucial for the attachment of spores, germlings, and appressoria, and they also contribute to signal transduction. The appressorium forms a penetration peg to penetrate existing defense barriers, such as the cuticle and the pectin-rich layers beneath it, likely employing turgor pressure along with host-induced enzymes (De Jong et al., 1997; Bechinger et al., 1999; Kou et al., 2016; Liu et al., 2022; Sugai et al., 2020; Wattad et al., 1997). In the course of interacting with the host, the ECM generates a variety of enzymes, such as cutinases, pectinases, cellulases, and xylanases. These enzymes facilitate the breakdown of the cuticle and cell wall, aiding in overcoming the host's defense mechanisms (Suzuki et al., 1998, 1999; Komiya et al., 2003; Zhao et al., 2025). Additionally, ECM activated by plant signals may contain toxins that assist in fungal colonization (Chowdhury et al., 2019; Jansson & Åkesson, 2003; Li et al., 2021; Zhang et al., 2021).

In fungal cells, external signals are eventually conveyed through pathways that include G-proteins, calcium-dependent signaling mechanisms, the cAMP–protein kinase cascade, and the MAPK pathway, leading to the development of appressoria and other infection structures. These mechanisms of signal transduction seem to be preserved among different pathogens that cause diseases like blight, powdery mildew, smut, anthracnose, ergot, fruit rot, wilt, and root rot. Nonetheless, simply triggering these intricate signaling pathways does not ensure the onset of disease; plants also detect signals from pathogens and react through their own signaling mechanisms. The interplay of these signals at the interface between host and pathogen ultimately dictates the result of the infection.

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