Oomycete Interactions with Plants: Infection Strategies and Resistance Principles

Stuart Fawke¹, Mehdi Doumane¹*, Sebastian Schornack¹#

¹Sainsbury Laboratory (SLCU), University of Cambridge, United Kingdom

*Current address: Department of Biology, École Normale Supérieure de Lyon, Lyon, France

#Address correspondence to Sebastian Schornack, sebastian.schornack@slcu.cam.ac.uk

Running head: Plant pathogenic oomycetes
Table of Contents

Summary ........................................................................................................... 3
Introduction ...................................................................................................... 3

Main Text

- Phylogeny .................................................................................................... 4
- Early life cycle stages: asexual reproduction and infection structures ............ 4
- Plants recognise oomycete-derived molecules .............................................. 5
- Effectors suppress host immunity ................................................................. 7
- How are effectors deployed in the host? ....................................................... 9
- Plant innate immunity .................................................................................. 11
- R gene-mediated resistance ....................................................................... 12
- S gene-mediated resistance ....................................................................... 13
- Future directions for developing oomycete resistant plants ....................... 15
- Potential for comparative pathogen-mutualist studies .................................. 17
- Summary ...................................................................................................... 18

Acknowledgements .......................................................................................... 18

Tables

- Table 1. Plant pathogenic oomycete genome sequence resources ............. 19
- Table 2. Lifestyle, host range and infection structures of important plant-infecting oomycete species ................................................................. 20
- Table 3. Examples of known oomycete elicitors ......................................... 21
- Table 4. Examples of effectors that suppress host immunity during interactions with oomycetes, together with their host targets and virulence effects .......... 21
- Table 5. Cloned resistance (R) and susceptibility (S) genes affecting oomycete-plant interactions ............................................................................. 22

Figure Legends ................................................................................................ 24
References ......................................................................................................... 25

Biographical Text ............................................................................................. 37
Summary
The Oomycota include many economically significant microbial pathogens of crop species. Understanding the mechanisms by which oomycetes infect plants and identifying methods to provide durable resistance are major research goals. Over the last few years, many elicitors that trigger plant immunity have been identified, as well as host genes that mediate susceptibility to oomycete pathogens. The mechanisms behind these processes have subsequently been investigated and many new discoveries made, marking a period of exciting research in the oomycete pathology field. This review provides an introduction to our current knowledge of the pathogenic mechanisms used by oomycetes, including elicitors and effectors, plus an overview of the major principles of host resistance: the established R gene hypothesis and the more recently defined susceptibility (S) gene model. Future directions for development of oomycete-resistant plants are discussed, along with ways that recent discoveries in the field of oomycete-plant interactions are generating novel means of studying how pathogen and symbiont colonizations overlap.

Abstract
The Oomycota include many economically significant microbial pathogens of crop species. Understanding the mechanisms by which oomycetes infect and identifying methods to provide durable resistance is a major research goal. Over the last few years many elicitors that trigger plant immunity have been identified as well as host genes that mediate susceptibility to oomycete pathogens. The mechanisms behind these processes have subsequently been investigated and many new discoveries made, marking a period of exciting research in the oomycete pathology field. This review provides an introduction to our current knowledge of the pathogenic mechanisms used by oomycetes, including elicitors and effectors, plus an overview of the major principles of host resistance: the established R gene hypothesis and the more recently defined susceptibility (S) gene model. Future directions for developing oomycete-resistant plants will be discussed, alongside how recent discoveries in the oomycete-plant interactions field are generating novel ways of studying how pathogen and symbiont colonisations overlap.

Introduction

The Oomycota are a distinct class of fungal-like eukaryotic microbes, many of which are highly destructive plant or animal pathogens. They share a range of morphological features with fungi, but possess various unique characteristics which set them apart (1). Cellulose is a major component of oomycete cell walls. By contrast, chitin, but not cellulose, is a major
cell wall component of true fungi. However, oomycetes also possess chitin synthases that are activated during tip morphogenesis (2, 3). Oomycetes are diploid during their vegetative mycelial stage, whereas fungi predominantly produce haploid thalli, although exceptions do exist (2, 4). Cells of oomycetes can be distinguished morphologically from true fungi by their mitochondria, possessing tubular cristae as opposed to the flattened cristae of fungi (5) or their hyphae which are always non-septate (6).

Typical structural features guided identification of oomycetes in the fossil record. The oldest existing evidence for oomycete-like structures dates back to the Devonian period, c. ~400-360 Ma (7) and there is evidence of oomycete parasitism occurring during the Carboniferous period, c. ~300 Ma (8). Molecular clock estimates position the origin of oomycetes as early as the Silurian period, c. ~430-400 Ma (9).

This review provides an overview of our current knowledge of oomycete plant pathogens. We introduce the elicitors, effector proteins and disease resistance and susceptibility principles involved in our current understanding of how oomycetes interact with their plant hosts. We also present strategies for developing oomycete-resistant crop plants and highlight the potential of oomycetes as tools to investigate common and contrasting mechanisms of pathogenic and mutualistic filamentous microbes.

**Phylogeny**

Analysis of conserved DNA sequences such as mitochondrial COX2 (10-12), LSU rDNA (13) and SSU rDNA (14) have confirmed that oomycetes belong outside the fungal kingdom, within the Chromalveolata. The Chromalveolata kingdom contains mainly photosynthetic species, a result of ancestral ‘enslavement’ of red algae (15), but oomycetes have since lost their chloroplasts (16). Availability of several sequenced genomes for some genera (see Table 1), in particular Phytophthora, has greatly facilitated multilocus assessment of oomycete taxonomic relationships (17). The Oomycota are broadly divided into two subclasses. The Saprolegniomycetidae, referred to as the ‘water moulds’, include the orders Eurychasmales, Leptomitales and Saprolegniales, whilst the Peronosporomycetidae are mostly plant pathogen orders and consist of the Rhipidiales, Pythiales and Peronosporales. The existence of early diverging genera of marine parasites within the mainly terrestrial Saprolegniales and Peronosporales orders has led evolutionary biologists to suggest that oomycetes made their migration onto the land and into the soil via parasitism of nematode hosts or by switching from colonisation of estuarine seaweed to the roots or shoots of early coastal vegetation (18).
Early life cycle stages: asexual reproduction and infection structures

Dispersal of oomycetes by wind or water is achieved through asexual sporangia. Germination of sporangia can either occur directly, forming invasive hyphae or indirectly, releasing motile zoospores, which are chemotactically and electrotactically attracted to the surfaces of host plants (19). Zoospores swim until reaching the plant surface at which point they shed their flagella and encyst, firmly attaching themselves to the plant surface via secretion of adhesion molecules (20), as visualised in Figure 1.

Upon germination of a zoospore, a germ tube emerges and grows across the plant surface until the development of an appressorium is induced by surface topology and/or hydrophobicity (6). In general, oomycete appressoria function in the penetration of the outermost, epidermal cell layers. Exceptions to this include Albugo candida, a leaf infecting pathogen of Arabidopsis thaliana, which enters through stomata and then forms appressoria in order to penetrate the mesophyll cells below (21) and Aphanomyces euteiches, which does not form distinct appressoria.

Oomycete plant pathogens exhibit biotrophic, necrotrophic or hemibiotrophic (a combination of both) lifestyles. Many biotrophic oomycetes are completely reliant on host tissues (obligate biotrophy). This is a feature of the downy mildews Hyaloperonospora arabidopsidis, H. parasitica, and Plasmopara viticola as well as A. candida that causes white rust. Hemibiotrophs commonly have the ability to survive in axenic culture (facultative) such as Phytophthora spp, as do necrotrophs like Pythium ultimum. A summary of the lifestyles of important plant-colonising oomycetes is provided in Table 2.

Obligate biotrophs such as H. parasitica must maintain a close interaction with their hosts whilst keeping the plant alive for their own survival, meaning that highly specific infection mechanisms exist, significantly restricting their host range. This is in contrast to hemibiotrophic pathogens, for example those of the Phytophthora genus, some of which have the ability to infect hundreds of different plant species, growing initially as a biotroph but later switching to a necrotrophic phase. Following penetration of the cell wall by appressoria, oomycetes generate vegetative hyphae that grow intercellularly and haustoria develop as side branches from intercellular and epicuticular hyphae, terminating inside penetrated host cells (22) (23) (Figure 1; Figure 2). Haustoria can be observed during colonisation by most obligate biotrophs (24) and have been implied in nutrient uptake in fungi where haustorium-specific sugar transporters have been described (25), although in oomycetes little is known about haustorium-specific transport processes. However, a
number of hemibiotrophs and necrotrophs do not form haustoria, for example *Aphanomyces euteiches* and *Pythium ultimum*.

**Plants recognise oomycete-derived molecules**

Elicitors are molecules which stimulate a defence response in a host plant (Table 3). Most of them constitute PAMPs (pathogen associated molecular patterns) (26) because they are structurally conserved and thought to be indispensable components or products of a pathogen’s lifecycle or infection process. Elicitors are perceived by some plants as a microbial signature, likely through peripheral receptors, some of which require BAK1/SERK3 for their activity (27, 28). The following paragraphs describe a number of oomycete elicitors and their receptors, if known.

The elicitor Pep-13 was isolated from *Phytophthora sojae* and is a thirteen amino acid peptide of a surface exposed stretch of a transglutaminase protein (29-31). Mutation in just one of these amino acids is sufficient to impair transglutaminase-mediated recognition of *P. sojae* and to avoid induction of plant-defence responses (29). Although Pep-13 was identified over 10 years ago, its plant receptor(s) have yet to be discovered.

Some parasitic oomycetes, including *Phytophthora* species, have lost the ability to synthesise their own sterols, which are essential molecules for many cellular functions. They must therefore acquire sterols from host cell membranes (32). *Phytophthora infestans* INF1 is a member of a family of conserved lipid transfer proteins with sterol-binding and elicitor capacity including Cryptogein from *Phytophthora cryptogea*, CAP1 from *Phytophthora capsici* and PAL1 from *Phytophthora palmivora*, amongst others. INF1 binds *in vitro* dehydroergosterol and catalyses sterol transfer between liposomes (33). However, there is still no *in vivo* evidence of INF1 involvement in sterol uptake and INF1-lacking *P. infestans* strains remain pathogenic (34, 35). INF1 is known to be secreted by *P. infestans* through its N-terminal signal peptide, initially localising to the extracellular space (36), and it has been shown by *in vitro* immunocytochemistry that the INF1-like Quercinin of *Phytophthora quercina* appears to be transported inside the host (29). INF1 was reported to interact with the cytoplasmic domain of NbLRK1, a lectin-like receptor kinase that is localised to the plasma membrane (37). However, requirement of BAK1/SERK3 for INF1-triggered immune responses rather points to a LRR containing receptor (27, 28), leaving open whether it is a receptor-like protein (RLP) or a receptor-like kinase (RLK). The identification of *SISOBIR1* as a required component for responses elicited by the *P. parasitica* INF1-like ParA1 (38) suggested that INF1 perception is mediated through a receptor-like protein (RLP) rather than
a receptor like-kinase (RLK), since SOBIR1 was previously reported to be a co-receptor of RLPs (39). Then, the discovery of ELR, a wild potato RLP that associates with BAK1/SERK3, mediating broad-spectrum recognition and induction of cell death, triggered by four *P. infestans* elicitors (INF1, INF2A, INF5 and INF6) as well as eleven elicitors of diverse other *Phytophthora* species, added a new chapter in our understanding of INF1 perception (40).

OPEL is a recently described secreted protein from culture filtrates of *Phytophthora parasitica* with homologs in other oomycetes but not in fungi (41). This 556 amino acid protein is inducibly expressed during plant invasion. Infiltration of OPEL proteins into *Nicotiana tabacum* leaves led to callose deposition, cell death, synthesis of reactive oxygen species (ROS) and induction of PTI response marker genes as well as salicylic acid-responsive defence genes (41); all characteristics of a plant defence response. OPEL is therefore considered a microbial signature that is recognised in tobacco leaves. Infiltration of OPEL also stimulates resistance to viruses, bacteria and the oomycete pathogen *P. parasitica*. OPEL contains three domains in addition to its signal peptide, a thaumatin-like domain, a glycine-rich protein domain and a glycosyl hydrolase (GH) domain with laminarinase activity. Recombinant OPEL protein infiltration resulted in enhanced plant immune response and resistance to *P. parasitica*. Chang et al. (41) conclude that the predicted laminarinase activity of OPEL triggers plant immune responses, presumably by generating degradation products in the apoplast that act as damage associated molecular patterns (DAMPs). However, the authors were unable to show any enzymatic activity from the wildtype OPEL protein using laminarin or 1,3-β-glucan as a substrate. OPEL might have a specific polysaccharide substrate in the plant cell wall whose degradation is detected by plant immunity. Alternatively, co-evolution of plant and oomycete may have led to perception of OPEL via its enzymatic active site.

The cellulose binding elicitor lectin (CBEL) of *P. parasitica* is an apoplastic elicitor that possesses two carbohydrate-binding modules belonging to family 1 (CBM1) domains, allowing binding to cellulose and lectin-like hemagglutinating activity (42). CBM1 domains occur commonly in oomycete and fungal proteins, although CBM1-containing fungal proteins function in plant cellulose degradation, whereas those of oomycetes (including CBEL) play a role in adhesion (43). There is downstream signalling following CBEL perception in tobacco cells, but not in cell wall-lacking protoplasts, suggesting that plant cell wall binding is required for CBEL-induced defence reactions (44). Alternatively, CBEL detection might require other cell wall-dependent processes such as polar exo- or endocytosis which cannot properly take place in non-polar protoplasts (45).
β-glucans represent PAMPs originating from cell wall fractions of filamentous pathogens (fungi and oomycetes). Soybean perceives branched heptaglucans with β(1-6) backbone linkages from *Phytophthora sojae*, and, in particular, its three non-reducing terminal glycosyl residues (46). Conversely, this glucan does not elicit defence responses in tobacco cells, but a linear β(1-3) glucan does (47). Branched glucan-chitosaccharides from cell wall fractions of *Aphanomyces euteiches* induce defence gene expression and nuclear calcium oscillation in *Medicago truncatula* root epidermis (48), similar, but not identical, to those elicited by lipochito-oligosaccharides produced by arbuscular mycorrhiza fungi.

**Effectors suppress host immunity**

In order to sustain an intimate association with the host plant, oomycetes must suppress immune responses triggered by their own elicitors. By secreting effector proteins that can act in many different cellular compartments, pathogens alter the plant’s physiological state to benefit colonisation. Descriptions of effector function are often defined by the available approaches used to study them. Here, we mention some recent effector studies that focus on the localisation and stability of effectors and their target proteins, as well as overall transcriptional changes and virulence effects, all of which are summarised in Table 4.

The *P. infestans* effector AVR3a suppresses perception of the PAMP, INF1, through stabilisation of the U-box protein CMPG1 (49). AVR3a was also found to interact with Dynamin-Related Protein 2 (DRP2), a plant GTPase implicated in receptor-mediated endocytosis, that, when overexpressed, attenuated PAMP-triggered ROS accumulation (50). It appears from these findings that AVR3a can suppress BAK1/SERK3-mediated immunity via two different methods.

*P. infestans* PexRD2 interacts with the kinase domain of MAPKKKε, a positive regulator of cell death associated with plant immunity. This in turn disrupts the signalling pathways triggered by, or dependent on, MAPKKKε, increasing the susceptibility of *N. benthamiana* to *P. infestans* (51).

When expressed in plant cells, *P. infestans* AVRblb2 displays an intriguing localisation at haustoria and renders plants more susceptible to infection. Furthermore, AVRblb2 prevents secretion of the plant defence protease C14, resulting in lower C14 levels in the apoplast and accumulation of C14-loaded secretory compartments around haustoria (52).

The nuclear-localized effector HaRxL44 of *H. arabidopsis* interacts with Mediator subunit 19a (MED19a), resulting in degradation of MED19a. The Mediator complex consists of
around 25 protein subunits and is broadly conserved in eukaryotes, functioning as a mediator in the interaction between transcriptional regulators and RNA polymerase II. MED19a was found to be a positive regulator of immunity against *H. arabidopsis* and responsible for transcriptional changes resembling jasmonic acid/ethylene (JA/ET) signalling when in the presence of HaRxL44. It was concluded that HaRxL44 attenuates salicylic acid–triggered immunity in Arabidopsis by degrading MED19, shifting the balance of defence transcription to JA/ET-signalling. (53).

Two *P. sojae* effectors, PsCRN63 and PsCRN115 (for crinkling- and necrosis-inducing proteins), which are suggested to be secreted, were shown to regulate plant-programmed cell death and H$_2$O$_2$ homeostasis. The effectors act through direct interaction with catalases to overcome host immune responses (54).

The identification of two putative membrane-associated NAC transcription factors (TF) as the host targets of the effector Pi03192 is one example of oomycete effectors targeting transcriptional responses. The effector interacts with NAC Targeted by Phytophthora (NTP) 1 and NTP2 at the endoplasmic reticulum (ER) membrane, where these proteins are localised. The proposed mechanism by which Pi03192 promotes disease progression is the prevention of relocalisation of NTP1 and 2 from the ER to the nucleus, that appears to be key for immunity. Few plant pathogen effectors have been shown to influence such relocalisation events or target transcriptional regulators of plant immunity (55).

Two effectors from *P. sojae*, PSR1 and PSR2, suppress RNA silencing by inhibiting the biogenesis of small RNAs (56). Very recently the host target of PSR1, PSR1-Interacting Protein 1 (PINP1), was identified and shown to regulate accumulation of microRNAs and small interfering RNAs in *Arabidopsis* (57). When overexpressed, PSR1 enhanced susceptibility of *Arabidopsis* to *P. capsici* and also enhanced susceptibility of *N. benthamiana* to *P. infestans*. A target for PSR2 has yet to be discovered, although PSR2 is known to be required for full virulence of *P. sojae* on soybean (56).

Recent research has also established that numerous *Phytophthora* and *Hyaloperonospora* effectors can suppress PTI against the bacterial PAMP derived peptide flg22 at different steps of the downstream signal cascade (58, 59). Other features of effector interference with plant defences are protease and peroxidase inhibition, targeting of the ubiquitination system, salicylate signalling or the disruption of plant cell wall to plasma membrane attachment (60-63).
How are effectors deployed in the host?

By definition, effectors are encoded by the oomycete but act inside the host. Accordingly, the majority of identified oomycete effectors carry an N-terminal signal peptide that mediates secretion from the microbe. A notable exception is the *P. sojae* effector PsIsc1, a putative isochorismatase that does not have a predicted secretory leader peptide but, nevertheless, can be detected in *P. sojae* secretion supernatants (62).

Once secreted, apoplastic effectors act in the apoplast surrounding plant and microbial cells, while cytoplasmic effectors enter the plant cell and would have to cross the plant cell wall and the plant plasma membrane or alternatively the extrahaustorial matrix and the extrahaustorial membrane (Fig. 2c). Fusions of the *P. infestans* effector AVR3a with RFP accumulate only at haustoria (23). These interfaces are presumably a specific site of secretion of AVR3a, or RFP is very stable in the extrahaustorial matrix space surrounding haustoria. Notably, a similar distribution has been observed when AVR3a was fused to GFP and secreted from *P. capsici* (64). Given this indirect evidence, haustoria have been hypothesised to be a site of translocation for cytoplasmic effectors. However, not all oomycetes form haustoria and studies have shown internalisation of effectors into plant cells even in the absence of the pathogen from which they originated (65), suggesting that specific microbial structures for delivery of effectors may not always be required.

The majority of cytoplasmic oomycete effectors characterised to date contain an RXLR (Arginine-any amino acid-Leucine-Arginine) motif following an N-terminal signal peptide, which is thought to allow translocation into plant cells (23, 66). The RXLR motif can be followed by an EER motif and, furthermore, similar motifs such as QXLR (67) and RXLQ (61) can replace the RXLR motif, or it can be absent such as in the case of ATR5 (68). A second class of effectors known as CRNs, named for their ‘crinkling and necrosis’-inducing activity (69), are also common in oomycetes and may perform a similar translocation function via conserved LXLFLAK motifs (64). It has been suggested that RXLRs may be an adaptation to facilitate biotrophy, because their expression is induced during pre-infection and biotrophic phases of infection (23), whereas certain other species may employ CRNs predominantly as a result of their adaptation to necrotrophy, e.g. *Pythium spp* (2). However, many biotrophic oomycete species exist which secrete both RXLRs and CRNs, implying that a connection between effector class and lifestyle is not easily defined.

There are two main experimental approaches that have been used in an attempt to conclusively demonstrate the function of host-targeting domains, such as RXLRs, in effectors. The first, cell re-entry assays, involves expression of a full-length effector protein
from a pathogen, including its secretion signal peptide, in a plant cell. Once expressed this effector passages through the plant secretory system and is secreted into the extracellular space (apoplast); its subsequent re-entry into the plant cell can then be traced microscopically via fusion to a fluorescent protein (70). Through the generation of mutations in specific domains suspected to function in delivery of effectors into plant cells and employing cell re-entry assays, it has been possible to identify putative domains required for entry (65, 71, 72). However, this assay cannot unequivocally demonstrate that when the effector is expressed it is assuredly secreted into the apoplast prior to re-entry. To address this weakness of cell re-entry experiments, a second assay was devised in which purified effector proteins, labelled by a fluorescent tag, are applied to plant tissues and their entry tracked via microscopy (65, 72, 73). The purified effector protein uptake assay into roots is currently under debate. Protein internalisation by root cells is non-specific (74) and fluorescent proteins are taken up by the plant at a comparable rate to their effector-fused derivatives (75). Thus, this assay cannot be used to properly assess specific effector entry. Conversely, Tyler et al (2013) observed differential uptake of fluorescent proteins when effector motifs implied in uptake were fused to them (76). A detailed list of supporting and conflicting experimental data on this topic has recently been published (77).

Whisson et al. (23) demonstrated that the N-terminus of the P. infestans AVR3a effector, i.e. the RXLR domain, is required for translocation into potato cells, implying that this domain functions as a leader sequence that mediates host cell targeting. The RXLR domains of oomycete effectors have been reported to bind extracellular phosphatidylinositol-3-phosphate (PI3P) to mediate effector endocytosis (72) with Bhattacharjee et al (78) producing data in support of strong RXLR-PI3P binding, albeit in the Plasmodium endoplasmic reticulum, when investigating the P. infestans host translocation motif of the candidate effector NUK10. However, their experiments, alongside others by Yaeno et. al in plants (79) also led them to conclude that this binding takes place inside the pathogen and is required for stabilisation and secretion of the effector. There have also been multiple publications claiming that, contrary to the idea that an N-terminal RXLR is required for PI3P binding, it may in fact be the C-terminal domain of the effector that is responsible. Wawra et al. (80) reported C-terminal mediated PI3P binding of AVR3a from P. infestans, whilst Sun et. al. (81) found similar binding properties within the Avh5 effector of P. sojae, although the latter concluded that both regions were involved in effector entry into cells. Notably, Wawra et al (54) showed that phospholipid binding of the RXLR effector AVR3a can occur even with denatured proteins but mutants in the C-terminus of AVR3a (79), known to impair phospholipid binding, have not been assessed in this study. Our idea of a conserved host-
targeting domain within effectors continues to be challenged by these conflicting findings as to their functional relevance.

**Plant innate immunity**

Oomycete-plant interactions are characterised by molecular-coevolution with each side battling for control over the other. Plant cell membrane-resident pattern recognition receptors (PRRs) expose their PAMP recognition domains to the apoplast to detect conserved oomycete PAMPs and subsequently trigger PAMP-triggered immunity (PTI). Intracellular disease resistance proteins mediate recognition of effectors entering the host cell and elicit effector triggered immunity (ETI). Both plant immune responses aim at interfering with pathogen ingress and spread. Researchers score for pH alkalinisation, callose deposition and defence gene activation as markers for PTI. ETI responses are often concomitant with a visible controlled cell death, the hypersensitive response (HR). However, some conserved PAMPs can also trigger cell death responses such as in the case of *P. infestans* INF1 when infiltrated as protein or when expressed inside *N. benthamiana* (28).

In order to fully colonise the host a pathogen must overcome plant immunity. As reported earlier, many effector proteins have been shown to suppress PTI responses (23, 61, 65, 82), namely three tested variants of the *P. infestans* effector AVR3a suppress flg22-triggered responses when overexpressed in planta (50). One way to avoid effector overexpression and achieve more targeted application is to deliver effectors via a bacterial pathogen, such as *Pseudomonas syringae* (61). This large scale investigation of candidate oomycete effectors and their effects on PTI utilised the type III secretion system of *P. syringae* to deliver candidate effectors. Since delivering effectors using *P. syringae* is still not a flawless experimental setup - the effector protein might block secretion of other *P. syringae* type III effectors thereby reducing *P. syringae* virulence and affecting subsequent symptoms - the authors followed up by generating stable transgenic plants expressing single effectors and showing that they enhance susceptibility to *H. arabidopsisid*.

While PTI is thought to be triggered by conserved PAMPs across a range of pathogen species, ETI provides race-specific resistance, because different races of a pathogen secrete different arrays of effectors and therefore may lack, or possess variants of, the effectors necessary to trigger ETI. Again, oomycetes have developed effectors to suppress this alternative recognition principle. Examples include *P. infestans* SNE1 and the *P. sojae* effectors CRN70 and Avr1k which have all been shown to suppress R3a/AVR3a-triggered HR in *N. benthamiana* leaves (83, 84), although these transient co-expression assays are
not always fully conclusive because the effector in question may, to some extent, suppress overall gene expression, including expression of the HR reporter constructs.

R gene-mediated resistance

According to the gene-for-gene model (85), a plant will be resistant to a pathogen when it possesses a dominant R gene that is complementary to the pathogen's avirulence (Avr) gene; this is referred to as an incompatible interaction. In a compatible interaction, there is no corresponding R gene for an Avr gene (or vice versa), resulting in disease. In the years shortly after the introduction of the 'gene-for-gene' hypothesis, Black, Mastenbroek and others generated eleven potato R gene differentials (86) via introgression and named them MaR1 to MaR11. The R1, R3a and R10 genes have been extensively and successfully used in European breeding programmes and R1 and R3a cloned to investigate their functions (87). The cytoplasmic RXLR effector AVR3a of Phytophthora infestans confers avirulence on potato plants carrying the R3a gene (25). Many other cloned R genes providing resistance to important oomycetes are listed in Table 5 (along with their cognate Avr genes, if known, in brackets).

The existence of PTI and ETI responses due to perception means that in order to retain the ability to infect a host species, pathogens constantly vary their repertoire of effector molecules to avoid Avr activity. As a result, R gene-based resistance, relying on presence of singular effectors which are not essential to the pathogen's success, can be easily overcome by rapid sequence diversification or loss. This has caused problems in an agricultural context where R genes were employed to provide resistance to crop pathogens because the resistance has only been durable if the required Avr gene is essential to the pathogen's success. However, there have been various attempts to improve the chances of durability, namely, stacking multiple R genes within one variety (88), and/or using variety mixtures (89) or multilines (90), as well as engineered R genes with extended recognition spectra (91, 92). The use of variety mixtures involves sowing several varieties containing different R genes and different parental backgrounds together in the same field. Multilines contain lines of the same variety but with different combinations of R genes, thereby creating a mosaic and preventing take-over of the field by a single pathogen isolate.

Identifying effectors which are required to maintain full pathogen virulence can aid the search for cognate disease resistance genes in wild varieties of host crop plant species (93). Several oomycete effectors have been shown to contribute to pathogen virulence. Variation in copy number of P. sojae Avr1 and Avr3a (94) as well as knock-down of
transcript levels of Avr3a (49), PsAvh172, PsAvh238 (95), PsAvr3b (96), PsCRN63 and PsCRN115 (97) negatively impact on virulence.

S gene-mediated resistance

All plant genes that facilitate infection and support compatibility can be considered susceptibility (S) genes. Mutation or loss of an S gene thus reduces the ability of the pathogen to cause disease. This can result in pathogen-specific resistance if the gene is involved in production of a component required for host penetration, or broad-spectrum resistance if the gene suppresses constitutive defences. The concept of susceptibility genes was first explored in 2002 (98) after the identification of PMR6 (powdery mildew resistance 6) in Arabidopsis (99). S genes that have been identified as susceptibility factors for colonisation by important oomycetes are included in Table 5. S genes can be classified into three groups based on the point at which they act during infection; early pathogen establishment, modulation of host defences and pathogen sustenance.

Early pathogen establishment: The Medicago truncatula mutant ram2 has altered cutin composition, a key component of the plant cuticle, due to a mutation in a gene encoding a cutin biosynthesis enzyme, glycerol-3-phosphate acyl transferase. ram2 mutants display reduced susceptibility to Phytophthora palmivora with significant disruption of appressoria formation (100). This example, together with others in plant-fungus interactions, implies that the leaf cuticle provides essential developmental cues for pathogenicity (101-103). Proteins involved in controlling cytoskeleton dynamics and vesicle trafficking, such as GTPase-activating proteins (GAP), also appear to be key susceptibility factors. For example, an ARF-GAP protein, AGD5, of A. thaliana has recently been found to be a susceptibility factor for H. arabidopsidis infection (104). It may be that rearrangements of the cytoskeleton, mediated by AGD5, ensure susceptibility to the adapted pathogen H. arabidopsidis.

Modulation of host defences: Although callose deposition is primarily an induced defence response that occurs at sites where the pathogen attempts to penetrate, providing a physical barrier to entry, it has also been implicated in suppression of PTI. Overexpression of PMR4 leads to increased callose deposition and is associated with complete resistance in A. thaliana to the non-adapted fungal pathogen Blumeria graminis (105). Surprisingly, a mutation causing loss-of-function of PMR4 also provides resistance to B. graminis, as well as the oomycete H. arabidopsidis, but via a different mechanism. The mechanism by which PMR4 acts as a susceptibility gene seems to lie in suppression of salicylic acid signalling which causes a moderate increase in defence gene expression (105).
A. thaliana plants are less susceptible to H. arabidopsidis in the absence of the gene IOS1 (impaired oomycete susceptibility) encoding a malectin-like, leucine-rich repeat receptor-like kinase (106). In support of this finding it appears that transcription of IOS1 promotes susceptibility and is localised to the area surrounding penetration by H. arabidopsidis, suggesting that it may either be a residual PAMP-triggered response, or a component of a defence mechanism that has been interfered with by the oomycete to benefit infection. In ios1 mutants PTI-responsive genes were delayed in their induction upon infection with H. arabidopsidis but their expression levels were increased, implying that IOS1 negatively regulates the activation of PTI responses, possibly through involvement in FLS2/BAK1 protein complex formation (107).

The mitogen-activated protein kinase 4 (MPK4) gene acts downstream of immune receptors to regulate the transduction of extracellular stimuli into adaptive, intracellular responses and has been found to act as a negative regulator of these defence responses (108). Silencing of MPK4 in Glycine max (soybean) leads to enhanced resistance to the downy mildew Peronospora manshurica (109). Suggestions have been made that GmMPK4 silencing causes increased lignin biosynthesis, which may indirectly provide a physical barrier at the epidermal cells such that the oomycete cannot penetrate into the mesophyll. Further evidence for the role of MPK4 as a susceptibility gene lies in a complex of BAK1/BRI1 (BRI1 associated receptor kinase 1, brassinosteroid insensitive 1), which is required for the activation of MPK4 (110). BRI1 was found to associate with BAK1 in vivo and both components appear to work cooperatively to negatively regulate cell death and defence responses to H. parasitica. The majority of susceptibility genes were identified through study of interactions between plants and H. arabidopsidis, and H. parasitica. Many of these S genes function in defence suppression (mutant plants exhibiting constitutive defence responses) that leads to dwarf phenotypes or developmental defects. However, there are some S genes for which mutant plants exhibit no significant dwarf phenotype and show no developmental defects. These include a number of genes encoding negative regulators of defence responses such as PTI, salicylic acid signalling and/or SAR (systemic acquired resistance), for example, ‘plant U-box E3 ubiquitin ligases’ (PUB22/23/24) and ‘suppressor of nim1-1’ (SON1) which are involved in ubiquitination and protein degradation (111, 112).

Other negative regulators of defence include ‘enhanced disease resistance 2’ (EDR2), ‘suppressor of npr1-1 inducible 1’ (SN1) and ‘constitutive defence without defect in growth and development 1’ (Cdd1) (113-116).

Pathogen sustenance: A. thaliana mutants have also been identified which display loss of susceptibility to H. arabidopsidis due to perturbations in enzymes that function in amino acid metabolism. For example, dmr1 carries a mutation in a gene encoding homoserine kinase,
an enzyme catalyst of the synthesis pathway for Met, Thr and Ile (117). When the activity of homoserine kinase is fully knocked out, the effect is lethal, but knockdown provides resistance to *H. arabidopsidis*. Other mutants, *rsp1* and *rsp2*, have disrupted aspartate kinase function which is again important for Met, Thr and Ile synthesis, but also for Lys. In an attempt to elucidate the mechanism of reduced susceptibility in these mutants, Thr and homoserine were applied exogenously, which resulted in reduced *H. arabidopsidis* conidiphore formation (118). This supports the hypothesis that metabolites downstream of, or induced by, Thr and homoserine are toxic to the oomycete. The availability of each of these amino acids has also been implicated in the induction of resistance (117, 118).

**Future directions for developing oomycete-resistant plants**

Strategies to tackle economic losses caused by oomycete pathogens are numerous and diverse in their approaches, but three main areas could be seen as having the greatest potential for success in the near future – tactical deployment of natural or engineered R genes, S gene knockouts/mutations and transgenic hairpin RNA silencing of essential pathogen transcripts.

Applying the R gene hypothesis to breeding for resistance leads to only short-lived success, being overcome quickly by the pathogen as it varies its effector repertoire. Identifying and accurately screening for new R genes using molecular markers is laborious, expensive, and sometimes problematic due to epistatic interactions between resistance genes. An alternative to marker-assisted screens for identification of novel R proteins are effector-based, high throughput, *in planta* expression assays (119). If combined with plant disease epidemiology studies and comparative genomics these expression assays could aid prioritisation of effectors present in emerging virulent strains as well as those abundant in numerous other isolates (120).

Only in the last few years have researchers begun to adopt structural biology to fully investigate functional relationships between interacting pathogen and plant proteins (121). Knowledge of how immune receptors function on a molecular level has already begun to fuel development of engineered receptors that detect a broader range of oomycete effectors (91, 92). The function of an R gene and its specificity for a given effector can also be validated via transient co-expression with effectors in plants that do not carry the candidate resistance gene. Once identified these R genes must be carefully applied in the field so as to extend the durability of the resistance they provide though techniques such as R gene stacking, variety mixtures or multilines. However, these techniques have their limitations when it
comes to implementation in a large scale agricultural context. Once stably engineered R
proteins with extended recognition spectra (91, 92) have been shown to perform well in the
field they may provide alternative solutions.

A second approach aims at removing key plant genes required for the infection. These S
gene mutation-based resistance mechanisms should provide much greater durability than R
genes because they involve a component that is essential for pathogen survival. Many of the
S genes identified to date in plant-oomycete interactions have been found through study of
model species A. thaliana-infecting downy mildews. There are, however, S genes that show
promise as a means to provide resistance to more economically significant oomycetes, for
example, ram2-mediated resistance to Phytophthora palmivora and Aphanomyces euteiches
spp. (100, 122).

The large majority of S genes are unfortunately involved in essential plant processes, which
constitutes a significant downside to their use in a disease resistance context. Knockouts of
some S genes, namely DMR1, are expected to result in lethal phenotypes (117). Mutation of
RAM2 in M. truncatula results in altered water permeability of the seed coat which might
affect its shelf life (100). For such S genes to be useful agriculturally therefore, different
alleles must be identified that encode proteins with reduced, but not fully abolished, activity.
To achieve this, “artificial evolution”, i.e. targeted mutagenesis, or assessment of natural
variation using haplotype-specific markers (123) could be applied.

Alongside discovering novel susceptibility gene alleles, it is important to combine this
research with a greater understanding of oomycete pathogenicity mechanisms. A number of
oomycete genomes have been sequenced to date (Table 1, including H. arabidopsidis, P.
ultimum, P. infestans, P. ramorum P. sojae and P. capsici (2, 124-126). The four
Phytophthora species here are all hemibiotrophs and therefore can be cultured in vitro,
making them more amenable to transformation and gene disruption. As a result these
species will, in the future, serve as tools to discover more about how oomycetes interact with
their hosts and, ultimately, which genes encode effectors, resistance proteins or
susceptibility proteins.

A third strategy, termed host-induced gene silencing, is based on transgenic plants, which
produce hairpin RNA constructs targeting pathogen transcripts essential for virulence. This
principle has been demonstrated to work in fungi and accumulating evidence suggests its
transferability to Phytophthora and Bremia (127-129)
Potential for comparative pathogen-mutualist studies

Our growing knowledge of oomycete interactions with plants opens up exciting possibilities to investigate the commonalities and differences between pathogenic and mutualistic lifestyles. For example, the important model legume species *Medicago truncatula* is able to be colonised by both arbuscular mycorrhizal fungi, such as *Rhizophagus irregularis*, as well as the oomycete pathogens *Aphanomyces euteiches* and *P. palmivora* (130). The advantage of a common host species for these distinct groups of filamentous microorganisms is the ability to genetically dissect common and contrasting elements required for their colonisation processes. Oomycete pathogens and mutualists share similarities with respect to intracellular structures in plants, i.e. they both feature host cell plasma membrane invaginations (haustoria and arbuscules, respectively, Figure 2), driven by the invading microbes, which penetrate the cell wall and then become surrounded by a specialised membrane (termed extra haustorial membrane and periarbuscular membrane, respectively, (131)). Whether arbuscules are translocation sites of the recently identified SP7 (132), or other effectors of arbuscular mycorrhiza fungi, remains to be clarified. In a recent publication by Rey et. al. (133), genetic elements of the common symbiosis signalling pathway required for arbuscule formation in *M. truncatula*, were found to have no functional overlap with the formation of *P. palmivora* haustoria, indicating that different mechanisms are operating during their formation. Common elements found in both mutualistic and pathogenic interface membrane formation are v-SNAREs of the VAMP72 family involved in exocytotic vesicle trafficking (134). Furthermore, marker localisation studies at oomycete haustoria suggest that rerouting of vacuolar-targeted late endosomal compartments, labelled by the small Rab7 type GTPase RabG3c, seems to contribute to extrahaustorial membrane formation (135). Notably, the corresponding Medicago Rab7a2 can be found in the cytoplasm of arbuscle containing root cells (136). It thus would be important to study distribution of this and other markers in a more comparative way using the same plant tissue for haustoria and arbuscules.

Summary

Considering the continued negative impact of oomycetes on agriculture, understanding their biology is imperative to reveal new strategies for their control. It is exciting to see that oomycete research is in full bloom and that the numbers of genetic, genomic and cell biology resources are continuously growing. Comparative studies with unrelated microbes that share colonisation strategies should enable us to extend our range of applicable resistance principles whilst maintaining the agronomic benefits of mutualist fungi.
Acknowledgements

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<thead>
<tr>
<th>Species</th>
<th>Genome size [Mb]</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Order Peronosporales</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Albugo laibachii</em></td>
<td>37.0</td>
<td>(137)</td>
</tr>
<tr>
<td><em>Albugo candida</em></td>
<td>45.3</td>
<td>(184)</td>
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<td><em>Bremia lactucae</em></td>
<td>Transcriptome only</td>
<td><a href="http://web.science.uu.nl/pmi/data/bremia/">http://web.science.uu.nl/pmi/data/bremia/</a></td>
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<tr>
<td><em>Hyaloperonospora arabidopsisis</em></td>
<td>81.6</td>
<td>(125)</td>
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<tr>
<td><em>Phytophthora cactorum</em></td>
<td>Transcriptome only</td>
<td>(185)</td>
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<td><em>Phytophthora capsici</em></td>
<td>64.0</td>
<td>(139)</td>
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<tr>
<td><em>Phytophthora cinnamomi</em></td>
<td>78.0</td>
<td><a href="http://genome.jgi.doe.gov/Phyci1/Phyci1.home.html">http://genome.jgi.doe.gov/Phyci1/Phyci1.home.html</a></td>
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<td><em>Phytophthora infestans</em></td>
<td>240.0</td>
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<td><em>Phytophthora ipomeae</em></td>
<td>Alignment to <em>P. infestans</em></td>
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<td><em>Phytophthora fragariae var. fragariae</em></td>
<td>73.6</td>
<td>(187)</td>
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<td><em>Phytophthora mirabilis</em></td>
<td>Alignment to <em>P. infestans</em></td>
<td>(186)</td>
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<td><em>Phytophthora parasitica</em></td>
<td>82.4</td>
<td><em>Phytophthora parasitica</em> Assembly Dev Initiative, Broad Institute (broadinstitute.org)</td>
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<tr>
<td><em>Phytophthora phaseoli</em></td>
<td>-</td>
<td>(186)</td>
</tr>
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<td><em>Phytophthora ramorum</em></td>
<td>65.0</td>
<td>(126)</td>
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<td><em>Phytophthora sojae</em></td>
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<td>(126)</td>
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<td><em>Pseudoperonospora cubensis</em></td>
<td>Transcriptome only</td>
<td>(140)</td>
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<td><strong>Order Pythiales</strong></td>
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<tr>
<td><em>Pythium ultimum</em></td>
<td>42.8</td>
<td>(2)</td>
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<tr>
<td>Species</td>
<td>Lifestyle</td>
<td>Hosts (organ)</td>
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<tr>
<td>------------------------------</td>
<td>-----------</td>
<td>-------------------------------------------------------------------------------</td>
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<tr>
<td>Albugo candida</td>
<td>B</td>
<td><em>Arabidopsis thaliana</em> and other <em>Brassicaceae</em> (leaves)</td>
</tr>
<tr>
<td>Aphanomyces euteiches</td>
<td>B</td>
<td><em>Medicago truncatula</em>, <em>Pisum sativum</em>, <em>Medicago sativa</em> (roots)</td>
</tr>
<tr>
<td>Hyaloperonospora arabidopsidis</td>
<td>B</td>
<td><em>Arabidopsis thaliana</em> (leaves)</td>
</tr>
<tr>
<td>Hyaloperonospora parasitica</td>
<td>B</td>
<td><em>Capsella bursa-pastoris</em> and <em>Brassicaceae</em> including <em>Arabidopsis thaliana</em> (leaves)</td>
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<td>Peronospora manshurica</td>
<td>B</td>
<td><em>Glycine max</em> (leaves)</td>
</tr>
<tr>
<td>Plasmopara viticola</td>
<td>B</td>
<td><em>Vitis spp</em> (leaves)</td>
</tr>
<tr>
<td>Phytophthora cinnamomi</td>
<td>HB</td>
<td>Very broad range: inc. most annual and herbaceous perennial species (roots)</td>
</tr>
<tr>
<td>Phytophthora capsici</td>
<td>HB</td>
<td><em>Capsicum annuum</em>, members of <em>Cucurbitaceae</em>, <em>Fabaceae</em>, and <em>Solanaceae</em> (stems and fruit)</td>
</tr>
<tr>
<td>Phytophthora infestans</td>
<td>HB</td>
<td>Potato, tomato, wild tobaccos (shoots)</td>
</tr>
<tr>
<td>Phytophthora palmivora</td>
<td>HB</td>
<td>Very broad range: inc. palm and fruit tree species, <em>Medicago truncatula</em>, <em>Nicotiana benthamiana</em> (roots, trunks,</td>
</tr>
<tr>
<td>Name</td>
<td>Type</td>
<td>Plant receptor</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>INF1</td>
<td>Protein, sterol-binding</td>
<td>BAK1/SERK3-dependent ELR</td>
</tr>
<tr>
<td>OPEL</td>
<td>Protein</td>
<td>Unknown monomeric 100 kDa integral plasma membrane</td>
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<tr>
<td>CBEL</td>
<td>Protein</td>
<td>Unknown, but cellulose-dependent</td>
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<tr>
<td>Pep-13</td>
<td>Peptide</td>
<td>Unknown</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>Unsaturated fatty acids</td>
<td>Unknown</td>
</tr>
<tr>
<td>Beta-glucans</td>
<td>Carbohydrate</td>
<td>Glucan-dependent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CEBiP CERK1</td>
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</table>

Table 3. Examples of known oomycete elicitors

<table>
<thead>
<tr>
<th>Effector (Oomycete species)</th>
<th>Known host target(s)</th>
<th>Virulence effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVR3a (P. infestans)</td>
<td>Stabilisation of potato CMPG1</td>
<td>When overexpressed in N. benthamiana, suppresses perception of INF1, attenuates flg22 and INF1-triggered ROS accumulation.</td>
<td>(49)</td>
</tr>
<tr>
<td></td>
<td>Interaction with Nicotiana benthamiana</td>
<td></td>
<td>(50)</td>
</tr>
<tr>
<td></td>
<td>Dynamin-Related Protein 2 (DRP2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PexRD2 (P. infestans)</td>
<td>Interaction with the kinase domain of potato MAPKKε</td>
<td>Suppressor of cell death triggered by MAPKKε: signalling pathway. When overexpressed, increases susceptibility of N. benthamiana to P. infestans</td>
<td>(51)</td>
</tr>
<tr>
<td>Gene Name</td>
<td>Description</td>
<td>Function</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td>AVRblb2 (P. infestans)</td>
<td>Associates with papain-like cysteine protease C14 from N. benthamiana and tomato</td>
<td>Prevents secretion of the plant defence protease C14 in N. benthamiana and tomato. When overexpressed, enhanced susceptibility of N. benthamiana plants to P. infestans</td>
<td>(52)</td>
</tr>
<tr>
<td>Pi03192 (P. infestans)</td>
<td>Interaction with the potato transcription factors NAC Targeted by Phytophthora (NT P) 1 and NTP2</td>
<td>Prevention of relocalisation of NTP1 and 2 from the ER to the nucleus, that appears to be key for immunity. Silencing of NTP1 or NTP2 in N. benthamiana increases susceptibility to P. infestans</td>
<td>(55)</td>
</tr>
<tr>
<td>HaRxL44 (H. arabidopsidis)</td>
<td>Degradation of Arabidopsis Mediator subunit 19a (MED19a), a mediator in the interaction between transcriptional regulators and RNA polymerase II</td>
<td>Attenuates salicylic acid–triggered immunity in Arabidopsis, shifting the balance of defence transcription to JA/ET-signalling</td>
<td>(53)</td>
</tr>
<tr>
<td>PsCRN63 (P. sojae)</td>
<td>Direct interaction with catalases from N. benthamiana (NbCAT1) and Glycine max (GmCAT1)</td>
<td>When overexpressed, cell death and accumulation of H2O2 in N. benthamiana leaves</td>
<td></td>
</tr>
<tr>
<td>PsCRN115 (P. sojae)</td>
<td>Interaction with Arabidopsis PINP1 helicase domain containing protein. Inhibition of the biogenesis of small RNAs</td>
<td>When coexpressed with PsCRN63, suppression of cell death and H2O2 accumulation in N. benthamiana leaves; suggested to suppress cell death by inhibiting PsCRN63-induced effects</td>
<td>(54)</td>
</tr>
<tr>
<td>PSR1 (P. sojae)</td>
<td>Interaction with Arabidopsis PINP1 helicase domain containing protein. Inhibition of the biogenesis of small RNAs</td>
<td>When overexpressed, enhanced susceptibility of N. benthamiana to Potato Virus X and P. infestans</td>
<td>(56)</td>
</tr>
<tr>
<td>PSR2 (P. sojae)</td>
<td>Target unknown. Inhibition of the biogenesis of small RNAs</td>
<td>When overexpressed, enhanced susceptibility of Arabidopsis to P. capsici</td>
<td>(57)</td>
</tr>
<tr>
<td>PsIsc1 (P. sojae)</td>
<td>Hydrolyses isochorismate (the direct precursor of salicylic acid)</td>
<td>Disruption of salicylate metabolism pathway. Suppression of salicylate-mediated innate immunity in N. benthamiana.</td>
<td>(62)</td>
</tr>
</tbody>
</table>

Table 5. Cloned resistance (R) and susceptibility (S) genes affecting oomycete plant interactions
<table>
<thead>
<tr>
<th>Species</th>
<th>Cloned R genes (cognate Avr genes)</th>
<th>Cloned S genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Albugo candida</em></td>
<td>Arabidopsis WRR4 (146)</td>
<td>Arabidopsis AGD5 (104), IOS1 (106), PUB22/23/24 (154, 155), SON1 (112), EDR2 (113, 114), SNI1 (115, 156), Cdd1 (116), DMR1 (117, 157), RSP1/2 (118), PMR4 (158), DMR6 (188, 189)</td>
</tr>
<tr>
<td><em>Hyaloperonospora arabidopsis</em></td>
<td>Arabidopsis RPP1 (147) (ATR1) (148, 149), RPP2 (150), RPP4 (150), RPP5 (151), RPP7 (150), RPP8 (152), RPP13 (153) (ATR13) (149),...</td>
<td></td>
</tr>
<tr>
<td><em>Peronospora manshurica</em></td>
<td>Soybean Rpm (159)</td>
<td>MPK4 (108, 109)</td>
</tr>
<tr>
<td><em>Phytophthora cinnamomi</em></td>
<td>Arabidopsis TIR1 (160)</td>
<td></td>
</tr>
<tr>
<td><em>Phytophthora infestans</em></td>
<td>Potato R1 (87, 161), R2 (162, 163) (AVR2) (163, 164), R3a (165) (Avr3a) (166), R3b (167) (Avr3b) (96), R4 and (AVR4) (168) (169), R6 and R7 (170), R10 and R11 (171), RB/Rpi-Bib1 (172, 173) (Avr-Bib1/IPI-O1) (119), Rpi-Bib2 (174), Ph-3 (175), Rpi-vnt1 (176), Rpi-bib3 (162), Rpi-abpt (162)</td>
<td>StREM1.3 and N. benthamiana REM1.3 orthologs (177)</td>
</tr>
<tr>
<td><em>Phytophthora palmivora</em></td>
<td></td>
<td>Medicago RAM2 (100), LATD (133)</td>
</tr>
<tr>
<td><em>Phytophthora sojae</em></td>
<td>Soybean Rps1d and (Avr1d) (178), Rps1b and (AVR1b) (179)</td>
<td></td>
</tr>
<tr>
<td><em>Plasmopara viticola</em></td>
<td>Grape Rpv1 Rpv2 (180), Rpv3 (181) (avrRpv3) (182), Rpv10 (183)</td>
<td></td>
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</table>
Figure 1. Infection strategies and lifestyles of selected oomycetes.
Figure 2 – Filamentous plant microbe interfaces and membrane barriers for effector translocation.
**Figure Legends**

**Figure 1.** Infection strategies and lifestyles of selected oomycetes.

(a) Typical asexual *Phytophthora* dispersal structures (b) leaf colonisation (c) root colonisation. Two alternative methods of germination (direct germination from deciduous sporangia or indirect germination from zoospores) are depicted. Other alternative germination strategies are not displayed. Following germination, depending on the species, oomycetes perform **Biotrophy**, e.g. *Hyaloperonospora arabidopsidis* or *Albugo laibachii*, the latter often entering through stomata and then forming appressoria, **Necrotrophy**, e.g. *Pythium ultimum*, or **Hemibiotrophy**, e.g. *Phytophthora sojae* or *Phytophthora palmivora*. Notably, oomycete entry occurs through epidermal cells or between cells. Cells which have been colonised by a biotrophic pathogen are highlighted in yellow, whilst those that are undergoing cell death as a result of necrotrophy are shaded grey. In the case of a hemibiotrophic oomycete colonising a root, the interaction is initially biotrophic whilst the oomycete spreads through the cortex, but once established, and hyphae have entered the endodermis and vasculature, necrotrophy can be observed.

**Figure 2 – Filamentous plant microbe interfaces and membrane barriers for effector translocation.**

Haustoria (a) and arbuscules (b) both represent invaginations of the plant cell protoplast caused by microbial ingrowth. Both are surrounded by specialised membranes termed extrahaustorial membrane (EHM) or periarbuscular membrane (PAM), labelled in red. Cytoplasmic effectors have to pass several membrane barriers (c). Originating in the pathogen cytosol (1.), effectors are thought to be secreted across the pathogen cell wall (2.) either into the space adjacent to the plant cell wall or into the extrahaustorial matrix/periarbuscular matrix (EHM/PAM). The EHM/PAM is an environment that may be modified by other pathogen-secreted molecules to stabilise the effector protein, or alternatively, contain host plant proteases which target effectors for hydrolysis. Some plant membrane molecules may act as receptors for effectors, assisting their transport to the host cell whilst effectors themselves may interact to aid translocation into the host cytosol. Movement across the host plasma membrane may or may not involve first crossing the plant cell wall (3a. and 3b. respectively) depending on where an effector is secreted from the microbe. This movement may occur either by endocytosis or via a translocon (pathogen-specific translocation mechanism). Focal host defence responses may inhibit the entry of effectors, whilst pathogen factors may prepare host cells for their uptake.
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**Biographical Text**

**Stuart Fawke** obtained a degree in Natural Sciences (2012) from the University of Birmingham and an MSc (2013) in Plant Genetics from the University of East Anglia, Norwich with a research project at The John Innes Centre. He is currently a Ph.D. student at the University of Cambridge, where he studies the role of cutin biosynthesis enzymes in plant-microbe interactions and development.

**Mehdi Doumane** obtained a BSc in Biology (2013) from the École Normale Supérieure (ENS) de Lyon, France. He is currently a MSc student at the École Normale Supérieure (ENS) de Lyon. In 2014 he undertook a four-month internship at the University of Cambridge, Sainsbury Laboratory in the group of Sebastian Schornack where he studied *Phytophthora*-plant interactions.

**Sebastian Schornack** obtained a diploma in genetics (2000) and a Ph.D. in Plant Genetics (2006) from Martin-Luther University Halle-Wittenberg, Germany, working on *Xanthomonas* TAL effectors and disease resistance genes and continued there for postdoctoral research with Prof. Thomas Lahaye, discovering the TAL effector code (2007). He then moved to the UK to for a postdoc with Prof. Sophien Kamoun at The Sainsbury Laboratory, Norwich (2008-2012), working on *Phytophthora infestans* effectors. In 2013 he moved to the University of Cambridge, where he is currently a Gatsby Group Leader and Royal Society UR Fellow in the Sainsbury Laboratory (SLCU). His main interests are common and contrasting principles of plant colonisation by pathogenic and beneficial filamentous microbes.