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DECIPHERING THE MECHANISMS OF HYPERPARASITISM: *Ampelomyces quisqualis*- MEDIATED SUPPRESSION OF POWDERY MILDEW

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Powdery mildew diseases pose a persistent challenge to crop production because of their wide host range, rapid epidemic development, and increasing difficulties associated with fungicide resistance and host resistance. In this context, hyperparasitism offers a distinctive biological control strategy in which one fungus directly recognises, penetrates and exploits another pathogenic fungus. *Ampelomyces quisqualis*, a naturally occurring intracellular mycoparasite of powdery mildew, exemplifies this specialised interaction. Its hyperparasitic process involves a coordinated sequence of host recognition, conidial germination, directed germ-tube growth, attachment, penetration and intracellular colonisation. Following invasion, hydrolytic enzymes such as β -1,3-glucanases, chitinolytic enzymes and proteases contribute to weakening host structures and facilitate progressive cellular degradation. The interaction subsequently shifts from relatively biotrophic colonisation towards destructive phases, resulting in the degeneration of powdery mildew hyphae and conidiophores. Importantly, *Ampelomyces* also targets reproductive structures, including conidiophores, developing conidia and immature chasmothecia, thereby reducing both asexual sporulation and sexual propagule formation. These effects can diminish secondary inoculum and interfere with successive disease cycles. However, the effectiveness of hyperparasitism is influenced by humidity, temperature, pathogen developmental stage, application timing, strain characteristics and formulation properties. Understanding these interconnected mechanisms provides a basis for improving the consistency of *Ampelomyces*-based biocontrol and integrating it with resistant cultivars, cultural practices, disease forecasting and judicious fungicide use. Thus, deciphering the biology of this intimate fungal interaction is essential for translating a naturally occurring hyperparasitic relationship into a more predictable and sustainable component of powdery mildew management.

Powdery mildew diseases are among the most widespread and recognisable fungal diseases of plants, affecting nearly 10,000 angiosperm species, including economically important cereals, fruits, vegetables, ornamentals and other high-value crops. Caused by obligate biotrophic fungi of the order Erysiphales, powdery mildews form conspicuous superficial colonies on aerial plant tissues, impairing photosynthesis and plant vigour, interfering with flowering and fruit development and ultimately reducing crop yield and quality. Crops such as wheat, barley, grapevine, cucumber and strawberry are particularly vulnerable, as favourable weather conditions can support rapid epidemic development through successive cycles of airborne conidia. Management largely depends on resistant cultivars and fungicides; however, the emergence of pathogen populations that overcome host resistance or develop reduced sensitivity to fungicides, together with the selection pressure imposed by repeated chemical applications, highlights the need for complementary and environmentally compatible approaches. Hyperparasitism, in which one organism parasitizes another parasite, represents an intriguing mechanism of biological control because fungal hyperparasites can directly recognise, penetrate and exploit pathogenic fungi rather than relying solely on competition or antibiosis. Among the best-known examples are *Ampelomyces* species, naturally occurring intracellular mycoparasites of powdery mildew fungi. Historically referred to mainly as *Ampelomyces quisqualis*, these mycoparasites colonise powdery mildew hyphae and reproductive structures, grow intracellularly within infected tissues and eventually produce pycnidia. This intimate interaction can suppress mildew development and reduce the production of conidia and overwintering inoculum, thereby disrupting subsequent disease cycles. The interaction involves several mechanisms, including the activity of hydrolytic enzymes such as exo- β -1,3-glucanase, which contributes to degradation of fungal cell-wall components during mycoparasitism. Understanding the molecular, cellular and ecological mechanisms by which *A. quisqualis* recognises, penetrates and exploits powdery mildew can therefore provide valuable insights into fungal hyperparasitism and support the development of more reliable, sustainable and environmentally compatible strategies for powdery mildew management.

Sequential Mechanism of Hyperparasitism by *Ampelomyces quisqualis*

The hyperparasitic action of *Ampelomyces quisqualis* against powdery mildew follows a well-organised sequence of events involving host recognition, conidial germination, directed germ-tube growth, attachment, penetration, intracellular colonisation, enzymatic degradation and eventual destruction of the pathogen. Upon recognising suitable powdery mildew

structures, *A. quisqualis* germinates and produces germ tubes that grow towards the host, attach to its surface and penetrate the fungal cell wall. The hyperparasite then proliferates within the pathogen, where hydrolytic enzymes facilitate the breakdown of structural components, leading to progressive cellular disorganisation and death. Colonisation of conidiophores and sexual reproductive structures further interferes with sporulation and reproductive development, thereby reducing the production of secondary inoculum and ultimately restricting the progression and spread of powdery mildew.

Host Recognition and Targeting

The interaction between *Ampelomyces quisqualis* and powdery mildew begins with the germination of *Ampelomyces* conidia on the plant surface, followed by the directed growth of germ tubes towards susceptible powdery mildew structures. This early stage is strongly influenced by the presence of the mycohost, suggesting that host-derived signals play an important role in recognition. Studies have shown that extracts from powdery mildew conidia can stimulate *Ampelomyces* germination, indicating the involvement of water-soluble chemical compounds released by the host. Consequently, the presence of living powdery mildew can enhance conidial germination compared with conditions where the host is absent. After germination, the emerging germ tubes grow preferentially towards nearby powdery mildew hyphae rather than randomly across the leaf surface, indicating that *Ampelomyces* can perceive and respond to physical and/or chemical cues associated with its host. Once contact is established, the hyperparasite forms a close association with the powdery mildew structure and develops at the site of penetration, marking the transition from host recognition to active colonisation.

The interaction is not limited to the vegetative hyphae of powdery mildew; *Ampelomyces* can recognise and colonise various developmental structures, including hyphae, conidiophores, conidia and immature sexual structures. At the point of host contact, appressorium-like structures may develop, indicating that the germ tube undergoes specialised differentiation to facilitate firm attachment and penetration. Entry into the host appears to involve both mechanical and enzymatic processes, with physical force assisting penetration and extracellular hydrolytic enzymes, particularly β -1,3-glucanase and other cell-wall-degrading enzymes, contributing to the weakening and degradation of the powdery mildew cell wall. Thus, host targeting occurs through a coordinated sequence of events involving chemical stimulation of conidial germination, directed germ-tube growth, physical contact and

attachment, followed by localised penetration. These processes provide the foundation for the subsequent intracellular growth and colonisation of *Ampelomyces* within the powdery mildew mycelium.

Penetration and Intracellular Colonization

Once *Ampelomyces quisqualis* establishes contact with a powdery mildew colony, its germ tube attaches to and penetrates the pathogen's intact hyphae through a combination of mechanical pressure and enzymatic activity. Appressorium-like structures may form at penetration sites, indicating localised differentiation of the germ tube, while extracellular lytic enzymes, particularly exo- β -1,3-glucanase and other cell-wall-degrading enzymes, contribute to weakening the powdery mildew cell wall. Following penetration, *Ampelomyces* hyphae enter the host cytoplasm and grow intracellularly, extending from one cell to another through septal pores. The interaction initially appears relatively biotrophic, but as the hyperparasite proliferates, progressive degeneration of the host cytoplasm occurs, eventually leading to the atrophy and destruction of invaded hyphae and conidiophores. With continued colonisation, *Ampelomyces* forms intracellular pycnidia within powdery mildew hyphae, conidiophores and immature sexual structures. These pycnidia subsequently mature and produce numerous conidia, completing the hyperparasitic cycle while simultaneously impairing the pathogen's vegetative growth and reproductive capacity. Thus, *A. quisqualis* does more than compete with powdery mildew on the plant surface; it directly invades the pathogen and exploits its cells as an intracellular niche, making penetration, intracellular colonisation and destruction central features of this specialised mycoparasitic interaction.

Enzymatic Degradation

A critical component of *Ampelomyces quisqualis* hyperparasitism is its ability to produce a diverse range of cell-wall-degrading and hydrolytic enzymes that support penetration and destruction of the powdery mildew host. Studies have demonstrated extracellular activities of β -1,3-glucanases, β -glucosidases, β -N-acetylglucosaminidase/chitinolytic enzymes and proteases, indicating that *A. quisqualis* possesses a versatile enzymatic arsenal capable of targeting different components of the fungal host. Among these, the *exgA* gene-encoded exo- β -1,3-glucanase is expressed during mycoparasitism, and enzyme-containing culture filtrates have been shown to degrade powdery mildew hyphal walls, providing evidence for its role in host cell-wall disruption. β -1,3-glucanases break down glucan polymers, chitinolytic enzymes target chitin-containing components and proteases degrade host proteins, collectively

weakening the fungal structure and facilitating access to the host interior. These enzymes are likely to act together rather than independently, with their complementary and potentially synergistic activities progressively compromising the powdery mildew cell wall and cellular integrity. Differences in the activities of chitinases, proteases and β -1,3-glucanases among *Ampelomyces* strains have also been associated with variations in mycoparasitic performance, suggesting that the coordinated action of multiple hydrolytic enzymes is an important factor determining the efficiency of pathogen penetration, intracellular colonisation and destruction.

Cellular Disruption and Pathogen Death

Ampelomyces progressively disrupts the structure and functioning of invaded powdery mildew cells, transforming an initially compatible, apparently biotrophic association into a destructive interaction. During the early stages of mycoparasitism, *Ampelomyces* hyphae grow intracellularly within the host without immediately causing complete loss of cellular integrity. However, as the hyperparasite continues to proliferate, the powdery mildew cytoplasm undergoes progressive disorganisation and eventual disintegration, impairing the physiological functions of the host and contributing to the cessation of conidiophore development and sporulation. With advancing colonisation, the interaction shifts towards a more necrotrophic phase, in which *Ampelomyces* exploits and destroys the increasingly weakened host cells. Parasitised powdery mildew structures subsequently show atrophy, severe cytoplasmic disruption and eventual collapse of hyphae and conidiophores. The formation of intracellular *Ampelomyces* pycnidia further damages reproductive structures and prevents the successful production of powdery mildew propagules. Thus, host death appears to result not primarily from a secreted toxin, for which convincing evidence is lacking, but from the combined effects of intracellular invasion, nutrient exploitation, progressive cellular degeneration and physical destruction. This transition from relatively biotrophic colonisation to necrotrophic destruction is a defining feature of *Ampelomyces* hyperparasitism and underpins its ability to suppress both the growth and reproductive capacity of powdery mildew fungi.

Reproductive Suppression

One of the most important outcomes of *Ampelomyces* hyperparasitism is the disruption of powdery mildew reproduction, as the hyperparasite targets not only vegetative hyphae but also the pathogen's reproductive structures. *Ampelomyces* can colonise conidiophores and developing conidia, forming intracellular pycnidia within these tissues and interfering with their normal development, maturation and sporulation. Its effects can also extend to the sexual

phase, where invasion of immature chasmothecia (formerly cleistothecia) may disrupt their development and reduce the formation of viable sexual propagules. This reproductive interference is particularly significant because powdery mildew epidemics depend largely on continuous production of airborne conidia for secondary spread. At the same time, sexual spores formed within chasmothecia contribute to pathogen survival and subsequent disease initiation. Consequently, *Ampelomyces* infection can reduce conidial production, impair chasmothecia development and compromise overwintering success. By simultaneously suppressing both asexual and sexual reproduction, the hyperparasite reduces the inoculum available for dispersal, limits successive infection cycles and ultimately slows disease progression. Thus, even when powdery mildew colonies continue to develop temporarily after colonisation, their reproductive capacity can be substantially weakened, highlighting reproductive interference as a key component of the biocontrol potential of *Ampelomyces*.

Harnessing Hyperparasitism for Disease Management

The effectiveness of *Ampelomyces quisqualis* as a biological control agent depends strongly on weather conditions, the developmental stage of powdery mildew and the timing of application. Adequate moisture is particularly important because conidial germination, germ-tube growth, penetration and intracellular colonisation are favoured by high relative humidity and actively developing mildew colonies. Temperature also influences its development, while rainfall and prolonged leaf wetness can facilitate conidial movement within the crop canopy. Therefore, *Ampelomyces* is generally more effective when applied during the early stages of disease development, before extensive sporulation and epidemic build-up. Once dense colonies are established and producing abundant secondary inoculum, the relatively slower development of the hyperparasite may result in only partial disease suppression. Field performance can consequently vary with environmental conditions, inoculum density, host–pathogen combinations and formulation characteristics. Protective polymers, oils and suitable adjuvants have therefore been investigated to improve conidial survival and minimise the effects of low humidity, temperature fluctuations and ultraviolet radiation.

The greatest potential of *A. quisqualis* lies not in completely replacing conventional fungicides, but in its integration into sustainable disease-management programmes. By directly attacking powdery mildew and reducing both pathogen growth and reproductive inoculum, *Ampelomyces* can complement resistant cultivars, cultural practices and judicious fungicide use, particularly where reduced chemical inputs are desirable. However, variable field efficacy,

dependence on favourable humidity, relatively slow development, strain variation and challenges in mass production and formulation remain important constraints. Future progress will depend on identifying highly effective strains, improving formulation and delivery systems, synchronising applications with pathogen development and environmental conditions, and clarifying the molecular basis of host recognition, penetration and intracellular colonisation. Integrating these insights with disease forecasting, precision application and compatible integrated disease-management programmes could make *Ampelomyces* a more reliable and durable component of sustainable powdery mildew management rather than simply an alternative to conventional fungicides.

Conclusion

The hyperparasitic interaction between *Ampelomyces quisqualis* and powdery mildew represents a highly specialized sequence of biological events, beginning with recognition of the pathogen and conidial germination, followed by directed germ-tube growth, attachment and penetration, intracellular colonization, enzymatic degradation, and progressive destruction of invaded fungal cells. Importantly, *A. quisqualis* also colonizes conidiophores and other reproductive structures, reducing conidium formation and interfering with sexual development, thereby decreasing secondary inoculum and restricting disease progression. This direct and targeted mode of action highlights its potential as a precise and sustainable biocontrol agent within integrated disease management. However, variable environmental conditions, strain-specific performance and formulation constraints remain important challenges. Future research integrating molecular, genomic, physiological and formulation-based approaches could facilitate the selection of highly effective *Ampelomyces* strains, improve application strategies and elucidate the mechanisms governing host recognition, penetration and intracellular destruction, ultimately enabling more predictable and mechanism-based hyperparasite-mediated management of powdery mildew diseases.

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