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Preface

Trichoderma is a genus of common filamentous fungi that display a remarkable range of life styles and interactions with plants, animals and other fungi. Because of their ability to stimulate plant growth and defense, some *Trichoderma* strains are used for biological control of plant diseases. In this Review, we discuss recent advances in molecular ecology and genomics that indicate that saprotrophy on fungal biomass (mycotrophy) and various forms of parasitism on other fungi (mycoparasitism), combined with broad environmental opportunism, may have driven the evolution of the present interactions of *Trichoderma* with plants and animals.

1. Introduction

Species of the filamentous ascomycete *Trichoderma* are among the most commonly isolated saprotrophic fungi. They are frequently found in soil and growing on wood, bark and other fungi, as well as on innumerable other substrates, demonstrating their high opportunistic potential and adaptability to various ecological conditions[1,2,3,4]. The nomenclature of these fungi (Box 1) is complicated because of their pleiomorphism, that is, some of them can exist in two morphologically and physiologically different stages. The sexual (teleomorphic) stage is known by the generic name *Hypocrea*, while the asexual (anamorphic or mitosporic) stage is called *Trichoderma*. Although several common species have lost their ability to reproduce sexually and have become clonal or agamospecies (e.g. *Trichoderma longibrachiatum, Trichoderma harzianum* and *Trichoderma parareesei*)[5,6,7,8], the majority of genetic diversity of the genus is represented by sexual forms[3,4,9,10], and some species are isolated equally frequently as both anamorphs and teleomorphs.

Most *Hypocrea* fruiting bodies are found associated with specific basidiomycete fungi; for example, *Hypocrea estonica* and *Hypocrea parestonica* always grow on *Hymenochaete* spp., *Hypocrea fomiticola* on *Fomes fomentarius*, and *Hypocrea pulvinata* on *Fomitopsis pinicola* and *Piptoporus betulinus* (Figure 1). Mycoparasitic species of *Hypocrea/Trichoderma* can degrade and grow within the resting structures (sclerotia) that are produced by a wide variety of plant pathogenic fungi such as *Sclerotinia* spp., *Sclerotium* spp., *Macrophomina phaseolina* and *Verticillium dahliae*[11]. These data support the hypothesis of Rossman *et al.*[12] that *Hypocrea* and some other Hypocreales evolved as biotrophic associates (i.e. parasites in a broad sense) of wood rotting fungi and later on explored the wood as an optional ecological niche. Some species such as *Hypocrea jecorina/Trichoderma reese*[13]*,* an important industrial producer of cellulolytic and hemicellulolytic enzymes[14], may have switched to using the pre-degraded wood rather than the host fungus itself[13]. Thus, the ability to antagonize, parasitize or even kill other fungi seems to be widespread among *Hypocrea/Trichoderma* spp. This property has initiated the use of of *Hypocrea/Trichoderma* strains for the antagonization and eventual killing of plant pathogens[15,16] (biological control, biocontrol) .

Although the genus *Hypocrea/Trichoderma* contains many species[3,4,9,10], research on mycoparasitism has been mostly performed with only a few of them such as *Trichoderma harzianum* sensu lato, *Hypocrea atroviridis/Trichoderma atroviride*, *Hypocrea virens/Trichoderma virens*, *Trichoderma asperellum* and *Trichoderma asperelloides*[15,16]. In the course of these studies, it was observed that *Hypocrea/Trichoderma* biocontrol strains can establish themselves in the plant rhizosphere, stimulate plant growth and elicit plant defense reactions against pathogens. These interactions with the plants have been shown to play important roles in some biocontrol strains and are therefore currently strongly exploited[15,16]. Moreover, some *Hypocrea/Trichoderma* strains were isolated as endophytes, i.e. as colonizers of intracellular plant compartments[17].

The recent sequencing of the genomes of two species that are widely used in biocontrol, *H. atroviridis* and *H. virens*[13], and the advent of associated "omics" technologies in *Hypocrea/Trichoderma* research[19,20] have shed new light on the ecology of the genus and the evolution of its traits. In this Review, we summarize recent insights from genomic analyses of *H. atroviridis* and *H. virens* and emphasize that mycotrophy in a broad sense (including mycoparasitism) seems to be a widespread property among *Hypocrea/Trichoderma* spp. and a key for a better understanding of the broad spectrum of opportunistic interactions with other organisms such as animals and plants.

2. A mosaic of mycotrophic interactions

The direct interactions between *Hypocrea/Trichoderma* spp. and other fungi are conventionally described as necrotrophic hyperparasitism or mycoparasitism[17]. This view is supported by a recent survey of >1,100 *Hypocrea/Trichoderma* strains comprising 75 molecularly defined species, which shows that all the species tested possess mycoparasitic potential against three causative agents of plant diseases, namely *Alternaria alternata, Botryotinia fuckeliana* (anamorph: *Botrytis cinerea)* and *Sclerotinia sclerotiorum* (I.S. Druzhinina, unpublished observations). However, because *Hypocrea/Trichoderma* spp. can also feed on dead fungal biomass, the life style of the genus may be better defined as mycotrophic rather than mycoparasitic, to include both biotrophic and saprotrophic nutritional strategies.

Sensing the presence of the prey

The genome sequencing of three *Hypocrea/Trichoderma* spp. (i.e. *H. jecorina, H. virens* and *H. atroviridis*)[13] and the application of transcriptomics[19,20] has recently provided several important insights into the molecular physiology of mycotrophy. Many genes that encode proteases and oligopeptide transporters are expressed before and at contact with the prey in different *Hypocrea/Trichoderma* species[20,21]. Most of these proteases belong to the subtilisin-like serine protease group, and genes encoding these enzymes are significantly overrepresented in expressed sequence tags (ESTs) derived from *T. harzianum* CECT 2413 grown under biocontrol conditions[21]. An abundance of genes encoding subtilisin-like serine proteases was also observed in an analysis of ESTs accumulated during the onset of contact between *H. atroviridis* and its fungal preys *Thanatephorus cucumeris* (anamorph: *Rhizoctonia solani*) and *S. sclerotiorum* [20]. Strains overexpressing one of these proteases (encoded by the gene *prb1*) from *H. atroviridis* exhibited enhanced mycoparasitic activity[22]. The actions of any of the mentioned proteases on the prey fungus may release oligopeptides that may then be bound by *H. atroviridis* receptors that sense nitrogen starvation[20]. Such a mechanism is reminiscent of that found in nematophagous fungi, where trapping of the prey is induced by oligopeptides from the host[23] (Figure 2). It has been suggested that class IV G-protein coupled receptors (GPCRs) present in *H. atroviridis*[20] could act as sensors for these oligopeptides[13]. *H. atroviridis, H. virens* and *H. jecorina* each have two paralogues that are members of the class IV GPCRs [13].

Yet there may be further GPCRs involved in sensing the prey. For example, GPR1 (protein identification number Triat2: 160995 in the JGI genome database), a member of the cAMP receptor-like GPCRs, is required for mycoparasitism in *H. atroviridis* [24]. Further signal transduction from any of these receptors occurs via a conserved G-protein signaling cascade (Figure 2) that comprises three Gα subunits, one Gβ subunit and one Gγ subunit. Loss-of-function mutants in the Gα subunit TGA1 in *H. atroviridis* displayed a complete loss of mycoparasitic overgrowth on three hosts (*R. solani, B. cinerea* and *S. sclerotiorum*), a strong reduction of chitinase activities and a decreased production of the antifungal compound 6-pentyl pyrone [25,26]. In contrast, the deletion of *tgaA* (a *tga1* homologue) in *H. virens* resulted only in a somewhat reduced mycoparasitic activity on *Athelia rolfsii* (anamorph*: Sclerotium rolfsii*)[27].

Mitogen-activated protein kinase (MAPK) pathways represent one of the most prominent signal transduction systems in fungi[28]. The *Hypocrea*/*Trichoderma* genomes harbor genes that encode three MAPKs: the so-called pathogenicity MAPK (TmkA/Tvk1), the cell-integrity kinase (TmkB) and the osmoregulatory MAPK (Hog1)[28]. Deletion of *tmkA (tvk1*) in a “P” strain of *H. virens* (“P” strains produce gliovirin and are effective against *Pythium* spp.) resulted in a loss of antagonism on *S. rolfsii*, but not on *R. solani*[29,30]. In contrast, deletion of *tmkA* in a *H. virens* “Q” strain (“Q” strains secrete copious amounts of gliotoxin and are effective against *R. solani*) resulted in further improved biocontrol against both *R. solani* and *P. ultimum* [31]. The different secondary metabolite profiles of the “P” and “Q” strains may explain the different result of deleting the pathogenicity-related MAPK gene. More complete information on the genome of “P” strains will help test this hypothesis. Similar to the *H. virens* “P” strains, the deletion of *tmk1* in *H. atroviridis* resulted in reduced mycoparasitism against *R. solani*, and increased production of chitinase and anti-fungal compounds[32]. The roles of the other two MAPKs, TmkB and Hog1, are less well understood because mutants for these genes are characterized by poor growth, which precludes successful antagonism. For example, *H. virens* mutants in TmkB were defective in mycoparasitism on *S. rolfsii*[33], and *H. atroviridis* mutants in Hog1 (which is involved in osmotic and oxidative stress tolerance) showed no mycoparasitic ability[34].

Attachment to the prey hyphae

While mycotrophy only requires an attachment to the fungal substrate, mycoparasitism typically requires coiling around the prey mycelium and formation of helix-shaped hyphae[17,18], which is dependent on the recognition of lectins from the fungal prey[35] (Figures 2 and 3). Yet plant lectins also induce coiling to a similar extent, suggesting that lectins are not determinants of specificity in the attachment of *Hypocrea/Trichoderma* to its prey[25]. However, coiling is not stringently correlated with mycoparasitism, as it has been observed that hyphae of some *Hypocrea/Trichoderma* can coil around themselves in the absence of the prey[36,37]. Moreover, spiral or helical hyphal elongations are diagnostic characteristic for many species, for example *Trichoderma spirale* and *Trichoderma helicum*[38].

Growth of *Hypocrea/Trichoderma* alongside of the host hyphae and formation of papillae-like structures most often precede mycoparasitic attack (Figure 3), and these events are independent of the prey species[25,39]. Cell wall degradation and penetration of the lumen occurs at points where papillae-like structures are formed [17,18,39]. These structures are similar to those induced in *T. harzianum* by tomato[39] and analogous to the appressoria of plant pathogenic fungi. In the rice blast fungus *Magnaporthe grisea*, glycerol generated from storage lipids serves to build up the turgor needed for the mechanical pressure to penetrate the plant cell wall[40]. The papillae-like structures of *Hypocrea/Trichoderma* may also build up glycerol for a similar purpose, as transcription of genes involved in lipid catabolism and osmoregulation increases during the contact stage of mycoparasitism in *H. atroviridis*[20].

Contact with and binding to a potential prey is not restricted to hyphae. Spores of *H. atroviridis* adhere to the hyphae of *Pythium ultimum* prior to germinating on them [36]. The mechanism of conidial affinity to the host mycelium is unknown, but could be mediated by hydrophobins, small amphiphilic proteins containing eight cysteines, of which *Hypocrea/Trichoderma* has the highest number among Ascomycota (as deduced from genomic sequences) [41].

Defense responses of **Hypocrea/Trichoderma**

Another event common to different *Hypocrea/Trichoderma* spp. is the induction of genes for the heat shock response, genes for oxidative stress response and genes for detoxification processes (such as those encoding ABC efflux transporters and the pleiotropic and multidrug drug resistance transporters) in the presence of the prey [19,20] (Figure 2). The fungal prey *R. solani* uses radical oxygen species as signaling molecules during sclerotia formation [42] and excretes antifungal metabolites[43], which may elicit the stress response that is observed in *Hypocrea/Trichoderma*. A knock-out in one of the genes encoding an ABC transporter (TAABC2) from *H. atroviridis* resulted in decreased biocontrol of *R. solan*i, thus providing support for the role of detoxification in mycoparasitism[44].

Killing the prey

The final death of the prey results from the synergistic action of antifungal secondary metabolites (Box 2) and cell-wall hydrolytic enzymes that are secreted by *Hypocrea/Trichoderma*. The importance of these molecules to the life style of the mycoparasite is reflected in the great number of genes encoding enzymes for their synthesis in *Hypocrea/Trichoderma* genomes[13]. As an example, *H. virens* contains the largest number (28) of nonribosomal peptide synthases known for any fungus. In addition, orthologous genes shared only between *H. atroviride* and *H. virens* but not present in *H. jecorina* seem to encode proteins for secondary metabolite synthesis[13], and may thus represent the machinery for the synthesis of as yet unknown antifungal compounds.

The cell wall accounts for approximately 30 % of the dry weight of the fungal cell and consists mainly of chitin, β-1,3-glucans and α-1,3/1,4-glucans[45]. Interestingly, *H. atroviridis* and *H. virens* have a very high number of chitinases (29 and 36, respectively)[13,46]. Enhancing chitinase activity by addition of a carbohydrate binding module (CBM) to the chitinases CHIT33 and CHIT42 increased the mycoparasitic ability of *T. harzianum*[47]. CBMs enable chitinases to bind more tightly to insoluble chitin substrates**.** Some *Hypocrea/Trichoderma* chitinases have evolved under positive selection[48], which is typical of a co-evolutionary arms race between host and pathogen. However, the deletion of certain chitinase genes in some *Hypocrea/Trichoderma* spp. did not result in loss of mycoparasitism or biocontrol[15,17], probably because of gene redundancy. *Hypocrea/Trichoderma* spp. also contain an expanded set of chitosanases of GH family 75 that hydrolyze chitosan, a partially deacetylated form of chitin[13].

The second most abundant polymer in fungal cell walls is β-1,3-glucan [45] with β-1,6-

branches, which is hydrolyzed by β-1,3-glucanases; genes encoding this type of enzymes seem to be overrepresented in the genomes of *Hypocrea/Trichoderma* spp., when compared to the genomes of other related fungi[13]. β-1,6-glucanases have been detected in the area of interaction between *Hypocrea/Trichoderma* and its prey. Overexpression of the β-1,6 glucanase BGN16.3 in *T. harzianum* CECT 2413 resulted in a more efficient biocontrol strain for inhibition of the growth of *B. cinerea*, *R. solani* and *Phytophthora citrophthora* [49]. In addition, *T. harzianum* and *H. virens* strains overproducing β-1,6 glucanases exerted more efficient biocontrol of *R. solani, B. cinerea* [48] and *Pythium ultimum*[51].

3. Animals as targets of an opportunist

Some of the traits that seem to have evolved in *Hypocrea/Trichoderma* in relation to mycotrophy may have functioned as preadaptations to allow parasitism or predation on animals. For example, several *Hypocrea/Trichoderma* spp. successfully antagonize and kill plant parasitic nematodes that occur in the rhizosphere[51]. Commercially relevant nematode pests in agriculture such as the root-knot nematode (*Meloidogyne*) and the cyst nematodes (*Heterodera* and *Globodera*), cannot be controlled by crop rotation due to their broad host range [51]. Thus, it is remarkable that different *Hypocrea/Trichoderma* species such as *T. harzianum* sensu lato can protect plants against the attack of *Meloidogyne incognita* by colonizing the eggs and second stage juveniles of the nematode [51]. The parasitism of nematode eggs requires the penetration of the eggshell, which is formed by several layers, including a thick chitinous one, that are considered to be a major barrier for infection [52]. Thus, the rich arsenal of chitinases of *Hypocrea/Trichoderma* may provide an advantage for opportunistic nematophagy. In addition, the high number of subtilisin-like S8 proteases possessed by *Hypocrea/Trichoderma* may be important for penetration of the nematode cuticle, which is composed of collagen-like and keratin-like proteins (Figure 4). Subtilisin and chemotrypsin proteases have been cloned from several *Hypocrea/Trichoderma* spp.[53,54,55], and the *H. atroviridis* alkaline subtilisin PRB1 and the *T. harzianum* chemotrypsin-like PRA1, which have an important role in mycoparasitism [20,52], also increased the ability to penetrate nematode eggs [53,54].

Some *Hypocrea/Trichoderma* spp. can cause invasive mycoses in mammals, including immunocompromised humans[56]. Although they are not a major threat to humans, they nevertheless pose difficult therapeutic challenges because of their resistance to most antifungal agents [57]. This remarkable resistance may be the result of the adaptation of *Hypocrea/Trichoderma* spp. to combat defense metabolites produced by prey fungi. So far, only two closely related species, *T. longibrachiatum* and *H. orientalis*, have been proven to infect immunocompromised patients[7]. However, it should be noted that whereas *T. longibrachiatum* is essentially clonal, *H. orientalis* forms a world-wide recombining population[8]; this may be relevant for antifungal therapy, as genes encoding factors for antibiotic resistance and virulence could be exchanged during sexual reproduction in *H. orientalis*. Clinical isolates of both species shared identical haplotypes with environmental strains, indicating a threat for nosocomial infections, as virtually any strain of these species may cause invasive mycoses.

There has been little attempt towards an understanding of the mechanisms by which particular members of the *Hypocrea/Trichoderma* genus infect human cells. All the infecting species can grow at 37 °C, but not all *Hypocrea/Trichoderma* strains that can grow at 37 °C are opportunistic human pathogens. When *T. longibrachiatum* is confronted with lung cell cultures, the human cells rapidly start to sediment and lose their adhesive properties, suggesting the action of proteases and/or secondary metabolites. No such effect was observed for *H. jecorina/T. reesei*, which was used as a non-pathogenic control [58].

4. In the rhizosphere

Why the rhizosphere?

The rhizosphere is among the preferred ecological niches for *Hypocrea/Trichoderma* spp. and provides opportunities for both biotrophy and saprotrophic nutrition on plant root exudates. This is illustrated by the fact that the highest species richness of this genus in a single habitat has been found in the rhizosphere of the coffee plant *Coffea arabica* in Ethiopian highland forests [59], whereas a similar survey in non-rhizosphere soil on Sardinia Island (Italy) showed remarkably poor diversity [60]. The affinity of *Hypocrea/Trichoderma* to the rhizosphere can be explained by two of their nutritional preferences. First, the roots of 92 % of the land plants are mycorrhized, and these mycorrhizal fungi are potential preys for a mycotroph. However, the interactions between arbuscular mycorrhizal (AM) fungi and *Hypocrea/Trichoderma* spp. are still poorly understood[61,62,63,64,65,66]: whereas some studies suggest a synergism between AM and *Hypocrea/Trichoderma*, others observed that these fungi attack AM and suppress root colonization. Moreover, even a reduction of *Hypocrea/Trichoderma* population density due to AM fungi was noted[66]. The interaction of *Hypocrea/Trichoderma* with ectomycorrhizal fungi has only been very scarcely studied[67]. Second, the plant roots and especially root tips are covered by a gel-like slimy capsule (mucigel), which is composed of highly hydrated polysaccharides such as pectins and hemicelluloses (particularly rhamnogalacturonans and arabinoxylans) that are secreted from the outermost cells of the root cap. These components are easily degradable targets for the *Hypocrea/Trichoderma* hemicellulases that -may have evolved for the utilization of polysaccharides that are released from predegraded wood by potential fungal preys. Indeed, successful establishment of *T. harzianum* CECT 2413 in the tomato rhizosphere requires an endopolygalacturonase [68].

Monosaccharides and disaccharides excreted by plant roots into the rhizosphere provide an important carbon substrate for mycorrhizae [69], and sucrose has a similar role for the establishment of *H. virens* in the rhizosphere [70]. As the genomes of *H. atroviridis, H. virens* and *H. jecorina* contain genes encoding intracellular (but not extracellular) invertases, sucrose must be taken up by a sucrose permease before being hydrolyzed. *H. virens* contains a highly specific sucrose transporter that is induced in the early stages of root colonization and has biochemical properties similar to plant sucrose carriers[71], which suggests an active sucrose transfer from plant to fungus. In addition, the genomes of *H. atroviridis* and *H. virens* encode a great number of major facilitator solute transporters [13] whose role in acquisition of other root exudates remains unknown. In summary, the presence of fungal preys and the availability of root-derived nutrients may have been major attractors for *Hypocrea/Trichoderma* ancestors to establish themselves in the rhizosphere and to develop interactions with plant roots.

Dialoging with the plant

Like fungi and animals, plants respond to the presence of other organisms by activating potential defense mechanisms. This is best understood for various plant pathogens that elicit a two-branched innate immune defense[72]. The first stage generally recognizes and responds to pathogen or microbe associated molecular patterns (PAMPs or MAMPs), which are molecules that are commonly found in microorganisms, and is known as PAMP triggered immunity (PTI), whereas the second stage responds to pathogen virulence factors and is called effector-triggered immunity (ETI). As with other microorganisms that are not plant pathogens, *Hypocrea/Trichoderma* spp. trigger induced systemic resistance (ISR) that culminates in the accumulation of components of the jasmonate and ethylene signaling pathways of ISR, such as hydroperoxide lyase, peroxidase and phenylalanine ammonia lyase (which induces lignification)[73]. For example, the action of fungal endopectinases on the mucigel releases oligogalacturonosides that activate plant defense mechanisms [68]. As a result of recognition of MAMPs and/or molecules released during initial stages of the interaction, the plant deposits increased callose and cellulose in its cell walls, and releases phenolic compounds that prevent further colonization, as observed during the early stages of root colonization by *T. asperelloides* on *Cucumis sativus* (cucumber)[74,75]. As *Hypocrea/Trichoderma* spp. are not plant pathogens, they are not expected to elicit the second stage of the plant innate immune system. However, systemic acquired resistance (SAR), normally associated with the second stage of the plant immune response, is induced by *Trichoderma asperellum* in cucumber plants in a concentration dependent manner, and may occur in the early stages of interactions with roots[75]. It must be noted that these effects have been studied in only a few *Hypocrea/Trichoderma* species and strains (the protoplast fusion hybrid *T. "harzianum"* T-22, *T. asperelloides* T203 (former *T. asperellum*) and *H. virens*) which are particularly effective in stimulation of plant defenses.

Several classes of *Hypocrea/Trichoderma* molecules, such as xylanases, peptaibols, swollenin and cerato-platanins, act as MAMPs. The endoxylanase EIX (also known as XYN2) from '*T. viride'* ATCC 52438 was the first *Hypocrea/Trichoderma* protein known to elicit ethylene formation in tobacco (*Nicotiana tabacum*) and tomato (*Solanum lycopersicum*) [76].

Unfortunately, the species identity of this strain has never been re-assessed by molecular methods and thus must be considered as uncertain. An effective biocontrol strain of *T. virens* secretes an endoxylanase that is identical to XYN2 (Ref.[77]), and genes encoding homologue enzymes are found in the genomes of *H. jecorina* GH 11 (protein identifier Trire2: 123818) and *H. virens* (Trive2: 72838). Remarkably, the catalytic activity of XYN2 is not required for eliciting the plant defense responses [78, 79], and thus the enzyme itself and not its reaction product must be acting as MAMP. In fact, to elicit the plant response, XYN2 binds to the plant LeEix receptor, a member of a superfamily of leucine-rich repeat receptor-like proteins of plants that also carry a signal for receptor-mediated endocytosis that is essential for proper induction of defense responses [80, 81]. In addition, binding of XYN2 to the plant receptors also causes alterations in membrane function that is required for eliciting the plant defense [82].

Blocking the synthesis of peptaibols (a group of non-ribosomal peptides, Box 2) in *H. virens* by disrupting the gene encoding the peptaibol synthase TEX1 results in strains that do not induce ISR in cucumber, although this can be overcome by addition of peptaibol mixtures [83]. The mechanism of action of peptaibols for ISR induction is not known but may be related to their ability to alter membrane function, as described for XYN2 (Ref.[82]).

Swollenin is a protein that carries a cellulose binding domain (CBM1) and can disrupt the crystalline cellulose structure of plant cell walls [84]. It contributes to root colonization in *T. asperellum* and induces local defense responses but not ISR [85]. Swollenin shows sequence similarity to expansins, which are plant proteins that facilitate expansion of the plant cell wall in roots and root hairs [86], and *Hypocrea/Trichoderma* may take advantage of this increase in root surface when establishing itself in the plant rhizosphere.

Cerato-platanins are small secreted proteins characterized by four cysteines that form two disulfide bonds. The *H. virens* cerato-platanin SM1 induces ISR in *Zea mays* (maize) and *Gossypium* sp. (cotton)[87], while the orthologue of SM1 in *H. atroviridis* (EPL1) is one of the major proteins constitutively secreted by the fungus [88]. Glycosylation of SM1 maintains the protein in a monomeric form which elicits ISR [89]. Deglycosylation leads to formation of an SM1 dimer which does not elicit ISR. It has been suggested that the plant may alter the aggregation state of SM1 by deglycosylation and ultimately affect its ability to induce defenses. *H. jecorina, H. virens* and *H. atroviridis* have three paralogues of *sm1* each, whereas most other fungi from related genera only have one, suggesting that cerato-platanins may be important for *Hypocrea/Trichoderma*. Other small secreted cysteine-rich proteins (SSCPs) are encoded in the *Hypocrea/Trichoderma* genomes[13] and may have a role in root colonization, similar to that described for small secreted proteins of the ectomycorrhizal basidiomycete *Laccaria bicolor*, which accumulate in the hyphae that colonize the plant root [91].

Promotion of plant growth

At least in some cases, the association of *Hypocrea/Trichoderma* with roots can promote plant growth (Figure 4). For example, *H. virens* increases the root system biomass and the lateral root growth rate of *Arabidopsis thaliana*. Auxin-mediated response pathways may have a role in mediating these effects, as plant mutants with defects in these pathways show reduced effects[92]. However, plant growth promotion may also be mediated by a decrease in the levels of the plant hormone ethylene[93]. *T. asperellum* T203 (later re-classified as *T. asperelloides*) possesses an α-1-aminocyclopropane-1-carboxylate (ACC) deaminase gene (*acc1*) that encodes an enzyme that cleaves ACC, a key intermediate in ethylene biosynthesis, and is expressed during interaction with roots of *Brassica napus* (canola) [94]. A knock-out in this gene reduced the ability of the fungus to promote root elongation. Because a sustained high level of ethylene inhibits root elongation, the ACC1 enzyme provides a mechanism for facilitating the formation of longer roots. Similar enzymes have been described in plant growth-promoting bacteria [95].

In addition, the *Hypocrea/Trichoderma* genomes contain many genes that encode nitrilases, as compared to other Ascomycota[13]. These nitrilases may have a role either in hydrolysing ß-cyano-L-alanin, a metabolite which is formed from cyanide released during the final step of ethylene biosynthesis, or in conversion of the plant metabolite indole-3-acetonitrile to indole-3-acetic acid (IAA), a plant root growth-promoting hormone [96].

5. Endophytism

Endophytic biotrophy (i.e. symptom-less growth inside plant tissue) is very common among bacteria and fungi. These microorganisms offer a wide range of benefits to the host including stimulation of plant growth, delaying onset of drought stress and preventing attacks of pathogens [97]. Only a few *Hypocrea/Trichoderma* species have been isolated as endophytes (Table 1), although it is likely that many other strains can behave as facultative endophytes. Almost all the isolated endophytes comprise new taxa and – with the exception of *H. stilbohypoxili* and *H. stromatica* – have no known teleomorphs. A phylogenetic analysis places them in a terminal position of their clades suggesting an evolutionarily recent development of endophytism in *Hypocrea/Trichoderma* [98,99,100]. Some species such as *T. hamatum* are detected both as endophytes and as common inhabitants of soil and rhizosphere, and such a behavior is known for many other opportunistic fungal genera as well[101]. It is therefore unclear whether any obligate endophytic *Hypocrea/Trichoderma* species exist. Interestingly, the mycelium of arbuscular mycorrhizae on the outer side of the colonized roots of *Solanum tuberosum* (potato) can be used by *Hypocrea/Trichoderma* mycoparasites to enter into the plant roots [102], which suggests that traits related to mycotrophy may facilitate the evolution of endophytism. No genomes from *Hypocrea/Trichoderma* strains that were isolated as endophytes have yet been sequenced.

6. Conclusions

The recent advent of genomic and transcriptomic data, combined with insights into molecular ecology and population genetics of *Hypocrea/Trichoderma*, have provided a wealth of information that allows a deeper understanding of this important fungal genus.

Until recently, it was commonly thought that most *Trichoderma* spp. were asexual soil fungi. This is in part also due to difficulties to mate *Hypocrea/Trichoderma* spp. under laboratory conditions. The application of population genetic methods, however, has now shown that many previously believed asexual *Trichoderma* species in fact display a history of sexual recombination, and only four of them could be proven to be clonal (strictly asexual) [5,6,7,8]. These conclusions are also reflected in the results from biodiversity surveys on *Hypocrea/Trichoderma* that led to the summary that of about 150 *Hypocrea/Trichoderma* species currently known and characterized by genetic markers, the main fraction comprises holomorphic species that grow on decaying wood or on basidiomycetes[3,4]. Mycotrophy is thus widespread in the genus.

The comparative analysis of the genomes from *H. jecorina, T. virens* and *T. atroviride* further expanded this finding to conclude that mycotrophy is in fact a very ancient trait of

Hypocrea/Trichoderma[13]: a phylogenetic analysis of 100 orthologues and syntenic proteins from *H. jecorina, T. virens* and *T. atroviride* (rooted against *Chaetomium* and *Gibberella*) and a whole genus phylogeny based on the RNA polymerase B subunit nucleotide sequence revealed *T. atroviride* at a position in the genus[13]. Yet its gene inventory already comprises several amplified gene families that are beneficial for competition and antagonism (for detailed description see ref.[13]) thus indicating a genetic predisposition for mycotrophy.

All these data suggest that mycotrophy is the basic property of *Hypocrea/Trichoderma*, and still the major life style for many of its species. However, several of its taxa seem to have evolved further towards new niches (*vide supra*), driven by the presence of genes enabling effective competition and opportunism[13]. This fact is nicely reflected by the findings that species that have found new niches or developed special traits (such as *T. longibrachiatum* to be able to colonize immunocompromised humans; or species that have so far only been isolated as endophytes), occur in terminal positions in the phylogenetic trees[7,98-100], and thus are the most recent taxa of the genus.

The presence of potential fungal preys and plant root-derived nutrients in the plant rhizosphere may have been the major attractors for the evolution of *Hypocrea/Trichoderma* ancestors towards colonizing the rhizosphere. Moreover, mycotrophy-related traits (such as certain proteases, chitinases and secondary metabolites) may have facilitated the evolution of further positive interactions of *Hypocrea/Trichoderma* with plants. We should note, however, that no components or mechanisms deployed by *Hypocrea/Trichoderma* strains are yet known that appear to have been specifically evolved for this process, because most of the components that have been described to date have either been shown to be also involved in other cellular functions (such as e.g. nutrition or competition) or contain orthologues in other fungi that have not been described to communicate with the plant[13]. Further studies of the interactions between plants, mycorrhizae and *Hypocrea/Trichoderma* strains are needed for a better understanding of these processes.

Finally, large-scale genome sequencing projects of additional *Hypocrea/Trichoderma* species (such as *T. harzianum, T. asperellum* and *T. longibrachiatum*) are currently undertaken by DOE Joint Genome Institute (http://www.jgi.doe.gov/fungi) and will enable a more comprehensive molecular-level analysis of the ecological diversity of the genus. This will not only help to understand the molecular basis of the opportunistic nature and the environmental successes of *Hypocrea/Trichoderma* spp. but will also improve their use in biotechnology, agriculture and other areas.

Box 1. Nomenclature of *Hypocrea/Trichoderma*

According to the International Code of Botanical Nomenclature (ICBN, article 59) [98], which also applies to fungi for historical reasons, the teleomorph (sexual stage) name should be used for fungal species wherein a complete (holomorphic) life cycle has been described. The anamorphic name should be used for confirmed agamospecies (clonal species) or when no sexual stage is known. In this Review, when the whole genus is considered, the term *Hypocrea/Trichoderma* is applied. Although we admit the modern trend to abolish the use of the name *Hypocrea* in favour of *Trichoderma* for the entire holomorph, this practice will not become accepted until the corresponding change is made in the ICBN.

Box 2. Secondary metabolites produced by *Hypocrea/Trichoderma* spp.

- **Non-ribosomal peptides**

Non-ribosomal peptides are synthesized by large modular enzymes known as non-ribosomal peptide synthetases (NRPSs). Peptaibols are 11-25-amino acid long linear peptides that are rich in α-aminoisobutyric acid and bear an acetylated N-terminus and a C-terminal amino alcohol [104]. They are amphipathic in nature and have antibiotic protperties because of their ability to self-assemble and form voltage-dependent ion channels in membranes. They act synergistically with cell wall hydrolases to antagonize other fungi by preventing cell-wall resynthesis, and thus potentially have a role in mycotrophy [105]. Another non-ribosomal peptide, gliotoxin, is produced by *Hypocrea virens* "Q" strains, which give very effective disease control of cotton seedling disease [106,107]. However, there are contradictory reports on the role of gliotoxin in mycotrophy under controlled conditions [108,109,110].

- **Polyketides**

Polyketides are synthesized by polyketide synthases (PKS). There are several NRPS-PKS hybrid enzymes encoded in the genomes of *H. atroviridis*, *H. virens* and *H. jecorina*[13], but their roles remain unknown.

- **Isoprenoid derived metabolites**

H. virens produces the fungistatic and anti-cancer steroid viridin, which can be reduced to viridiol, which has herbicidal properties [111]. A gene cluster putatively involved in viridin biosynthesis is present in *H. virens* [112]*.* In addition, *T. arundinaceum* and *T. brevicompactum* produce the trichothecenes harzianum A and trichodermin, respectively, the latter being highly fungitoxic and phytotoxic and formed by a cascade of reactions of which the trichodiene synthase TRI5 catalyzes the first step [113].

- **Pyrones**

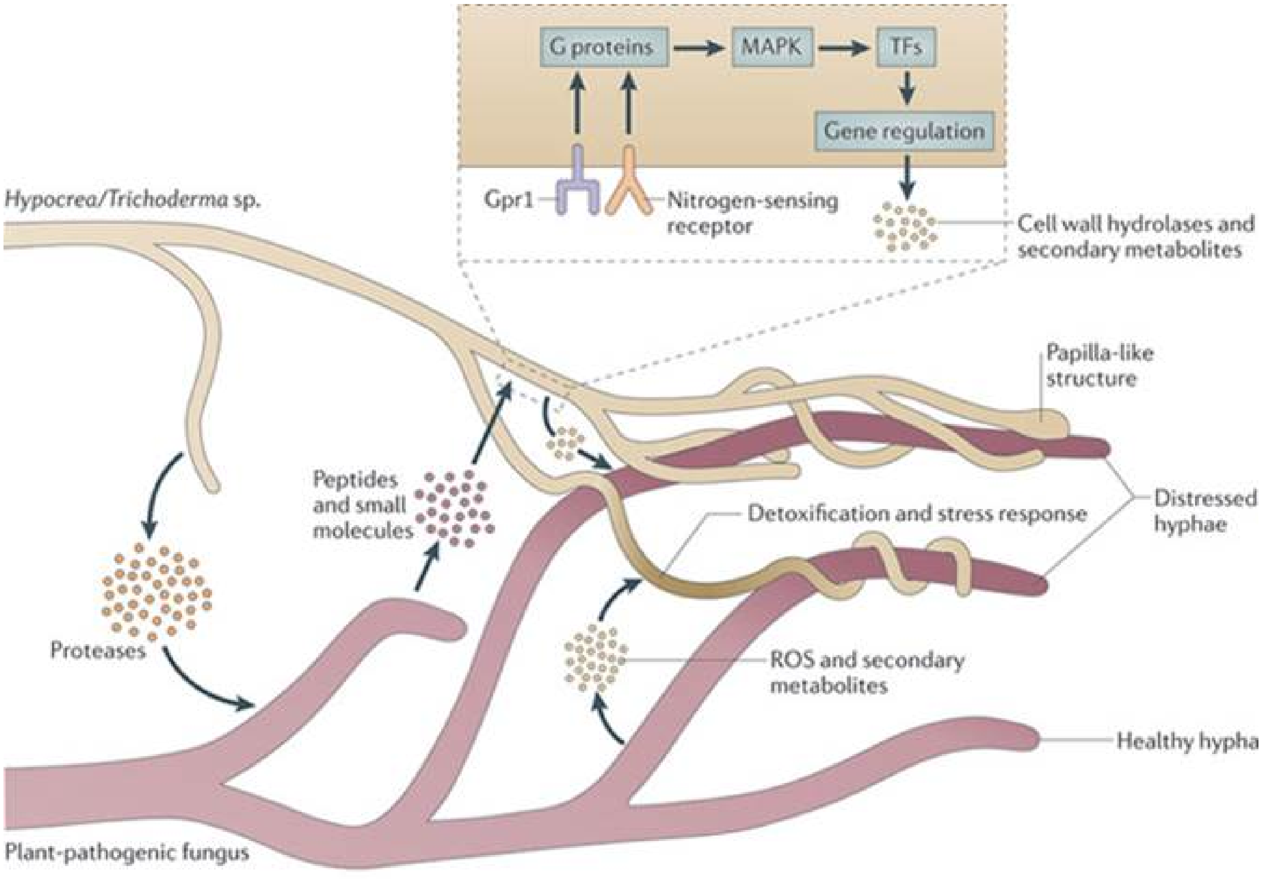
6-pentyl-2H-pyran-2-one (6-PP) is a volatile component (“coconut aroma”) with antifungal activity that is produced by *H. atroviridis* [114].

Figure Legends

Figure 1.

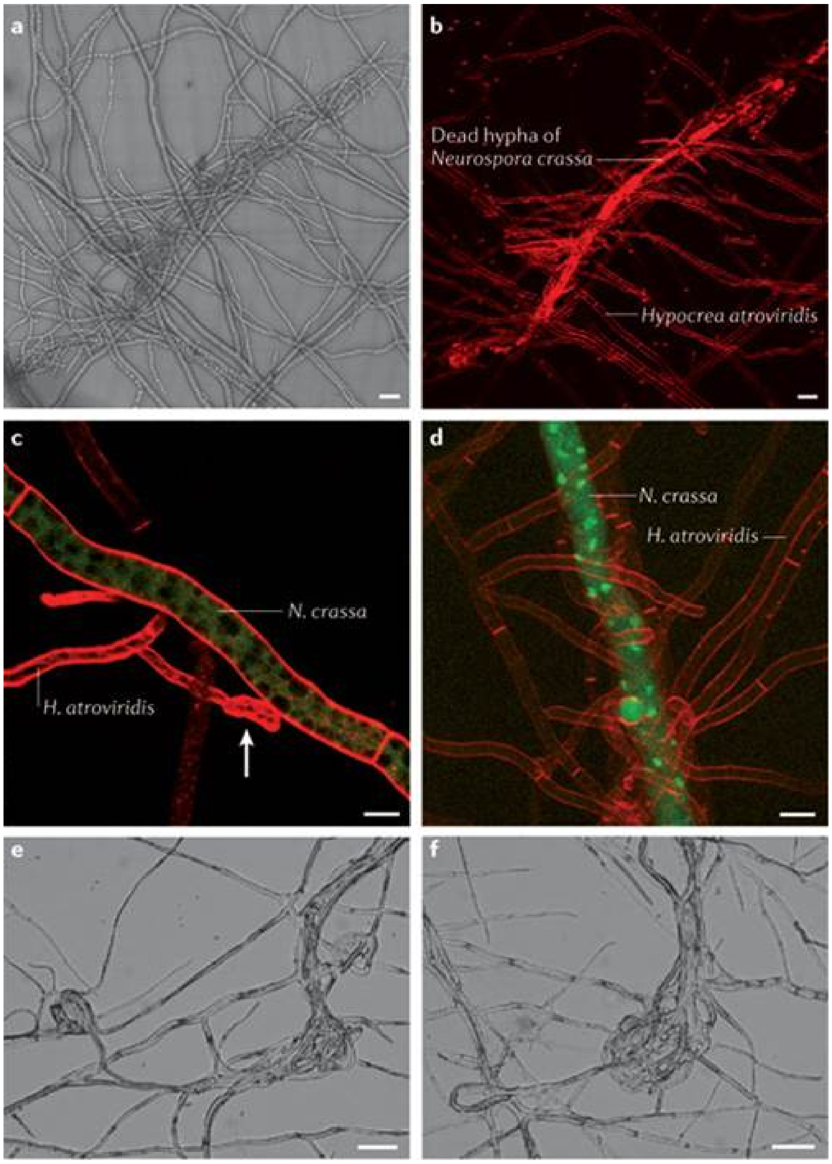
Mycotrophy of *Hypocrea/Trichoderma* spp. The figure shows several examples of *Hypocrea/Trichoderma* spp. growing on various fungal preys: **(A)** *Hypocrea thelephoricola* growing on *Steccherinum ochraceum;* **(B)** *Hypocrea lixii* on *Phellinus sp.;* **(C)** *Hypocrea protopulvinata* on *Fomitopsis* sp.*;* **(D)** *Hypocrea sulphurea* on *Exidia* sp.; **(E)** *Hypocrea parestonica* on *Hymenochaete* sp.; **(F)** *Hypocrea pulvinata* on *Piptoporus betulinus*. The letters H, T and FS indicate, respectively, *Hypocrea* (the sexual stage), *Trichoderma* (the asexual stage) and the fungal prey substratum. Photographs taken from Refs.[3,4] by courtesy of Walter M. Jaklitsch, University of Vienna, Vienna, Austria.

Figure 2



Mycoparasitic interactions of *Hypocrea/Trichoderma* within the soil community: *Hypocrea/Trichoderma* recognizes the plant pathogenic fungus (a prey) via small molecules released by the pathogen, and possibly also by peptides released by the action of its own proteases (which are secreted prior contact). These molecules may bind to G-protein coupled receptors, such as GPCR-1, or nitrogen sensing receptors, thereby eliciting in *Hypocrea/Trichoderma* a signaling cascade comprising G-proteins and MAP kinase (MAPK), which may ultimately modulate the activities of as yet unknown transcription factors (TFs). These factors then enhance the already constitutive expression of genes encoding enzymes for biosynthesis of secondary metabolites and for cell wall lysis. Once close to the prey, lectins and proteins harbouring carbohydrate binding domains (CBMs) may collaborate in the attachment of *Hypocrea/Trichoderma* to the prey. At the same time, the plant pathogen responds by forming secondary metabolites and reactive oxygen species (ROS), which elicit a stress response and detoxification in *Hypocrea/Trichoderma*.

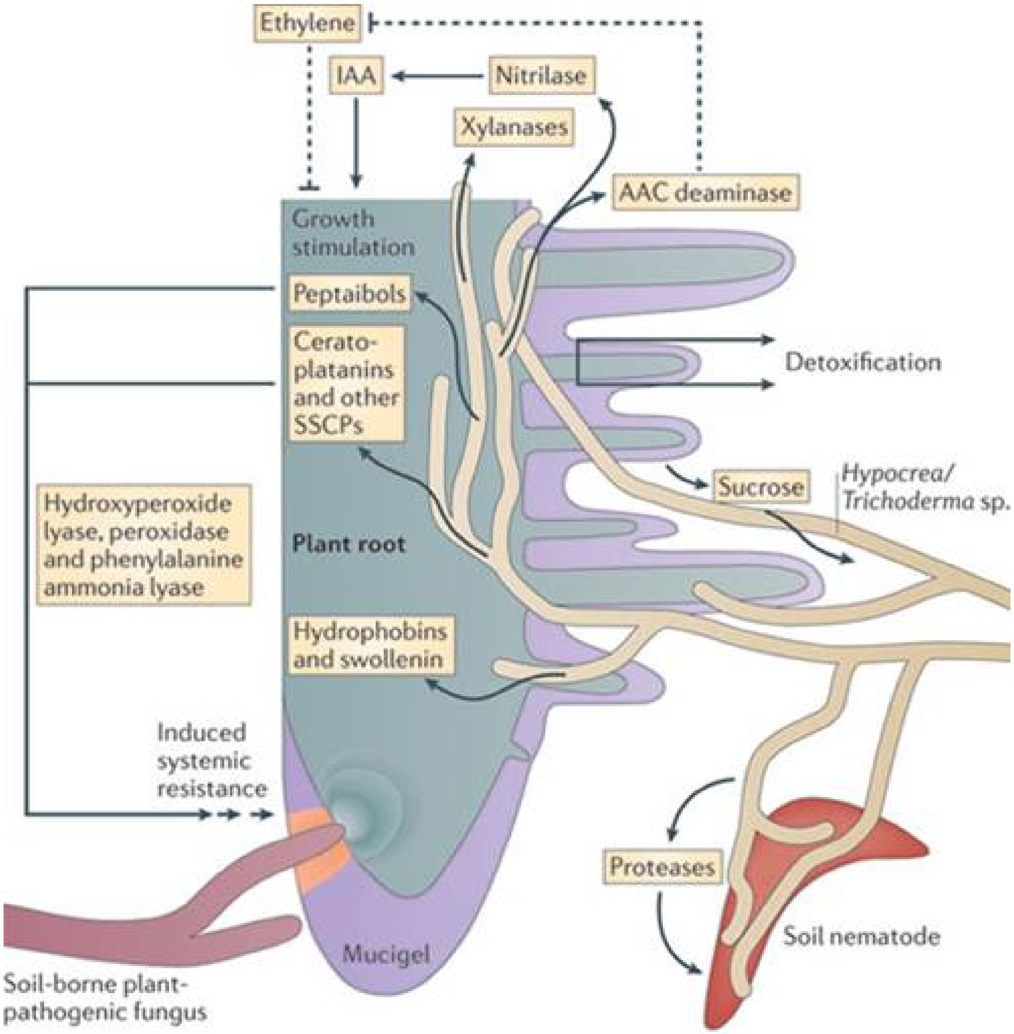
Figure 3



Mycotrophy of *Hypocrea atroviridis*. **(A, B)** *H. atroviridis* grows around, enwraps and attaches to a dead hyphal fragment of the fungus *Neurospora crassa* (**A**, brightfield image; **B**, confocal image). Hyphae were stained with the membrane-sensitive dye FM4-64, which stains the membranes of intact hyphae and the complete hyphal compartment of dead hyphal fragments (because the cell wall and the plasma membrane are then permeable). Scale bars = 20 µm. **(C)** Attachment of *H.*

atroviridis to a *Neurospora crassa* strain that expressed cytosolic GFP, showing formation of papillae-like structures (arrow). Membranes were stained with FM4-64. Scale bar = 20 µm. **(D)** *H. atroviridis* hyphae grows towards and around a *N. crassa* hypha. Membranes were stained with FM4-64, and a *N. crassa* strain expressing both cytosolic GFP and nuclear-specific H1 GFP was used. Scale bar = 10 µm. **(E, F)** Brightfield differential interference contrast images of *H. atroviridis* growing in coils around its own hyphae. Scale bars = 50 µm. Images from Ref.[22].

Figure 4



Interactions of *Hypocrea/Trichoderma* in the rhizosphere. *Hypocrea/Trichoderma* releases several components that trigger induced systemic resistance (ISR) and systemic acquired resistance (SAR) in the plant. Only such effects are shown which occur in the rhizosphere and where the *Hypocrea/Trichoderma* component is known (for an update on further positive effects such as resistance to abiotic plant stresses, enhancement of photosynthetic efficiency and improved nitrogen usage, see Ref.[73]). Peptaibols and the cerato-platanin SM1 induce a systemic resistance in the plants, which culminates in the synthesis of hydroperoxide lyase, peroxidase and phenylalanine ammonia lyase (which induces lignification). The xylanase XYN2 and the 1-aminocyclopropane-1-carboxylic-acid (AAC) deaminase elicit ethylene formation, which leads to enhanced root growth; the constitutively secreted nitrilase may aid in the formation of the auxin 3-indole acetic acid (IAA). Attachment of *Hypocrea/Trichoderma* to the plant roots requires hydrophobins and swollenin. Finally, *Hypocrea/Trichoderma* benefits from the plant roots by receiving sucrose as a carbon source, which enables faster growth. The nematophagy of *Hypocrea/Trichoderma* likely involves subtilisin-like S8 proteases and chitinases.

Table 1. Endophytic *Hypocrea/Trichoderma* spp.

Species	Putatively obligate	Host plant	Location
T. amazonicum[98]	yes	*Hevea* spp.	Peru
T. carribeum var. *equatoriale*[199]	yes	*Theobroma* spp.	Tropical America
T. evansii[100]	yes	*Lophira alata*	Cameroun
	yes	*Cola verticillata*	Cameroun
	yes	*Theobroma gileri*	Peru
T. hamatum[97]	no	*Theobroma cacao*	not available
T. cf. *kongiiopsis*[99]	no	*Theobroma* spp.	not available
T. martiale[117]	yes	*Theobroma cacao*	Brazil
T. ovalisporum[99]	yes	*Banisteropsis carpii*	Ecuador
T. paucisporum[118]	yes	*Theobroma cacao*	Ecuador
T. scalesiae[115]	yes	*Scalesia pedunculata*	Galapagos Islands
H. stilbohypoxili[99]	no	*Fagus* sp.	UK
T. taxii[119]	yes	*Taxus mairei*	China
T. theobromicola[118]	yes	*Theobroma cacao*	Peru

Glossary (in alphabetic order):

Appressorium: a flattened, hyphal pressing structure from which an infection peg that enters the host emerges.

Biotrophy: nutrition on another living organism. Includes the broad spectrum of parasitic or symbiotic interactions.

Callose: a ß-1,3-linked polysaccharide of the plant cell wall that is formed in response to wounding, including infections by pathogens.

Ethylene ($H_2C=CH_2$): a gaseous unsaturated hydrocarbon which acts as a plant hormone that promotes growth and development.

G-protein coupled receptors: receptors that possess seven transmembrane helices, bind an extracellular signaling molecule and transmit this binding by activating a G-alpha protein.

Hemicellulolytic: ability of an organism to use plant hemicelluloses, such as xylans and pectins, as carbon sources.

Induced systemic resistance: a process in which plants respond to a non-pathogenic microbe with a jasmonate/ethylene-dependent signalling cascade. The result is systemic expression of a broad spectrum and long-lasting ability to mount a faster and stronger defense when challenged by a pathogen.

Lectins: sugar-binding proteins that are highly specific for the respective sugar moiety and have a role in recognition of cells and proteins.

Mycorrhiza: usually symbiotic or weakly parasitic association between a fungus and the roots of vascular plants; mycorrhizae are found in most plants.

Mycosis: a fungal infection of animals or humans.

Nematophagous: (fungi) that are specialized in trapping and digesting nematodes.

Opportunistic : being able to rapidly adapt to occupy a newly arising ecological niche.

Parasitism: Resembles predation except that the host (an organism being adversely affected) is not killed outright but exploited over some period of time; may be considered as “weak” predation.

Predation: occurs when one population affects another adversely and benefits itself from the interaction. Ultimately a predator kills its prey and consumes part or the entire prey organism.

Saprotrophy: Extra-cellular digestion of dead or decayed organic matter.

Symbiosis: “Living together”. Interaction between two organisms that live together without harming one another. Includes MUTUALISM and COMMENSALISM.

Systemic acquired resistance: a mechanism of induced defense by a plant that confers long-lasting protection against a broad spectrum of microorganisms. It involves production of the signal molecule salicylic acid which then leads to the accumulation of pathogenesis-related proteins that are thought to contribute to resistance.

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