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REVIEW ARTICLE

Friends or foes? Emerging insights from fungal interactions with plants

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One sentence summary: The diversity of fungal–plant interactions are reviewed as a function of biochemical, physiological and evolutionary adaptation, which are interconnected at various stages.

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ABSTRACT

Fungi interact with plants in various ways, with each interaction giving rise to different alterations in both partners. While fungal pathogens have detrimental effects on plant physiology, mutualistic fungi augment host defence responses to pathogens and/or improve plant nutrient uptake. Tropic growth towards plant roots or stomata, mediated by chemical and topographical signals, has been described for several fungi, with evidence of species-specific signals and sensing mechanisms. Fungal partners secrete bioactive molecules such as small peptide effectors, enzymes and secondary metabolites which facilitate colonization and contribute to both symbiotic and pathogenic relationships. There has been tremendous advancement in fungal molecular biology, omics sciences and microscopy in recent years, opening up new possibilities for the identification of key molecular mechanisms in plant–fungal interactions, the power of which is often borne out in their combination. Our fragmentary knowledge on the interactions between plants and fungi must be made whole to understand the potential of fungi in preventing plant diseases, improving plant productivity and understanding ecosystem stability. Here, we review innovative methods and the associated new insights into plant–fungal interactions.

Keywords: plant–fungal interactions; advanced microscopy; phytopathogenic and symbiotic fungi; plant receptors; plant defence response; crop productivity

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INTRODUCTION

Fungi play a major role in natural ecosystems and in modern agriculture based on their nutritional versatility and various interactions with plants. Fungi are important decomposers and recyclers of organic materials; they positively or negatively interact with plant roots in the rhizosphere or with aboveground plant components. The interactions between plants and their associated fungi are complex and the outcomes diverse. Since several fungi can combine different lifestyles—saprophytic, pathogenic or symbiotic—their boundaries are often not clear-cut (Grigoriev 2013).

Plants are able to mount successful defences, and in nature are generally resistant to most pathogens; hence, symbiotic and neutral associations dominate and parasitic associations are considered to be the exception (Staskawicz 2001). The plant genotype determines its metabolic secretions which serve as important signals for recruitment of fungi into the rhizosphere of the plant. Plant receptors and expression patterns of defence-related proteins which interact with specific fungus-derived molecules may determine the outcome of an interaction. The use of advanced microscopy techniques to characterize organisms, even down to the single molecule level, has led to the development of completely novel assays for probing such interactions. A mutational shift in either the genes of the fungal pathogen or host receptor can alter plant–pathogen interactions from resistant to susceptible or vice-versa (Stracke *et al.* 2002; Giraldo and Valent 2013). Beneficial microbes have evolved strategies to suppress or mask the defence responses of the host plant, allowing them to epiphytically or endophytically colonize their hosts (Zamioudis and Pieterse 2012). Interestingly, pathogenic and symbiotic fungi establish obligate relationships with plants using colonization patterns that are common in several aspects, including feeding structure development and its physical separation from the nutrient source (Corradi and Bonfante 2012). However, the outcomes of these interactions are contrasting as in one case the plant is rewarded (symbiosis) while in the other the plant suffers (parasitism). Both types of interactions can be viewed from an evolutionary perspective: in the case of biotrophic pathogens it is the fungal component that has evolved to become a successful parasite, whereas in the other case the fungus has evolved along with the plant partner to become a successful symbiont. To understand the complex interplay of signals between fungi and host plants, we need to decode the functions of both microbial and plant signals and their respective receptors, as well as their roles in triggering plant immunity. All of these contributing factors are involved in successful fungal colonization of host plants as well as their resistance capabilities. This article highlights the progressive development in the understanding of plant–fungal interactions based on innovative methods and recent discoveries.

DIVERSITY AND EVOLUTION OF PLANT–FUNGAL INTERACTIONS—FROM SYMBIONTS TO PATHOGENS

Fungi are much older than plants and plant–fungal interactions are considered to be as old as the evolutionary period of higher plants, particularly the terrestrial vascular plants (Humphreys *et al.* 2010; Field *et al.* 2012). Even the colonization of land by plants is believed to be with the help of fungal partners (Redecker, Kodner and Graham 2000) and these associations date back to 400–460 million years ago, the time period in which vascular plants evolved (Remy *et al.* 1994; Kemen and Jones 2012). Beneficial plant–fungal interactions provide stability to both partners, but harmful interactions may result in host destabilization (Fig. 1) resulting in survival pressure and faster plant evolution (Jones and Dangl 2006). However, most plant–fungal interactions promote plant growth and development, with the fungus potentially acting as symbiotic partner that improves plant foraging, acquisition of soil resources (nutrients and water) and stress tolerance. In turn, the plants deliver carbohydrates to the fungus (Buscot *et al.* 2000) contributing to a stable association between the interaction partners. There is a wide variety of symbiotic plant–fungal interactions which include endophytic and mycorrhizal fungi. The Greek word 'mycorrhizal', literally meaning 'fungus roots', was introduced in 1885 by Frank (Trappe 2005), and mycorrhiza is defined as the symbiotic interaction between a fungus and the roots of a plant. However, endophytes show symptomless growth inside living tissues of roots, stems or leaves until senescence of the host plant, at which point the fungi may become slightly pathogenic (Brundrett 2004). Since fungal endophytes are abundant, most plants on earth likely host one or more with the benefit of increased resistance to pathogens, herbivores and/or stress (Strobel and Daisy 2003).

Mycorrhizae differ from endophytic associations primarily based on nutrient transfer at their interface and by a synchronized plant–fungal development (Brundrett 2004). Most, if not all, land plant species host mycorrhizal fungi for efficient nutrient uptake and around 6000 fungal species in the *Glomeromycota*, *Ascomycota* and *Basidiomycota* have been identified as mycorrhizal (Wang and Qiu 2006; Bonfante and Anca 2009). The positive impacts of the plant root–fungal symbiotic relationship (improved nutrient status of the plant and its improved resistance to biotic and abiotic stresses) likely enabled plants to move from an aquatic environment, in which nutrient resources are directly available, to terrestrial habitats where depletion zones rapidly develop after element absorption by roots (Corradi and Bonfante 2012).

Depending on the plant and fungal partners, mycorrhizas can either be endomycorrhizas or ectomycorrhizas in which the hyphae of the fungal partners are intracellular, penetrating into root cells or extracellular, surrounding plant lateral roots or penetrating between root cells, respectively (Bonfante and Anca 2009). About 80% of plants present today on our planet are associated with endomycorrhizal fungi of the phylum *Glomeromycota*, many of which are obligate biotrophic mycorrhizal symbionts (Karandashov *et al.* 2004). These fungi typically form highly branched haustoria-like intracellular structures called arbuscules and hence are called 'arbuscular mycorrhizae' (AM) (Buscot 2015). *Glomeromycota* have remained associated with plants throughout evolution and have existed for more than 400 million years morphologically unaltered (Wang and Qiu 2006; Parniske 2008). In contrast, other mycorrhizal fungi have polyphyletic lineages that represent parallel or convergent evolution (Cairney 2000; Brundrett 2002; Bruns and Shefferson 2004). The hypothesis that ectomycorrhizal fungi evolved polyphylogenetically from multiple saprophytic species is supported by a recent study. Kohler *et al.* (2015) generated a reconciled evolutionary tree for molecular clock analysis, including 49 fungal species with saprophytic or symbiotic lifestyles, showing that ectomycorrhizal fungi likely evolved from multiple lineages fewer than 200 million years ago. Further, analysis of 16 gene families associated with plant cell wall degradation in ancestral white-rot wood decaying fungi and ectomycorrhizal lineages showed that all symbionts in these families have substantial gene loss. In particular, those enzymes associated with lignin degradation were lost in ectomycorrhizal fungi, while endomycorrhizal

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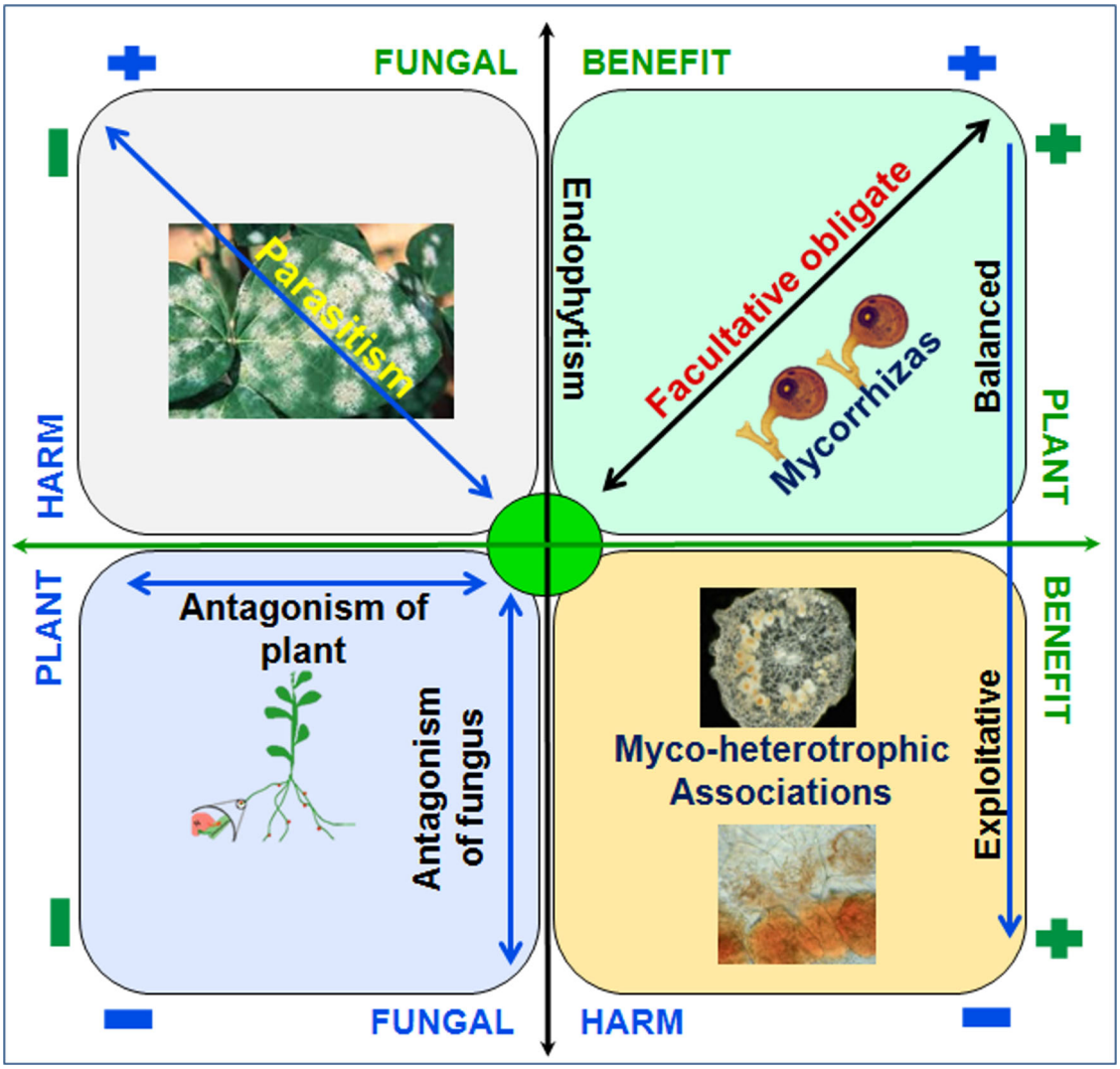

Figure 1. Comparison of different plant–fungus interactions. Mutualistic associations occupy the mutual benefit (+ +) quadrant in diagrams contrasting the relative benefits (+) or harm (–) to two interacting organisms. The figure was redrafted with permission from Adjunct Associate Professor Mark Brundrett, Plant Biology, University of Western Australia; (http://mycorrhizas.info/download/pdf/symb-assoc.pdf).

ericoid and orchid fungi maintained an extensive repertoire of cell wall-degrading enzymes (CWDEs) (Kohler *et al.* 2015; Venturini and Delledonne 2015). Evolutionary gene loss and the parallel birth of genes that specifically contribute to the establishment of symbiosis might be accompanied by analogous genomic duplication in host plants for which upcoming genome sequences may soon lead to deeper insights (Venturini and Delledonne 2015).

Some fungal species developed further, breaking the fine balance of mutual benefit to become plant pathogens classified as biotrophs, hemibiotrophs and necrotrophs (Fig. 1), each having different modes of interaction with their host plants (Gardiner, Kazan and Manners 2013). Wounds and leaf stomata are the usual route through which pathogens gain access into the plant interior, however, secreted fungal CWDEs and specific infection structures in many cases support penetration. Necrotrophic pathogens, which often show a broad host range, rapidly cause substantial tissue damage. Host cells are killed by a combination of CWDEs, reactive oxygen species (ROS), and/or toxins for which the virulence of several pathogens has been correlated with toxin synthesis (Wang *et al.* 2014a). These activities lead to membrane destruction in the host and release of its nutrients, which is followed by the colonization and decomposition of the host plant (Wolpert, Dunkle and Ciuffetti 2002). Biotrophic pathogens, in contrast, are obligate parasites that do not produce toxins but often secrete effectors to suppress the host immune system (Perfect and Green 2001). Biotrophs can only complete their life cycles in living host cells which leads to disease symptoms after a relatively long period following infection. These fungi show host specificity and interact with the host through specialized biotrophic hyphae at an interfacial zone where both interaction partners actively secrete biomolecules (Yi and Valent 2013). Ectoparasitic powdery mildews for example develop highly specialized infection structures, such as primary and appressorial germ tubes on the plant cuticle, which allow the pathogen to breach the cell wall using a combination of mechanical force and CWDEs (Takahashi 1985; Pryce-Jones, Carver and Gurr 1999). After plant cell wall penetration, the plasma membrane is indented surrounding the newly formed nutritional cell, the haustorium (Horbach *et al.* 2011). Consequently, a close metabolic interaction between the host plant and the biotrophic pathogen is established and the

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fungus aims to block host defences to sustain the host processes it requires for feeding and growth (Giraldo *et al.* 2013; Yi and Valent 2013). Hemibiotrophic pathogens are intermediate between the necrotrophic and the biotrophic lifestyles, initially growing as biotrophs and later switching to a necrotrophic lifestyle (Struck 2006; Gardiner, Kazan and Manners 2013). The biotroph–necrotroph switch in hemibiotrophs depends on molecular and physiological factors. Several hemibiotrophs require extended periods of biotrophic colonization to establish infection, while for others hours suffice for successful infection, and the switch to necrotrophy is rapid (Kabbage, Yarden and Dickman 2015). One possible explanation for this divergence is that biotrophy requires a sufficient amount of time to thwart host defences and suppress programmed cell death through effector secretion. Thus, there is disease onset despite accumulation of host defences, suggesting that the increased pressure from plant defences may trigger the change from biotrophy to necrotrophy. On the other hand, it is conceivable that once a defence limit has been reached, the pathogen detects that it no longer has the advantage and converts to necrotrophy as a more viable infection strategy. Therefore, at certain times, the fungal-induced demise of the host cell may be the result of expanding plant defences. It is possible that both the maintenance and length of the biotrophic stage are not completely reliant on how adequately the fungus manages host barriers. On the contrary, the change to necrotrophy could also relate to the fungal requirement for improved nutrient acquisition (Kabbage, Yarden and Dickman 2015).

Biotrophy is thought to have been an old way of life for fungal pathogens, while necrotrophy would be a more recent evolutionary achievement (Pieterse *et al.* 2009). In this context, hemibiotrophs would reflect the transition between these nutritional strategies (Horbach *et al.* 2011). Plant immunity to necrotrophs varies depending on the fungal species and may be antagonistic to, similar to, or distinct from the immune responses to biotrophs. In general, necrotrophs are viewed as brute force pathogens, having restricted their physiological interaction with their host based on their poorly developed infection-related morphogenesis, and the multitude of biochemical compounds they deploy that overwhelm the plant. In most cases, the infection strategy of necrotrophic fungi is less complex than that of obligate biotrophs.

The term 'appressorium' (=adhesion organ) has first been used in the 19th century (Frank 1883), and Emmett and Parbery (1975) defined it as 'all structures adhering to host surfaces to achieve penetration, regardless of morphology'. Appressoria formed by typical necrotrophs, such as *Alternaria*, *Botrytis*, *Cercospora*, *Fusarium*, *Helminthosporium*, *Ramularia*, *Rhynchosporium*, *Sclerotinia* or *Verticillium* species, are inconspicuous, and infection hyphae formed within the host are rather uniform. Furthermore, appressoria may as well appear as discrete swollen, lobed or dome-shaped cells, separated from the germ tube by a septum as in rust uredinio—and aeciosporelings, in *Magnaporthe grisea* and *Colletotrichum* species, and in many other plant pathogens (Deising *et al.* 2000; Horbach *et al.* 2011).

The interactions between plants and their pathogens are subject to parallel or coevolution, wherein pathogens must find innovative strategies to successfully colonize their hosts, and plants must identify new detection methods and more robust defence mechanisms to ward off pathogen attacks. The particular morphological and biochemical toolkits evolved and used by fungi in developing their relationship with host plants have evolved convergently and divergently to include complex components that take advantage of and control host pathways. Indeed, basic developmental branches contain species equipped with a range of host reaches and species with assorted trophic ways of life (Horbach *et al.* 2011).

ADVANCED MICROSCOPIC METHODS FOR STUDYING PLANT–FUNGAL INTERACTIONS

Microscopy underpins many studies of plant–fungal interactions. The use of light microscopy (LM) to study fungi goes back to Hooke (1665) who first described and illustrated *Phragmidium mucronatum* (parasitic rose rust) and the saprophytic *Mucor,* followed by Malpighi (1675,1679) who documented a variety of fungi. The relative transparency of fungi in bright field (light) microscopy was initially overcome using contrast-enhancing dyes and differential staining (Von Gerlach 1858), which can sometimes alter sample integrity and viability. Other optical modes based on different light–sample interactions, including fluorescence (Heimstadt 1911; Reichert 1911), polarization (Nicol 1828), dark-field (Lister 1830), phase contrast (Zernike 1955) and differential interference contrast (Nomarski 1955) microscopy were developed to improve contrast of samples without staining. In the past several decades we have witnessed the birth of new technology and techniques that have improved microscopic contrast, resolution and depth of field (Table S1, Supporting Information).

The development of bright fluorescent labels for biological molecules, including chemical dyes, fluorophore-coupled antibodies and fluorescent proteins (FPs; 2008 Nobel Prize in Chemistry to M. Chalfie, O. Shimomura, R. Y. Tsien), has revolutionized fluorescence microscopy, spawning new methods with improved contrast and resolution for tracking plant–fungal interactions (i.e. Hood and Shew 1996). Signal captured from the fluorescence of individual molecules enables resolution that depends only on the ability to differentiate individual points of light. The confocal microscope (CM; Minsky 1988) uses pinhole technology which dramatically improves contrast over epifluorescence (wide-field) by rejecting out of focus light and enabling optical sectioning (focus into different sample depths). The induction of specific *Trichoderma* genes on plant surfaces to view initiation of the mycoparasitic gene expression cascade *in vivo* is an excellent example of modern CM (Lu *et al.* 2004), as is the mycoparasitic attack of *T. atroviride* which induces tip growth arrest, tip swelling and cell lysis in *Botrytis cinerea* (Fig. 2). The advent of two-photon laser excitation further improves depth of field and resolution of 3D confocal image reconstructions and enables single molecules within live cells and tissues to be tracked in real-time (reviewed in Howard 2001). Techniques that have evolved alongside fluorescence and confocal microscopes include bimolecular fluorescence complementation (BiFC) and the so-called four letter F-words (reviewed in Ishikawa-Ankerhold, Ankerhold and Drummen 2012)—Förster resonance energy transfer (FRET), fluorescence recovery after photobleaching (FRAP), fluorescence loss in photobleaching (FLIP), fluorescence localization after photobleaching (FLAP) and fluorescence lifetime imaging microscopy (FLIM). FLIM provides additional imaging contrast by measuring decay times of the fluorophores, which are often sensitive to their local environment. FRET relies on energy transfer between two fluorescent molecules, thus probing molecular interactions at Ångstrom resolution, and so can be used to track plant–pathogen protein–protein interactions (Hayward, Goguen and Leong 2010). BiFC, albeit at lower resolution, has been used to visualize protein interactions at the

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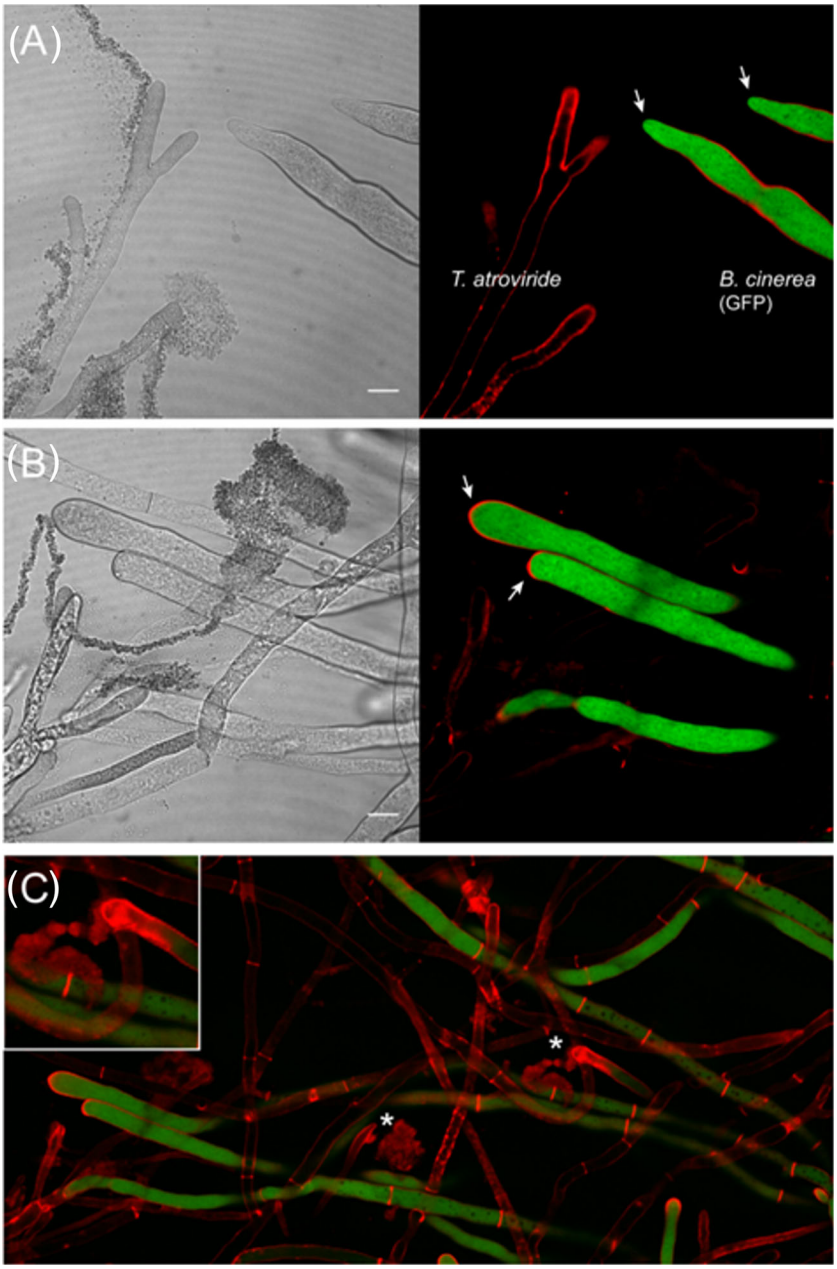

Figure 2. Mycoparasitic attack of *T. atroviride* induces tip growth arrest, tip swelling and cell lysis in *B. cinerea*. (**A**) Before *B. cinerea* (expressing cytoplasmic GFP; Schumacher *et al.* 2012) is attacked by *T. atroviride*, the apical cell wall extends quickly and hence shows only weak staining with the chitin-specific fluorescent dye Congo Red (arrowheads). (**B**) As soon as there is an attack, hyphal tip growth arrests, leading to tip swelling and increased deposition of chitin in the apical cell wall (arrowheads). (**C**) Tip lysis of the prey hypha results in the release of cytoplasm into the surroundings (asterisks and inset), which can be used as nutrient substrate by *T. atroviride*. Scale bars, 20 μm.

subcellular level in *Arabidopsis in situ* (Walter *et al.* 2004) and shortly after was successfully applied to fungal cells (Hoff and Kück 2005). FRAP, FLIP and FLAP all rely on photobleaching by intense laser light followed by tracking various parameters to produce kinetic information for a subset of molecules (Ishikawa-Ankerhold, Ankerhold and Drummen 2012). These methods hold promise for myriad future applications in studying plant pathogens.

During the past decade superresolution techniques (Hell 2007; Huang *et al.* 2008), the subject of the 2014 Nobel Prize in Chemistry to Eric Betzig, Stefan W. Hell and William E. Moerner, have been developed to overcome the diffraction limit (Abbé 1873) for fluorescent samples. 'True' super-resolution (SR) methods include near-field scanning optical microscopy (NSOM; Synge 1928) and structured illumination microscopy (SIM; Gustafsson 2000). Stimulated emission depletion (STED; Hell and Wichmann 1994), which relies on confocal technology, is considered a deterministic functional SR method and stochastic functional SR methods encompass localization microscopy, including photoactivated localization microscopy (PALM; Betzig *et al.* 2006; Hess, Giririjan and Mason 2006) and stochastic optical reconstruction microscopy (STORM; Rust, Bates and Zhuang

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2006). Such techniques, generally requiring specialized instrumentation and expertise, are most often used to accurately estimate object size and resolve ultrastructure not available to other fluorescence microscopy modes. Recently PALM was used to image Cse4 at the centromere of budding yeast at 50 nm resolution in 3D, showing compaction in anaphase and how a chaperonin stabilizes the nucleosome (Wisniewski *et al.* 2014). To date these methods have mostly been applied to yeast, but would offer single molecule resolution to plant–pathogen experiments traditionally pursued with CM (i.e. Lu *et al.* 2004).

Fourier transform infrared (FTIR) microspectroscopy (Coates, Offner and Siegler 1953), offering spatially resolved chemical information on the bulk specimen at micron resolution, has been successfully applied to studying the effects of spruce wood-degrading brown and white rot (Fackler *et al.* 2010). Recently X-ray tomography (Kirkpatrick and Baez 1948) has been applied to the 3D reconstruction of yeast (Zheng *et al.* 2012), and while the resolution has yet to match that from TEM studies, this method may hold promise for future studies of plants and their pathogens.

Electron microscopy (EM), including transmission (T) and scanning (S) modes, has been a gold standard in biological imaging for decades (Knoll and Ruska 1932; Zworykin, Hillier and Snyder 1942). TEM of immunogold labelled (Coons, Creech and Jones 1941), sectioned samples provides high contrast images of intracellular and interface regions, offering insight into plant–fungal interactions at the molecular level (Howard 2001). For instance, Diagne-Leye *et al.* (2013) recently used LM, TEM and SEM to show how *Moesziomyces penicillariae*, a smut fungus, adapts to the short life cycle of pearl millet. Cryo-SEM images of sample surfaces when combined with focused ion beam milling and energy-dispersive X-ray spectroscopy, can be used to localize and identify fungal and plant secretions in response to their interaction (Dahms and Kaminskyj 2008). While EM offers ultra high resolution, high vacuum instruments preclude live sample imaging which has led to the development of environmental SEM for probing hydrated and uncoated samples, but not live cells. Atomic force microscopy (AFM; Binnig, Quate and Gerber 1986), another surface scanning method, images live biological specimens under ambient conditions at higher resolution than cryo-SEM (Dahms and Kaminskyj 2008). AFM in force mapping or quantitative imaging mode gives additional information on mechanical and molecular surface properties of the sample, appropriate for probing cell spring constants, cell wall elasticity and specific molecular surface interactions between plants and their pathogens, for instance interaction forces between spores and plant surfaces in the context of host invasion (Adams *et al.* 2012).

Correlative microscopy (reviewed in Caplan *et al.* 2011; Czymmek and Dahms 2015 (in press)) combines data from more than one microscope to yield information that extends individual modes, scales and dimensions. Fully integrated microscopes enable simultaneous data collection using two or more different microscopic modes, for example confocal-AFM which simultaneously probes the inside and outside of the specimen. A very new example is near-field interferomic IR atomic force microscopy, developed at ALS Berkeley (Bechtel *et al.* 2014), to examine the ultrastructure and chemical composition of fungal exudates (Gough *et al.* 2015). Many of the researchers developing advanced microscopy methods have extensive equipment and expertise, making collaborative relationships the key for success but which often render ground-breaking results. There are so many plant pathogen questions appropriate for high resolution imaging that the opportunities are boundless.

PHYSIOLOGY OF PLANT–FUNGAL INTERACTIONS—FUNGAL DISEASE DEVELOPMENT IN PLANTS

The interaction of a pathogen with a host is characterized by a series of sequential events called the disease cycle which result in the development and perpetuation of disease (Daly 1984) (Fig. 3). A general disease cycle comprises the following phases: (1) Spread and contact in which fungi are spread and come into contact with an appropriate host plant by environmental mechanisms such as wind, water, insects or by active growth as with some root-infecting fungi (Travadon *et al.* 2012), (2) Prepenetration, including spore germination, pathogen attachment to host structures and recognition events that are triggered by signals from the host as well as environmental factors (Tucker and Talbot 2001), (3) Entry of pathogens into the plant through natural openings, wounds, or by direct penetration that can involve specialized penetration structures such as appressoria (Pryce-Jones, Carver and Gurr 1999) or through insect-caused wounds

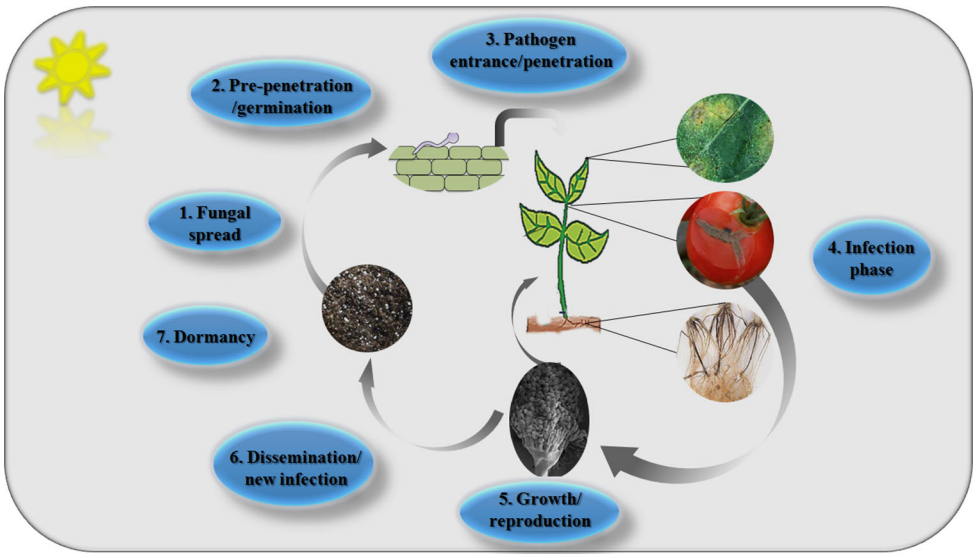

Figure 3. Disease cycle. For details see text.

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such as *Grosmannia clavigera* attack on lodgepole pines (Diguistini *et al.* 2011) and *Ophiostomata ulmi* attack on Dutch elm (D'Arcy 2000), (4) Infection and invasion whereby the pathogen establishes contact with host cells and may spread from cell to cell thereby resulting in visible symptoms, (5) Reproduction in which an immense number of fungal spores are produced from infected host tissues, (6) Spore dissemination from the site of reproduction to other susceptible host surfaces or new plants and (7) Dormancy, helping the pathogen to survive under unfavourable conditions (Brown and Ogle 1997).

Plants respond to pathogen infection with defence reactions as well as changes in other physiological processes such as respiration, photosynthesis, nutrient translocation, transpiration, growth and development, many of which are related to primary carbon metabolism (Berger, Sinha and Roitsch 2007). The plant's respiration is one of the first processes to be affected upon pathogen infection, accompanied by metabolic changes such as increased enzymatic activity of the respiratory pathway, an accumulation of phenolics, and an increased activity of the pentose pathway (Sharma 2004). Tomato plants attacked by the necrotrophic fungus *B. cinerea* exhibit coordinated regulation of defence and carbohydrate metabolism, along with a correlation between the gene expression regulation magnitude and symptom development (Berger *et al.* 2004). The attack by a biotrophic pathogen additionally brings about a metabolic sink at the infection site, changing the pattern of supplement translocation inside of the plant and bringing on a net flood of supplements into infected leaves to fulfil the pathogen's requirements. Therefore, the consumption, redirection and maintenance of photosynthetic products by the pathogen trick the plant's developmental programming, and further diminish the plant's photosynthetic effectiveness (Agrios 2005). In addition, pathogen-derived biomolecules such as some enzymes and toxins may increase membrane permeability in plant cells, resulting in an uncontrollable loss of useful substances such as electrolytes as well as an inability to inhibit the inflow of undesirable substances (Agrios 2005). Pathogens can discharge plant hormones themselves, or trigger an increase or decrease in synthesis or degradation of plant hormones, exasperating hormone offset. This can bring about a mixture of symptoms, for example, the formation of adventitious roots, gall development and epinasty (the down-turning of petioles) (Agrios 2005). Mayerhofer, Kernaghan and Harper (2013) found that the aggregate biomass of endophyte-inoculated plants was reduced compared to non-inoculated controls, although individually, shoot biomass, root biomass and nitrogen focus reactions were neutral. In contrast, dark septate endophytes evoked an overall increase in root biomass (Alberton *et al.* 2010), shoot, root and total biomass as well as nitrogen and phosphorus content in the host plant (Newsham 2011). Several pathogens have a direct adverse effect on plant reproduction as they directly attack and kill flowers, fruits or seeds, interfere with their production, or interfere directly or indirectly with the propagation of their host plant (Clay *et al.* 1989). Physiological changes in plants upon challenge with fungal pathogens are also reflected at the transcriptional and translational levels. Enhanced mRNA levels and elevated protein synthesis in infected plant cells upon pathogen attack reflects the increased production of defence-related substances, enzymes and other proteins (Samborski *et al.* 1978; Agrios 2005).

HOST DEFENCE AGAINST FUNGAL INVASION

During the invasion of host plants, fungi have to overcome a plethora of host defensive physical and chemical barriers categorized as constitutive or inducible (Miedes *et al.* 2014). The constitution and the chemical nature of the plant surface hinder pathogen invasion and hence are important for defence. Structural compounds such as the cuticle of aerial plant parts serve as a constitutive barrier to direct penetration, and cuticle waxes that repel water prevent fungal spore germination (Sharma 2004; Freeman and Beattie 2008). The triggers for inducible barriers generally reside within the fungi and are known as effectors molecules. Effectors are secreted by fungi to interfere with the basal plant defence responses, but plants have evolved mechanisms to recognize such molecules. Effector recognition by the plant triggers defence responses known as effector-triggered immunity (ETI) which results in hypersensitive responses (HR) and the biosynthesis of pathogenesis-related (PR) proteins (Cui *et al.* 2014). Other inducible defence barriers in plants include phytohormonal signalling culminating in expression of defence related genes, cross-linking of cell wall proteins and production of ROS and phenolics (Mellersh *et al.* 2002; Singh *et al.* 2013). Cell wall fortifications that strengthen plant mechanical barriers and restrict the developing pathogen, such as lignification, suberinization, deposition of callose and hydroxyproline-rich glycoproteins, can be observed at penetration sites (Schenk *et al.* 2000). Lignin and callose make the plant cell wall more resistant to CWDEs and prevent the diffusion of pathogen-produced toxins (Sattler and Funnell-Harris 2013; Eggert *et al.* 2014). Callose deposition at penetration sites further prevents haustoria formation and penetration (Ellinger *et al.* 2013), whereas suberin is secreted by vascular parenchyma cells forming vessel coating material that blocks colonization of the vascular system by vascular wilts (Robb *et al.* 1991).

To overcome these barriers, fungi deploy a variety of strategies. They secrete enzymes to degrade the physical barriers and detoxify some chemical components of plants towards which some other fungal species may be susceptible (Bisen *et al.* 2015). Fungi also secrete chemical messengers that interfere with the signalling process of the host and thereby overcome the chemical barrier of the plant. For invasion of different plant parts, tissue-specific barriers have to be overcome, for example the lignin barrier to fungi entering through roots is associated with a greater chemical arsenal compared to that associated with leaves (Underwood 2012). Biotrophic fungi like rusts have adopted specialized strategies to conceal their identity by changing the physicochemical properties of proteins normally recognized by plant receptors (Underwood 2012), whereas symbiotic AM fungi not only interfere with host defence signalling (Volpin *et al.* 1995) but may also use other soil microbes like helper bacteria to suppress host defence responses (Lehr *et al.* 2007).

BIOCHEMICAL AND GENETIC ASPECTS OF PLANT–FUNGAL INTERACTIONS

Cell-wall degrading enzymes and effectors

Plant cell walls consist of cellulose, hemicelluloses, pectin and lignin. Consequently the lignocellulose-degrading enzyme system of fungi mainly comprises peroxidases and laccases for the degradation of lignin, and glycoside hydrolases such as cellulases, hemicellulases and pectinases for the degradation of the polysaccharides cellulose, hemicelluloses and pectin, respectively (Kubicek 2013).

A recent genomic analysis of 103 fungi revealed a larger number of carbohydrate active enzymes (CAZymes), such as

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carbohydrate esterases and PL1 pectate lyases, in fungal phytopathogens compared to saprophytic fungi that efficiently degrade dead lignocellulosic material but cannot colonize living plants (Zhao *et al.* 2013). Accordingly, the hemibiotrophic pathogens *Fusarium graminearum* and *M. oryzae* showed an upregulation of genes encoding CWDEs during infection of their plant hosts (Kawahara *et al.* 2012; Zhao *et al.* 2013) and the necrotrophic pathogen *B. cinerea* showed a correlation between virulence and certain CWDEs (i.e. pectinases and xylanases) (Brito, Espino and González 2006; Fernandez-Acero *et al.* 2010). In contrast to fungal necrotrophs and hemibiotrophs, most biotrophs, which depend on living plant tissues for their nutrition, encode fewer plant CWDEs in their genomes and completely lack glycoside hydrolase family 6 (GH6) endoglucanase and cellobiohydrolase activities (Zhao *et al.* 2013).

However, many of the proteins secreted by fungal plant pathogens are small effectors that do not encode catalytic activities. These effectors help the pathogens establish themselves in the host by deregulating plant immune responses and by facilitating host colonization (Rovenich, Boshoven and Thomma 2014). Fungal effectors may be secreted into the plant's extracellular compartment (apoplastic effectors) or may reside in the cytoplasm and accumulate in the biotrophic interfacial complex, a plant membrane-rich structure associated with invasive fungal hyphae (Giraldo *et al.* 2013). Apoplastic effectors are highly diverse and include protease inhibitors that target host proteases, proteins protecting fungal cell walls against plant chitinases or against detection by the plant, and small molecules that minimize ROS levels. Cytoplasmic effectors are recognized by host plant resistance (R) proteins thereby triggering the HR, a reaction characterized by rapid cell death in the local infection region with the aim of blocking pathogen growth and spread (Giraldo and Valent 2013).

The genome of the biotrophic maize pathogen *Ustilago maydis* predicts encoding of ~550 secreted proteins, of which many are virulence effectors that are upregulated during host colonization (Djamei and Kahmann 2012). Recent studies suggested that *U. maydis* is able to sense and adapt to the host plant and secrete different specific effector cocktails, i.e. a first set of 'core' effectors for suppressing plant defence during the penetration stage followed by a second set of cell-type and organ-specific effectors for infecting different plant tissues (Skibbe *et al.* 2010; Djamei and Kahmann 2012). Many effector-encoding genes are arranged in clusters in the *U. maydis* genome and analyses of the largest effector gene cluster, cluster 19A, revealed that its 23 genes are differentially induced when different plant organs are colonized. Deletion of the complete cluster 19A abolished tumor formation in maize plants, whereas strains deleted for individual effector genes only showed minor reduction in virulence (Kamper *et al.* 2006; Brefort *et al.* 2014).

Most known effectors are proteins but there are also examples of metabolites. Metabolic effectors include host-selective toxins produced by *Cochliobolus, Alternaria* and some *Pyrenophora* species (Walton and Panaccione 1993; Martinez, Oesch and Ciuffetti 2004; Tsuge *et al.* 2013), fuminosin mycotoxins in *Fusarium verticillioides* (Arias *et al.* 2012) and pyrichalasin H and Ace1 (Avirulence conferring enzyme 1) of *M. oryzae*. The *ACE1* gene encodes a cytoplasmic hybrid protein with both a polyketide synthase and a non-ribosomal peptide synthetase domain (PKS-NRPS) and is specifically expressed during penetration. Ace1 is supposed to synthesize the actual effector, a still unknown secondary metabolite, which is secreted and recognized by rice resistance gene *Pi33* (Collemare *et al.* 2008; Yi and Valent 2013). Recent studies added non-coding small RNAs that are delivered into host cells to suppress plant immunity for a subset of pathogen effectors. In *B. cinerea*, some small RNAs were shown to silence *Arabidopsis* and tomato genes involved in immunity by hijacking the host RNA interference machinery (Weiberg *et al.* 2013).

While pathogens are detrimental to the host plant, the mycorrhizal interaction is a mutualistic relationship in which both partners benefit. Nevertheless, plant tissues must still be disrupted by the fungal partner during root colonization. Interestingly, in contrast to saprophytes and nectrotrophic plant pathogens, the genomes of mycorrhizal fungi such as the ectomycorrhizal (ECM) fungus *Laccaria bicolor* and the AM fungus *Rhizophagus irregularis* (*Glomus intraradices*) show an extreme reduction in enzymes for plant cell wall degradation and toxin synthesis (Martin *et al.* 2008; Tisserant *et al.* 2013; Zhao *et al.* 2013; Kohler *et al.* 2015). A key factor in the symbiotic interaction between mycorrhiza fungi and plants is their ability to mobilize organic forms of nitrogen and phosphorus for the plant host in exchange for photosynthetically derived sugars. This is also reflected in the transcriptomes of the ECM fungi *L. bicolor* and *Tuber melanosporum*, which express a core set of genes related to nutrient cycling during symbiosis (Martin *et al.* 2010). In addition, gene families encoding small secreted proteins are expanded and are among the most expressed during colonization of the plant host (Martin *et al.* 2008). One of those effectors, *L. bicolor* MiSSP7 (mycorrhiza-induced small secreted protein 7), is necessary for the establishment of symbiosis with host trees and the respective gene has the highest upregulation during root colonization. MiSSP7, secreted by the fungus upon reception of plant root-derived signals, moves to the nucleus of plant cells and modulates the expression of host genes associated with oxidative stress, defence, root architecture and cell wall modification (Plett *et al.* 2011). MiSSP7 is further able to counter the negative impacts of jasmonic acid (JA), a plant hormone involved in defence signalling, on fungal colonization of host tissues by repressing JA-induced gene transcription (Plett *et al.* 2014).

Similar to pathogens and ECM fungi, effectors are also used by AM fungi to bypass the plant defence system. *G. intraradices* secretes a highly expressed effector, SP7, to help establish symbiosis by dampening the plant immune response. SP7 enters host plant cells, moves into the nucleus and there interacts with the PR transcription factor ERF 19 (Ethylene Response Factor 19) to repress plant defence signalling (Kloppholz, Kuhn and Requena 2011). Similar findings emerged from recent comprehensive studies of eight symbiotic species in which the gene expression in free-living mycelia and established mycorrhiza was compared. A large proportion of the up and downregulated genes in mycorrhizal roots turned out to be lineage-specific 'orphans' missing a functional annotation but encoding short proteins with predicted secretion signals, i.e. putative effectors (Kohler *et al.* 2015; Venturini and Delledonne 2015).

Biochemical plant defences against fungal invasion

Plant defence responses to pathogen attack frequently result in a HR, the local accumulation of phytoalexins, and an enhancement of several enzyme activities (including β-1, 3- glucanase, chitinase, peroxidase, lipoxygenase and catalase) (Lebeda *et al.* 2001). Cell death during HR is thought to be dependent on the balanced production of nitric oxide (NO) and ROS (Delledonne *et al.* 2001), active signalling molecules in disease resistance and plant–necrotrophic pathogen interactions (Sarkar *et al.* 2014). These defence reactions aim to isolate the invading fungus in a location lacking a sufficient supply of nutrients required for

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survival and hence prevent spreading of the pathogen (Bolwell *et al.* 2002).

The rapid, transient production of huge amounts of ROS, the so-called oxidative burst, induces a large number of PR proteins. PR proteins are divided into 17 different families (PR1 to PR17) based on their primary structure, serological relationship and biological activities (Christensen *et al.* 2002; Sels *et al.* 2008). While to some a definite function such as β-1, 3-glucanase activity (PR-2), osmotin (PR5), protease inhibitor (PR6), endoproteinase (PR-7), peroxidase (PR-9), chitinases activity (PR-3, PR-8, PR-11), defensin (PR-12), thionin (PR-13), lipid-transer protein (PR-14), oxalate oxidase (PR-15 and PR-16) could be assigned, this is less clear for others (Van Loon and Van Strien 1999; Ghosh 2006; Kim *et al.* 2009; Laluk and Mengiste 2011; Rather *et al.* 2015). A ribonuclease-like function has been suggested for PR-10 (Lurie *et al.* 1997). Some of PR4 proteins show chitinase, RNase, and/or DNase activities as well as antifungal properties, whereas others exhibit RNase and antifungal activities (Bertini *et al.* 2012; Bai *et al.* 2013) or RNase and DNase, but no chitinase activities (Guevara-Morato *et al.* 2010). The transcriptional regulation of PR protein-encoding genes is also heterogeneous, with low constitutive or undetectable expression under normal physiological conditions and induction upon injury, pathogen attack and environmental stress (Sabater-Jara *et al.* 2010).

The accumulation of PR proteins at the infection site is usually associated with systemic acquired resistance (SAR), a long-lasting, broad-spectrum whole plant immunity that protects distal undamaged tissues against subsequent invasion by pathogens (Durrant and Dong 2004). In induced resistance processes, biochemical pathways depending on salicylic acid (SA) or jasmonic acid (JA) and ethylene (ET) act in parallel (Sticher, Mauch-Mani and Metraux 1997). These plant hormones function as signalling molecules triggering the synthesis of transcription factors in the plant cell. The JA pathway induces defensin synthesis, leads to induction of osmotin, proline-rich glycoproteins, synthesis of phytoalexins (Wasternack 1997) and proteinase inhibitors (Stiche, Mauch-Mani and Metraux 1997). SA and JA are each involved in controlling basal resistance against different pathogens. Some studies have shown that the hormone signal may depend on the type of pathogen, with SA preferentially regulating the defence responses against biotrophic pathogens and JA and ET regulating the response to fungal necrotrophs (Mengiste 2012) (Fig. 4). Interestingly, beneficial microorganisms such as rhizobacteria and symbiotic fungi can also induce JA and ET-mediated induced systemic resistance (ISR), in this case associated with priming for enhanced defence rather than direct defence activation (Pieterse *et al.* 2009).

The gene for gene relationship

When studying the genetics of flax and the rust pathogen *Melampsora lini*, Harold Henry Flor developed the gene for gene relationship (Flor 1942) that was later renamed ETI. In this model, a one-to-one relationship between an avirulence (*avr*) gene in the pathogen (leading to expression of a suite of effector proteins) and a cognate resistance (R) gene in the plant (leading to expression of resistance proteins) is envisioned to trigger a signal transduction cascade which affects race-specific resistance in the plant (Flor 1971). A classic example is the *Cf9* and *avr9* genes described for the tomato-*Cladiosporum fulvum* pathosystem in which the fungal race-specific *avr9* gene product induces HR on tomato plants carrying the complimentary resistance gene *Cf9*. In contrast, fungal races virulent on *Cf9* genotypes of tomato do not produce the effector as they lack the *avr9* gene. By introducing the *avr9* gene into a *C. fulvum* race virulent to *Cf9* tomato genotypes, van den Ackerveken, van Kan and de Wit (1992) demonstrated that *avr9* is a true *avr* gene obeying the gene-for-gene hypothesis. Subsequently, a number of *Cf* and *avr* genes were isolated and evidence emerged that each effector has a particular role, such as the binding and modification of host proteins or a passive role masking the pathogen (Wulff *et al.* 2001).

SIGNALLING IN PLANT–FUNGAL INTERACTIONS —PLANT RECEPTORS THAT ORCHESTRATE DEFENCE AND SYMBIOSIS

For a plant to ensure appropriate cellular responses, it must distinguish between fungal friend and foe on multiple levels. A first layer in the perception of microbes relies on the sensing of microbial-associated molecular patterns (MAMPs) or pathogen-associated molecular patterns (PAMPs) through plant cell surface-localized receptor proteins called pattern recognition receptors (PRR; De Wit 2007; Dodds and Rathjen 2010) (Fig. 5). The resulting PAMP-triggered immunity (PTI) allows protection to non-adapted pathogens and limited basal immunity to host-adapted microbes. As described above, by secreting effectors, host-adapted microbes are able to suppress PTI but can be counteracted by ETI. However, evidence has accumulated that a distinction between PAMPs/MAMPs and effectors, and hence PTI and ETI, is not always clear-cut. Both plasma membrane-resident receptors as well as cytoplasmic resistance proteins resembling Nod-like receptors (NLR) are capable of mediating recognition (Thomma, Nurnberger and Joosten 2011; Bohm *et al.* 2014) (Fig. 5).

The perception of fungal interactors by pattern recognition receptors

Plants recognize MAMPs/PAMPs by small epitopes that provide ligands for plasma membrane-localized receptors. These PRRs are highly specific and sensitive and allow plant cells to perceive a specific molecular pattern at subnanomolar concentrations (Boller and He, 2009). Plant PRRs comprise receptor kinases and receptor-like proteins (RLPs). While the former consist of an extracellular domain, a membrane-spanning region, and an intracellular serine/threonine or tyrosine kinase domain, RLPs lack the intracellular signalling domain (Han, Sun and Chai. 2014). Of the >600 receptor-like kinases (RLKs) and >50 RLPs in the genome of the model plant *Arabidopsis thaliana*, the FLAGELLIN-SENSING 2 (FLS2) PRR is the best characterized. FLS2 is a multi-domain transmembrane leucine-rich repeat RLK that recognizes the highly conserved 22-amino acid flg22 epitope of eubacterial flagellin (Chinchilla *et al.* 2006). *Arabidopsis* can also recognize fungal PAMPs such as ethylene-inducing xylanase (EIX) via orthologues of LeEIX1/2. The transmembrane RLPs LeEIX1 and LeEIX2 have first been identified in tomato where they mediate perception of the cell wall-derived ethylene-inducing xylanase (Eix) from *Trichoderma* fungi (Fritz-Laylin *et al.* 2005; Kaku *et al.* 2006). Another defined PAMP-receptor pair is Ave1-Ve1. Ave1 is a conserved protein found in various fungal species that is perceived by the tomato leucine-rich repeat RLP Ve1 (de Jonge *et al.* 2012). Further examples of unraveled PRRs are the barley kinase RPG1, which *in vitro* interacts with two proteins from *Puccinia graminis* f.sp. *tritici* to confer resistance to stem rust, the *Arabidopsis* leucine-rich repeat RLP RBPG1, which recognizes fungal endopolygalacturonases, and the *Arabidopsis* RLP30

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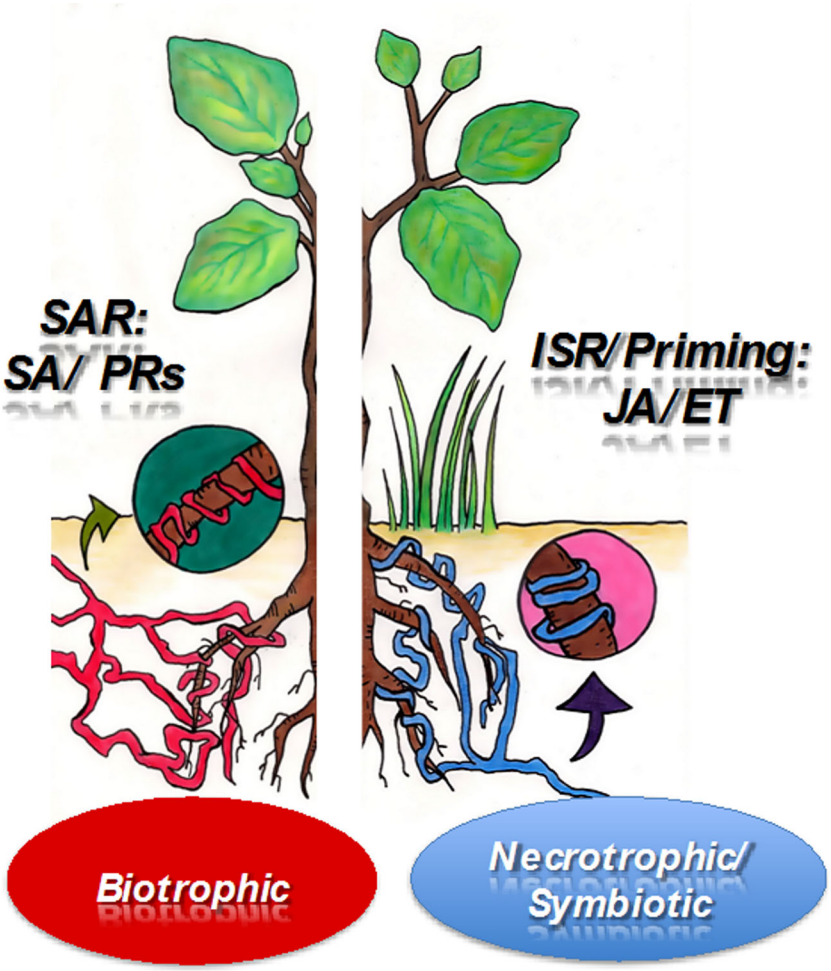

Figure 4. Schematic representation of induced immune responses in plants. SAR is a long-lasting and broad-spectrum induced disease resistance and evidence accumulated that the SA-induced pathway is primarily triggered by fungal biotrophic pathogens while the pathway induced by necrotrophic and symbiotic fungi relies on JA and ET as signalling molecules and is designated as ISR (Induced Systemic Resistance) (adopted from Pieterse *et al.* 2009; redrafted with permission).

(Receptor-like protein 30) receptor, whose ligand is the proteinaceous elicitor *sclerotinia* culture filtrate elicitor1 (SCFE1) produced by *Sclerotinia sclerotiorum* (Monaghan and Zipfel 2012; Wang *et al.* 2014b). Rice and *Arabidopsis* perceive fungal chitin through the lysine motif (LysM) RLK CERK1 (chitin elicitor receptor kinase 1) which induces CERK1 dimerization, essential for the activation of downstream signalling (Miya *et al.* 2007; Wan *et al.* 2008).

Ligand-induced signalling through PRRs, especially RLPs that lack an intracellular signalling domain, may require additional partners to activate respective immune responses. BAK1 (BRI1-associated kinase 1) forms ligand-induced heteromers with several receptor kinases in *Arabidopsis* and is among the major regulators of bacterial FLS2-mediated signalling. BAK1 is also important for resistance to obligate biotrophic and hemibiotrophic fungal pathogens such as Ve1-mediated resistance of tomato to *Verticillium* wilt and has been suggested to regulate Eix1 in response to ethylene-induced xylanase (Monaghan and Zipfel 2012; Han *et al.* 2014). The receptor-like cytoplasmic kinase BIK1 is a component of the FLS2–BAK1 immune receptor complex where it is directly phosphorylated by BAK1. Upon phosphorylation, BIK1 dissociates from the receptor complex to activate downstream signalling and plant immunity (Fig. 5). Further, BIK1 is essential for mediating *Arabidopsis* resistance to necrotrophic pathogens and is induced during *B. cinerea* infection (Wang *et al.* 2014a). BAK1 and BIK1 may also associate with other PRRs such as CERK1 to control PAMP responses; however, knowledge on how different PRRs may converge on those central regulators remains fragmentary and more details on the underlying molecular mechanisms are reviewed elsewhere (Zipfel 2008; Monaghan and Zipfel 2012; Bohm *et al.* 2014; Wang *et al.* 2014a).

The SYM pathway

The recognition of AM fungi by the host plant during mycorrhiza formation is mediated by the common symbiosis (SYM) pathway partly shared with *Rhizobium*-legume symbiosis (Bonfante and Genre 2010). The symbiosis receptor-like kinase (SYMRK) is a central component of this pathway as it perceives rhizobial Nod factors as well as fungal AM signals and transduces these to the cytoplasm by phosphorylating respective substrates. SYMRK has been found to physically interact with various proteins such as the small basic intrinsic protein 2 (SIP2) mitogen-activated protein kinase kinase (MAPKK) and the E3 ubiquitin ligase SEVEN IN ABSENTIA4 (SINA4), which modulates symbiosis signalling by negatively regulating SYMRK abundance at the plasma membrane (Bapaume and Reinhardt 2012; Tax and Kemmerling 2012). Further evidence suggests that SYMRK, together with

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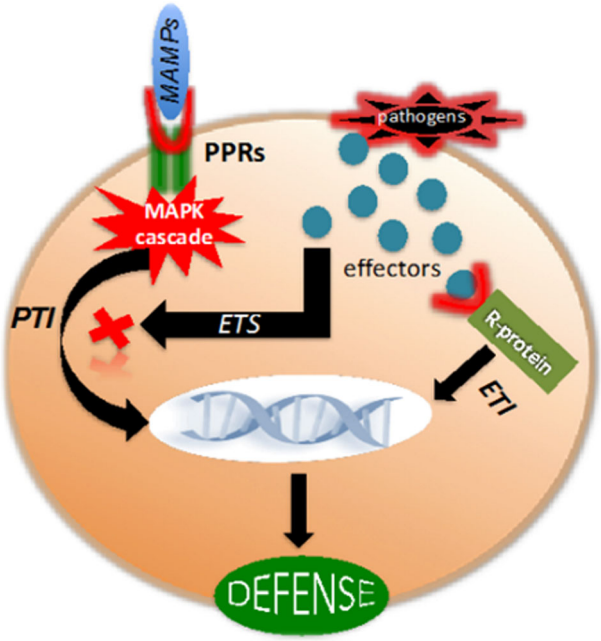

Figure 5. Signalling in plant–fungal pathogen interaction. The first defence line of plants is based on receptor proteins located in the plasma membrane. PRRs recognize conserved microbial structures (MAMPs/PAMPs) which lead to activation of PTI via calcium signalling and MAPK cascades. Pathogens interfere with PTI through effectors, inducing susceptibility known as effector-triggered susceptibility (ETS) by blocking the PTI response. On the other hand, effector recognition by plant R proteins triggers an immune reaction designated as effector-triggered immunity (ETI) (adopted from Kazan and Lyons 2014; redrafted with permission).

interactors such as SINA4, resides in membrane microdomains that serve as signalling platforms (Bapaume and Reinhardt 2012).

Interestingly, recent studies with rice knockout mutants of CERK1 revealed a bifunctional nature of CERK1 in both defence and symbiosis, as mutants were impaired not only for chitin-triggered immune responses against fungal and bacterial pathogens but also for AM symbiosis (Miyata *et al.* 2014). CERK1 was suggested to be involved in the perception of undecorated chitin tetrasaccharides and pentasaccharides, fungal symbiotic signals of AM fungi that elicit Ca^{2+} spiking (Delaunois *et al.* 2014; Zhang *et al.* 2015). The role of CERK1 as a molecular switch in rice plants that activates either defence or symbiotic responses, depending on the infecting microbe, further indicates a close evolutionary relationship between these processes and evidences different receptor partners that enable CERK1 to recognize variable ligands (Miyata *et al.* 2014; Zhang *et al.* 2015).

Besides receptors at the plasma membrane, proteins localized to the endoplasmic reticulum and the nuclear envelope are essential for symbiotic signalling including Ca^{2+} channels and a calcium ATPase involved in Ca^{2+} spiking. The Ca^{2+} signal is suggested to be decoded by a nuclear-localized calcium- and calmodulin-dependent protein kinase (CCaMK) which then phosphorylates respective transcription factor targets to trigger the expression of symbiosis-related genes (Bapaume and Reinhardt 2012; Singh and Parniske, 2012). Ca^{2+} signalling seems to be a common way for plants to open a dialogue with their fungal interactors as transiently elevated intracellular Ca^{2+} levels are also observed during pathogen attack and during interaction with the beneficial root-colonizing biocontrol fungus *T. atroviride* (Navazio *et al.* 2007).

Escape from plant defence

To escape detection and plant defence, plant-associated microbes may interfere with plant signalling processes by secreting effectors that physically interact and inhibit the kinase activity of PRRs or BAK1. Such an effect has been shown for the AvrPro and AvrPtoB effectors from the bacterium *Pseudomonas syringae* (Boller and He 2009) and accordingly, fungi manipulate receptor function. For example, fungi employ LysM effectors that bind soluble chitin fragments and sequester them from detection by plant chitin receptors as demonstrated for pathogens *C. fulvum* and *M. oryzae*. Symbiotic microbes use effectors as well. An effector from the AM fungus *G. intraradices* is taken up by the host and functions through modification of defence-related gene expression in the nucleus (Bapaume and Reinhardt 2012).

SIGNALLING IN PLANT–FUNGAL INTERACTIONS—SIGNALS AND PATHWAYS

The recognition of appropriate plant hosts is among the most critical steps in the interaction of fungi with plants and often begins before direct contact between the partners. Fungi sense and respond to chemical and physical cues through differentiation, movement to an appropriate infection site, and/or formation of invasion-related structures (Hoch and Staples 1991; Kumamoto 2008; Bonfante and Genre 2010).

The following section summarizes the current knowledge on the signals as well as signalling pathways involved in plant–fungus interactions with a focus on the fungal interaction partner. However, the facts that many more interaction-relevant genes from plants than from fungi have been examined to date and that our insights into pathogenic compared to mutualistic and saprotrophic relationships is more advanced, are reflected in this section.

Diffusible chemicals from root exudates

A variety of compounds contributing to plant–fungal communication is released by plant roots into their surroundings, i.e. the rhizosphere. These include low molecular weight substances such as ions, free oxygen, amino acids, organic acids, sugars, phenolics and other secondary metabolites as well as high molecular weight exudates such as mucilage (polysaccharides) and proteins (Bais *et al.* 2006). The rhizosphere hence attracts both beneficial as well as detrimental microbes by representing a carbon-rich environment; on the other hand, volatiles emitted from plant roots act as belowground defence substances that exert antimicrobial and antiherbivore activity (Baetz and Martinoia 2014).

Root exudates can be produced both constitutively (so-called phytoanticipins) as well as in response to stimuli such as pathogen attack (so-called phytoalexins) (Baetz and Martinoia 2014). In barley for example, the exudation of phenylpropanoids, plant phenolics with antifungal activity, is specifically induced upon attack by the soil-borne pathogen *F. graminearum* (Boddu *et al.* 2006). Similarly, the production of different volatile antimicrobials, mainly terpenes, which contribute to the plant's induced systemic resistance, is triggered in barley roots during attack by *Cochliobolus sativus* and *Fusarium culmorum* (Fiers *et al.* 2013). Fungal pathogens release volatile substances as well, such as the sesquiterpene-derived trichotecene toxins from *F. culmorum* that are potent inhibitors of protein synthesis and inhibit the activation of plant defence response genes prior to any physical contact with the pathogen (Fiers *et al.* 2013).

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Besides contributing to chemical warfare between plants and their pathogens, root exudates are equally important as signalling molecules in the communication of plants with symbiotic microbes. Root colonization by AM fungi is initiated upon perception of root exudates by the presymbiotic fungal mycelium. The responsible compounds have been identified as strigolactones, carotenoid-derived plant hormones that are present in the exudates of plants from diverse taxa and hence can be regarded as general essential signalling compounds for the establishment of AM symbiosis (Akiyama and Hayashi 2006; Steinkellner *et al.* 2007; Bonfante and Genre 2010). In AM fungi, strigolactones act as hyphal branching factors thereby stimulating root colonization (Akiyama, Matsuzaki and Hayashi 2005); however, they are only needed in the presymbiotic stage and not for intracellular fungal development (Koltai 2014). Analysis of the effect of the strigolactone analogue GR24 on fungi other than AM such as ECM, biocontrol fungi of the genus *Trichoderma*, and the pathogens *B. cinerea* and *Cladosporium* sp. revealed unaltered branching patterns and suggests strigolactones as specific signals for AM (Steinkellner *et al.* 2007).

Flavonoids represent another group of metabolites that are found in the root exudates of various plants and that contribute to signalling in plant–fungus interactions. While various flavonoids stimulate hyphal growth of AM fungi in the presymbiotic stage (Steinkellner *et al.* 2007), both positive and negative effects of flavonoids on fungal phytopathogens have been reported. In a range of root pathogens, spore germination and hyphal growth is inhibited in the presence of flavonoids (Hassan and Mathesius 2012), whereas flavonoids from the exudates of pea and bean have a stimulatory activity on their associated pathogen, *Fusarium solani formae specialis*. Specific inhibitors demonstrated that cAMP-dependent protein kinase (PKA) signalling is involved in this flavonoid-stimulated spore germination (Ruan, Kotraiah and Straney 1995) and confirmed that root-excreted flavonoids have the potential to initiate interactions with pathogens that have developed an ability to cope with their inhibitory action. In the case of *F. solani* f.sp. *pisi*, the isoflavonoid pisatin induces *pda1* expression which encodes a pisatin demethylase that detoxifies pisatin and so is a virulence factor of this fungus (Khan *et al.* 2003). Similarly, the germination of spores is stimulated by root exudates in *F. oxysporum*, with the fungus showing chemotropic growth towards tomato roots (Rodriguez-Galvez and Mendgen 1995; Turrà *et al.* 2014). Recent studies revealed that class III peroxidases (POX) secreted by tomato roots function in chemotropic sensing by *F. oxysporum* via a pheromone receptor homologue and mitogen-activated protein kinase (MAPK) signalling (Turrà *et al.* 2014).

Root exudates are rich in carbohydrates such as the disaccharide sucrose, an important signalling molecule in various processes such as the activation of plant immune responses. Sucrose degradation by plant cells themselves yields a carbon source for beneficial microbes during plant–microbe associations (Koch 2004). While most mycorrhizal fungi rely on the monosaccharides provided, as they lack sucrolytic enzymes, the sucrose-hydrolyzing enzyme invertase is expressed during infection in several fungal plant pathogens (Voegele *et al.* 2006). Similarly, the rhizosphere-competent biocontrol fungi *T. virens* and *Metarhizium robertsii* employ invertase to metabolize sucrose and in the case of *T. virens*, invertase activity is crucial for the control of root colonization (Vargas, Crutcher and Kenerley 2011; Liao *et al.* 2013).

The fact that the precontact communication between fungi and roots not only involves plant- but also fungus-derived signals is well exemplified by the presymbiotic phase of mycorrhiza formation between AM fungi and host plants. The nature of diffusible AM fungal signalling molecules, the 'Myc' factors, which induce molecular responses in host roots required for early mycorrhization, has only recently been revealed. They are a mixture of diffusible sulphated and non-sulphated lipochitooligosaccharides (LCOs), similar to the Nod factors known from rhizobia bacteria, and are able to stimulate AM formation and root branching (Maillet *et al.* 2011).

Oxylipins

The role of oxylipins, a group of oxygenated lipid secondary metabolites, in cross-kingdom signalling has gained much attention during recent years. Both plants and fungi are affected by endogenous oxylipin-mediated signals as well as those produced by the interacting partner (Borrego and Kolomiets 2012). Plant- and fungus-derived oxylipins are structurally similar and hence it is not surprising that they can partly substitute for one another. For example, fungal oxylipins influence processes in infected plant tissues by mimicking endogenous signalling molecules (Brodhagen *et al.* 2008) and can manipulate host lipid metabolism and alter plant defence responses (Tsitsigiannis and Keller 2007; Brodhagen *et al.* 2008). On the other side, plant-derived oxylipins (e.g. jasmonates, JA discussed earlier) have direct effects on the reproduction and secondary metabolite production in fungi (Burow *et al.* 1997) or by influencing the survival of fungal overwintering structures (Calvo *et al.* 1999). Oxylipins are also implicated in promoting disease progression and pathogenicity by inducing JA-responsive genes (Thatcher, Manners and Kazan 2009). Although JA-mediated defence responses are often involved in resistance against necrotrophic pathogens, jasmonate signalling mediated by the JA perception protein coronatine insensitive 1(COI1) in *A. thaliana* has been shown to be responsible for susceptibility to wilt disease caused by *F. oxysporum*. Such evidence suggests that the fungus can hijack defence-independent aspects of the JA-signalling pathway to promote disease (Thatcher, Manners and Kazan 2009).

Recognition of oxylipins has long been speculated to involve G protein-coupled receptors (GPCRs). GPCR activation frequently is associated with cAMP signalling and recently, plant oxylipins were found to stimulate a burst in cAMP in *Aspergillus nidulans* which was lost upon deletion of the *gprD* GPCR-encoding gene (Affeldt, Brodhagen and Keller 2012). In the soil-borne plant pathogen, *Aspergillus flavus* grown at different densities, endogenous oxylipins mediate a developmental shift affecting spore and slerotia production and the biosynthesis of the mycotoxin aflatoxin, processes found to be regulated by the *A. flavus* GprD homologues, GprC and GprD. Based on the assumption that endogenous oxylipins are likely similar to exogenous, plant-derived oxylipins, the authors speculated that GprC and GprD could also be important for fungal–host interactions (Affeldt, Brodhagen and Keller 2012).

Reactive oxygen species (ROS)

Oxylipin-mediated signalling during plant–fungus interaction is tightly connected to ROS-stimulated cell signalling. ROS act as signalling molecules mediating defence gene expression by redox control of transcription factors or by interacting with other signalling components such as phosphorylation cascades. Further, lipid derivatives such as oxylipins can be generated by ROS action through non-enzymatic oxygenation (Reverberi *et al.* 2012). ROS production is not limited to the plant since invading fungi produce superoxide. Fungal nicotinamide adenine

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dinucleotide phosphate (NADPH) oxidase enzymes mediate ROS production, and ROS accumulating at the plant–fungus interface act as signals for triggering attack and counterattack responses. In *M. grisea*, a local oxidative burst is elicited during plant infection by the action of Nox1 and Nox2 NADPH oxidases associated with appressorium formation (Egan *et al.* 2007). On the other hand, fungal pathogens have to overcome the plant's defensive oxidative burst, for example by employing ROS scavenging enzymes and modifying ROS accumulation in the host. In *M. oryzae*, the pathogenicity factor DES1 (defence suppressor 1) is essential for scavenging extracellular ROS within host cells and regulates counterdefence responses (Chi *et al.* 2009). A basic leucine zipper (bZIP) transcription factor, yes-associated protein (Yap1), is used by *U. maydis* to function as a redox sensor which prevents the accumulation of hydrogen peroxide produced by plant NADPH oxidases and allows the fungus to counteract early host defences (Molina and Kahmann 2007). Recent evidence suggests that secondary metabolites contribute to the fungal antioxidant defence in response to elevated ROS levels. Several transcription factors associated with the stress-activated protein kinase (SAPK)/MAPK pathway were found to coordinate the expression of genes, including those for antioxidant and secondary metabolism, thus controlling metabolic processes with cellular stress response (Hong, Roze and Linz 2013).

ROS are further involved in plant–symbiont interactions such as the association of perennial ryegrass with the endophyte *Epichloe festucae*. ROS produced by the *E. festucae* NoxA NADPH oxidase have a critical role in regulating hyphal growth within the plant host and in maintaining the mutualistic interaction. Strikingly, disruption of components of the NADPH oxidase complex, including NoxA, NoxR and rho-related C3 botulinum toxin substrate (RacA), lead to a reversal of the mutualistic interaction to become antagonistic, with fungal mutants showing unrestricted growth in planta (Tanaka *et al.* 2006). Increased ROS levels and alterations in the pattern of antioxidative enzymes in mycorrhizal roots are observed in the interaction of plant hosts with AM fungi. The degradation of ROS involved in plant signalling cascades by catalase in AM was suggested to represent a possible mechanism for avoiding the activation of defence response genes (Garcia-Garrido and Ocampo 2002). Similarly, ROS are involved in the symbiotic association of ECM fungi with their host plants. In the *Castanea sativa-Pisolithus tinctorius* system, oxidative bursts in the plant are induced during early contact with the fungus, although massive root colonization by the fungus does not induce cell death at the infection site. Circumvention of the common host defence response by the fungus was attributed to temporal activation of catalase by which host cell damage during mycorrhiza establishment could be prevented (Baptista *et al.* 2007).

Physical, biochemical and chemical plant surface signals

In the foliar rice pathogen *M. oryzae,* appressorium formation is triggered in response to the hydrophobicity and hardness of the host surface and plant-derived signals such as cutin monomers and leaf waxes ((Liu *et al.* 2011; Perez-Nadales *et al.* 2014). A *M. oryzae* cutinase mutant shows reduced pathogenicity, and this defect can be rescued by supplementation with cutin monomers (Skamnioti and Gurr 2007). Similar signals are used by other plant pathogens including anthracnose fungi of the genus *Colletotrichum* and the corn smut fungus *U. maydis* (Kim *et al.* 1998; Lanver *et al.* 2014). In the latter, cutin monomers and a hydrophobic surface trigger the production of secreted CWDEs and virulence-related effectors which depend on two plant surface sensors, the tetraspanin protein synthetic high osmolarity sensitive 1 (Sho1) and the signalling mucin multicopy suppression of a budding defect2 (Msb2) (Lanver *et al.* 2014). Similarly, Msb2 and Sho1 in *M. oryzae* are involved in recognizing different physical and chemical signals present on rice leaves, thereby triggering appressorium formation by acting as upstream sensors of the Pmk1 MAPK pathway. While Msb2 is crucial for sensing surface hydrophobicity and cutin molecules, Sho1 is more important for recognizing wax components (Liu *et al.* 2011). Msb2 also plays an important role in the root-infecting, non-appressorium-forming *F. oxysporum,* where it regulates invasive growth and plant infection by phosphorylating the Fmk1 MAPK in response to surface cues (Perez-Nadales and Di Pietro 2011).

MAPKs are organized as cascades consisting of three interlinked protein kinases, MAPK kinase kinase (MAP3K), MAPK kinase (MAP2K) and MAPK, that are sequentially activated by phosphorylation (Widmann *et al.* 1999). In plant pathogenic fungi, MAPKs regulate the mechanical and enzymatic penetration of the host plant, while the plant uses MAPK signalling for activation of immunity (Fig. 5). Hence, the MAPK cascades of both partners contribute to a highly interconnected molecular dialogue between plant and fungus (Hamel *et al.* 2012). In all plant pathogenic fungi studied so far, including appressorium- and non-appressorium-forming pathogens, necrotrophs and biotrophs, the orthologue of the *Saccharomyces cerevisiae* mating pathway Fus3/Kss1 MAPK is required for pathogenicity (Rispail *et al.* 2009). In *M. oryzae,* Pmk1 (pathogenicity MAPK) stimulates appressorium formation and is further required for infectious growth of the fungus inside the plant (Xu and Hamer 1996). In support of an essential role of the Pmk1 MAPK cascade in pathogenicity, mutants lacking the upstream components MAP3K Mst11 or the MAP2K Mst7 or the Ste12 transcription factor, a downstream target of Pmk1, are non-pathogenic in rice (Park *et al.* 2002; Zhao *et al.* 2005). While the Pmk1 MAPK cascade regulates late stages of appressorium formation, penetration and infectious growth, the cAMP-PKA signalling pathway controls surface recognition in *M. oryzae* (Zhao and Xu 2007; Li, Zhou and Xu 2012). The membrane protein Pth11, a GPCR that is involved in recognizing surface hydrophobicity, has been suggested to function upstream of the cAMP-PKA pathway. *PTH11* gene deletion mutants are reduced in virulence and appressorium formation on hydrophobic surfaces but still form appressoria in the presence of exogenous cAMP (DeZwaan *et al.* 1999). Cross-talk between cAMP and Pmk1 signalling is evidenced by the overlapping roles of the Pth11 receptor and the signalling mucin Msb2 (which acts upstream of the Pmk1 MAPK cascade) in sensing surface hydrophobicity and regulation of appressorium formation (Xu and Hamer 1996; Liu *et al.* 2011).

In *U. maydis* virulence-related processes such as filamentation and appressorium formation in response to cutin monomers and surface hydrophobicity are mediated by the Kpp2 MAPK (Mendoza-Mendoza *et al.* 2009) and a complex cross-talk of MAPK signalling with the cAMP pathway (Bolker 2001). The two pathways appear to be connected at the Gpa3 G protein subunit and the perforin 1 (Prf1) transcription factor. Prf1, which regulates the pheromone-induced expression of the *a* and *b* mating type genes, carries sequence motifs specific for PKA- and MAPK-dependent phosphorylation, which are essential for its function (Bolker 2001).

Evidence for the involvement of cutin monomers in symbiotic plant–fungal interactions came from genetic screens for plant mutants that are deficient in mycorrhiza formation. These

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screens identified two genes, protein farnesyltransferase *(RAM)1* and *RAM2: RAM2* encodes a glycerol-3-phosphate acyl transferase involved in the production of cutin monomers whose induction upon mycorrhizal colonization is regulated by the transcription factor RAM1 (Gobbato *et al.* 2012; Wang *et al.* 2012). Both *RAM1* and *RAM2* specifically affect hyphopodia and arbuscule formation, whereas they are not involved in the induction of the earlier strigolactone-mediated branching response in AM fungi (Murray *et al.* 2013). Interestingly, RAM2-deficient plants are not only unable to be colonized by AM fungi but are also defective in colonization by the pathogenic oomycete *Phytophtora palmivora* (Wang *et al.* 2012). The fact that roots do not contain a cuticle and hence in general lack cutin suggest that cutin signals act as specific cues between roots and AM fungi that have been hijacked by pathogenic oomycetes to facilitate plant invasion (Geurts and Vleeshouwers 2012).

Infection of host plants via natural openings is common among rust fungi, such as *Uromyces* and *Puccinia* species. These fungi form appressoria exactly over the guard cells of stomata. Studies on the bean rust *Uromyces appendiculatus* revealed that fungal germ tubes receive topographical signals from the leaf surface upon contact sensing (thigmotropism) resulting in oriented growth towards stomata. On an inert substrate surface, appressorium formation could be triggered by a simple ridge with a specific height showing that the orientation and differentiation events are mediated entirely by the topography of the plant surface and not by any chemical signals (Hoch *et al.* 1987; Brand and Gow 2009). Further, studies showed that mechanosensitive ion channels, which open in response to membrane-affecting physical stimuli, respond to topographical information for thigmotropic growth and appressorium formation in *U. appendiculatus* by transducing the membrane stress induced by the leaf topography into an influx of ions such as Ca^{2+} (Zhou *et al.* 1991).

Ca^{2+} signalling also triggers appressorium formation in *Colletotrichum lagenarium* and *C. gloeosporioides* where hard-surface contact primes the conidia to germinate and differentiate (Kim *et al.* 1998; Sakaguchi *et al.* 2008). Similarly, proteins involved in Ca^{2+} signalling are required for appressorium formation, turgor generation and host penetration in *M. oryzae* (Liu and Kolattukudy 1999), making the rice blast fungus a well-explored model for the interplay of various signalling pathways in pathogenic development.

Signalling pathways in symbiotic fungi

In contrast to fungal pathogens, where virulence-associated signal transduction pathways are well characterized, only few studies are available from symbiotic fungi. In the ECM fungus *Tuber borchii*, the conserved orthologue of the *S. cerevisiae* Fus3/Kss1 MAPK becomes activated during interaction of the fungus with its host plant *Tilia americana (*Menotta *et al.* 2006). Further support for a role of MAPK signalling in mycorrhiza formation includes: (i) the identification of a Ste20-like serine/threonine kinase, a MAPK kinase kinase kinase (MAP4K) involved in the mating pathway of *S. cerevisiae*, among the genes being activated in the fungus *Hydnangium* sp. during the presymbiotic phase of the ectomycorrhizal association with *Eucalyptus grandis* (da Silva Coelho *et al.* 2010); and (ii) a MAP3K-encoding gene being among those with the highest upregulation in a genome-wide transcriptome analysis of the endomycorrhizal fungus *G. intraradices* (Tisserant *et al.* 2012).

Accordingly, the fungal stress-activated MAPK SakA plays an essential role in the establishment and maintenance of the mutualistic interaction between endophytic *E. festucae* and perennial ryegrass. Deletion of *sakA* switched the interaction from mutualistic to pathogenic, accompanied by dramatic changes in fungal gene expression including down-regulation of several genes associated with secondary metabolism and upregulation of genes encoding hydrolytic enzymes and transporters (Eaton *et al.* 2010).

Genome analysis of *L. bicolor*, the first ECM fungus to be sequenced, revealed a significant expansion in several gene families known to be involved in signal transduction pathways, such as protein kinases and small guanosine triphosphatases (GTPases) of the Ras-family, compared with saprophytic and parasitic basidiomycetes (Martin *et al.* 2008). Although this expansion may indicate important roles of these protein families in the establishment and development of the mycorrhizal association, this requires further study.

Two-component systems, which typically comprise a membrane-bound histidine kinase for sensing specific environmental cues and a response regulator for transmitting the signal to a downstream pathway (e.g. MAPK), are important regulators of pathogenicity in fungal pathogens (Catlett, Yoder and Turgeon 2003). They regulate virulence and stress responses in *C. heterotrophus* and *F. graminearum* (Oide *et al.* 2010) and in *Alternatia brassicicola* (Cho *et al.* 2009). An involvement of two-component systems in ECM symbiosis is evidenced by studies on *Pisolithus tinctorius*. A histidine kinase transcript was found to be induced in the early symbiotic interaction with *Eucalyptus globulus* (Voiblet *et al.* 2001), as well as in response to plant metabolites such as pinelactone (Herrera-Martinez *et al.* 2014). Pretreatment of the fungus with the histidine kinase inhibitor closantel blocked the colonization of plant roots by *P. tinctorius*, further supporting an essential role of two-component signalling systems in the early stages of ECM symbiosis (Herrera-Martinez *et al.* 2014).

THE ROLE OF FUNGAL METABOLIC DIVERSITY IN PLANT–FUNGAL INTERACTIONS

Metabolic diversity, including the production of secondary metabolic products, significantly contributes to the ability of fungi to colonize and penetrate plants. The metabolites required during the interaction with plants are not considered essential to cellular life of the fungus but essential to access the cellular contents of the host for growth and development (Keller, Turner and Bennett 2005). Surfeit of secondary metabolites like polyketides (e.g. aflatoxin and fumonisins), terpenes and non-ribosomal peptides (e.g. sirodesmin, peramine and siderophores such as ferricrocin) are major components of filamentous fungi (Keller, Turner and Bennett 2005). Although being chemically disparate, only few biosynthetic pathways are involved in secondary metabolism, often in conjunction with specific stages of morphological differentiation like sporulation and hyphal elongation. Further, the production of such compounds differs not only with fungal strain but also in context with the balance between elicited biosynthesis and biotransformation rates (Vinale *et al.* 2009). Though the various roles of secondary metabolites in fungal biology are hard to pin down the most probable benefit they confer is empowering the fungus to survive in its niche thereby providing an added advantage over its other counterparts. Moreover, secondary metabolite production is not only species specific but also governed by interactions with the host as in case of certain isolates of *M. grisea,* where identification of specific resistant rice cultivars is achieved by an unidentified

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secondary metabolite (Collemare *et al.* 2008). Similarly, virulence potential of *C. heterostrophus*, *C. miyabeanus*, *F. graminearum* and *A. brassicicola* on their particular host plants is governed by certain secondary metabolites associated with iron uptake (Oide *et al.* 2006).

Genes involved in secondary metabolism are often clustered in fungal genomes and diversify with time due to several phenomena such as gene duplication (GD) and horizontal gene transfer (HGT). The diversity of fungal metabolic pathways, recently reviewed by Steindorff *et al.* (2015), allows fungi to sense nutrients and environmental changes differently. *Trichoderma harzianum* is one of the most common fungal root colonizers in agricultural fields. Among different *Trichoderma* species it represents the highest metabolic diversity which is associated with its numerous beneficial effects on plants such as growth promotion and enhancement of stress resistance (Kubicek *et al.* 2003; Singh *et al.* 2011; Keswani *et al.* 2014). Genomic studies revealed that metabolic diversity in fungi is more often brought about through GD compared to HGT (Wisecaver, Slot and Rokas 2014), but genes acquired by HGT are often associated with virulence and constantly subjected to GD and gene loss (Jaramillo, Sukno and Thon 2015). The fungal genus *Fusarium,* which comprises species known to infect agricultural crops as well as many non-pathogens, represents an example for the role of HGT in acquiring diversity. The experimental transfer of a pathogenicity chromosome of *F. oxysporum* f. sp. *lycopersici* into a non-pathogenic strain transformed the latter into a tomato pathogen (Ma *et al.* 2013) suggesting a role of HGT in generating diversity in nature and the polyphyletic origins of host specificity in *Fusarium*. It is therefore concluded that fungal metabolic diversity determines the outcome of the fungus-plant interactions for both beneficial and harmful fungi and that evolution through either GD or HGT or both leads to their metabolic diversity.

PLANT–FUNGAL INTERACTIONS AND CROP PRODUCTIVITY

Plant health and productivity significantly depend on microbial activity in the rhizosphere and on the microbes directly interacting with the plant. The latter affect nutrient availability in soil and plant–microbe partnerships can improve stress tolerance in the host plant, provide disease resistance and promote biodiversity of plants (Morrissey *et al.* 2004). On the other hand, 70–80% of all plant diseases are caused by fungi, and it is proposed that approximately 10 000 fungal species may induce diseases in plants (Agrios 2005). A recent survey among 495 international experts in plant pathology led to a list of the 10 most important phytopathogenic fungi on a scientific and economic level (Dean *et al.* 2012). This list is headed by *M. oryzae* with its economic importance since over one-half of the world's population relies on rice as a staple food, followed by *B. cinerea* that causes severe pre and post harvest damage, and *Puccinia* spp., which cause rust diseases on wheat. For fighting these and other phytopathogens, conventional agriculture relies heavily on chemicals which unfortunately cause environmental and health issues. Harnessing beneficial plant-associated microbes such as growth-promoting rhizobacteria, mycorrhizal fungi and microbial antagonists has long been neglected. These organisms can improve plant performance and crop productivity by inducing systemic resistance to phytopathogens and insect herbivores in the plant, and some biocontrol agents such as mycoparasitic fungi can also directly attack fungal plant pathogens (van der Heijden, Bardgett and Van Straalen 2008). Symbiotic AM fungi also act as natural fertilizers, enhancing plant yield, and as bioprotectants against pathogens and toxic stresses (van der Heijden, Bardgett and Van Straalen 2008). Many important agricultural crops such as maize, potato, sunflower, wheat and soybean benefit from AM fungi, especially under nutrient-limiting conditions, since extensive hyphal networks in the soil improves the efficient uptake of orthophosphate and other minor nutrients. Studies with potatoes grown with AM fungi revealed that the plants required only 38% of phosphate fertilizer normally used, while rice grown with AM fungi gave 20% increase in yield (Reid 2011). AM fungi are further reported to reduce damage caused by soil-borne plant pathogens (Azcon-Aguilar and Berea 1996), and the activity of mycorrhizal fungi can even be enhanced by combining them with other beneficial microbes such as growth-promoting rhizobacteria or fungal biocontrol agents such as *Trichoderma* spp. (Colla *et al.* 2014).

Marketed fungal biocontrol agents include fungi such as *Ampelomyces quisqualis* (AQ10) for controlling powdery mildew on e.g. strawberry, tomato and grape, *Coniothyrium minitans* (Contans WG) for the control of *S. sclerotiorum* in a variety of crops, non-pathogenic *F. oxysporum* strains (Fusaclean, Biofox) for suppressing pathogenic strains or *Trichoderma* spp., the latter being the most frequent and best studied fungal biocontrol agents applied in agriculture (Butt and Copping 2000). *Trichoderma* strains used for biocontrol can establish themselves in the plant rhizosphere and act as opportunistic avirulent plant symbionts (Harman *et al.* 2004). They are marketed world-wide as biopesticides for the control of soil-borne and foliar fungal pathogens such as *Rhizoctonia, Pythium*, *Sclerotinia, Botrytis* and *Alternaria*. They are also biofertilizers and growth enhancers based on their ability to directly attack plant pathogenic fungi (direct antagonism or mycoparasitism) and promote plant growth, elicit plant defences against pathogen attack and environmental stress, and improve or maintain soil productivity (indirect antagonism) (Harman *et al.* 2004; Woo *et al.* 2014). The plant's reaction to a biocontrol agent is similar to rhizobacteria-elicited ISR and results in the induction of a systemic change in the expression of plant genes involved in the scavenging of ROS, response to stress, biosynthesis of oxylipin and ethylene, photosynthesis, photorespiration and metabolism of carbohydrates (Djonovic *et al.* 2007; Shoresh and Harman 2008). Similar to mycorrhiza, increased nutrient uptake and improvement of plant growth, development and yield has been reported for *Trichoderma*-treated plants (Yedidia *et al.* 2001) which may result from improved micronutrient solubility and the production of hormone-like substances (auxins, indole-3-acetic acid) by the fungus (Contreras-Cornejo *et al.* 2009; Druzhinina *et al.* 2011).

Modification of the plant defence system against microbial pathogens may be an interesting approach for improving disease resistance in crops. Modern biotechnologies have focused on enhancing plant resistance against fungal pathogens by using genetic engineering (van der Biezen 2001). The most common genes for this purpose are those encoding chitinases, glucanases, peroxidases and antifungal proteins from *Trichoderma* species (Nicolás *et al.* 2014). The expression of high levels of a *T. harzianum* endochitinase-encoding gene in tobacco and potato plants resulted in transgenic lines highly tolerant or completely resistant to the foliar pathogens *Alternaria alternata*, *A. solani*, *B. cinerea* and *R. solani*, without visible effects on plant growth and development (Lorito *et al.* 1998). Other fungal genes have also been used in different plants and for different purposes including endopolygalacturonase II from *A. niger* (*AnPGII*) in tobacco (Lionetti *et al.* 2010; Cona *et al.* 2014; Tomassetti *et al.* 2014); laccase III (*LAC*) from *Trametes versicolor* (Furukawa *et al.* 2013);

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phytase (*phyA2*) from *Aspergillus niger* in maize (Chen *et al.* 2008; Huang *et al.* 2014); cellobiohydrolase I (*CBHI*) and *CBHII* (Harrison *et al.* 2011); clitocypin (*Clt*; a cysteine protease inhibitor) from *Clitocybe nebularis* in potato plants (Šmid *et al.* 2015); cadmium factor 1 from *S. cerevisiae* (*ScYCF1*, a yeast ATP-binding cassette [ABC] transporter for cadmium detoxification) in poplar trees (Shim *et al.* 2013) and *Brassica juncea* (Indian mustard; Mondal *et al.* 2007); and genes encoding β-xylosidase/α-arabinosidase, feruloyl esterase and acetylxylan esterase from *A. nidulans* in *A. thaliana* (Pogorelko *et al.* 2011).

Fungi are prolific producers of secondary metabolites, some of which contribute to their beneficial effects on plants. Besides, a direct antibiotic activity against plant pathogenic fungi and insects, secondary metabolites of beneficial fungi such as endophytes and biocontrol agents positively affect plants as a function of growth promotion, yield increase and elicitation of defence responses against pathogens. Secondary metabolites such as 6-pentyl-alpha-pyrone, peptaibols, harzianum A and aspinolides produced by certain *Trichoderma* species act as elicitors of plant defence against pathogens and often also show positive effects on plant growth and development (Vinale *et al.* 2012; Malmierca *et al.* 2014). Similarly, endophytic *Phomopsis* sp. or *Muscodor albus* produce mixtures of volatile chemicals which effectively inhibit and kill pathogenic fungi, nematodes and certain insects (Strobel 2006; Singh *et al.* 2011). Based on these activities, microbial metabolites can be used as active ingredients in agrochemicals for crop protection of which strobilurin-based fungicides are the most successful (Kim and Hwang 2007; Kim *et al.* 2007). They have been developed by chemical modification using natural metabolites such as strobilurin A, which is biosynthesized by the wood-rotting fungus *Strobilurus tenacellus,* as a lead substance. Strobilurins interfere with fungal mitochondrial respiration and the commercialized substances have a wide antifungal spectrum effective against all major groups of plant pathogenic fungi (Kim and Hwang 2007).

Plants may benefit from the interaction with beneficial fungi in several ways including defence against pathogen attack, improvement of nutrient uptake and stress resistance which often results in better growth and crop yield. Some fungal biocontrol agents or natural substances derived thereof are already used in the field but there is still potential for improvement so that such microbes could become a realistic alternative to the heavy fungicide regimens used in agriculture at present. The potential of mycorrhizal fungi should be considered in modern agriculture to maximally benefit from their positive effects on crop productivity and ecosystem sustainability.

EFFECT OF CLIMATE CHANGE ON PLANT–FUNGAL INTERACTIONS AND ITS IMPLICATIONS

Climate change relates to major changes in temperature, precipitation or wind patterns, among other effects, taking place over several decades or longer (Harvell *et al.* 2002). The emission of anthropogenic greenhouse gas is considered the major factor accounting for climate change and has resulted in rising levels of carbon dioxide and an increase in the global average temperature (Harvell *et al.* 2002). The impact of climate change on agriculture, ecosystem health, human safety, food production and food security is significant (Garrett *et al.* 2006). For instance, it has been suggested that climate change has a negative impact on agriculture as a result of (a) reduced yields in warmer regions due to heat stress, (b) damage to crops, (c) soil erosion, (d) inability to cultivate land caused by heavy precipitation events and (e) land degradation and desertification resulting from increasing drought (Paterson, Lima and Sariah 2013).

Climate change may drive the emergence of novel fungal disease and preexisting pathogens that are already present in the environment (Anderson *et al.* 2004). Based on its direct link to agriculture and ecosystem health, understanding the impact of climate change on plant–fungal interactions is a priority (Chakraborty, Tiedemann and Teng 2000; Anderson *et al.* 2004; Garrett *et al.* 2006; Pautasso *et al.* 2012; Altizer *et al.* 2013). For instance, climate change may be linked to the emergence of aggressive, 'stripe' rust on wheat (*P. graminis*) and *M. oryzae* on rice (Olsen *et al.* 2011). Climate change may also provide more susceptible and suitable trees for the infection by *Phytophthora ramorum* (Pautasso *et al.* 2012), which is largely spread by human activities. Here, we focus on one vector-borne tree disease—the outbreak of mountain pine beetle—and its fungal associates.

Climate change can alter the distribution and abundance of arthropod vectors, increasing the frequency of vector-borne diseases. One of the recent examples in a forest ecosystem is the large scale outbreak of the mountain pine beetle (*Dendroctonus ponderosae*; MPB) in Canada and north western USA. The MPB outbreak that started in the late 1990s has destroyed over 18 million ha of conifer forests in western Canada and is by far the largest devastation in recorded history (Carroll *et al.* 2004). MPB is a native pest having a distribution from northern Mexico to central British Columbia (BC), including south western Alberta, south western Saskatchewan and most of the western United States such as Colorado, Idaho and Montana (Carroll *et al.* 2004). MPB primarily attacks and kills lodgepole pine (*Pinus contorta* var. *latifolia*), but its host range encompasses other pines such as jack pine (*P. banksiana*), western white pine (*P. monticola*), whitebark pine (*P. albicaulis*) and ponderosa pine (*P. ponderosa*) as well as natural lodgepole-jack pine hybrids (Safranyik and Carroll 2006; Cullingham *et al.* 2011). MPB attacks damaged trees, and the infestation follows a cyclical pattern but it occasionally erupts into large-scale outbreaks (Carroll *et al.* 2004; Safranyik *et al.* 2010). MPB forms a symbiotic relationship with several species of blue stain fungi such as *Grosmannia clavigera, Leptographium longiclavatum* and *Ophiostoma montium* (Klepzig and Six 2004; Lee *et al.* 2006). The combined effects of blue-stain fungal colonization, the mass attack of MPBs and subsequent larval feeding can kill a tree quickly, within months (Klepzig and Six 2004). Western Canada (the southern interior regions of British Columbia and in the northern Rocky Mountains in the USA) has experienced a long period of consecutive MPB epidemics, with reports in the early 1900s, 1960s and mid-1970s to mid-1980s, possibly a result of prolonged drought and warm summer (Safranyik and Carroll 2006). However, the impact of the current MPB epidemic is unprecedented compared to the past epidemics and has led to substantial economic losses and ecological damage (Kurz *et al.* 2008).

The major influential factor of current MPB outbreaks in Canada is linked to climate change (Fig. 6). Temperature rise in British Columbia has altered habitats that have been traditionally climatically unsuitable to MPB outbreak (Carroll *et al.* 2004). MPB infestations in British Columbia from 1998 to 2003 also coincided spatially with ideal habitats based on GIS analysis, suggesting that recent global warming has induced MPB range expansion (Carroll *et al.* 2004). Climate change (warm summer and milder winter) enables MPB to expand and to colonize habitats of greater latitude and higher elevations, even though the success of fire suppression, and the availability of mature and overmature lodgepole pine populations are also thought to have created ideal conditions that help account for the

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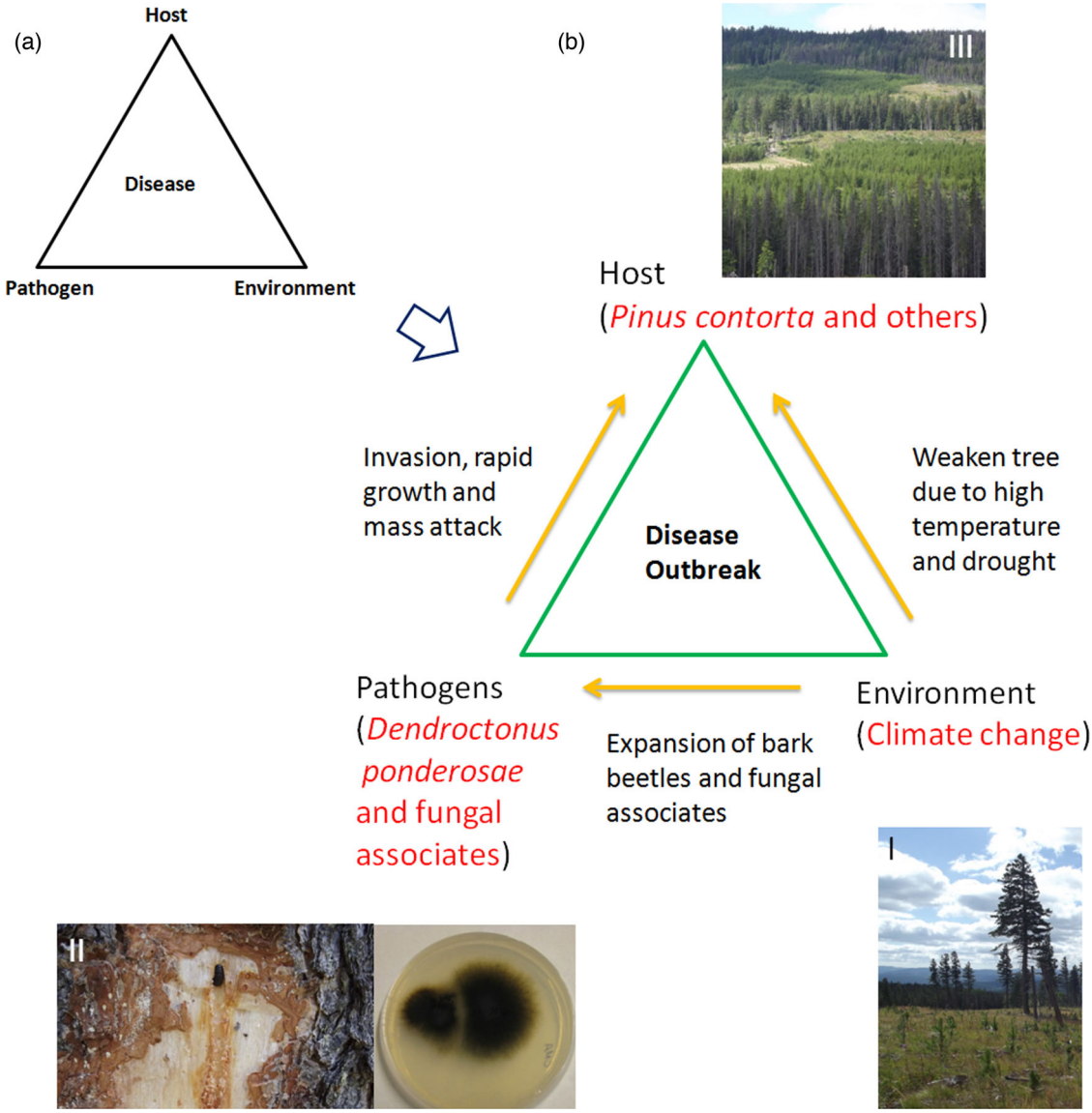

Figure 6. (a) Disease triangle illustrating the interactions among pathogen, host and the environment. The triangle serves as a conceptual model describing the environmental factors that may affect the host–pathogen interaction and favour disease in the development of an epidemic. Climate directly and indirectly affects plant health by altering abiotic conditions. It also has an influence on forest health by directly acting on various pathogens (insect pests and fungal symbionts). (**b**) The interactions among *G. clavigera*, its vector mountain pine beetle (MPB) and their host trees (*P. contorta*) under climate change. (I) Climate change causing e.g. drought may stress the trees. (II) Mountain pine beetle builds galleries in the infected conifer during range expansion and mass attack. A culture of *Grosmannia clavigera* is isolated from infected conifer. (III). Many lodgepole pines are killed during the epidemics in BC, Canada.

magnitude of the current outbreak (Stahl *et al.* 2006; Raffa *et al.* 2008). MPB outbreaks also impact forest productivity: according to Environment Canada, the forest was a carbon sink from 1990 to 2002 but was converted to a carbon source due to MPB outbreaks (Kurz *et al.* 2008).

G. clavigera and *L. longiclavatum* are the major ophiostomatoid blue stain fungi (Ophiostomatales, Ascomycota) that appear to be exclusively associated with the MPB (Lee *et al.* 2006). *G. clavigera* has been identified as a primary and aggressive invader of sapwood and it is commonly isolated from the MPB mycangia (Yamaoka, Hiratsuka and Maruyama 1995; Solheim and Krokene, 1998). *G. clavigera* can kill mature or young lodgepole pine in the absence of MPB when inoculated at a density similar to that of a beetle mass attack (Yamaoka, Hiratsuka and Maruyama 1995). *L. longiclavatum* also caused necrotic tissue around inoculation points, both on the phloem and the sapwood (Lee *et al.* 2006). With the expansion of the MPB range to higher latitudes and elevations give these fungal pathogens access to lodgepole pine, jack pines and their hybrids normally beyond their natural distribution range (Tsui *et al.* 2012, 2014). It is hypothesized that the fungal symbionts can also adapt to the novel environment, as *L. longiclavatum* has been suggested to be more cold tolerant than *G. clavigera* (Rice and Langor 2009; Roe *et al.* 2011).

Since climate change may lead to the spread of plant-destroying organisms, renewed efforts to monitor the occurrence of pests and diseases and to control their transport is necessary to reduce this growing threat to global food security and forest systems (Bebber, Ramotowski and Gurr 2013). In addition to ecological information, climatic data and spatiotemporal models, genetic variation data from pests and diseases

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may be useful to predict the movement pattern and expansion pathway of pests and pathogens.

CONCLUSIONS

Studies on plant–fungal interactions are showing resurgence based on recent in depth insights into the evolutionary relationships between fungi and plants, and the development of new methods for their exploration. For a long time scientists used fairly reductionist approaches to study such interactions. However, plant–fungal interactions are by far more complex than previously thought, with not only a single fungal interactor, but rather a whole plant-associated microbiome. We have made significant progress in understanding the roles of fungi as major interactors with plants, but much remains to be explored, especially regarding associations of biotrophic fungal pathogens and/or non-culturable fungi. Having just entered the 'omics' and superresolution era, we are poised to take advantage of the associated genomics, transcriptomics, proteomics, metagenomics and advanced microscopy tools to open up new avenues for exploring plant–microbe interactions.

To date, the interactions of fungi with plants are broadly classified as mycorrhizal, parasitic or endophytic, with a large number of fungal associations playing significant roles in plant development and health. It still remains a challenge to understand how a fungal partner alters its life style to assimilate with a plant host, in particular the adaptation of endophytes into parasites and vice versa. A detailed understanding of fungal diversity and the influence of fungi on plant biology will not only improve scientific knowledge on ecosystem function but will be crucial for controlling plant pathogens and exploiting the potential of plant beneficial fungi to ensure global food availability.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSRE online.

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