Fungal and oomycete effectors – strategies to subdue a host

SHAWKAT ALI1,2 AND GUUS BAKKEREN1

1Agriculture and Agri-Food Canada, Pacific Agri-Food Research Center, Summerland, BC V0H 1Z0, Canada
2Department of Botany, University of British Columbia, Vancouver, BC V6T 1Z4, Canada; current address: Horticulture R&D Center, Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu Québec J3B 3E6, Canada

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Abstract: Molecular studies focusing on the interface between microbes and plant hosts have provided major insights into the basis underlying pathogenesis, symbiosis and plant defence and resistance mechanisms. A more recent focus on microbes, facilitated by the generation of complete genome sequences, has uncovered the sheer number of protein effectors microbes deliver in this interface as well as inside host cells to manipulate the plant immune system. Although studies on the characterization and roles of bacterial effectors are further advanced, in this review we focus on the current knowledge of fungal and oomycete effectors and their roles. Examples are given of effectors disarming plant defence enzymes, such as the apoplastic effector AVR2 from Cladosporium fulvum which inhibits the tomato defence cysteine protease. Other effectors interfere with the perception by the host of microbes exposing molecular determinants such as Phytophthora infestans INF1 protein. Many effectors alter gene expression induced by the host during defence, exemplified in fungi by Ustilago maydis Pit2 suppressing maize defence genes. Effectors recognized by resistance gene products, either directly or indirectly, and eliciting defence, represent the classical avirulence genes and almost 50 have now been cloned from fungi and oomycetes. Evolutionary adaptations and arms races have produced diversification in both pathogen and host, and in pathogens, are the cause of breaking crop resistance in agricultural settings. Molecular insight provides valuable information for applications. For example, some effectors are crucial for pathogenesis, thereby revealing targets for disease control and others interact with host resistance gene products and could be used to screen germplasm for novel sources of disease resistance. Variation among effectors will likely yield diagnostic tools for pathogen race identification. The study of model systems is providing insight into avenues by which other, major plant diseases can potentially be controlled.

Keywords: avirulence genes, disease resistance, effector proteins, microbe–plant interactions

Résumé: Les études moléculaires ciblant l’interface entre les microorganismes et les plantes hôtes ont ouvert de nouvelles perspectives sur les bases sous-jacentes de la pathogenèse, de la symbiose ainsi que des mécanismes de défenses et de résistance. Une nouvelle approche des microorganismes, facilitée par la génération de séquences entières de génomes, a permis de découvrir l’importance du nombre de protéines effectrices que les microorganismes sécrètent dans cette interface ainsi que dans les cellules hôtes afin de modifier le système immunitaire de la plante. Bien que les études sur la caractérisation et les rôles des effecteurs microbiens soient avancées, dans cet exposé, nous mettons l’accent sur les connaissances actuelles relatives aux effecteurs fongiques et à ceux des oomycètes, ainsi que sur leurs rôles. On y présente des exemples d’effecteurs neutralisant les enzymes de défense de plantes, comme l’effecteur apoplastique AVR2 de Cladosporium fulvum qui inhibe chez la tomate les mécanismes de défense dépendant de la protéase à cystéine. D’autres effecteurs modifient la perception que l’hôte a de microorganismes exposant des déterminants moléculaires comme la protéine INF1 de Phytophthora infestans. Un grand nombre d’effecteurs altèrent l’expression du gène induite durant la phase de défense, phénomène illustré chez les champignons par la protéine Pit-2 d’Ustilago maydis qui inactive les gènes de défense du maïs. Les effecteurs reconnus, directement ou indirectement, par les produits géniques de résistance suscitant la défense sont les gènes classiques d’avirulence et presque 50 ont à ce jour été clonés à partir de champignons et d’oomycètes. Les adaptations évolutives et les « courses aux armements » ont induit la diversification tant chez l’hôte que chez les agents pathogènes et, chez ces derniers, elles sont la cause de l’effondrement de la résistance des cultures commerciales. Les perspectives moléculaires fournissent des données précieuses en vue d’applications utiles. Par exemple, certains effecteurs sont essentiels à la
pathogenèse, déterminant de ce fait des cibles de lutte contre les maladies des plantes; d’autres interagissent avec les produits géniques de la résistance de l’hôte et pourraient être utilisés pour analyser les germoplasmes afin d’y décéler de nouvelles sources de résistance aux maladies. La variation observée chez les effecteurs contribuera de toute évidence au développement d’outils de diagnostic permettant l’identification de races pathogènes. L’étude de modèles ouvré de nouvelles voies grâce auxquelles d’autres maladies importantes pourront être traitées.

**Mots clés:** gènes d’avirulence, interactions microorganismes plantes, protéines effectrices, résistance aux maladies

**Introduction**

Among the myriad of microbes larger organisms encounter, there are a few that have adapted to overcome the many barriers and defence mechanisms of these organisms to become pathogens. The more tools such pathogens have at their disposal, the more virulent they are on a given host. Over the last 10 years, tremendous progress has been made in the field of plant–microbe interactions and the discovery of many pathogen and host genes involved in these interactions has provided some insight into the molecular mechanisms that decide the outcome of such encounters i.e. plant disease or host defence and resistance. Pathogens flood their hosts with numerous proteins and compounds to counteract the defence and ensure proliferation. The host, on its part, resists and the uncovering of the increasingly astonishing ways each interacting organism tries to decide the outcome of this delicate balance in their favour, promises novel strategies to help plants boost their defence responses. Although the focus of this paper is on fungal and oomycete effectors, many recent discoveries into their roles have emerged from work on bacterial systems, some of which will be highlighted because they might direct research on fungal and oomycete effectors.

**Strategies used by pathogens to infect host plants**

Pathogens use different strategies to enter their host plants. Fungi and oomycetes enter either through natural openings or directly through the plant epidermal cells by mechanical and chemical means, or expand their hyphae on the top of, within or between the plant cells (Jones & Dangl, 2006). Pathogens need to adhere to the plant before penetrating the plant cuticle. Fungal hyphae and spores use mucilaginous and adhesive substances at their tips, and the intermolecular forces between plant and pathogen are responsible for the close contact. Most rust fungi enter plants through stomata by developing appressoria over the stomata to penetrate into the cavity below, while ascomycetes such as the rice blast fungus *Magnaporthe grisea* and the powdery mildews, such as *Blumeria graminis* f. sp. *hordei* (*Bgh*), penetrate the cuticle of plants directly by means of an appressorium. For direct penetration of the cuticle, *M. grisea* uses turgor pressure in its melanized appressorium while *Bgh* penetrates cell walls by the combined activity of cellulases and turgor pressure (Pryce-Jones et al., 1999; Talbot, 2003). Other pathogens secrete cell wall-degrading enzymes, such as cutinases, cellulases, pectinases and wax degrading enzymes for penetration of their host. After gaining entrance to the host plants, pathogens require additional ‘tools’ to neutralize the defence reaction of the host and to gain access to nutrients.

**Resistance in plants to pathogens**

Resistance in plants to pathogens is often categorized in three types; nonhost, race-nonspecific and race-specific resistance. Nonhost resistance is defined by the resistance of an entire plant species against a specific non-adapted pathogenic microbe and is the most common and durable form of disease resistance exhibited by plants. It is the result of both preformed and inducible defence mechanisms and seems to be under complex genetic control which can involve multiple defence factors that individually may segregate within host species without compromising overall resistance (Heath, 2002; Mysore & Ryu, 2004). Race-nonspecific resistance is known as general, quantitative or partial resistance of a plant against a species of an adapted pathogen. It is generally durable and is mostly controlled by genes with incremental/additive effects, such as combinations of wheat leaf rust resistance gene *Lr34* and several ‘slow-rusting’ genes (Singh & Rajaram, 1992). Race-specific resistance is qualitative, usually controlled by dominant avirulence (*Avr*) genes in the pathogen and corresponding dominant resistance (*R*) genes in a specific plant genotype or cultivar of an otherwise susceptible host species. This resistance is of the ‘gene-for-gene interaction’ type and is often easily overcome by the pathogen.

Preformed physical barriers can include waxy cuticular surface layers of the leaves and thick and stable cell walls that most microbes are not equipped to penetrate. Even after successful penetration, the pathogen needs to overcome the biochemical barriers that include low pH of the apoplast, broad-spectrum antimicrobial compounds and ‘defence’ enzymes that degrade microbial cell walls
Cell wall components such as actin microfilaments play an important role in defence against fungal penetration and whose disruption results in loss of nonhost resistance against several nonhost fungi (Kobayashi et al., 1997; Heath, 2002; Mysore & Ryu, 2004). Biochemical barriers consist of many peptides, proteins and non-proteinaceous secondary antimicrobial metabolites which may determine the host range of some pathogens (Broekaert et al., 1995; Morrissey & Osbourn, 1999), and fungitoxic substances on the surface of leaves in sufficient concentration to inhibit the germination of spores (Agrios, 2005). Phytoanticipins, preformed antimicrobial compounds such as tannins and fatty acid-like dienes, often present in high concentration in cells of young fruits, leaves or seeds, or saponins, are responsible for resistance to pathogens that lack the corresponding detoxifying enzymes (Osbourn et al., 1996; Pedras & Ahiahonu, 2005). Other classes of antimicrobial compounds are proteases, such as cysteine proteases secreted into the apoplast by tomato and potato (Lucas, 1998; Kruger et al., 2002) and protease inhibitors, such as a cysteine protease inhibitor produced by pearl millet (Joshi et al., 1999).

Some of the above-mentioned defence systems are preformed or could be kicked into higher gear, or represent inducible mechanisms only activated in the host when attacked. Examples are induced physical defences, such as the formation of cell wall deposits (papilla) directly under the penetration sites of pathogens; this can stop up to 90% of the penetrations (papilla) directly under the penetration sites of pathogens (Collins et al., 2003). The production of phytoalexins is often induced upon attack (Hammerschmidt, 1999). Pathogenesis-related (PR) defence proteins such as chitinases and β-1,3 glucanase, (per-)oxidases, proteinase inhibitors, thionins and lipid-transfer proteins, are induced in response to pathogen attack and the accumulation of these proteins is usually associated with the acquisition of systemic resistance in plants against a wide range of other pathogens. These are specific families of proteins, currently in 17 classes (van Loon et al., 2006) although a larger group of “inducible defence-related proteins”, including phenylalanine ammonia lyase, one of the key enzymes involved in the synthesis of aromatic compounds, like phytoalexins, is often included in the PR proteins.

Pathogen-associated molecular patterns

In addition to preformed and inducible physical and biochemical barriers, plants also have evolved a surveillance system to recognize various pathogen surface-exposed and cytoplasmic molecules known as pathogen (microbe) associated molecular patterns: PAMPs or MAMPs (Shiu & Bleecker, 2003). These are highly conserved molecules of microbes that are perceived by host receptors (PAMP recognition receptors or PRRs) at an early stage of infection. Recognition results in induction of PAMP-triggered immunity (PTI). Examples of surface-exposed PAMPs that have been shown to be capable of triggering PTI are bacterial flagellin (Felix et al., 1999), lipopolysaccharides (LPS; Meyer et al., 2001; Erbs & Newman, 2003), lipooligosaccharides from Gram-negative bacteria, chitin from cell walls of higher fungi (Bartnicki-Garcia, 1968; Ren & West, 1992), invertase from Saccharomyces cerevisiae (Basse et al., 1992), and 1,3-1,6-hepta-β-glucoside from the cell walls of Phytophthora (Sharp et al., 1984a, 1984b). Examples of cytoplasmic PAMPs that induce host defence are cold shock protein (CSP) and elongation factor Tu (Ef-Tu; Felix & Boller, 2003; Kunze et al., 2004).

Pathogen effectors and their delivery during plant–microbe interactions

Pathogens secrete a wide range of so-called ‘effectors’, small molecules and proteins that can modify host cell structures or functions. Effectors from several groups of pathogens such as bacteria, oomycetes and fungi can enter plant cells (Huang et al., 2003; Chisholm et al., 2006; Kamoun, 2007). Bacteria use the Type II, Type IV and Type III Secretion System (T3SS) to deliver 20 to hundreds of protein effectors into the host plant (Lindeberg et al., 2006; Cunnac et al., 2009). Oomycetes and fungi likely secrete even more protein effectors; computational analysis of their genomes has revealed hundreds (Kamper et al., 2011; Schirawski et al., 2010), even more than 1700 of potential effectors for rust fungi (Duplessis et al., 2011).

Predicted effectors from oomycetes, in addition to a N-terminal signal peptide (SP), carry a host targeting signal (HTS) next to the SP that contains conserved RXLR and a dEER motifs that can target them into host cells in the absence of the pathogen (Rehmny et al., 2005; Tyler et al., 2006; Whisson et al., 2007; Jiang et al., 2008; Dou et al., 2008b; Haas et al., 2009; Kale et al., 2010), while effectors from fungi have an N-terminal SP and in some cases an RXLR-like motif (Dean et al., 2005; Kamper et al., 2006; Kale et al., 2010; Schirawski et al., 2010). Many examples exist where predicted effectors with a SP are shown to be delivered into the host cytoplasm (Kemen et al., 2005; Catananzari et al., 2006; Mosquera et al., 2009; Doehlemann et al., 2009; Khang et al., 2010).
2010) and some can do so through N-terminal sequences without the help of the pathogen (Rafiqi et al., 2010; Kale et al., 2010). Recently, Kale et al. (2010) showed that the conserved RXLR motif from oomycetes and the RXLR-like motif from other fungi bind specifically to phospholipids, in particular phosphatidylinositol-3-phosphate (PI3P) on the surface of the plasma membrane and likely enter the cell through lipid raft-mediated endocytosis; results on lipid-binding assays have been controversial (Gan et al., 2010). In a search for possible equivalent motifs in other fungi, Godfrey et al. (2010) showed that small secreted proteins from haustoria-forming fungal pathogens share a conserved Y/F/WxC motif in addition to an N-terminal secretion signal although no function has yet been ascribed to these motifs.

**Roles of effectors in infection**

**Breaking the physical barrier**

After landing on the surface of plants, pathogens either enter their host through natural openings (stomata and hydathodes) or penetrate the surface directly. Bacteria use natural openings or wounds to enter the apoplast for colonization. Pathogenic bacteria sense compounds released during photosynthesis from stomata and move towards them. The PRR of the guard cells can sense PAMPs such as bacterial flagellin or lipopolysaccharides or lipooligosaccharides, which induces the closure of stomata (Gohre & Robatzek, 2008). Pathogenic bacteria, such as *Pseudomonas syringae*, produce coronatine, a phytotoxin that mimics jasmonic acid (JA) to interfere with salicylic acid (SA) and abscisic acid (ABA) signalling for reopening stomata to get access to the host apoplast (Melotto et al., 2006). It is not yet known whether MAMP-associated defence pathways also close stomata to euukaryotic pathogens and whether these pathogens, such as cereal rusts, use effectors in a similar way to overcome this hurdle. Powdery mildew fungi, such as *Bgh*, penetrate the cuticle of plant cells directly by forming an appressorium. It has been shown that effectors AvrA10 and AvrK1 increase the penetration efficiency of *Bgh* on susceptible barley cultivars but the exact mechanism is not yet known (Ridout et al., 2006). After getting into the plant apoplast, the next physical barrier is the plant cell wall. To promote nutrient leakage from the cytosol into the apoplast, lytic enzymes that degrade the cell wall locally are secreted. At the same time, pathogens secrete effector molecules to suppress the host defence that is activated by danger-associated molecular patterns (DAMPs) generated from degrading cell wall molecules and signalling damage to the plant integrity and hence potential danger of infection (Jha et al., 2007).

**Disarming plant defence enzymes**

Plants produce antimicrobial enzymes such as proteases, hydrolases, glucanases and chitinases that can degrade the cell wall of invading pathogenic fungi in the apoplast, without detrimental effect to the plant itself (Lucas, 1998). This has a dual role in defence; the degradation of cell walls can attenuate fungal growth while on the other hand the molecules released from degraded cell walls serve as elicitors for the induction of host defence. Pathogens use effector molecules either to stop the delivery of these antimicrobial enzymes and compounds by preventing their secretion, or by inhibiting their activity after they are secreted (Bent & Mackey, 2007), and/or by inhibiting downstream signalling. Bacteria secrete plant cell wall-degrading enzymes locally in order to construct the T3SS and use effectors such as HopP1 to suppress defence induced by DAMPs in the *N. benthamiana* apoplast (Gust et al., 2007; Oh et al., 2007). HopP1 either sequesters or processes the fragments that are produced during cell wall degradation so that they cannot function as DAMPs (Gohre & Robatzek, 2008).

Examples from fungi are represented by the apoplastic fungus *Cladosporium fulvum* which secretes effector AVR2, a cysteine protease inhibitor, during infection that binds directly to RCR3, a tomato cysteine protease, to protect the fungus from the deleterious effect of the enzyme. AVR2 also promotes virulence for other fungal pathogens that cause diseases in tomato, such as *Verticillium dahliae* and *Botrytis cinerea*, when expressed heterologously in *Arabidopsis* (van Esse et al., 2008). Similarly, *C. fulvum* effector AVR4 binds to chitin of fungal cell walls to protect it from tomato host chitinases (van den Burg et al., 2006). AVR4 can also protect chitin against plant chitinases in the cell wall of other fungi, such as *Trichoderma viride* and *Fusarium solani* f. sp. *phaseoli* (van den Burg et al., 2006). In this way, AVR4 not only protects the fungi from hydrolysis by plant chitinases but also keeps chitin fragments from eliciting PTI (Libault et al., 2007). Effector ECP6 from *C. fulvum* has a Lys-M domain that binds to carbohydrates including chitin and protects the pathogen from plant chitinases or may be involved in scavenging of chitin fragments that are released during cell wall degradation by plant chitinases, thus preventing them from inducing PTI (Bolton et al., 2008).

The oomycete pathogen, *P. infestans*, is known to secrete a suite of Cysteine and Kazal family protease inhibitors. The tomato papain-like protease, PIP1, which is induced by SA, is blocked by the EPIC2B inhibitor of *P. infestans* (Tian & Kamoun, 2005; Tian et al., 2007; van Esse et al., 2008). PIP1 is related to RCR3, and AVR2 from *C. fulvum* apparently can also inhibit...
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PIP1 and two other plant cysteine proteases, aleurain and TDI65 (Rooney et al., 2005; Shabab et al., 2008; van Esse et al., 2008). P. infestans EPIC1 and EPIC2B can also bind and inhibit RCR3, similar to AVR2, but unlike AVR2, these EPICs do not elicit an HR on Cf-2/Rcr3 pimp tomato plants, suggesting that P. infestans evolved stealthy effectors that can inhibit tomato proteases without activating defence responses (Song et al., 2009). These findings show that effectors from different pathogens can target the same apoplastic enzymes to increase pathogen fitness in the host (Shabab et al., 2008). Recently, another apoplastic tomato and potato papain-like cysteine protease, C14, was shown to be targeted by EPIC1 and EPIC2B (Kaschani et al., 2010). Other P. infestans effectors, EPII and EPI10, target the subtilisin-like serine protease of tomato P69B, a PR protein (Tian et al., 2004; Tian & Kamoun, 2005; Tian et al., 2007). AVRP123, a secreted protein from the flax rust fungus M. lini, also shows similarity to Kazal serine protease inhibitors (Catanzariti et al., 2006). The soybean pathogen, P. sojae, secretes glucanase inhibitor proteins, GIP1 and GIP2, that target endo-β-1,3-glucanaseA of the host plant to protect the pathogen during infection and also to prevent PTI induced by oligoglucosides (Rose et al., 2002) as discussed next.

**Suppression of receptor activation**

The recognition of PAMPs by plant PRRs leads to the activation of defence responses against the pathogen. It was proposed in the beginning that resistance induced by PRRs is only basic and not as strong as that induced by resistance genes, but it is clear now, at least in the case of FLS2 (the flagellin receptor), that the contribution of PRRs towards overall resistance is huge (He et al., 2006; de Torres et al., 2006; Hann & Rathjen, 2007). It is therefore not surprising that pathogens have evolved to target with their effectors these signalling pathways in order to reduce resistance responses. Insight into the molecular mechanisms involved has emerged over the last few years. For example, several effectors target the PRRs directly to jam all the downstream resistance responses (Jamir et al., 2004; Jones & Dangl, 2006; Blocker et al., 2008) such as P. syringae AVR Pto and AVR Pto B which can also suppress nonhost HR in N. benthamiana induced by FLG22 or P. infestans INF1 (Hann & Rathjen, 2007).

Several oomycete RXLR effectors can suppress host cell immunity in a similar manner. AVR3A from P. infestans can block an HR induced by INF1 (Bos et al., 2009) as can several random P. infestans effectors (Oh et al., 2009). Similarly, AVR1B from P. sojae also suppresses programmed cell death triggered by the mouse protein, BAX, in plants and yeast (Dou et al., 2008a) and M. oryzae AvrPiz-t can also suppress mouse BAX protein-mediated programmed cell death in tobacco leaves (Li et al., 2009). ATR1 and ATR13 from *Hyaloperonospora arabidopsidis* increased the virulence of *P. syringae* DC3000 on susceptible *Arabidopsis* when these effectors were delivered by the *P. syringae* T3SS, and ATR13 seemed to target PTI by suppressing callose accumulation and ROS production (Sohn et al., 2007). In the genus *Phytophthora*, effectors such as *P. infestans* IpiO1 bind to a membrane-spanning lectin receptor kinase and seem to prevent downstream defence signalling (Bouwmeester et al., 2011).

**Suppression of R gene-triggered resistance**

Pathogenic fungi such as *Fusarium oxysporum* f. sp. lycopersici (Fol) can avoid host defences by evolving effectors that can suppress *R* gene-triggered resistance (Houterman et al., 2008). AVR3 and AVR2 are required for full virulence on tomato plants but they are also recognized by tomato lines that have resistance genes I-3 or I-2, respectively, triggering an HR and cause arrest of the pathogen (Huang & Lindhout, 1997; Rep et al., 2004, 2005). In contrast, AVR1 is a small cysteine-rich secreted protein that is recognized by resistance gene I or I-1 but is not required for virulence. It seems generally true that effectors that contribute to full virulence are maintained in the species and tellingly, AVR3 is present in all Fol strains analysed, while AVR1 is present only in Fol strains that are virulent on I-3 lines. Houterman et al. (2008) showed that AVR1 actually suppresses the resistance triggered by I-2 and I-3, as the transformation of Avr1 to Fol strains that were avirulent on I-2 or I-3 became virulent on these lines. It was proposed that Fol strains acquired Avr1 as a mechanism of partial functional redundancy so that they can avoid the consequences of losing Avr3 and probably Avr2 that are required for full virulence (Stergiopoulos & de Wit, 2009). In the flax rust fungus, *M. lini*, the interaction of AvrL567 in strain CH5-89 with the flax cultivar ‘Barnes’ that contains the L7 gene is inhibited by an inhibitor gene and thus results in a lower virulence reaction (Lawrence et al., 2010). *P. infestans* secreted effector SNE1 can suppress programmed cell death resulting from the interaction of several Avr-R protein interactions (Kelley et al., 2010).

**Alteration of the plant defence transcriptome**

Bacterial effectors have been identified many years before fungal and oomycete effectors and it is therefore not surprising that a better understanding of the impact of such
effectors on the host transcriptome during infection has come from the study of bacteria–plant interactions. For example, the perception of PAMPs such as bacterial flagellin changes the expression of at least 1000 genes in Arabidopsis (Zipfel et al., 2004). Microbes have evolved to become pathogens in part because their effectors inhibit the activation of defence genes. Effectors can have an indirect effect on transcription by dephosphorylating MAPK signalling components in order to suppress the defence response triggered by the recognition of PAMPs by PRRs (reviewed in Gohre & Robatzek, 2008). Another mechanism by which effectors have been shown to affect the plant transcriptome is by causing changes in RNA stability. For example, P. syringae HopU1, a mono-ADP-ribosyltransferase, acts on glycine-rich RNA-binding proteins such as Arabidopsis AtGRP7 and AtGRP8 which are RNA chaperones and thus, HopU1 changes the plant transcriptome by reducing transcript levels and hence affects the production of defence response proteins (Fu et al., 2007). Some pathogen effectors act as transcription factors and can induce the expression of host genes for the benefit of the pathogen. For example, the AVRBS3 family of effectors from X. campestris pv. vesicatoria has plant nuclear localization signals and binds to a ‘upa-box’ (upregulated by AVRBS3) that is found in the promoter of Upa20, a master regulator of cell size inducing hypertrophy, and several other host genes, ensuring proper nutrient supply for pathogen multiplication (Szurek et al., 2002; Gurlebeck et al., 2006; Kay et al., 2007); however, in resistant plant cultivars, the promoter of Bs3 also carries a ‘upa-box’ and, thus, binding of the AVRBS3 effector induces transcription of Bs3 and cell death (Kay et al., 2007). This shows that under selection pressure, plants can evolve to recognize effectors and use them for their own defence.

Several studies in fungal and oomycete pathosystems have reported on changes in host transcriptomes: in compatible interactions, initial defence reactions supposedly triggered by recognition of PAMPs, are the result of the upregulation of genes in defence pathways; at later infection stages, these are suppressed and this effect is proposed to be caused by suites of secreted pathogen effectors. Such a scenario has been demonstrated in the smut fungus Ustilago maydis–maize interaction (Doehlemann et al., 2008). In this latter system, effectors likely even reprogram specific developmental pathways in the host to create the best niche for their own development, depending on the age and part of the plant infected (Skibbe et al., 2010). One particular effector, Pit2, was deduced to suppress defence genes (Doehlemann et al., 2011). In the M. oryzae–rice interaction, changes in the host transcriptome were noted including upregulation of susceptibility genes and downregulation of defence genes and these effects on gene transcription were attributed to the actions of secreted effectors, although no particular effector could be identified to cause specific effects (Mosquera et al., 2009).

**Killing of host cells**

Necrotrophic pathogens produce several phytotoxins in addition to cell wall hydrolysing enzymes in order to kill the host tissue for colonization during infection. Some necrotrophic fungi produce proteinaceous effectors, also called host selective toxins (HST), that are required for infection on susceptible host plants that have the corresponding dominant receptor gene (Wolpert et al., 2002). This represents a situation opposite to the classical gene-for-gene interaction in which a dominant gene is required for disease resistance rather than susceptibility. Two wheat necrotrophs, Stagonospora nodorum (Sn) and Pyrenophora tritici-repentis (Ptr), produce several host-specific peptide effectors, such as PtrTOXA, SnTOX1, SnTOX2 and SnTOX4, that, when recognized by their corresponding dominant susceptibility genes in wheat (TsN1, Snm1, Smn2 and Smn4), cause disease (Liu et al., 2004, 2006; Friesen et al., 2007; Abeysekara et al., 2009; de Wit et al., 2009; Manning et al., 2009). PtrTOXA, the best-studied effector, has an N-terminal secretion signal, followed by an RGD domain (Arg-Gly-Asp-containing loop) for host targeting and a C-terminal domain with effector function (Sarma et al., 2005; Manning et al., 2007). After entering the host cell, PtrTOXA was reported to enter the chloroplast and bind to ToxABP1, thereby interfering with functions of the chloroplast and affecting photosystem I and II function in a light-dependent manner (Manning et al., 2007). PtrTOXA is an ortholog to SnTOX1 and both effectors are recognized by the same wheat susceptibility gene, Tsn1 (Liu et al., 2006).

Some pathogenic bacteria, oomycetes and fungi produce NEP1-like (necrosis-inducing protein) proteins (NLPs), effectors that are toxic to only dicotyledonous plants, possibly because of the different molecular compositions of dicot and monocot cell membranes (Pemberton & Salmond, 2004; Gijzen & Nurnberger, 2006; Ottmann et al., 2009). The common hepta-peptide motif, GHRHDWE, and two conserved cysteine residues make NLPs structurally similar to actinoporins, cytolytic toxins from marine organisms. Hemibiotrophs, such as P. infestans and P. sojae, produce NLPs such as NP1 and PiNPP1, in the late necrotrophic phase which could contribute to disease development with their cytolytic activities (Qutob et al., 2002; Kanneganti et al., 2006).
Suppression of host defence by symbionts

Symbiotic microorganisms also secrete effectors into host plants to suppress host defence responses, a condition essential for their lifestyle. Many rhizobial strains use the T3SS to deliver effectors into host cells to suppress host defence responses in a similar way that pathogenic bacteria do (Deakin & Broughton, 2009). This could indicate that this ancient delivery system is used for different purposes or that symbiotic organisms have evolved their effectors such as to overcome host defence responses. In the fungal kingdom, the genome of *Laccaria bicolor*, an ectomycorrhizal fungus, revealed more than 3000 predicted secreted proteins of which 10% were small secreted proteins (SSPs) of the effector-type (Martin et al., 2008; Martin & Selosse, 2008). Some of these SSPs are homologous to rust fungus ‘haustoria-expressed secreted proteins’ (HESPs) and are differentially expressed during infection. One effector, MiSSP7, was recently shown to localize to host cell nuclei upon cell entry where it altered host transcription and was shown to be necessary for promoting symbiosis and evading the host defence (Plett et al., 2011).

Gene-for-gene or R-Avr gene interaction

Adapted pathogens secrete effector molecules into the host plant to surmount physical barriers, neutralize preformed antimicrobial compounds, and overcome PTI. As a result, during co-evolution of plant host and adapted pathogen, plants have developed sophisticated recognition systems, usually encoded by *R* genes to recognize these effectors or their action and trigger defence responses; this induced resistance has been called effector-triggered immunity (ETI) and leads to a rapid and enhanced defence response in the host plant often including an HR (Jones & Dangl, 2006). These effectors represent avirulence (AVR) factors since they activate the plant defence systems, usually encoded by *R* genes to recognize these effectors or their action and trigger defence responses; this induced resistance has been called effector-triggered immunity (ETI) and leads to a rapid and enhanced defence response in the host plant often including an HR (Jones & Dangl, 2006). These effectors represent avirulence (AVR) factors since they activate the plant defence system and make the pathogen unable to cause disease when the plant has the ‘recognizing’ *R* gene. This genetically superimposed *R* and *Avr* interaction has been known for many years as ‘gene-for-gene’ resistance, or host/cultivar-level resistance, as particular cultivars of the host with a certain *R* gene product recognize an *Avr* gene product from a particular race of the pathogen. This concept was formulated after genetic studies on two fungal pathosystems: the *Melampsora lini*–*flax* (Flor, 1942) and the *Ustilago tritici*–wheat interactions (Oort, 1944). As mentioned, some *Avr* genes encode effectors that suppress host defence or carry out other essential functions and, thus, act as virulence factors when the plant does not have the ‘recognizing’ resistance gene. ETI represents the qualitative, secondary layer of resistance and has led to an evolutionary arms race between the pathogen and the plant in which the pathogen either mutates or discards effectors to avoid recognition by the host or develops new effectors to suppress ETI, while the plant develops new *R* genes to recognize the mutated or new effectors (Jones & Dangl, 2006; de Wit, 2007; Bent & Mackey, 2007).

*Avr* (-triggering effector) genes

Because, until recently, the term avirulence gene/factor featured prominently in the literature, we will discuss these below but would prefer the term ‘*Avr*-triggering effector genes’. The first *Avr* gene was cloned from a bacterium in 1984 (Staskawicz et al., 1984), which was followed by the cloning and characterization of more than 40 bacterial *Avr* genes in the following decades (van ‘t Slot & Knogge, 2002; Mudgett, 2005). The cloning and characterization of *Avr* genes from fungi and oomycetes lagged behind because of these organisms’ larger genome sizes and sometimes inefficient transformation systems. The first *Avr* gene from a fungus was isolated from *C. fulvum* in 1991 (van Kan et al., 1991) and the first oomycete *Avr* gene was cloned from *P. sojae* and *P. infestans* in 2004 (Shan et al., 2004). In the following years, most fungal *Avr* genes were cloned from ascomycetes, although more recently, a few have been reported from a basidiomycete, the flax rust fungus, *M. lini* (Catanzariti et al., 2006; Table 1).

*Avr* genes isolated and characterized to date differ among one another both in sequence and function, and those from different plant pathogens do not seem to share many common features, other than the ones already mentioned. Exceptions are found among some family members, e.g. in *Pseudomonas* and *Xanthomonas* species (Lahaye & Bonas, 2001; Deslandes et al., 2003). And it is true that among genomes from related species, sometimes even among genera, homologous effectors are found, but these, or not all of these, have proven avirulence functions.

Most fungal and oomycete *Avr* genes characterized to date encode small proteins (28–311 amino acids), except the *ACEI* of *M. grisea* (see below) and most have a secretion signal/protein transport motif at the N-terminus (Ellis et al., 2006), although there are exceptions (see further).

**Fungal *Avr* (-triggering effector) genes**

*Cladosporium fulvum*

This fungus is an apoplastic pathogen of the ascomycete subgroup that reproduces asexually and causes leaf mold of tomato (de Wit et al., 1997; Joosten & de Wit, 1999; Thomma et al., 2005). Four avirulence genes have been cloned: *Avr2*, *Avr4*, *Avr4E* and *Avr9*, which all encode small secreted cysteine-rich effector proteins that are
<table>
<thead>
<tr>
<th>Protein</th>
<th>Organism</th>
<th>Length aa residues (mature)</th>
<th>Number of cysteines</th>
<th>SP length (aa)</th>
<th>Biological activity/homology</th>
<th>Protein localization</th>
<th>Role in virulence/pathogenicity</th>
<th>Corresponding R gene</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>AVR2</td>
<td>C. fulvum</td>
<td>78 (58)</td>
<td>8</td>
<td>20</td>
<td>Induces HR in the presence of Tomato Rcr3, Protease inhibitor</td>
<td>Apoplast</td>
<td>Inhibits Rcr3 and other proteases against chitinases</td>
<td>Cf-2</td>
<td>Stergiopoulos et al., 2010</td>
</tr>
<tr>
<td>AVR4</td>
<td>C. fulvum</td>
<td>135 (86)</td>
<td>8</td>
<td>18</td>
<td>Induces HR, Chitin-binding, orthologs in some other fungi</td>
<td>Apoplast; fungal cell wall chitin</td>
<td>Protects against chitinases</td>
<td>Cf-4</td>
<td>Joosten et al., 1994; Stergiopoulos et al., 2010; van den Burg et al., 2006</td>
</tr>
<tr>
<td>AVR4E</td>
<td>C. fulvum</td>
<td>121 (101)</td>
<td>6</td>
<td>10</td>
<td>Induces HR</td>
<td>Apoplast</td>
<td>Unknown</td>
<td>Hcr9-4E</td>
<td>Westernink et al., 2004</td>
</tr>
<tr>
<td>AVR9</td>
<td>C. fulvum</td>
<td>63 (28)</td>
<td>6</td>
<td>23</td>
<td>Induces HR, Carboxypeptidase inhibitor</td>
<td>Apoplast</td>
<td>Unknown</td>
<td>Cf-9</td>
<td>van den Ackerveken et al., 1993</td>
</tr>
<tr>
<td>ECP1</td>
<td>C. fulvum</td>
<td>96 (65)</td>
<td>8</td>
<td>23</td>
<td>Induces HR, Tumor-necrosis factor receptor induces HR</td>
<td>Apoplast</td>
<td>Disruption leads to reduced virulence</td>
<td>Cf-Ecp1</td>
<td>van den Ackerveken et al., 1993</td>
</tr>
<tr>
<td>ECP2</td>
<td>C. fulvum</td>
<td>165 (143)</td>
<td>4</td>
<td>22</td>
<td>Induces HR</td>
<td>Apoplast</td>
<td>Unknown</td>
<td>Cf-Ecp2</td>
<td>van den Ackerveken et al., 1993</td>
</tr>
<tr>
<td>ECP4</td>
<td>C. fulvum</td>
<td>119 (101)</td>
<td>6</td>
<td>18</td>
<td>Induces HR</td>
<td>Apoplast</td>
<td>Unknown</td>
<td>Cf-Ecp4</td>
<td>Bolton et al., 2008</td>
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<tr>
<td>ECP5</td>
<td>C. fulvum</td>
<td>115 (98)</td>
<td>6</td>
<td>17</td>
<td>Induce necrosis,</td>
<td>Apoplast</td>
<td>Unknown</td>
<td>Cf-Ecp5</td>
<td>Bolton et al., 2008</td>
</tr>
<tr>
<td>ECP6</td>
<td>C. fulvum</td>
<td>222 (199)</td>
<td>8</td>
<td>23</td>
<td>LysM-domains; chitin-binding, ortholog found in different pathogen and nonpathogenic sp.</td>
<td>Apoplast</td>
<td>Knock-down leads to reduced virulence</td>
<td>Unknown</td>
<td>de Jonge &amp; Thomma, 2009</td>
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<tr>
<td>ECP7</td>
<td>C. fulvum</td>
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<td>6</td>
<td>–</td>
<td>Unknown</td>
<td>Apoplast</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Bolton et al., 2008</td>
</tr>
<tr>
<td>NIP1</td>
<td>R. secalis</td>
<td>82 (60)</td>
<td>10</td>
<td>22</td>
<td>Non-specific toxin/induces necrosis and plasma-membrane H+ ATPase</td>
<td>Probably in apoplast</td>
<td>Not required for virulence</td>
<td>Rrs-1</td>
<td>Rohe et al., 1995</td>
</tr>
<tr>
<td>NIP2</td>
<td>R. secalis</td>
<td>109 (?)</td>
<td>7 (6)</td>
<td>16</td>
<td>Non-specific toxin/induces necrosis in several plant spp.</td>
<td>Probably in apoplast</td>
<td>Not required for full virulence</td>
<td>Unknown</td>
<td>Rohe et al., 1995; Stergiopoulos &amp; de Wit, 2009</td>
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<tr>
<td>NIP3</td>
<td>R. secalis</td>
<td>115 (?)</td>
<td>9 (8)</td>
<td>17</td>
<td>Non-specific toxin/induces necrosis in several plant species</td>
<td>Probably in apoplast</td>
<td>Not required for full virulence</td>
<td>Unknown</td>
<td>Rohe et al., 1995; Stergiopoulos &amp; de Wit, 2009</td>
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<tr>
<td>AVRA10</td>
<td>B. graminis</td>
<td>286</td>
<td>4</td>
<td>–</td>
<td>&gt; 30 paralogs in Bgh and other f. sp. No N-terminal SP, induces HR</td>
<td>Probably in cytoplasm</td>
<td>Unknown</td>
<td>Mla10</td>
<td>Ridout et al., 2006</td>
</tr>
<tr>
<td>AVRK1</td>
<td>B. graminis</td>
<td>177</td>
<td>3</td>
<td>–</td>
<td>&gt; 30 paralogs in Bgh and other f. sp. No N-terminal SP, induces HR</td>
<td>Probably in cytoplasm</td>
<td>Unknown</td>
<td>Mk1</td>
<td>Ridout et al., 2006</td>
</tr>
<tr>
<td>AVRL567 (A, B and C)</td>
<td>M. lini</td>
<td>150 (127)</td>
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<td>23</td>
<td>Unknown, induces HR, functional RXLR like motif</td>
<td>Cytoplasm</td>
<td>Unknown</td>
<td>L5, L6 and L7</td>
<td>Dodds et al., 2004; Kale et al., 2010; Rafiqi et al., 2010</td>
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<tr>
<td>AVRM</td>
<td><em>M. lini</em></td>
<td>314 (286)</td>
<td>1</td>
<td>28</td>
<td>Unknown, induces HR, RXLR-like motif</td>
<td>Cytoplasm</td>
<td>Unknown</td>
<td>M</td>
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<tr>
<td>AVRP123</td>
<td><em>M. lini</em></td>
<td>117 (94)</td>
<td>11</td>
<td>23</td>
<td>Induces HR, Kazal Serine protease inhibitor peptide</td>
<td>Probably in cytoplasm</td>
<td>Unknown</td>
<td>P, P1, P2 and/or P3</td>
<td></td>
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<td>AVRP4</td>
<td><em>M. lini</em></td>
<td>95 (67)</td>
<td>7</td>
<td>28</td>
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<td>Probably in cytoplasm</td>
<td>Unknown</td>
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<td></td>
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<tr>
<td>Avr-Pita1</td>
<td><em>M. oryzae</em></td>
<td>223 (176)</td>
<td>8</td>
<td>16</td>
<td>Homology to metallo-proteases, RXLR-like motif</td>
<td>Cytoplasm</td>
<td>Not required for virulence on rice</td>
<td>Pita</td>
<td></td>
</tr>
<tr>
<td>Avr-Pita2</td>
<td><em>M. oryzae</em></td>
<td>224 (?)</td>
<td>8</td>
<td>16</td>
<td>Homology to metallo-proteases</td>
<td>Probably in apoplast</td>
<td>Probably not required for virulence on rice</td>
<td>Pi-ta</td>
<td></td>
</tr>
<tr>
<td>Avr-Pita3</td>
<td><em>M. oryzae</em></td>
<td>226 (?)</td>
<td>8</td>
<td>16</td>
<td>Homology to metallo-proteases</td>
<td>Probably in cytoplasm</td>
<td>Probably not required for virulence on rice</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>PWL1</td>
<td><em>M. oryzae</em></td>
<td>147 (124)</td>
<td>2</td>
<td>23</td>
<td>Glycine-rich hydrophilic protein</td>
<td>Biotrophic interfacial complex</td>
<td>Unknown</td>
<td>Unknown</td>
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<tr>
<td>PWL2</td>
<td><em>M. oryzae</em></td>
<td>145 (124)</td>
<td>2</td>
<td>21</td>
<td>Glycine-rich hydrophilic protein</td>
<td>Cytoplasm</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
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<tr>
<td>PWL3</td>
<td><em>M. oryzae</em></td>
<td>137 (116)</td>
<td>0</td>
<td>21</td>
<td>Glycine-rich hydrophilic protein</td>
<td>Probably in apoplast</td>
<td>Non-functional</td>
<td>Unknown</td>
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<tr>
<td>PWL4</td>
<td><em>M. oryzae</em></td>
<td>138 (117)</td>
<td>0</td>
<td>21</td>
<td>Glycine-rich hydrophilic protein</td>
<td>Probably in apoplast</td>
<td>Non-functional</td>
<td>Unknown</td>
<td></td>
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<tr>
<td>ACE1</td>
<td><em>M. oryzae</em></td>
<td>4035</td>
<td>43</td>
<td>–</td>
<td>Hybrid polyketide synthase/nonribosomal peptide synthetase</td>
<td>Not secreted</td>
<td>Unknown</td>
<td>Pi33</td>
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<tr>
<td>AVR1-CO39</td>
<td><em>M. oryzae</em></td>
<td>not cloned yet</td>
<td>–</td>
<td>–</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Pi-CO39(t)</td>
<td></td>
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<tr>
<td>AVR-Pia</td>
<td><em>M. oryzae</em></td>
<td>85 (66)</td>
<td>2</td>
<td>19</td>
<td>Induces HR</td>
<td>Probably in cytoplasm</td>
<td>Unknown</td>
<td>Pia</td>
<td></td>
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<td><em>M. oryzae</em></td>
<td>70 (51)</td>
<td>3</td>
<td>19</td>
<td>Induces HR</td>
<td>Probably in cytoplasm</td>
<td>Unknown</td>
<td>Pii</td>
<td></td>
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<tr>
<td>AVR-Pi</td>
<td><em>M. oryzae</em></td>
<td>70 (51)</td>
<td>3</td>
<td>19</td>
<td>Induces HR</td>
<td>Probably in cytoplasm</td>
<td>Unknown</td>
<td>Pii</td>
<td></td>
</tr>
<tr>
<td>AVR-Pi/km/kp</td>
<td><em>M. oryzae</em></td>
<td>113 (92)</td>
<td>3</td>
<td>21</td>
<td>Induces HR</td>
<td>Probably in cytoplasm</td>
<td>Unknown</td>
<td>Pik</td>
<td></td>
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<tr>
<td>AVRLm1</td>
<td><em>L. maculans</em></td>
<td>205 (183)</td>
<td>1</td>
<td>22</td>
<td>Induces HR</td>
<td>Probably in cytoplasm</td>
<td>Required for full virulence</td>
<td>Rlm1</td>
<td></td>
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<tr>
<td>AVRLm6</td>
<td><em>L. maculans</em></td>
<td>144 (124)</td>
<td>6</td>
<td>20</td>
<td>Unknown, functional RXLR-like motif</td>
<td>Probably in apoplast</td>
<td>Unknown</td>
<td>Rlm6</td>
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</table>

(Continued)
Table 1. (Continued.)

<table>
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<tr>
<th>Protein</th>
<th>Organism</th>
<th>Length aa residues (mature)</th>
<th>Number of cysteines</th>
<th>SP length (aa)</th>
<th>Biological activity/homology</th>
<th>Protein localization</th>
<th>Role in virulence/pathogenicity</th>
<th>Corresponding R gene</th>
<th>References</th>
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<tbody>
<tr>
<td>AVRLm4-7</td>
<td>L. maculans</td>
<td>143 (122)</td>
<td>8</td>
<td>21</td>
<td>Unknown</td>
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<td>Rlm4 and/or Rlm7</td>
<td>Parlange et al., 2009</td>
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<td>AVR3</td>
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<td>284 (189)</td>
<td>6 or 8</td>
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<td>Unknown</td>
<td>Xylem</td>
<td>Required for full virulence</td>
<td>I-3</td>
<td>Rep et al., 2004; Stergiopoulos &amp; de Wit, 2009</td>
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<td>AVR4</td>
<td>F. oxysporum f. sp. lycopersici</td>
<td>232 (172)</td>
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<td>20</td>
<td>Unknown</td>
<td>Xylem</td>
<td>Probably not required for virulence</td>
<td>Unknown</td>
<td>Houterman et al., 2007; Stergiopoulos &amp; de Wit, 2009</td>
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<td>F. oxysporum f. sp. lycopersici</td>
<td>163 (144)</td>
<td>3 (2)</td>
<td>19</td>
<td>Induces HR, unknown, functional RXLR like motif</td>
<td>Xylem</td>
<td>Required for full virulence</td>
<td>I-2</td>
<td>Houterman et al., 2007; Stergiopoulos &amp; de Wit, 2009; Kale et al., 2010</td>
</tr>
<tr>
<td>AVR1</td>
<td>F. oxysporum f. sp. lycopersici</td>
<td>242 (184)</td>
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<td>17</td>
<td>Unknown</td>
<td>Xylem</td>
<td>Suppression of I-2 and I-3 resistance</td>
<td>I or I-1</td>
<td>Houterman et al., 2007; Stergiopoulos &amp; de Wit, 2009</td>
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<tr>
<td>ATR1NWWb</td>
<td>H. arabidopsis</td>
<td>311 (296)</td>
<td>–</td>
<td>15</td>
<td>Induces HR, RXLR domain</td>
<td>Cytoplasm</td>
<td>Suppress host defense</td>
<td>RPP1Wb and RPP1Nd</td>
<td>Rehmany et al., 2005; Sohn et al., 2007</td>
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<tr>
<td>ATR13</td>
<td>H. arabidopsis</td>
<td>187 (168)</td>
<td>–</td>
<td>19</td>
<td>Induces HR, RXLR domain</td>
<td>Cytoplasm</td>
<td>Suppress host defense</td>
<td>RPP13</td>
<td>Allen et al., 2004; Sohn et al., 2007</td>
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<tr>
<td>AVR1-b1</td>
<td>P. sojée</td>
<td>138 (117)</td>
<td>1</td>
<td>21</td>
<td>Induces HR, suppresses BAX-induced cell death, RXLR domain</td>
<td>Probably cytoplasm</td>
<td>Unknown</td>
<td>Rps1b and Rpsk1</td>
<td>Shan et al., 2004; Dou et al., 2008a,b</td>
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<tr>
<td>AVR1a</td>
<td>P. sojée</td>
<td>121 (98)</td>
<td>–</td>
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<td>Unknown</td>
<td>Unknown</td>
<td>Rps1a</td>
<td>Qutob et al., 2009</td>
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<tr>
<td>AVR3a</td>
<td>P. sojée</td>
<td>111 (91)</td>
<td>–</td>
<td>20</td>
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<td>Unknown</td>
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<td>Rps3a</td>
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<tr>
<td>AVR3c</td>
<td>P. sojée</td>
<td>220 (200)</td>
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<td>20</td>
<td>Induces HR, RXLR domain</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Rps3c</td>
<td>Dong et al., 2009</td>
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<tr>
<td>AVR3a</td>
<td>P. infestans</td>
<td>147 (126)</td>
<td>–</td>
<td>21</td>
<td>Induces HR, suppresses INP1-induced HR, RXLR domain, interact with CMPG1 ubiquitin E3 ligase</td>
<td>Cytoplasm</td>
<td>Required for full virulence</td>
<td>R3a</td>
<td>Armstrong et al., 2005; Bos et al., 2006, 2009, 2010</td>
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<tr>
<td>MfAVR4</td>
<td>M. fijiensis</td>
<td>121 (100)</td>
<td>10</td>
<td>21</td>
<td>Chitin-binding peritrophin-A, induces HR or necrosis in Cf4 tomato</td>
<td>Probably in apoplast</td>
<td>Protect fungi against chitinases</td>
<td>Cf4 and Hcr9</td>
<td>Stergiopoulos et al., 2010</td>
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<td>MfECP2</td>
<td>M. fijiensis</td>
<td>161 (142)</td>
<td>4</td>
<td>19</td>
<td>Induces HR or necrosis in CIEcp2 tomato</td>
<td>Probably in apoplast</td>
<td>Promote virulence by interacting with host cell target causing necrosis</td>
<td>Ecp2</td>
<td>Stergiopoulos et al., 2010</td>
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</tbody>
</table>
recognized in tomato by Cf2, Cf4, Cf4E and Cf9 resistance proteins, respectively (Table 1; de Wit et al., 1997; Joosten & de Wit, 1999; Thomma et al., 2005). The virulence function of AVR2 and AVR4 has been discussed earlier. AVR4E is a 101 amino acid (aa) protein but does not have a known virulence function (Westerink et al., 2004). Several field isolates that overcome Cf4E-mediated resistance reveal point mutations in AVR4E or a complete loss of the Avr4E gene, indicating that loss of this effector does not affect fitness of the pathogen significantly (Stergiopoulos et al., 2007). Avr9 encodes a 28 aa mature protein with six cysteine residues after it is processed by plant and fungal proteases at its C- and N-termini (van Kan et al., 1991; van den Ackerveken et al., 1993). Structurally, AVR9 is similar to carboxy-peptidase inhibitor but no definitive function has been identified so far (van Kan et al., 1991; van den Ackerveken et al., 1993; van den Hooven et al., 2001). All natural strains of \textit{C. fulvum} that overcome Cf9 resistance lack Avr9, suggesting that it is not required for full virulence (Stergiopoulos et al., 2007). \textit{C. fulvum} deleted for Avr9 is fully virulent on tomato plants, but the heterologous expression of Avr9 in tomato plants makes it more susceptible to \textit{C. fulvum} strains lacking Avr9, indicating some (redundant) virulence function (Marmeisse et al., 1993; de Wit et al., 2009).

Besides Avr effectors, four extracellular cysteine-rich proteins (ECP), such as ECP1, ECP2, ECP4 and ECP5, have been cloned and characterized from \textit{C. fulvum} that induce an HR in tomato lines containing the corresponding Cf-Ecp resistance genes (Lauge et al., 2000; de Kock et al., 2005). Ecp6 and Ecp7 have been cloned but the corresponding tomato lines that recognize these genes have not yet been identified (Bolton et al., 2008). ECPs are present in all strains of \textit{C. fulvum} and are secreted during infection. They contain an even number of cysteine residues that are most likely involved in disulphide bridge formation and secondary structure formation to protect them from apoplastic proteases (Luderer et al., 2002). Three of the ECPs, ECP1, ECP2 and ECP6, have virulence functions on host plants, based on data showing that deleting or suppressing expression of these genes reduced virulence (Lauge et al., 1997; Bolton et al., 2008). Orthologs for AVR4 and ECP6 have been identified in several fungal species because of the presence of CMB14 and lysin motif (LysM, carbohydrate-binding) domains in these proteins (Bolton et al., 2008). Orthologs of AVR4 and ECP2 have been identified in \textit{Mycosphaerella fijiensis} that causes black Sigatoka disease of banana (Stergiopoulos et al., 2010). The \textit{M. fijiensis} ortholog of AVR4 induces an HR in tomato lines containing the corresponding Cf4 gene and binds to chitin of fungal cell walls to protect against cell wall degradation, similar to \textit{C. fulvum} AVR4 (Stergiopoulos et al., 2010). Similarly, \textit{M. fijiensis} ECP2 is a functional ortholog of \textit{C. fulvum} ECP2 and is recognized by Cf2 of tomato to induce an HR, while in the absence of Cf2, it promotes virulence on tomato plants (Stergiopoulos et al., 2010).

\textbf{Rynchosporium secalis}

The imperfect fungus \textit{R. secalis} causes leaf scald disease on barley by secreting low molecular-weight toxic proteins. The genes for three of these effectors, designated as necrosis-inducing proteins NIP1, NIP2 and NIP3, have been cloned (Table 1). They encode small secreted proteins with toxicity in a genotype non-specific manner on barley and related cereal plant species (Hahn et al., 1993; Rohe et al., 1995; Steiner-Lange et al., 2003). Mature NIP1 is a 60 aa protein with 10 cysteine residues that are involved in intramolecular disulphide bond formation. NIP1 triggers specific defence responses without an HR on barley cultivars that have the corresponding resistance gene, \textit{Rrs1} (Lehnackers & Knogge, 1990). The injection of NIP1 into leaves of barley and other cereal plant species causes scald-like lesion formation (Wevelsiep et al., 1991; van’t Slot et al., 2007). A \textit{nip1} disruption mutant of \textit{R. secalis} is slightly less virulent than the wild type on susceptible plants, demonstrating a (minor) role in virulence (Knogge & Marie 1997). Virulent strains of \textit{R. secalis} overcome \textit{Rrs1} resistance of barley either by a point mutation in the ORF that results in a single aa substitution or by jettison of the \textit{Nip1} gene (Rohe et al., 1995). It has been shown that NIP1 interacts with a single plasma membrane receptor (different from \textit{Rrs1}) that is involved both in necrosis and defence induction (van’t Slot et al., 2007). A field population study of this pathogen showed a positive diversifying selection on the \textit{Nip1} locus, as three out of the 14 isoforms gained virulence on \textit{Rrs1} barley lines and a high deletion frequency was observed in the \textit{Nip1} locus compared with \textit{Nip2} and \textit{Nip3} (de Wit et al., 2009). The deletion frequency of \textit{Nip1} was higher than the occurrence of the point mutation that gains virulence indicating a reduced fitness penalty for the loss of the \textit{Nip1} gene. \textit{Nip2} encodes a 109 aa protein with a predicted secretion signal of 16 aa and a mature protein with 6 cysteine residues, while \textit{Nip3} encodes a 115 aa protein with a predicted secretion signal of 17 aa and a mature protein with 8 cysteine residues (de Wit et al., 2009).

\textbf{Magnaporthe oryzae}

\textit{M. oryzae} (formerly known as \textit{M. grisea}) is a filamentous ascomycete fungus that causes rice blast disease,
destructive to rice production worldwide, but can also cause disease in many other members of graminaceous plants (Kato et al., 2000; Couch & Kohn, 2002). More than 40 resistance genes have been identified in rice controlling the blast fungus and several of them have been extensively used in resistant rice lines in the past few decades (Bryan et al., 2000; Chen et al., 2006). These resistant rice lines are overcome quickly in the field by the emergence of new races of the pathogen through various mechanisms in the pathogen, such as deletion of Avr genes from the genome (Yoshida et al., 2009), changes in their gene expression (Kang et al., 2001; Fudal et al., 2005) or point mutations in their genes (Orbach et al., 2000) resulting in escaping recognition by R genes (Kolmer, 1989; Leach et al., 2001; McDonald & Linde, 2002). Eight cultivar- and species-specific Avr genes have been cloned and characterized from M. oryzae: Avr-Pita, Avr1-CO39, Ace1, Pwl1, Pwl2, AvrPitz-t, Avr-Pia, Avr-Pii and Avr-pik/km/kp (Table 1; Valent et al., 1991; Farman & Leong, 1998; Orbach et al., 2000; Bohnert et al., 2004; Collemare et al., 2008; Li et al., 2009; Miki et al., 2009; Yoshida et al., 2009).

The AVR-Pita effector shows similarity to fungal metalloproteases of the deuterolysin family and is not required for full virulence on rice plants (Jia et al., 2000; Orbach et al., 2000). Avr-Pita encodes a 223 aa protein that is predicted to be secreted and needs to be processed into a 176 aa active form (AVR-Pita 176) to interact directly with the LRR (leucine-rich repeat) domain of the PITA resistance protein and trigger PITA-mediated defence responses, as assayed by yeast 2-hybrid assays, in vitro binding analyses and direct expression in rice cells (Jia et al., 2000). In certain strains of M. oryzae, Avr-Pita undergoes spontaneous mutations in the laboratory and also under field conditions, such as deletion, point mutation, and the insertion of transposons, all resulting in overcoming Pita resistance in rice cultivars (Orbach et al., 2000; Kang et al., 2001; Zhou et al., 2007; Kang et al., 2008). Avr-Pita is located close to the telomere of chromosome 3 in the genome of M. oryzae and this may be responsible for the genetic instability of this gene. Avr-Pita was renamed Avr-Pita1 after identification of Avr-Pita2 and Avr-Pita3 (Khang et al., 2008). Avr-Pita2 is recognized by the rice Pita gene and elicits the defence response while Avr-Pita3 is not recognized by Pita (Khang et al., 2008).

Avr1-Co39 was isolated from M. oryzae isolate 4091-5-8 pathogenic on weeping lovegrass and specifies avirulence on rice cultivar CO39 that contains the resistant gene, Pi-CO39(t) in a gene-for-gene manner (Valent et al., 1991; Chauhan et al., 2002). Isolates virulent on rice cultivar ‘CO-39’, lack Avr1-CO39 in most of the cases (Farman et al., 2002). It has been shown that Avr1-CO39 is a species-specific rather than a cultivar-specific type of Avr gene (Zheng et al., 2011). The Pwl genes stop this pathogen from causing disease on weeping lovegrass and finger millet in a species-specific manner, but they still can infect rice (Kang et al., 1995; Sweigard et al., 1995). PWL effectors are small glycine-rich secreted proteins that are evolving fast and belong to a gene family designated as PWL1-PWL4. Pwl2 strains virulent on weeping lovegrass appear due to spontaneous mutations, predominantly by genetic rearrangement and large deletions (Kang et al., 1995; Sweigard et al., 1995). In the three homologs of Pwl2, identified by homology searches, only Pwl1 is the functional homolog while Pwl3 and Pwl4 are not functional; however, Pwl4 is functional only when expressed under the control of the Pwl1 or Pwl2 promoter, while Pwl3 is not functional in that case (Kang et al., 1995). Three new Avr genes, Avr-Pia, Avr-Pii and Avr-Pik/km/kp, have been isolated from M. oryzae by association genetics, i.e. by looking for polymorphisms among 1032 predicted secreted proteins in field isolates (Yoshida et al., 2009).

An Avr gene from a different class, Ace1, encodes a 4035 aa polyketide synthase (PKS) fused to a nonribosomal peptide synthetase (NRPS); these are two different classes of enzymes that are probably involved in the production of a secondary metabolite that triggers Pi33-mediated resistance in rice cultivars (Bohnert et al., 2004). M. grisea genome analysis revealed that Ace1 is present in a cluster of 15 genes of which 14 encode enzymes such as a second PKS-NRPS (Syn2), two enoyl reductases (Rap1 and Rap2) and a putative Zn(II)(2)/Cys(6) transcription factor (BC2) which probably all play a role in secondary metabolism (Collemare et al. 2008). Ace1 and all other genes in the cluster are specifically expressed during penetration into the host plant, defining an infection-specific gene cluster, which suggests that Ace1 might have a role in virulence; however, an Ace1 disruption mutant did not show any reduction in virulence (Bohnert et al., 2004; Fudal et al., 2005).

**Leptosphaeria maculans**

This ascomycete fungus causes blackleg (phoma stem canker) disease on oilseed rape (Brassica napus). Genetic analysis of the interaction has revealed at least nine avirulence genes designated as AvrLm1–AvrLm9 that are recognized by corresponding resistance genes Rlm1–Rlm9 of the host (Balesdent et al., 2002; Rouxel & Balesdent, 2005; Yu et al., 2005; Fitt et al., 2006). Seven of these nine genes are present in two unlinked clusters (AvrLm1-2-6 and AvrLm 3-4-7-9) while the remaining two
are individual genes (Balesdent et al., 2002). *AvrLm1*, *AvrLm6* and *AvrLm4-7* have been cloned by a map-based strategy and all encode small putative secreted proteins that have no similarity to sequences in public databases (Table 1; Gout et al., 2006; Fudal et al., 2007; Parlange et al., 2009). Both *AvrLm1* and *AvrLm6* are located in a gene-poor, AT-rich, non-coding heterochromatin-like region as solo genes in stretches of 269 and 131 kb, respectively, which contain a number of degenerated nested copies of long terminal repeat (LTR) retrotransposon (Gout et al., 2006; Fudal et al., 2007). Also, both *AvrLm1* and *AvrLm6* are single copy genes that encode small proteins of 205 and 144 aa, respectively, with an N-terminal secretion signal but no other conserved motif or any similarity to each other. The expression of both genes is strongly induced during early leaf infection but they are also expressed in culture media at relatively low levels when not in contact with the host (Gout et al., 2006; Fudal et al., 2007). *AvrLm1* has only one cysteine residue and is likely taken up by the host cell (Gout et al., 2006), while *AvrLm6* contains six cysteine residues that make disulphide bridges that could provide stability in the apoplastic environment (Fudal et al., 2007). Strains virulent on *Rlm1* oilseed rape cultivars lack the *AvrLm1* gene. Repeat-induced point (RIP) mutation, a common mechanism to inactivate genes in ascomycetes, and deletion were also responsible for gain of virulence on *AvrLm1* cultivars (Fudal et al., 2009). Similar to the *AvrLm1* and *AvrLm6* loci, the isolated *AvrLm4-7* gene was located to a 238 kb genetic locus having multiple LTR retrotransposons; this gene induces a resistance response in plants having either *Rlm7* or *Rlm4*. Within the locus, this single gene is embedded in a gene-poor, AT-rich, recombination-deficient 60 kb isochor (Parlange et al., 2009). It encodes a small cysteine-rich secreted protein of 143 aa with a predicted N-terminal secretion signal of 21 aa. Like other effectors from this fungus, the expression of *AvrLm4-7* is induced in the plant but is also expressed at low levels in culture media (Parlange et al., 2009). The partial or complete loss of *AvrLm4-7* in field isolates is responsible for gain of virulence on both *Rlm4* and *Rlm7* cultivars, while a point mutation that changes a glycine to an arginine residue, can overcome recognition by *Rlm4* (Parlange et al., 2009).

**Fusarium oxysporum**

*F. oxysporum* f. sp. *lycopersici* (Fol) infects tomato roots via wounds or by direct penetration after which it colonizes xylem vessels. Several small cysteine-rich proteins were identified from xylem sap during *Fol* infection, and three of them induced resistance in an *R* gene-specific manner. They are coded for by avirulence gene SIX (secreted in xylem) 1 to 4 (Table 1; Rep et al., 2004; Houterman et al., 2007, 2008, 2009). *Fusarium* genome comparisons revealed that all the SIX genes are located on the same chromosome that is absent from non-pathogenic isolates. The transfer of this chromosome to non-pathogenic strains converted them to pathogens (Ma et al., 2010). The function of three of these genes in virulence has been discussed in the previous section.

**Blumeria graminis**

Powdery mildews are biotrophic ascomycete fungi that cause diseases on various mono- and dicotyledonous plant species, including food and feed crops and ornamental plants (Bushnell, 2002). They are obligate biotrophs that need a living host for growth and reproduction and produce intracellular feeding structures, the haustoria, in the epidermis of their host plants (Yarwood, 1957; Glawe, 2008). *Bgh* causes powdery mildew on barley and is the most thoroughly studied powdery mildew fungus. It interacts with its host in a ‘gene-for-gene’ manner (Both et al., 2005; Zhang et al., 2005). Genetic analysis revealed that there are more than 85 dominant or semi-dominant mildew (*Ml*) resistance genes that recognize different *Bgh* races including 28 highly similar genes at the *Mla* locus on barley chromosome 5 (Jensen et al., 1980). Seven *Mla* genes at this locus have been cloned and they all encode closely related intracellular coiled-coil, nucleotide-binding site, leucine-rich repeat (CC-NBS-LRR) type R proteins that recognize different *Bgh* avirulent effector proteins (Haltermann et al., 2001; Haltermann & Wise, 2004). Two of these effectors, AVRa10 and AVRk1, that are recognized by the barley R proteins, Mla10 and Mlk1, respectively, inducing an HR, have been cloned; however, they are also virulence factors in that they promote infection on susceptible barley cultivars (Table 1; Ridout et al., 2006). Both *Avr* genes belong to multi-gene families that have more than 30 paralogs and they also have orthologs in other *formae speciales* that are pathogenic on other grasses (Ridout et al., 2006). The predicted AVRa10 and AVRk1 effectors lack an N-terminal secretion signal or host targeting sequence, but it has been shown by fluorescence microscopy that the Mla10 protein is present intracellularly, both in the cytoplasm and the nucleus of invaded barley cells. Assuming a necessary interaction with effectors, this means that AVRa10 is secreted from the fungus by non-endomembranous pathways and taken up by the cell by an as yet unknown mechanism (Bieri et al., 2004; Shen et al., 2007). Using a novel approach to gene silencing, Nowara et al. (2010) showed that by
expressing double-stranded or antisense RNA sequences in barley but targeted to the Bgh-specific Avr10 and AvrK1 effector genes, they could reduce fungal development in hosts lacking the corresponding Mla10 resistance gene, confirming their role in virulence. Interestingly, no clear release from R-gene recognition could be seen by silencing the Avr10 gene using this system which they termed ‘Host-Induced Gene Silencing’ or HIGS. Besides Avr10 and AvrK1, two other Avr genes, Avra22 and Avra12, have been mapped recently (Skamnioti et al., 2008).

**Melampsora lini**

Another obligate biotrophic fungus, but one that belongs to the basidiomycetes, is *M. lini* that causes flax rust disease not only in flax but also in other species of the genus *Linum*. A number of flax R proteins have been analysed; these are highly polymorphic cytoplasmic (Toll/Interleukin1 Receptor) TIR-NBS-LRR proteins that recognize effector proteins that are delivered into flax cells during colonization. This interaction triggers an HR in a ‘gene-for-gene’ manner, arresting growth of the fungus (Lawrence et al., 2007). The genetic analysis of *M. lini* with its host plant flax (the basis of the original ‘gene-for-gene’ theory by Flor in 1942) revealed at least 30 Avr genes and corresponding R genes (Ellis et al., 1997). Several Avr genes have been cloned from *M. lini*, mainly from four different loci: AvrL567, AvrM, AvrP123 and AvrP4 (Table 1). These genes encode haustoria-expressed secreted proteins (HESPs), suggesting that they have virulence functions, but elicit defence responses in hosts that have the corresponding R genes (Catanzariti et al., 2006). The AvrL567 locus has a cluster of three polymorphic Avr genes: AvrL567A, AvrL567B and AvrL567C. All three encode 127 aa mature proteins after cleavage of a 23 aa SP and are recognized directly by the L5, L6 and L7 proteins inside the cell (Dodds et al., 2004). The mature AVR proteins are highly polymorphic with at least one or more aa substitutions in the exposed surface of the proteins, suggesting functional interactions (Ellis et al., 2007; Wang et al., 2007). Some of the isolates harbouring these AVR proteins became virulent by defeating matching plant resistance genes, indicating that genes in the AvrL567 locus are under positive diversifying selection (Dodds et al., 2006). The analysis of six flax rust isolates revealed 12 members of the AvrL567 effector gene family, including the three previously isolated AvrL567A-C genes. Seven of these caused avirulence while five belonged to virulent strains and could no longer be recognized by L5, L6 or L7 (Dodds et al., 2006). The AvrM gene family is a small family that consists of five avirulence paralogs, AvrMA to AvrME, and one virulent one, AvrM, which is not recognized by any known flax R protein (Catanzariti et al., 2006). These AvrM proteins do not have any known homologs and are highly variable both in sequence and size due to deletions or insertions of DNA, or to premature termination of the protein because of the location of stop codons (Catanzariti et al., 2006).

AvrP123 is a small cysteine-rich protein that contains the characteristic CX7CX6XYX3CX2-3C signature of the Kazal serine protease inhibitor family, suggesting its role as an inhibitor of host proteases. This is similar to the function of *C. fulvum* AVR2 that inhibits the Rcr3 cysteine protease in the tomato apoplast (Catanzariti et al., 2006). AvrP4 also encodes a small cysteine-rich protein of 67 aa after cleavage of a 28 aa SP. The 28 aa C-terminal part of AvrP4 has 6 cysteine residues with a spacing consensus of a typical ‘cysteine-knotted’ peptide, similar to the *C. fulvum* AVR9 protein (van den Hooven et al., 2001). AvrP4 is expressed only *in planta* while AvrM is expressed both *in planta* and *in vitro* (Stergiopoulos & de Wit, 2009). Agroinfiltration of AvrP4 and AvrM genes in flax plants with matching resistance genes results in a HR indicating that the produced effectors are functional in host cells and are, therefore, likely translocated into the host during infections. This is in agreement with the predicted cytoplasmic location of P and M resistance proteins in flax (Anderson et al., 1997). Using a yeast 2-hybrid system, the AvrM-A and -D effectors were shown to interact directly with the M resistance protein resulting in fast necrosis, and that a globular C-terminal domain, variable among alleles, is important for this interaction (Catanzariti et al., 2010).

**Oomycete Avr (-triggering effector) genes**

Oomycetes belong to the kingdom Stramenopila and are evolutionarily related to algae and include some well-known plant pathogens. Many effector genes have been isolated, some of which have proven avirulence functions (Table 1).

**Phytophthora infestans**

*P. infestans* causes late blight disease in potato and tomato and was responsible for the ‘Irish Famine’ in 1840. Many effectors have been identified and isolated from *P. infestans* such as INF1 ‘elicitin’, a secreted protein that elicits hypersensitive cell death and is used in many current functional assays (Kamoun et al., 1997; Kamoun, 2006; Haas et al., 2009). However, only one race-specific avirulence gene, Avr3a has been cloned. Avr3a encodes a cytoplasmic RXLR effector and when
transiently expressed lacking its SP, in potato cells, R3a-mediated cell death ensues illustrating its recognition in the cytoplasm and avirulence function (Armstrong et al., 2005). The mature secreted protein is 147 aa in length and allelic polymorphic residues distinguishing functionality were identified in various pathogen races: one allelic variant Avr3A^K80/M103 (abbreviated as Avr3A^KI) causes avirulence on potato plants expressing R3a with the lysine residue at position 80 being critical for recognition, while the virulent allele has two aa substitutions, glutamate at position 80 and methionine at position 103 (designated as Avr3A^E80/M103 or Avr3A^EM). In the absence of R3a, Avr3A^KI strongly suppresses P. infestans INF1 eliciting-induced cell death in plants, thereby illustrating a major virulence function. The virulent allele Avr3a^EM cannot induce an R3a-mediated HR but can still (weakly) suppress INF1-induced cell death suggesting that this effector has various parts with distinct functions that likely act through amino acid interactions with host proteins (Bos et al., 2006). These two distinct activities have been separated through saturated high-throughput mutant screens revealing important aa residues (Bos et al., 2009). Deletions or mutations in the C-terminal residue at tyrosine 147 maintained the R3A-mediated HR activity while it caused the loss of the ability to suppress INF1-induced cell death. Molecular analysis of its cell death-suppressing function showed that Avr3A^KI interacts and stabilizes the host ubiquitin E3-ligase CMPG1 which is required for INF1-induced cell death (Bos et al., 2010). The effector Avr3A likely targets CMPG1, interfering with the ubiquitin proteasome system to block signal transduction upon pathogen perception (Gilroy et al., 2011). P. infestans Avr3a is located in a region syntenic to a part in the genome of another oomycete, Hyaloperonospora arabidopsidis which harbours avirulence effector ATR1^{N^D/W^B} (see below), suggesting that this locus is ancient in these oomycetes.

The P. infestans genome sequence revealed many predicted secreted effectors and targeted searches for function revealed novel Avr activities for some effectors interacting with specific resistance genes found in certain potato species to induce hypersensitive cell death (Oh et al., 2009). Another secreted effector, SNE1, likely is delivered into the plant host nucleus where it orchestrates the suppression of the effects of cell death-inducing effectors secreted during the biotrophic phase (Kelley et al., 2010).

Phytophthora sojae

P. sojae causes root and stem rot of soybean, resulting in huge damage to soybean production in North America (Shan et al., 2004). Four avirulence genes, designated Avr1b-1, Avr1a, Avr3a and Avr3c, have been cloned from this pathogen; they are recognized by soybean resistance genes Rps1b, Rps1a, Rps3a and Rps3c, respectively (Table 1; Shan et al., 2004; Dong et al., 2009; Qutob et al., 2009). Avr1b-1 was cloned by map-based cloning and is predicted to encode a secreted protein of 138 aa with a RXLR motif. Interestingly, this gene requires another gene, Avr1-b2, at the locus for accumulation of Avr1b-1 mRNA. Virulent P. sojae isolates such as P6497 and P9073, harbour a complete Avr1b-1 gene but cannot accumulate Avr1b-1 mRNA like avirulent strains (Shan et al., 2004). In addition to recognition by Rps1B, Avr1B-1 is also recognized in plants having the Rps1L resistance gene resulting in limited cell death and showing that such effectors might have multiple targets (Kamoun, 2006). As R proteins from the Rps1 gene clusters are cytoplasmic, it is assumed that Avr1-B is recognized inside the host cytoplasm (Bhattacharyya et al., 1997; Shan et al., 2004).
cell death themselves and are hence possible avirulence factors (Wang et al., 2011).

**Hyaloperonospora arabidopsidis**

*H. arabidopsidis*, formerly known as (*Hyalol*)peronospora parasitica, is an obligate oomycete pathogen that causes downy mildew disease on the model plant *Arabidopsis*. From this pathogen, two avirulence genes have been cloned and characterized: (*Arabidopsis thaliana* recognized 1), Atr1 and Atr13 (Table 1). The product of the Atr1 gene is recognized by *Arabidopsis* R proteins from two ecotypes, Niederzenn (RPP1	extsuperscript{Nd}) and Wassilewskija (RPP1	extsuperscript{WsB}) and hence the effector allele is called Atr1	extsuperscript{NdWsB}. Transient expression of Atr1	extsuperscript{NdWsB} in *Arabidopsis* leaves by particle bombardment triggered cell death. Extensive searches for allelic variants found that RPP1	extsuperscript{Nd} recognized the product of a single allele of Atr1, while RPP1	extsuperscript{WsB} recognized the products of four different diverged alleles and provided resistance to a wide range of isolates (Rehmany et al., 2005). Atr1	extsuperscript{NdWsB} encodes a 311 aa protein with a predicted secretion signal and a conserved RXLR motif. Atr1	extsuperscript{NdWsB} is a highly polymorphic protein and six different alleles that differ in about one-third of all residues were identified in eight isolates; intense diversifying selection and recombination played an important role in the evolution of this locus (Rehmany et al., 2005; Kamoun, 2006). Recent molecular studies have shown that binding of RPP1 occurs at the LRR domain and is a prerequisite for HR induction, while the actual induction is facilitated by the TIR domain (Krasilëva et al., 2010).

ATR13 is recognized by resistance protein RPP13 and triggers a HR when transiently expressed in *Arabidopsis* cells using particle bombardment. The ATR13 gene encodes a 187 aa effector protein for which no related sequences have been found in public databases (Allen et al., 2004). ATR13 revealed apparent domain structures: in addition to the N-terminal SP and an RXLR motif, it has a heptad leucine-isoleucine repeat motif that is required for RPP13 recognition, followed by an imperfect direct repeat of 4 × 11 aa which lies between aa residues 93 and 136. All pathogen Atr13 effector alleles as well as identified alleles of the interacting host RPP13 resistance gene reveal a high level of aa polymorphisms among their protein products and apparently have been under intense diversifying selection suggesting a co-evolutionary arms race at these loci (Allen et al., 2004). Both ATR1 and ATR13 suppress basal defence responses of host plants when delivered by the *P. syringae* T3SS, revealing a virulence function (Sohn et al., 2007).

**Concluding remarks**

Over the last two decades, the study of different fungal, oomycete and bacterial pathogen effectors and their function during the interaction with host plants has led to remarkable insights into the molecular basis underpinning plant diseases. These effectors facilitate a pathogen’s entry into the host by suppressing defence responses at multiple levels. Examples include interference with recognition of PAMPs by PRRs and/or downstream signalling thereby evading basal immunity that otherwise would be sufficient to stop infection. Effectors also have roles in establishing feeding interactions and/or nutrient leakage from the host to the benefit of the pathogens. These effectors function at the interface of pathogen and host plant or inside plant cells. Inadvertently, when recognized by components of the host surveillance system, they can trigger defence responses, revealing avirulence or elicitor functions. The sequencing of complete genomes of many bacterial, fungal, and oomycete plant pathogens and subsequent computational analyses have revealed a myriad of effectors, many of which are potential avirulence genes that can trigger ETI. These then have the potential of revealing novel resistance potential, in particular resistance genes in varied germplasm if suitable assays can be designed. This has become an important goal of many research laboratories and has already led to the discovery of novel resistance genes (van der Hoorn et al., 2000; Torto et al., 2003; Stergiopoulos et al., 2010). The identification of complete sets of pathogen effectors, their functions, and their host targets will advance the knowledge of molecular plant–pathogen interactions and spark the design of novel disease control strategies.

**References**


Fungal and oomycete effectors


