



## UvA-DARE (Digital Academic Repository)

### Host-specificity factors in plant pathogenic fungi

Li, J.; Cornelissen, B.; Rep, M.

**DOI**

[10.1016/j.fgb.2020.103447](https://doi.org/10.1016/j.fgb.2020.103447)

**Publication date**

2020

**Document Version**

Final published version

**Published in**

Fungal Genetics and Biology

**License**

CC BY

[Link to publication](#)

**Citation for published version (APA):**

Li, J., Cornelissen, B., & Rep, M. (2020). Host-specificity factors in plant pathogenic fungi. *Fungal Genetics and Biology*, 144, Article 103447. <https://doi.org/10.1016/j.fgb.2020.103447>

**General rights**

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

**Disclaimer/Complaints regulations**

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.



# Host-specificity factors in plant pathogenic fungi

Jiming Li, Ben Cornelissen, Martijn Rep\*

Molecular Plant Pathology, University of Amsterdam, Amsterdam 1098 XH, the Netherlands

## ARTICLE INFO

### Keywords:

Host specificity  
Virulence genes  
Avirulence genes  
Plant pathogenic fungi

## ABSTRACT

Fortunately, no fungus can cause disease on all plant species, and although some plant-pathogenic fungi have quite a broad host range, most are highly limited in the range of plant species or even cultivars that they cause disease in. The mechanisms of host specificity have been extensively studied in many plant-pathogenic fungi, especially in fungal pathogens causing disease on economically important crops. Specifically, genes involved in host specificity have been identified during the last few decades. In this overview, we describe and discuss these host-specificity genes. These genes encode avirulence (Avr) proteins, proteinaceous host-specific toxins or secondary metabolites. We discuss the genomic context of these genes, their expression, polymorphism, horizontal transfer and involvement in pathogenesis.

## 1. Introduction

Plant diseases caused by fungi are recognized as a major threat to food security (Doehlemann et al., 2017; Fisher et al., 2012; Pennisi, 2010). For example, wheat stem rust caused by *Puccinia graminis*, Asian soybean rust caused by *Phakopsora pachyrhizi*, rice blast caused by *Magnaporthe oryzae*, and banana black sigatoka caused by *Mycosphaerella fijiensis* have resulted in serious yield losses in human history (Pennisi, 2010).

Most plant pathogenic fungal species have a narrow range of plant species in which they cause disease, a phenomenon we here call 'host species specificity'. The collective host range of some fungal species, such as *Fusarium oxysporum* (*F. oxysporum*), can be very large, but then individual strains are often limited to infect one or a few plant species only (Borah et al., 2018; Pietro et al., 2003). Based on host range, strains within such fungal species are commonly classified into different pathotypes or *formae speciales*. In addition, fungal species or *formae speciales* are sometimes divided into different races depending on the particular cultivars of a plant species that they are able to infect. This phenomenon we here call 'host cultivar specificity'. For example, *F. oxysporum* is classified into more than 100 *formae speciales* based on host species specificity, including the tomato-infecting strain *F. oxysporum* forma specialis (f. sp.) *lycopersici* (Fol), while Fol itself is subdivided into three races based on host cultivar specificity (Takken and Rep, 2010). The molecular basis of host cultivar specificity has been extensively studied in many plant pathogenic fungi, while the molecular basis of host species specificity is less well understood (de Wit, 2016; Lanver et al., 2017; Lo Presti et al., 2015; Prasad et al., 2019;

Selin et al., 2016; Yan and Talbot, 2016). In this review, the term 'host specificity' includes both host species specificity and host cultivar specificity.

To understand the genetic basis of host specificity in plant pathogenic fungi, the emergence of a molecular understanding of plant immunity over the last three decades has been essential. To defend themselves against fungal pathogens, plants have evolved two layers of immunity (Jones and Dangl, 2006). The first layer of immunity responds to pathogen-associated molecular patterns (PAMPs) common to many microbes, including non-pathogens, and this defense system is called PAMP-triggered immunity (PTI) (Jones and Dangl, 2006). To suppress PTI responses, pathogens secrete molecules called effectors to facilitate colonization. These effectors are commonly secreted proteins but can also be metabolites. Some effectors target plant susceptibility (S) proteins, resulting in effector-triggered susceptibility (ETS) (van Schie and Takken, 2014). For example, necrotrophic effectors of *Stagonospora nodorum* (*S. nodorum*) are able to interact with wheat susceptibility gene products (Oliver et al., 2012). The second layer of defense comprises plant resistance (R) proteins that directly or indirectly recognize pathogen-produced effectors, resulting in effector-triggered immunity (ETI) (Jones and Dangl, 2006). Among identified R proteins, most are nucleotide-binding leucine-rich repeat proteins (NLRs) (Kourelis and van der Hoorn, 2018). To evade effector recognition by plant R proteins, pathogens can undergo loss or mutation of the corresponding effector genes (Jones and Dangl, 2006; Wang and Wang, 2018). As a result, pathogens and plants are evolving in a perpetual arms race. Most effector genes are located in repeat-rich regions. For example, all known effector genes in *Leptosphaeria maculans* (L.

\* Corresponding author at: Box 94215, 1090 GE Amsterdam, the Netherlands.  
E-mail address: [m.rep@uva.nl](mailto:m.rep@uva.nl) (M. Rep).

<https://doi.org/10.1016/j.fgb.2020.103447>

Received 16 March 2020; Received in revised form 14 August 2020; Accepted 15 August 2020

Available online 20 August 2020

1087-1845/© 2020 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

*maculans*) are located in AT-rich isochores, mainly consisting of de-generated transposable elements (TEs) (Rouxel and Balesdent, 2017). AT-rich isochores are sculpted by repeat-induced point mutation (RIP) acting on repetitive elements by  $G \rightarrow A$  and  $C \rightarrow T$  mutations, a process that inactivates duplicated sequences (Rouxel et al., 2011; Rouxel and Balesdent, 2017).

This arms race between pathogens and plants results in relatively fast evolution of effectors, R and S proteins (Jones and Dangl, 2006; Wang and Wang, 2018). In general, effectors promote virulence and are therefore virulence factors. Effectors that are recognized by R proteins are (also) called avirulence (Avr) factors (Avr proteins) (Stotz et al., 2014). These avirulence and virulence factors determining resistance and susceptibility of plants, respectively, are considered to be host-specificity factors and have been identified in many plant pathogenic fungi (de Wit, 2016; Lanver et al., 2017; Prasad et al., 2019; Selin et al., 2016; Toruño et al., 2016; Yan and Talbot, 2016). The virulence factors reviewed here are known as proteinaceous toxins, therefore they are referred as proteinaceous host-specific toxins hereafter. In addition to pathogen-secreted proteins that can determine host specificity, secondary metabolites can also act as host-specificity determinants, such as the host-selective toxins (HSTs) of *Alternaria alternata* and *Verticillium dahliae* (Chen et al., 2018; Tsuge et al., 2013; Zhang et al., 2019).

Here we first describe host-specificity factors in plant pathogenic fungi, including avirulence proteins, proteinaceous host-specific toxins and secondary metabolites. Then, involvement of horizontal transfer and the genomic context in the acquisition and evolution of host-specificity genes is addressed.

## 2. Avirulence proteins determining host specificity

Avirulence proteins determining host cultivar specificity have been identified in various fungal species (Table 1). Below we provide a brief overview of these cases, including involvement in pathogenesis, polymorphism and interaction with the corresponding resistance proteins.

### 2.1. *Fusarium oxysporum*

*F. oxysporum* is a soil-borne (presumably) asexual fungal species complex, which causes vascular wilt and root rot (Edel-Hermann and Lecomte, 2019). Avirulence genes in *F. oxysporum* determining host cultivar specificity have been identified in several *formae speciales*, including Fol (Takken and Rep, 2010), *F. oxysporum* f. sp. *melonis* (Schmidt et al., 2016) and *F. oxysporum* f. sp. *niveum* (Niu et al., 2016). Here, we will review the avirulence (AVR) genes identified in Fol and *F. oxysporum* f. sp. *melonis*, which infect tomato and melon, respectively.

#### 2.1.1. *F. oxysporum* f. sp. *lycopersici* (Fol)

*F. oxysporum* f. sp. *lycopersici* (Fol) can be divided into three races based on their capability to infect tomato cultivars containing different resistance genes to Fol (Takken and Rep, 2010). Race 1 contains three AVR genes, notably AVR1, AVR2 and AVR3. The protein encoded by resistance gene *I* recognizes the product of AVR1, upon which the immune system is activated in the plant (Houterman et al., 2008). Avr1 also suppresses recognition of Avr2 and Avr3 by resistance proteins I-2 and I-3, respectively (Houterman et al., 2008). Race 2 evolved from race 1 by deletion of a chromosomal region containing AVR1, likely due to a recombination event between two TEs bordering the fragment (Biju et al., 2017). The I-2 resistance gene was introduced into tomato cultivars to protect them against race 2. The I-2 protein recognizes Avr2 (Houterman et al., 2009; Ma et al., 2015). Single point mutations in AVR2 subsequently emerged such that the gene product was no longer recognized by I-2, resulting in race 3 (Houterman et al., 2009). Resistance gene I-3 against race 3 was introduced in tomato cultivars, and the corresponding AVR3 gene, the product of which is recognized by the I-3 protein, was identified in Fol as well (Rep et al. 2004).

Among the three Fol AVR genes known, AVR1 is not required for full

virulence on susceptible hosts (Houterman et al., 2008), whereas AVR2 and AVR3 are (Houterman et al., 2009; Rep et al., 2004). For activation of I-2-mediated resistance, not only Avr2 is required, but also a Fol protein called Secreted in xylem 5 (Six5). Like Avr2, Six5 is required for full virulence (Houterman et al., 2009; Ma et al., 2015). All three Fol AVR genes encode small, secreted proteins with multiple cysteines, and are located on a single accessory chromosome with high density of repetitive elements (Ma et al., 2010; Schmidt et al., 2013).

#### 2.1.2. *F. oxysporum* f. sp. *melonis*

*F. oxysporum* f. sp. *melonis* is divided into race 0, race 1, race 2, and race 1,2. So far, only one avirulence gene has been identified, *AvrFom2*, whose product is recognized by the protein encoded by the melon R gene *Fom2* (Schmidt et al., 2016). *AvrFom2* is a small secreted protein with two cysteine residues and without recognizable domains (Schmidt et al., 2016). However, it does show an overall low similarity to ToxA of *Pyrenophora tritici-repentis* (discussed below), and the cysteine residues that form the characteristic cysteine knot in ToxA are conserved (Schmidt et al., 2016). *AvrFom2* is located in a lineage-specific region of the Fom001 genome and resides close to transposons (Schmidt et al., 2016; van Dam et al., 2017). The gene is absent in race 2 isolates (Schmidt et al., 2016).

### 2.2. *Cladosporium fulvum*

*C. fulvum* (*Passalora fulva*) is a non-obligate biotrophic fungal species and the causal agent of tomato leaf mold (de Wit, 2016). Already in the 1970s, it was found that the gene-for-gene relation between tomato and *C. fulvum* is based on the interaction of specific fungal products with specific resistance proteins in tomato (van Dijkman and Kaars Sijpesteijn, 1973).

The first fungal Avr protein was identified in *C. fulvum* in 1991 (van Kan, 1991). Until now, ten AVR genes of *C. fulvum*, which encode small cysteine-rich proteins, have been identified, notably Avr2 (Luderer et al., 2002), Avr4 (Joosten et al., 1994), Avr4E (Westerink et al., 2004), Avr5 (Mesarich et al., 2014), and Avr9 (van Kan, 1991), Ecp1 (Laugé et al., 1997), Ecp2-1 (Laugé et al., 1997), Ecp4 (Lauge et al., 2000), Ecp5 (Lauge et al., 2000) and Ecp6 (Bolton et al., 2008). Recognition of the encoded proteins in tomato is mediated by the cognate Cf resistance proteins Cf-2, Cf-4, Cf-4E, Cf-5 and Cf-9, Cf-Ecp1, Cf-Ecp2-2, Cf-Ecp4, Cf-Ecp5 and Cf-Ecp6, respectively. These *C. fulvum* Avr proteins and other apoplastic effector proteins have been extensively reviewed previously (de Wit, 2016; Rivas and Thomas, 2005; Stergiopoulos and de Wit, 2009). In a recent study, nine newly identified small secreted proteins were found to be recognized by specific wild accessions of tomato, but the corresponding Cf immune receptor genes in these accessions are still unknown (Mesarich et al., 2018).

All *C. fulvum* Avr proteins identified are less than 300 amino acids in size and contain an even number of at least four cysteine residues (Mesarich et al., 2018, 2014). A virulence function for Avr2, Avr4, Avr5 and Ecp6 has been demonstrated (Mesarich et al., 2014; Stergiopoulos and de Wit, 2009). To avoid recognition by R proteins, several types of sequence modifications have occurred in *C. fulvum* AVR genes including gene deletions, gene disruption by insertion of a transposon-like element, and nonsynonymous amino acid substitutions (Stergiopoulos and de Wit, 2009). The frequency of such mutations may have been enhanced by proximity of these AVR genes to repetitive elements (de Wit et al., 2012).

### 2.3. *Leptosphaeria maculans*

*L. maculans* is a hemi-biotrophic ascomycete responsible for stem canker of oilseed rape (Petit-Houdenot and Fudal, 2017). To date, eight AVR genes from *L. maculans* have been identified, *AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm4-7*, *AvrLm5-9* (*AvrLmJ1*), *AvrLm6*, *AvrLm10* and *AvrLm11*, and all are located in repeat-rich, gene-poor genomic regions

**Table 1**  
Host-specificity factors in plant pathogenic fungi.

Fungal species	Host	Host-specificity genes	Corresponding resistance or susceptibility genes	Genome location of host-specificity genes	References
<b>Avirulence proteins determining host cultivar specificity</b>					
<i>Fusarium oxysporum</i> f. sp. lycopersici	Tomato	AVR1, AVR2, AVR3	I-1, I-2, I-3	Accessory chromosome	(Ma et al., 2010; Takken and Rep, 2010)
<i>Fusarium oxysporum</i> f. sp. melonis	Melon	AvrFom2	Fom2	Close to repetitive elements	(Schmidt et al., 2016)
<i>Cladosporium fulvum</i> (Passalora fuba)	Tomato	AVR2, AVR4, AVR4E, AVR9, Ecp1, Ecp2, Ecp4, Ecp5, and Ecp6	Cf-2, Cf-4, Cf-4E, Cf-9, Cf-Ecp1, Cf-Ecp2, Cf-Ecp4, Cf-Ecp5 and Cf-Ecp6	Close to repetitive elements	(de Wit, 2016; Iakovidis et al., 2020)
<i>Leptosphaeria maculans</i>	Oilseed rape	AvrLm1, AvrLm2, AvrLm3, AvrLm4-7, AvrLm5-9, AvrLm6, AvrLm10 and AvrLm11	Rlm1, Rlm2, Rlm3, Rlm4, Rlm7, Rlm5, Rlm9, Rlm6, Rlm10, and Rlm11	Repeat-rich regions	(Ghanbaria et al., 2018; Petit-Houdenot et al., 2019; Plissommeau et al., 2018; Rouxel and Balesdent, 2017)
<i>Magnaporthe oryzae</i>	Grass species	Avr-Pt-54, Avr-Pt-9, Avr-Pt-a, Avr-Pt-b, Avr-Pt-i, Avr-Pt-k/km/kp, Avr-Pt-ta1/2/3, Avr-Pt-z-t, Avr1-CO39, Ace1	Pt-54, Pt-9, Pt-a, Pt-b, Pt-i, Pt-k/km/kp, Pt-ta1/2/3, Pt-z-t, Pt-CO39, Pt33	Close to repetitive elements	(Fernandez and Orth, 2018)
<i>Rhynchosporium secalis</i>	Barley	Nip1	Rrs1	Present on a large chromosome	(Hahn, 1993; Mohd-Assaad et al., 2019)
<i>Melampsora lini</i>	Flax	AvrL567, AvrM, AvrP123, AvrP4, AvrL2-A, and AvrM14-A	L5, L6, L7, M, P, P1, P2, P3, P4, L2, M14	Mostly located at recombination hot-spots	(Lorrain et al., 2019; Petre et al., 2014)
<i>Puccinia graminis</i> f. sp. tritici	Wheat	AvrSr35; AvrSr50	Sr35; Sr50	-	(Chen et al., 2017; Salcedo et al., 2017)
<i>Blumeria graminis</i> f. sp. hordei	Barley	AvrK1, AvrA10, AvrA1, AvrA13, AvrA7, AvrA9, AvrA22	Mk1, Mla10, Ml a1, Mla13, Mla7, Mla9, Mla22	Close to repetitive elements	(Bourras et al., 2018; Saur et al., 2019)
<i>Blumeria graminis</i> f. sp. tritici	Wheat	AvrPm3a2/2, AvrPm2, AvrPm3b2/c2, AvrPm3d3	Pm3a, Pm3f, Pm3m, Pm3b, Pm3c, Pm3d	Close to repetitive elements	(Bourras et al., 2019, 2018)
<i>Verticillium dahliae</i>	Tomato	Ave1	Vz1	Close to repetitive elements	(de Jonge et al., 2012)
<b>Avirulence proteins determining host species specificity</b>					
<i>Magnaporthe oryzae</i>	Grass species	Pw12, PWT3, PWT4	Rwt3 and Rwt4	-	(Inoue et al., 2017; Sweigard et al., 1995; Takabayashi et al., 2002)
<i>Blumeria graminis</i> f. sp. tritici	Wheat	AvrPm3b2/c2, AvrPm3d3	Pm3b, Pm3c, Pm3d	Close to repetitive elements	(Bourras et al., 2019)
<b>Proteinaceous host-specific toxins determining host cultivar specificity</b>					
<i>Stagonospora nodorum</i>	Wheat	SnTox1, SnToxA, SnTox3	Snn1, Tsn1, Snn3	Repeat-rich regions	(Friesen et al., 2006; Liu et al., 2012, 2009)
<i>Pyrenophora tritici-repentis</i>	Wheat	ToxA and ToxB	Tsn1 and Tsc2	Close to repetitive elements	(Friesen et al., 2006; Martinez et al., 2004)
<b>Secondary metabolites determining host cultivar specificity</b>					
<i>Cochliobolus carbonum</i>	Corn	HC-toxin	hm/hm	Repeat-rich region	(Walton, 2006)
<i>Cochliobolus heterostrophus</i>	Corn	T-toxin	Texas male sterile cytoplasm (cms-T)	Repeat-rich regions	(Inderbitzin et al., 2010; Yang et al., 1996)
<b>Secondary metabolites determining host species specificity</b>					
<i>Verticillium dahliae</i>	Cotton, olive and okra	VdDj5 and VdDj6	-	Lineage-specific genomic region	(Chen et al., 2018; Zhang et al., 2019)
<i>Alternaria alternata</i>	A number of crops	AM-toxin, AF-toxin, AK-toxin, ACT-toxin, ACR-toxin, AAL-toxin, AT-toxin	-	Accessory chromosome	(Akimitsu et al., 2014; Tsuge et al., 2013)

(Ghanbarnia et al., 2018; Petit-Houdenot et al., 2019; Rouxel and Balesdent, 2017). For example, *AvrLm2* (Ghanbarnia et al., 2015), *AvrLm4-7* (Parlange et al., 2009) and *AvrLm5-9* (Plissonneau et al., 2018; Van de Wouw et al., 2014) reside within AT-rich isochores of the genome, while *AvrLm3* (Plissonneau et al., 2016) and *AvrLm10* (Petit-Houdenot et al., 2019) are located in sub-telomeric regions. *AvrLm11* is located on a dispensable mini-chromosome, which is frequently lost during meiosis (Balesdent et al., 2013).

AVR genes of *L. maculans* are commonly inactivated by the RIP mechanism which usually targets duplicated sequences (Daverdin et al., 2012). In addition, gene deletion (such as of *AvrLm1* and *AvrLm11*) (Balesdent et al., 2013; Gout et al., 2006), single nucleotide polymorphisms (SNPs) (such as in *AvrLm4-7* and *AvrLm2*) (Ghanbarnia et al., 2015; Parlange et al., 2009), long terminal repeat insertion, single base pair deletions, and low expression *in planta* have contributed to regaining virulence on oilseed rape (Daverdin et al., 2012).

Complex interactions between Avr proteins and R proteins have been demonstrated in *L. maculans*. For instance, *AvrLm1* can be recognized by both Rlm1 and Rlm3 (Gout et al., 2006; Larkan et al., 2013). Similarly, *AvrLm4-7* is recognized by Rlm4 and Rlm7 (Parlange et al., 2009). Like in *Fol*, where Avr1 suppresses I-2 and I-3-mediated resistance, *AvrLm4-7* suppresses recognition of *AvrLm3* and *AvrLm5-9* by Rlm3 and Rlm9, respectively (Ghanbarnia et al., 2018; Plissonneau et al., 2016). *AvrLm3* is highly conserved in the fungal population despite its sub-telomeric location, implying its importance for fitness (Plissonneau et al., 2016).

#### 2.4. *Magnaporthe oryzae*

*M. oryzae* causes disease on more than 50 grass species, but individual strains can only infect one or a few species. Both host cultivar specificity and host species specificity factors have been identified in *M. oryzae* (Inoue et al., 2017; Wang et al., 2017), including the Avr-Pita family (Orbach et al., 2000), Avr1-CO39 (Farman et al., 2002; Farman and Leong, 1998), Pwl2 (Sweigard et al., 1995), PWT3/4 (Inoue et al., 2017) and Ace1 (Böhnert et al., 2004). Several Avr factors contribute to host species specificity. Pwl2 functions as a host species specificity factor preventing infection of weeping lovegrass (Sweigard et al., 1995), while PWT3 and PWT4 from *Avena*-infecting isolates are recognized by the corresponding resistance proteins Rwt3 and Rwt4 in wheat, respectively (Inoue et al., 2017; Takabayashi et al., 2002).

Most *M. oryzae* AVR genes encode small secreted proteins (less than 200 aa), excepted ACE1 which encodes a polyketide synthase/peptide synthetase of 4035 aa potentially involved in the biosynthesis of a secondary metabolite (Wang et al., 2017). The three-dimensional structures of Avr1-CO39 and Avr-Pia have been determined (de Guillen et al., 2015). These two effectors, together with AvrPiz-t and ToxB, an effector from the wheat tan spot pathogen *Pyrenophora tritici-repentis* (*Ptr*), have the same structures, and they are named MAX-effectors (*Magnaporthe* Avr and ToxB like) (de Guillen et al., 2015). Most AVR genes are located near the end of chromosomes and/or are surrounded by transposons (Wang et al., 2017).

Diverse mechanisms contribute to loss of the avirulence function of effectors in *M. oryzae*. For example, point mutations and insertions in and deletions of *Avr-Pita* permit the fungus to avoid triggering a resistance response mediated by Pita (Orbach et al., 2000). In addition, transposon insertion in *Avr-Pi9* (Wu et al., 2015), *Ace1* (Fudal et al., 2005), *Avr-Pi-zt* (Li et al., 2009) and *Avr-Pib* (Zhang et al., 2015), and segmental deletion in *Avr-Pib* (Zhang et al., 2015) render the pathogen virulent.

The molecular interactions of seven pairs of *M. oryzae* Avr and rice R proteins have been studied, including Pi-ta/Avr-Pita, Piz-t/AvrPiz-t, Pik/Avr-Pik, Pia/Avr-Pia, Pi-CO39/Avr1-CO39, Pi54/Avr-Pi54, and Pii/Avr-Pii (Wang et al., 2017). Three modes of interaction have been demonstrated, including a direct interaction between one R and one Avr protein, i.e., Pi54/Avr-Pi54 (Ray et al., 2016), two R proteins

interacting with one Avr protein, i.e., Pi-k1/Pi-k2/Avr-Pik (Ashikawa et al., 2008), and two cooperating R proteins recognizing two Avr proteins, i.e., RGA4/RGA5/Avr-Pia/Avr1-CO39 (Cesari et al., 2013; Okuyama et al., 2011).

#### 2.5. *Rhynchosporium secalis*

*R. secalis* is the causal agent of leaf scald on barley. Three low molecular weight necrosis-inducing peptides (NIPs), designated Nip1 to Nip3, function as non-specific toxins on barley (Wevelsiek et al., 1991). Nip1 is also a race-specific elicitor of defense responses in barley cultivars carrying the resistance gene *Rrs1*. The amino acid sequence encoded by *NIP1* contains a secretory signal peptide and a cysteine-rich mature protein of 60 residues (Rohe et al., 1995). Strains of *R. secalis* virulent on *Rrs1*-containing plants either lack *NIP1* or carry alleles with point mutations that translate into single amino acid substitutions (Rohe et al., 1995; Schürch et al., 2004). *NIP1* was lost with a high frequency (45%) among 614 isolates from different geographic populations on four continents, and 14 types of DNA polymorphisms were found, indicating diversifying selection (Schürch et al., 2004). A recent study shows that the *NIP1* gene family evolved mainly through point mutations and copy number variation (Mohd-Assaad et al., 2019). *NIP1* is present on a large chromosome that is not likely to be dispensable (von Felten et al., 2011).

#### 2.6. *Melampsora lini*

The flax rust fungus *M. lini* is an obligate biotrophic basidiomycete that infects flax (*Linum usitatissimum*) and other species of the genus *Linum* (Lawrence et al., 2007). To date, avirulence genes have been identified in six loci in *M. lini*, encoding AvrL567 (Dodds et al., 2004), AvrM (Catanzariti et al., 2006a), AvrP123 (Catanzariti et al., 2006b), AvrP4 (Catanzariti et al., 2006b), AvrL2-A (Anderson et al., 2016) and AvrM14-A (Anderson et al., 2016), respectively. AvrL567-A is recognized by resistance proteins L5, L6 and L7, whereas AvrL567-B is recognized most strongly by L5, weakly by L6 and not at all by L7 (Dodds et al., 2004). The gene encoding AvrL567-C co-segregates with the virulence phenotype, and this version is not recognized by L5, L6, or L7 (Dodds et al., 2004). AvrP4 and AvrP123 are cysteine-rich proteins, whereas AvrM does not contain cysteine residues at all. *AvrL2-A* and *AvrM14-A* were identified by map-based cloning (Anderson et al., 2016). *AvrM14-A* is not related to AvrM and is recognized by both the flax M1 and M4 resistance proteins (Anderson et al., 2016). *AvrM14-A* shows homology with the nudix hydrolase superfamily and is the first rust avirulence protein for which a biochemical function could be predicted from the protein sequence (Anderson et al., 2016). The AvrL2 protein family has no homology to proteins with known function.

The three-dimensional structures of AvrL567, AvrM and AvrP have been determined. They exhibit completely different structures, but they all display surface polymorphic residues involved in the recognition by the flax resistance proteins L5/L6/L7, M and P, respectively (Lorrain et al., 2019; Ve et al., 2013; Wang et al., 2007; Zhang et al., 2018). Except for *AvrL2-A*, all avirulence genes identified are located at recombination hot-spots (Anderson et al., 2016). *AvrL2-A* has been proposed to be located in a centromere or other heterochromatic repeat-rich region (Anderson et al., 2016). All the identified genes have undergone diversifying selection (Anderson et al., 2016; Barrett et al., 2009; Ellis et al., 2007).

#### 2.7. *Puccinia graminis*

*Puccinia graminis* f. sp. *tritici* (Pgt) causes wheat stem rust, which has posed a threat to wheat production recently (Singh et al., 2011). Two avirulence factors have been identified in Pgt, AvrSr35 and AvrSr50, which are recognized by resistance proteins Sr35 and Sr50, respectively (Chen et al., 2017; Salcedo et al., 2017). *AvrSr50* encodes a 132-amino

acid protein which interacts with Sr50 directly (Chen et al., 2017; Salcedo et al., 2017). The origin of isolates virulent on Sr35-containing plants is associated with the insertion of a miniature inverted transposable element (MITE) in *AvrSr35* (Salcedo et al., 2017). A switch to virulence towards Sr50 was due to the exchange of a whole chromosome between two haploid nuclei, resulting in loss of the avirulence allele (Chen et al., 2017). In addition, the Pgt protein PGTAUSPE-10-1 causes cell death in a host line carrying resistance gene Sr22. Therefore, PGTAUSPE-10-1 might be the avirulence factor corresponding to Sr22 (Upadhyaya et al., 2014). Through mutational genomics approaches, *AvrSr27* has also been identified, and identification of *AvrSr5* is underway (Dodds et al., 2019).

## 2.8. *Blumeria graminis*

### 2.8.1. *B. graminis* f. sp. *hordei* (Bgh)

*B. graminis* f. sp. *hordei* (Bgh) causes powdery mildew on barley, and interacts with its host in a gene-for-gene manner (Zhang et al., 2005). So far, seven avirulence genes have been identified in Bgh, namely *AvrK1*, *Avra1*, *Avra13*, *Avra7*, *Avra9*, *Avra10* and *Avra22*, corresponding to barley resistance genes *mlk1*, *m1a1*, *m1a13*, *m1a7*, *m1a9*, *m1a10* and *m1a22*, respectively (Lu et al., 2016; Ridout et al., 2006; Saur et al., 2019). *Avra7*, *Avra9*, *Avra10*, and *Avra22* were identified in a recent study and these proteins interact directly with their respective plant R proteins (Saur et al., 2019). Virulence of isolates is predominately associated with non-synonymous SNPs and loss of expression of avirulence genes (Saur et al., 2019). *Ava10* and *Ava22* share homology and are co-maintained in pathogen populations in the form of balanced polymorphism (Saur et al., 2019). *Avra10* and *Avra22* probably have evolved through two opposing selective pressures: sequence conservation to maintain their virulence function and sequence diversification to escape recognition by *Mla10* and *Mla22*, respectively (Saur et al., 2019).

### 2.8.2. *B. graminis* f. sp. *tritici* (Bgt)

*B. graminis* f. sp. *tritici* (Bgt) is the causal agent of powdery mildew on wheat (Bourras et al., 2018). Four genes encoding avirulence factors have been identified in Bgt: *AvrPm3<sup>a2/f2</sup>*, *AvrPm2*, *AvrPm3<sup>b2/c2</sup>* and *AvrPm3<sup>d3</sup>*. The encoded products are recognized by R proteins *Pm3a/3f*, *Pm2*, *Pm3b/3c* and *AvrPm3d*, respectively (Bourras et al., 2019, 2015; Praz et al., 2017). The avirulence genes all encode small, secreted proteins with conserved cysteines residues, and they all are highly expressed in haustoria (Bourras et al., 2019, 2015; Praz et al., 2017). *AvrPm2* belongs to a small gene family encoding structurally conserved RNase-like effectors (Praz et al., 2017). Recognition of *AvrPm3<sup>a2/f2</sup>*, *AvrPm3<sup>b2/c2</sup>* and *AvrPm3<sup>d3</sup>* by the respective resistance proteins is suppressed by the ribonuclease-like effector *SvrPm3<sup>a1/f1</sup>* (Bourras et al., 2019). *AVRPm3<sup>d3</sup>* shows a high level of copy number variation in mildew isolates, while *AVRPm3<sup>b2/c2</sup>* is present as a single copy only (Bourras et al., 2019). Although *Pm3* alleles share more than 97% sequence identity on the protein level, *AvrPm3<sup>a2/f2</sup>*, *AvrPm3<sup>b2/c2</sup>*, and *AvrPm3<sup>d3</sup>* share low sequence identity (Bourras et al., 2019). In the fungal genome all these avirulence genes are surrounded by TEs (Bourras et al., 2019, 2015; Praz et al., 2017). In 185 isolates, 10 non-synonymous mutations were found across the *AVRPm3<sup>b2/c2</sup>* gene, while 17 non-synonymous mutations were found for *AVRPm3<sup>d3</sup>* (Bourras et al., 2019). *AVRPm3<sup>b2/c2</sup>* and *AVRPm3<sup>d3</sup>* homologs have also been found in rye and *Dactylis* mildews. The products of the homologs are recognized by the resistance proteins *Pm3b*, *Pm3c* and *Pm3d* in wheat, demonstrating that *AvrPm3*-*Pm3* interactions also determine host species specificity in cereal mildews.

## 2.9. *Verticillium dahliae*

*V. dahliae* is an asexual soil-borne, xylem-invading plant pathogen that causes vascular wilt diseases in over 200 dicotyledonous plant

species, such as tomato (Klosterman et al., 2009). So far, identification of only one avirulence protein, *Ave1*, has been published. *Ave1* is a 134 aa secreted protein recognized by *Ve1* in tomato, and expression of *Ave1* is induced during host colonization (de Jonge et al., 2012). Intriguingly, no SNP was found in 85 *Ave1* alleles from *Verticillium* strains isolated from various host plants and different geographical locations (de Jonge et al., 2012). The presence of numerous *Ave1* orthologs in plants, absence of orthologs in fungi other than *Fol*, *Colletotrichum higginsianum*, and *Cercospora beticola*, and the association of *Ave1* with a flexible genomic region containing various TEs suggest that *Verticillium* acquired *Ave1* from plants through horizontal gene transfer (HGT) (de Jonge et al., 2012). In a recently study, *Ave1* was shown to play a role in niche colonization by suppressing microbes with antagonistic activities (Snelders et al., 2020).

In another study of *V. dahliae* (personal communication with Jinling Li, unpublished data), a duplicated defoliation-specific gene, encoding a small secreted protein, was found. It was demonstrated that this effector gene is required for the defoliation of cotton and olive. Application of this heterologously produced protein to cotton seedlings also induced defoliation, indicating that the protein is directly responsible for the defoliation symptoms.

## 3. Virulence genes determining host cultivar specificity

### 3.1. *Stagonospora nodorum*

The ascomycete fungus *S. nodorum* (also known as *Parastagonospora nodorum*) is a major necrotrophic pathogen of wheat causing leaf and glume blotch (Liu et al., 2009). So far, three *S. nodorum* genes encoding host selective toxins, notably *SnToxA* (Liu et al., 2012), *SnTox1* (Liu et al., 2009) and *SnTox3* (Liu et al., 2009) have been identified. The genes reside on three different chromosomes (Liu et al., 2012). The toxins induce cell death and necrosis as an outcome of their interaction with their cognate dominant susceptibility gene products (*ToxA-Tsn1*, *Tox1-Snn1* and *Tox3-Snn3*). Below these host-specific interactions are summarized.

#### 3.1.1. *SnTox1-Snn1*

Although the *SnTox1-Snn1* interaction was the first to be characterized (Liu et al., 2004), the toxin gene was identified only in 2012 (Liu et al., 2012). The mature *SnTox1* contains 100 amino acids including 16 cysteine residues, all predicted to be involved in disulfide bridges necessary for the activity/stability of the protein. The C-terminus shows similarity to the chitin-binding domain of *Avr4* of *C. fulvum* (van Esse et al., 2007). Later, it was shown that *SnTox1* binds chitin of the fungal cell wall, protecting the pathogen from chitinase degradation (Liu et al., 2016). *SnTox1* is highly expressed at 3 days post infection (dpi), which correlates with the onset of necrotic lesion development. Different from other effector genes in *S. nodorum*, which are located in gene poor and repeat-rich regions, *SnTox1* is located in a gene-rich region and no obvious repeats or AT-rich sequences were identified within the 300 kb region containing *SnTox1* (Liu et al., 2012). Among 159 Sn isolates from around the globe, 11 *Tox1* isoforms were found, suggesting diversifying selection on *SnTox1* (Liu et al., 2012).

#### 3.1.2. *SnToxA-Tsn1* and *PtrToxA-Tsn1*

*SnToxA* is highly similar to *PtrToxA* identified in *P. tritici-repentis* (Ciuffetti et al., 1997), having only two amino acid differences, and both are recognized by the wheat protein *Tsn1* (Faris et al., 2010). Mature *SnToxA* is a 13.2 kDa protein containing two cysteine residues as well as an RGD-containing vitronectin-like motif that is present in a solvent-exposed loop in the active protein (van Esse et al., 2007). The regions upstream and downstream of *SnToxA* contain repetitive, AT-rich regions, but these AT-rich regions have not been found in the corresponding flanking regions of *P. tritici-repentis* (Friesen et al., 2006). By sequencing 95 *S. nodorum* *ToxA* and 54 *P. tritici-repentis* *ToxA*

amplicons from geographically diverse populations, 11 haplotypes were found in *S. nodorum* and only one haplotype in *P. tritici-repentis* (Friesen et al., 2006). The gene has likely undergone HGT from *S. nodorum* to *P. tritici-repentis* based on the following observations: (1) the presence of an almost identical 11 kb region containing SnToxA/PtrToxA in both species; (2) the presence of high sequence diversity of SnToxA in *S. nodorum* and monomorphism in Ptr; (3) absence of PtrToxA in related species; (4) the recent emergence of tan spot (Friesen et al., 2006; Liu et al., 2006).

### 3.1.3. SnTox3-Snn3

SnTox3 was identified by partial purification and sequencing of the protein (Liu et al., 2009). SnTox3 encodes a 230 amino acid pre-protein consisting of a 20 amino acid signal sequence and a predicted pro-domain of approximately 30 amino acids, resulting in a mature protein of ~18 kDa (Liu et al., 2009). SnTox3 contains six cysteine residues, each being predicted to be involved in the formation of a disulfide bridge critical to the structure and function of the protein (Liu et al., 2009). Like SnTox1, SnTox3 is highly expressed at 3 dpi when lesions start to develop. SnTox3 interacts with a wheat pathogenicity related-1 protein (Breen et al., 2016). SnTox3 is also flanked by AT-rich sequences, containing long terminal repeat retrotransposons. By sequencing SnTox3 from 245 isolates, eleven haplotypes were identified resulting in four amino acid polymorphisms (Liu et al., 2009).

### 3.2. *Pyrenophora tritici-repentis* (*P. tritici-repentis*)

The ascomycete *Pyrenophora tritici-repentis* (Ptr) causes tan spot and chlorosis on wheat. Two Ptr host-specific genes encoding host-specific toxins have been identified, including Ptr-ToxA (Ciuffetti et al., 1997) and Ptr-ToxB (Strelkov et al., 1999). Ptr-ToxA and Ptr-ToxB interact specifically with the products of the host susceptibility genes *Tsn1* and *Tsc2*, respectively. It is likely that Ptr-ToxA originated from *S. nodorum* (see above) (Friesen et al., 2006). Ptr-ToxA is a single domain protein having a  $\beta$ -sandwich fold with two antiparallel  $\beta$ -sheets composed of four strands each enclosing the hydrophobic core (Sarma et al., 2005). Unlike Ptr-ToxA, which is present as a single copy in the genome, Ptr-ToxB is present in multiple copies in the genome of some races of Ptr, and the number of copies is proportional to virulence (Martinez et al., 2004). Ptr-ToxB encodes a 64 amino acid host-selective toxin (Martinez et al., 2001), contains four cysteine residues involved in the formation of two disulfide bridges (Nyarko et al., 2014) and is a heat-stable protein (Strelkov et al., 1999). All Ptr-ToxB loci are associated with retrotransposons (Martinez et al., 2004). Ptr-toxb is a related single copy gene from a non-pathogenic strain and shares 86% identity with Ptr-ToxB (Martinez et al., 2004). Both Ptr-ToxB and Ptr-toxb adopt a  $\beta$ -sandwich fold stabilized by two disulfide bonds, but differ in the dynamics of one sandwich half. The absence of toxic activity of Ptr-toxb is attributed to the more open structure close to one disulfide bond, higher flexibility, and different residues in an exposed loop of Ptr-toxb (Nyarko et al., 2014).

## 4. Secondary metabolites determining host specificity

### 4.1. *Cochliobolus carbonum*

*Cochliobolus carbonum* (*C. carbonum*) causes Northern Corn Leaf Spot and is virulent on *hm/hm* corn (Johal and Briggs, 1992). The virulence of the fungus is due to production of HC-toxin, a cyclic tetrapeptide (Panaccione et al., 1992). *Hm1* and *Hm2* both encode a carbonyl reductase that inactivates HC-toxin (Johal and Briggs, 1992). It has been hypothesized that HC-toxin alters the expression of plant defense genes by inhibiting histone deacetylases (Ransom and Walton, 1997; Walton, 2006). The production of HC-toxin is governed by the single locus *TOX2* (Panaccione et al., 1992). It contains *HST1*, encoding the 570 kDa non-ribosomal peptide synthetase key enzyme. *TOX2* is

duplicated in toxin-producing isolates of the fungus, but is completely absent from the genomes of toxin-non-producing isolates (Panaccione et al., 1992). Disruption of all copies of *HST1* resulted in abolished HC-toxin production and loss of host-selective pathogenicity. Other genes involved in the processing of biosynthetic intermediates and possibly secretion include *TOXA* (major facilitator superfamily transporter) (Pitkin et al., 1996), *TOXC* (fatty acid synthase subunit) (Ahn and Walton, 1997) and *TOXF* (amino acid transaminase) (Cheng et al., 1999). Except for one copy of the pathway-specific regulator gene *TOXE*, all other *TOX2* genes are located in an approximately 600 kb repeat-rich region (Ahn and Walton, 1996; Condon et al., 2013).

### 4.2. *Cochliobolus heterostrophus*

*Cochliobolus heterostrophus* (*C. heterostrophus*) causes Southern Corn Leaf Blight. There are two known races, race T and race O, with race T producing a HST called T-toxin. Race T is highly virulent on corn carrying Texas male sterile cytoplasm, while Race O only shows mild virulence. So far, nine genes have been shown to be required for T-toxin production (Baker et al., 2006; Inderbitzin et al., 2010; Rose et al., 2002; Yang et al., 1996), and these genes are located at two unlinked loci, designated as *Tox1A* and *Tox1B*. Appropriately, these genes are absent in Race O. The nine known *Tox1* genes encode two polyketide synthases (PKS), a decarboxylase, five dehydrogenases and an unknown protein (Inderbitzin et al., 2010). The genes do not reside in a single cluster, but reside alone or in small groups in four distinct AT-rich regions (Inderbitzin et al., 2010). Together, these *Tox1* regions comprise less than 5% of the 1.2 Mb of race T-specific DNA (Inderbitzin et al., 2010).

### 4.3. *Verticillium dahliae*

As mentioned above, *V. dahliae* is a vascular wilt pathogen which can infect nearly 200 plant species. However, it causes defoliation in a few hosts only, including cotton, olive and okra (Milgroom et al., 2016). Recently, by using comparative genomics, seven genes associated with the defoliation pathotype, *VdDf1-VdDf7*, were discovered in a lineage-specific genomic region (G-LSR2) of *V. dahliae* Vd991 (Chen et al., 2018; Zhang et al., 2019). *VdDf5* and *VdDf6* are critical for the defoliation phenotype (Zhang et al., 2019). The *VdDfs* are involved in the production of NAE 12:0, that cause defoliation either by altering abscisic acid sensitivity, hormone disruption or sensitivity to the pathogen (Zhang et al., 2019). Phylogenetic analysis of the region comprising all seven protein-coding genes suggests that G-LSR2 has been acquired from *F. oxysporum* f. sp. *vasinfectum* through horizontal transfer (Chen et al., 2018).

### 4.4. *Alternaria alternata*

*Alternaria alternata* (*A. alternata*) is a ubiquitous, mostly saprophytic fungus present in dead plant material but is also known as a weak pathogen causing opportunistic diseases in a number of crops (Akimitsu et al., 2014). HSTs in *A. alternata* have been extensively reviewed before (Akimitsu et al., 2014; Meena et al., 2017). So far, there are seven known diseases caused by *A. alternata* in which HSTs are responsible for pathogenesis. Accordingly, *A. alternata* is classified into seven different pathotypes, each producing a distinct host-specific toxin: AM-toxin (apple pathotype), AF-toxin (strawberry pathotype), AK-toxin (Japanese pear pathotype), ACT-toxin (tangerine pathotype), ACR-toxin (rough lemon pathotype), AAL-toxin (tomato pathotype), or AT-toxin (tobacco pathotype). Interestingly, HST genes in *A. alternata* are located on conditionally dispensable chromosomes (CDCs) as gene clusters (Hu et al., 2012). Chemical structures of HSTs from six pathotypes have been determined, excluding that of AT-toxin of the tobacco pathotype. AAL-toxin is a structural analog of sphingolipid precursors, and thereby efficiently inhibits eukaryotic ceramide synthases. Appropriately,

resistance in tomato is conferred by the ceramide synthase Asc-1 encoded within the *Alternaria* stem canker (*Asc*) locus (Abbas et al., 1994; Brandwagt et al., 2000; Spassieva et al., 2002).

## 5. Horizontal gene and chromosome transfer and host specificity

HGT or horizontal chromosome transfer (HCT) has likely occurred in several plant pathogenic fungi, including *Colletotrichum gloeosporioides* (He et al., 1998), *S. nodorum*/*P. tritici-repentis* (Friesen et al., 2006), *F. oxysporum* (Ma et al., 2010; van Dam et al., 2017; Vlaardingerbroek et al., 2016) and *A. alternata* (Akimitsu et al., 2014).

The outbreak of tan spot disease on wheat in 1941 was caused by *P. tritici-repentis*. The high virulence of *P. tritici-repentis* is due to the presence of a HST, ToxA, and, as mentioned above, it is likely that the *ToxA* gene was transferred from *S. nodorum* to *P. tritici-repentis* in a recent event (Friesen et al., 2006).

In *F. oxysporum*, HCT of pathogenicity chromosomes has been shown in three *formae speciales*: Fol (Ma et al., 2010; Vlaardingerbroek et al., 2016), *F. oxysporum* f. sp. *radicis-cucumerinum* (Forc) (van Dam et al., 2017) and *F. oxysporum* f. sp. *melonis* (Fom) (Li et al., 2020). In all three cases, following transfer of an accessory chromosome containing effector genes from a pathogenic strain to non-pathogenic *F. oxysporum*, the recipient strain becomes pathogenic to the host species of the chromosome donor strain.

As mentioned above, in *A. alternata*, all HST-encoding genes are located on CDCs (Mehrabi et al., 2011). It has been suggested that these CDCs are transferrable between different pathotypes in *A. alternata* (Akagi et al., 2009). Indeed, HCT between different pathotypes has been demonstrated through protoplast fusion experiments. For example, by fusion of a tomato pathotype with a strawberry pathotype, the resulting strain was found to be pathogenic on both tomato and strawberry (Akagi et al., 2009).

Often, virulence or host-determining genes are clustered, so HGT or HCT can cause previously non-pathogenic microbes to become pathogenic, or pathogens to expand or change host range. In *A. alternata* and *F. oxysporum*, genes determining host range – a secondary metabolite gene cluster or virulence genes together with transcription factors – are located on a single chromosome, therefore single chromosome transfer is sufficient to expand host range. This may be an important mechanism for asexual fungi to generate genetic variation and adapt to a changing environment.

## 6. Potential role of genome localization of host-specificity genes in adaptation

From the findings discussed above, host-specificity genes appear to be predominately located in AT-rich isochores (such as avirulence genes in *L. maculans* and *AvrPm3<sup>a2/f2</sup>*, *AvrPm3<sup>b2/c2</sup>* and *AvrPm3<sup>d3</sup>* in *B. graminis* f. sp. *tritici*), and/or TE-rich lineage-specific chromosomes (such as *AVR1*, *AVR2*, and *AVR3* in *Fol*). These repeat-rich genomic compartments are believed to evolve more rapidly than other parts of the genome. Based on this, the concept of a ‘two-speed genome’ has been proposed (Croll and McDonald, 2012; Raffaele and Kamoun, 2012). The gene-poor, repeat-rich genomic compartment could serve as a ‘cradle’ for adaptive evolution (Croll and McDonald, 2012). Evidence for a higher diversification rate has been found for almost all the host-specificity genes listed above. For example, in *L. maculans*, avirulence genes are located in AT-rich isochores and these genes have an exceptionally high mutation rate due to RIP (Rouxel and Balesdent, 2017).

Modern crop management practices have accelerated the arms race between pathogens and plants (Möller and Stukenbrock, 2017). Cultivars with new resistance genes may be introduced in each growing season, which poses great pressure on pathogens to rapidly adapt to the new cultivar. Since avirulence factors that are recognized by resistance proteins are generally located in repeat-rich genomic regions, a high

frequency of point mutations, deletions, duplications, silencing or rearrangement of avirulence genes can result in rapid emergence of strains that evade recognition by resistance proteins. If loss of or changes in avirulence genes have no or little fitness cost, these strains will quickly become dominant in the pathogen population. The compartmentalization of a genome thus allows pathogens to harbor fast-evolving genes without affecting the stability of core genes.

## 7. Conclusions and perspectives

Both proteins and secondary metabolites can determine host specificity in plant pathogenic fungi. With an increased rate of publication of pathogen genome sequences, the rate of discovery of genes determining host cultivar-specificity will likewise increase, by comparing strains of different races within a species or *forma specialis*. Identification of host species-specificity factors may require more effort, depending on the fungal species involved. Regarding the latter, it would be interesting to test the hypothesis of Schulze-Lefert and Panstruga, who proposed that PTI plays a major role in non-host resistance of evolutionary divergent non-host plant species, while ETI would be more dominant as a non-host resistance mechanism in more closely related plant species (Schulze-Lefert and Panstruga, 2011). To test whether ETI indeed plays a dominant role in non-host resistance of closely related plant species, we propose to identify effectors eliciting an ETI response in non-host plant species closely related to host species in fungal pathogens such as *M. oryzae* or *F. oxysporum*.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

J.L. was financially supported by the China Scholarship Council program (File number: 201504910768). The authors thank Slavica Janevska for providing comments on the manuscript and both reviewers for their constructive, critical and detailed comments.

## Author contributions

Conceptualization: JL and MR; Investigation: JL; Writing of the original draft: JL; Review & editing: JL, BC, and MR.

## References

- Abbas, H.K., Tanaka, T., Duke, S.O., Porter, J.K., Wray, E.M., Hodges, L., Sessions, A.E., Wang, E., Merrill Jr, A.H., Riley, R.T., 1994. Fumonisin- and AAL-toxin-induced disruption of sphingolipid metabolism with accumulation of free sphingoid Bases. *Plant Physiol.* 106, 1085–1093. <https://doi.org/10.1104/pp.106.3.1085>.
- Ahn, J.-H., Walton, J.D., 1997. A fatty acid synthase gene in *Cochliobolus carbonum* required for production of HC-toxin, Cyclo (d-Prolyl-1-Alanyl- d-Alanyl-1-2-Amino-9,10-Epoxy-8-Oxodecanoyl). *Mol. Plant-Microbe Interact.* 10, 207–214. <https://doi.org/10.1094/MPMI.1997.10.2.207>.
- Ahn, J.H., Walton, J.D., 1996. Chromosomal organization of TOX2, a complex locus controlling host-selective toxin biosynthesis in *Cochliobolus carbonum*. *Plant Cell.* <https://doi.org/10.2307/3870290>.
- Akagi, Y., Akamatsu, H., Otani, H., Kodama, M., 2009. Horizontal chromosome transfer, a mechanism for the evolution and differentiation of a plant-pathogenic fungus. *Eukaryot. Cell* 8, 1732–1738. <https://doi.org/10.1128/EC.00135-09>.
- Akimitsu, K., Tsuge, T., Kodama, M., Yamamoto, M., Otani, H., 2014. *Alternaria* host-selective toxins: determinant factors of plant disease. *J. Gen. Plant Pathol.* 80, 109–122. <https://doi.org/10.1007/s10327-013-0498-7>.
- Anderson, C., Khan, M.A., Catanzariti, A.-M., Jack, C.A., Nemri, A., Lawrence, G.J., Upadhyaya, N.M., Hardham, A.R., Ellis, J.G., Dodds, P.N., Jones, D.A., 2016. Genome analysis and avirulence gene cloning using a high-density RADseq linkage map of the flax rust fungus, *Melampsora lini*. *BMC Genomics* 17, 667. <https://doi.org/10.1186/s12864-016-3011-9>.
- Ashikawa, I., Hayashi, N., Yamane, H., Kanamori, H., Wu, J., Matsumoto, T., Ono, K., Yano, M., 2008. Two adjacent nucleotide-binding site-leucine-rich repeat class genes



- are required to confer Pkm-specific rice blast resistance. *Genetics* 180, 2267–2276. <https://doi.org/10.1534/genetics.108.095034>.
- Baker, S.E., Kroken, S., Inderbitzin, P., Asvarak, T., Li, B.-Y., Shi, L., Yoder, O.C., Turgeon, B.G., 2006. Two polyketide synthase-encoding genes are required for biosynthesis of the polyketide virulence factor, T-toxin, by *Cochliobolus heterostrophus*. *Mol. Plant-Microbe Interact.* 19, 139–149. <https://doi.org/10.1094/MPMI-19-0139>.
- Balesdent, M.-H., Fudal, I., Ollivier, B., Bally, P., Grandaubert, J., Eber, F., Chèvre, A.-M., Leflon, M., Rouxel, T., 2013. The dispensable chromosome of *Leptosphaeria maculans* shelters an effector gene conferring avirulence towards *Brassica rapa*. *New Phytol.* 198, 887–898. <https://doi.org/10.1111/nph.12178>.
- Barrett, L.G., Thrall, P.H., Dodds, P.N., van der Merwe, M., Linde, C.C., Lawrence, G.J., Burdon, J.J., 2009. Diversity and evolution of effector loci in natural populations of the plant pathogen *Melampsora lini*. *Mol. Biol. Evol.* 26, 2499–2513. <https://doi.org/10.1093/molbev/msp166>.
- Biju, V.C., Fokkens, L., Houterman, P.M., Rep, M., Cornelissen, B.J.C., 2017. Multiple evolutionary trajectories have led to the emergence of races in *Fusarium oxysporum* f. sp. *lycopersici*. *Appl. Environ. Microbiol.* 83, e02548–e2616. <https://doi.org/10.1128/AEM.02548-16>.
- Böhnert, H.U., Fudal, I., Dìoh, W., Tharreau, D., Notteghem, J.-L., Lebrun, M.-H., 2004. A putative polyketide synthase/peptide synthetase from *Magnaporthe grisea* signals pathogen attack to resistant rice. *Plant Cell* 16, 2499–2513. <https://doi.org/10.1105/tpc.104.022715>.
- Bolton, M.D., van Esse, H.P., Vossen, J.H., de Jonge, R., Stergiopoulos, I., Stulemeijer, I.J.E., van den Berg, G.C.M., Borrás-Hidalgo, O., Dekker, H.L., de Koster, C.G., de Wit, P.J.G.M., Joosten, M.H.A.J., Thomma, B.P.H.J., 2008. The novel *Cladosporium fulvum* lysin motif effector Ecp6 is a virulence factor with orthologues in other fungal species. *Mol. Microbiol.* 69, 119–136. <https://doi.org/10.1111/j.1365-2958.2008.06270.x>.
- Borah, N., Albarouki, E., Schirawski, J., 2018. Comparative methods for molecular determination of host-specificity factors in plant-pathogenic fungi. *Int. J. Mol. Sci.* 19, 863. <https://doi.org/10.3390/ijms19030863>.
- Bourras, S., Kunz, L., Xue, M., Praz, C.R., Müller, M.C., Kälin, C., Schläfli, M., Ackermann, P., Flückiger, S., Parlange, F., Menardo, F., Schaefer, L.K., Ben-David, R., Roffler, S., Oberhaensli, S., Widrig, V., Lindner, S., Isaksson, J., Wicker, T., Yu, D., Keller, B., 2019. The AvrPm3-Pm3 effector-NLR interactions control both race-specific resistance and host-specificity of cereal mildews on wheat. *Nat. Commun.* 10, 2292. <https://doi.org/10.1038/s41467-019-10274-1>.
- Bourras, S., McNally, K.E., Ben-David, R., Parlange, F., Roffler, S., Praz, C.R., Oberhaensli, S., Menardo, F., Stirnweis, D., Frenkel, Z., Schaefer, L.K., Flückiger, S., Treier, G., Herren, G., Korol, A.B., Wicker, T., Keller, B., 2015. Multiple avirulence loci and allele-specific effector recognition control the Pm3 race-specific resistance of wheat to powdery mildew. *Plant Cell* tpc.15.00171. <https://doi.org/10.1105/tpc.15.00171>.
- Bourras, S., Praz, C.R., Spanu, P.D., Keller, B., 2018. Cereal powdery mildew effectors: a complex toolbox for an obligate pathogen. *Curr. Opin. Microbiol.* 46, 26–33. <https://doi.org/10.1016/j.mib.2018.01.018>.
- Brandwagt, B.F., Mesbah, L.A., Takken, F.L.W., Laurent, P.L., Kneppers, T.J.A., Hille, J., Nijkamp, H.J.J., 2000. A longevity assurance gene homolog of tomato mediates resistance to *Alternaria alternata* f. sp. *lycopersici* toxins and fumonisin B1. *Proc. Natl. Acad. Sci.* 97, 4961–4966. <https://doi.org/10.1073/pnas.97.9.4961>.
- Breen, S., Williams, S.J., Winterberg, B., Kobe, B., Solomon, P.S., 2016. Wheat PR-1 proteins are targeted by necrotrophic pathogen effector proteins. *Plant J.* 88, 13–25. <https://doi.org/10.1111/tpj.13228>.
- Catanzariti, A.-M., Dodds, P.N., Lawrence, G.J., Ayliffe, M.A., Ellis, J.G., 2006. Haustorially expressed secreted proteins from flax rust are highly enriched for avirulence elicitors. *Plant Cell* 18, 243–256. <https://doi.org/10.1105/tpc.105.035980>.
- Cesari, S., Thilliez, G., Ribot, C., Chalvon, V., Michel, C., Jauneau, A., Rivas, S., Alaux, L., Kanzaki, H., Okuyama, Y., Morel, J.-B., Fournier, E., Tharreau, D., Terauchi, R., Kroj, T., 2013. The Rice Resistance Protein Pair RGA4/RGA5 Recognizes the *Magnaporthe oryzae* Effectors AVR-Pia and AVR1-CO39 by Direct Binding. *Plant Cell* 25, 1463–1481. <https://doi.org/10.1105/tpc.112.107201>.
- Chen, J.-Y., Liu, C., Gui, Y.-J., Si, K.-W., Zhang, D.-D., Wang, J., Short, D.P.G., Huang, J.-Q., Li, N.-Y., Liang, Y., Zhang, W.-Q., Yang, L., Ma, X.-F., Li, T.-G., Zhou, L., Wang, B.-L., Bao, Y.-M., Subbarao, K.V., Zhang, G.-Y., Dai, X.-F., 2018. Comparative genomics reveals cotton-specific virulence factors in flexible genomic regions in *Verticillium dahliae* and evidence of horizontal gene transfer from *Fusarium*. *New Phytol.* 217, 756–770. <https://doi.org/10.1111/nph.14861>.
- Chen, J., Upadhyaya, N.M., Ortiz, D., Spersneider, J., Li, F., Bouton, C., Breen, S., Dong, C., Xu, B., Zhang, X., Mago, R., Newell, K., Xia, X., Bernoux, M., Taylor, J.M., Steffenson, B., Jin, Y., Zhang, P., Kanyuka, K., Figueroa, M., Ellis, J.G., Park, R.F., Dodds, P.N., 2017. Loss of AvrSr50 by somatic exchange in stem rust leads to virulence for Sr50 resistance in wheat. *Science* (80-). 358, 1607–1610. <https://doi.org/10.1126/science.aao4810>.
- Cheng, Y.-Q., Ahn, J.-H., Walton, J.D., 1999. A putative branched-chain-amino-acid transaminase gene required for HC-toxin biosynthesis and pathogenicity in *Cochliobolus carbonum* The GenBank accession number for the nucleotide sequence reported in this paper is AF157629. *Microbiology* 145, 3539–3546. <https://doi.org/10.1099/00221287-145-12-3539>.
- Ciuffetti, L.M., Tuori, R.P., Gaventa, J.M., 1997. A single gene encodes a selective toxin causal to the development of tan spot of wheat. *Plant Cell* 9, 135–144. <https://doi.org/10.1105/tpc.9.2.135>.
- Condon, B.J., Leng, Y., Wu, D., Bushley, K.E., Ohm, R.A., Otiillar, R., Martin, J., Schackwitz, W., Grimwood, J., MohdZainudin, N., Xue, C., Wang, R., Manning, V.A., Dhillon, B., Tu, Z.J., Steffenson, B.J., Salamov, A., Sun, H., Lowry, S., LaButti, K., Han, J., Copeland, A., Lindquist, E., Barry, K., Schmutz, J., Baker, S.E., Ciuffetti, L.M., Grigoriev, I.V., Zhong, S., Turgeon, B.G., 2013. Comparative genome structure, secondary metabolite, and effector coding capacity across *Cochliobolus* pathogens. *PLoS Genet.* 9, e1003233. <https://doi.org/10.1371/journal.pgen.1003233>.
- Croll, D., McDonald, B.A., 2012. The accessory genome as a cradle for adaptive evolution in pathogens. *PLoS Pathog.* 8, e1002608. <https://doi.org/10.1371/journal.ppat.1002608>.
- Daverdin, G., Rouxel, T., Gout, L., Aubertot, J.-N., Fudal, I., Meyer, M., Parlange, F., Carpezat, J., Balesdent, M.-H., 2012. Genome structure and reproductive behaviour influence the evolutionary potential of a fungal phytopathogen. *PLoS Pathog.* 8, e1003020. <https://doi.org/10.1371/journal.ppat.1003020>.
- de Guillen, K., Ortiz-Vallejo, D., Gracy, J., Fournier, E., Kroj, T., Padilla, A., 2015. Structure analysis uncovers a highly diverse but structurally conserved effector family in phytopathogenic fungi. *PLoS Pathog.* 11, e1005228. <https://doi.org/10.1371/journal.ppat.1005228>.
- de Jonge, R., Peter van Esse, H., Maruthachalam, K., Bolton, M.D., Santhanam, P., Saber, M.K., Zhang, Z., Usami, T., Lievens, B., Subbarao, K.V., Thomma, B.P.H.J., 2012. Tomato immune receptor Ve1 recognizes effector of multiple fungal pathogens uncovered by genome and RNA sequencing. *Proc. Natl. Acad. Sci.* 109, 5110–5115. <https://doi.org/10.1073/pnas.1119623109>.
- de Wit, P.J.G.M., 2016. *Cladosporium fulvum* effectors: weapons in the arms race with tomato. *Annu. Rev. Phytopathol.* 54, 1–23. <https://doi.org/10.1146/annurev-phyto-011516-040249>.
- de Wit, P.J.G.M., van der Burgt, A., Ökmen, B., Stergiopoulos, I., Abd-Elsalam, K.A., Aerts, A.L., Bahkali, A.H., Beenen, H.G., Chettri, P., Cox, M.P., Datema, E., de Vries, R.P., Dhillon, B., Ganley, A.R., Griffiths, S.A., Guo, Y., Hamelin, R.C., Henrissat, B., Kabir, M.S., Jashni, M.K., Kema, G., Klaubauf, S., Lapidus, A., Levasseur, A., Lindquist, E., Mehrabi, R., Ohm, R.A., Owen, T.J., Salamov, A., Schwelm, A., Schijlen, E., Sun, H., van den Burg, H.A., van Ham, R.C.H.J., Zhang, S., Goodwin, S.B., Grigoriev, I.V., Collemare, J., Bradshaw, R.E., 2012. The genomes of the fungal plant pathogens *Cladosporium fulvum* and *Dothistroma septosporium* reveal adaptation to different hosts and lifestyles but also signatures of common ancestry. *PLoS Genet.* 8, e1003088. <https://doi.org/10.1371/journal.pgen.1003088>.
- Dodds, P., Upadhyaya, N., Oriz, D., Li, F., Chen, J., Miller, M., Luo, M., Mago, R., Ellis, J., Spersneider, J., Nguyen-Phuc, H., Bouton, C., Steffenson, B., Hirsch, C., Silverstein, K., Park, R., Kanyuka, K., Figueroa, M., 2019. Using virulence mutants to identify Avr genes in the wheat stem rust fungus, *Puccinia graminis* f. sp. *Tritici*. In: 30th Fungal Genetics Conference.
- Dodds, P.N., Lawrence, G.J., Catanzariti, A.-M., Ayliffe, M.A., Ellis, J.G., 2004. The *Melampsora lini* AvrL567 avirulence genes are expressed in haustoria and their products are recognized inside plant cells. *Plant Cell* 16, 755–768. <https://doi.org/10.1105/tpc.020040>.
- Doehlemann, G., Ökmen, B., Zhu, W., Sharon, A., 2017. Plant Pathogenic Fungi. *Microbiol. Spectr.* 5. <https://doi.org/10.1128/microbiolspec.FUNK-0023-2016>.
- Edel-Hermann, V., Lecomte, C., 2019. Current status of *Fusarium oxysporum* formae speciales and races. *Phytopathology* 109, 512–530. <https://doi.org/10.1094/PHYTO-08-18-0320-RVW>.
- Ellis, J.G., Dodds, P.N., Lawrence, G.J., 2007. The role of secreted proteins in diseases of plants caused by rust, powdery mildew and smut fungi. *Curr. Opin. Microbiol.* 10, 326–331. <https://doi.org/10.1016/j.mib.2007.05.015>.
- Faris, J.D., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J.P., Meinhardt, S.W., Rasmussen, J.B., Xu, S.S., Oliver, R.P., Simons, K.J., 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proc. Natl. Acad. Sci.* 107, 13544–13549. <https://doi.org/10.1073/pnas.1004090107>.
- Farman, M.L., Eto, Y., Nakao, T., Tosa, Y., Nakayashiki, H., Mayama, S., Leong, S.A., 2002. Analysis of the structure of the AVR1-CO39 avirulence locus in virulent rice-infecting isolates of *Magnaporthe grisea*. *Mol. Plant-Microbe Interact.* 15, 6–16. <https://doi.org/10.1094/MPMI.2002.15.1.6>.
- Farman, M.L., Leong, S.A., 1998. Chromosome walking to the AVR1-CO39 avirulence gene of *Magnaporthe grisea*: Discrepancy between the physical and genetic maps. *Genetics*.
- Fernandez, J., Orth, K., 2018. Rise of a cereal killer: The biology of *Magnaporthe oryzae* biotrophic growth. *Trends Microbiol.* 26, 582–597. <https://doi.org/10.1016/j.tim.2017.12.007>.
- Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., Gurr, S.J., 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484, 186–194. <https://doi.org/10.1038/nature10947>.
- Friesen, T.L., Stukenbrock, E.H., Liu, Z., Meinhardt, S., Ling, H., Faris, J.D., Rasmussen, J.B., Solomon, P.S., McDonald, B.A., Oliver, R.P., 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. *Nat. Genet.* 38, 953–956. <https://doi.org/10.1038/ng1839>.
- Fudal, I., Böhnert, H.U., Tharreau, D., Lebrun, M.-H., 2005. Transposition of MINE, a composite retrotransposon, in the avirulence gene ACE1 of the rice blast fungus *Magnaporthe grisea*. *Fungal Genet. Biol.* 42, 761–772. <https://doi.org/10.1016/j.fgb.2005.05.001>.
- Ghanbarnia, K., Fudal, I., Larkan, N.J., Links, M.G., Balesdent, M.-H., Profotova, B., Fernando, W.G.D., Rouxel, T., Borhan, M.H., 2015. Rapid identification of the *Leptosphaeria maculans* avirulence gene AvrLm2 using an intraspecific comparative genomics approach. *Mol. Plant Pathol.* 16, 699–709. <https://doi.org/10.1111/mpp.12228>.
- Ghanbarnia, K., Ma, L., Larkan, N.J., Haddadi, P., Fernando, W.G.D., Borhan, M.H., 2018. *Leptosphaeria maculans* AvrLm9: a new player in the game of hide and seek with AvrLm4-7. *Mol. Plant Pathol.* 19, 1754–1764. <https://doi.org/10.1111/mpp.12658>.
- Gout, L., Fudal, I., Kuhn, M.-L., Blaise, F., Eckert, M., Catolico, L., Balesdent, M.-H., Rouxel, T., 2006. Lost in the middle of nowhere: the AvrLm1 avirulence gene of the Dothideomycete *Leptosphaeria maculans*. *Mol. Microbiol.* 60, 67–80. <https://doi.org/10.1111/j.1365-2958.2006.05076.x>.

- Hahn, M., 1993. Cultivar-specific elicitation of barley defense reactions by the phytotoxic peptide NIP1 from *Rhynchosporium secalis*. *Mol. Plant-Microbe Interact.* 6, 745. <https://doi.org/10.1094/MPMI-6-745>.
- He, C., Rusu, A.G., Poplawski, A.M., Irwin, J.A.G., Manners, J.M., 1998. Transfer of a supernumerary chromosome between vegetatively incompatible biotypes of the fungus *Colletotrichum gloeosporioides*. *Genetics* 150, 1459–1466.
- Houterman, P.M., Cornelissen, B.J.C., Rep, M., 2008. Suppression of Plant Resistance Gene-Based Immunity by a Fungal Effector. *PLoS Pathog.* 4, e1000061. <https://doi.org/10.1371/journal.ppat.1000061>.
- Houterman, P.M., Ma, L., van Ooijen, G., de Vroomen, M.J., Cornelissen, B.J.C., Takken, F.L.W., Rep, M., 2009. The effector protein Avr2 of the xylem-colonizing fungus *Fusarium oxysporum* activates the tomato resistance protein I-2 intracellularly. *Plant J.* 58, 970–978. <https://doi.org/10.1111/j.1365-313X.2009.03838.x>.
- Hu, J., Chen, C., Peever, T., Dang, H., Lawrence, C., Mitchell, T., 2012. Genomic characterization of the conditionally dispensable chromosome in *Alternaria arborescens* provides evidence for horizontal gene transfer. *BMC Genomics* 13, 171. <https://doi.org/10.1186/1471-2164-13-171>.
- Iakovidis, M., Soumpourou, E., Anderson, E., Etherington, G., Yourstone, S., Thomas, C., 2020. Genes encoding recognition of the *Cladosporium fulvum* effector protein Ecp5 are encoded at several loci in the tomato genome. *G3* 10, 1753–1763. <https://doi.org/10.1534/g3.120.401119>.
- Inderbitzin, P., Asvarak, T., Turgeon, B.G., 2010. Six new genes required for production of T-Toxin, a polyketide determinant of high virulence of *Cochliobolus heterostrophus* to maize. *Mol. Plant-Microbe Interact.* 23, 458–472. <https://doi.org/10.1094/MPMI-23-4-0458>.
- Inoue, Y., Vy, T.T.P., Yoshida, K., Asano, H., Mitsuoka, C., Asuke, S., Anh, V.L., Cumagun, C.J.R., Chuma, I., Terauchi, R., Kato, K., Mitchell, T., Valent, B., Farman, M., Tosa, Y., 2017. Evolution of the wheat blast fungus through functional losses in a host specificity determinant. *Science* (80-) 357, 80–83. <https://doi.org/10.1126/science.aam9654>.
- Johal, G., Briggs, S., 1992. Reductase activity encoded by the HM1 disease resistance gene in maize. *Science* (80-) 258, 985–987. <https://doi.org/10.1126/science.1359642>.
- Jones, J.D.G., Dangl, J.L., 2006. The plant immune system. *Nature* 444, 323–329. <https://doi.org/10.1038/nature05286>.
- Joosten, M.H.A.J., Cozijnsen, T.J., De Wit, P.J.G.M., 1994. Host resistance to a fungal tomato pathogen lost by a single base-pair change in an avirulence gene. *Nature* 367, 384–386. <https://doi.org/10.1038/367384a0>.
- Klosterman, S.J., Atallah, Z.K., Vallad, G.E., Subbarao, K.V., 2009. Diversity, pathogenicity, and management of *Verticillium* Species. *Annu. Rev. Phytopathol.* 47, 39–62. <https://doi.org/10.1146/annurev-phyto-080508-081748>.
- Kourelis, J., van der Hoorn, R.A.L., 2018. Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. *Plant Cell* 30, 285–299. <https://doi.org/10.1105/tpc.17.00579>.
- Lanver, D., Tollot, M., Schweizer, G., Lo Presti, L., Reissmann, S., Ma, L.-S., Schuster, M., Tanaka, S., Liang, L., Ludwig, N., Kahmann, R., 2017. *Ustilago maydis* effectors and their impact on virulence. *Nat. Rev. Microbiol.* 15, 409–421. <https://doi.org/10.1038/nrmicro.2017.33>.
- Larkan, N.J., Lydiate, D.J., Parkin, I.A.P., Nelson, M.N., Epp, D.J., Cowling, W.A., Rimmer, S.R., Borhan, M.H., 2013. The Brassica napus blackleg resistance gene LepR3 encodes a receptor-like protein triggered by the *Leptosphaeria maculans* effector AVR1ML1. *New Phytol.* 197, 595–605. <https://doi.org/10.1111/nph.12043>.
- Lauge, R., Goodwin, P.H., de Wit, P.J.G.M., Joosten, M.H.A.J., 2000. Specific HR-associated induction of secreted proteins from *Cladosporium fulvum* occurs in both host and non-host plants. *Plant J.* 23, 735–745. <https://doi.org/10.1046/j.1365-313x.2000.00843.x>.
- Laugé, R., Joosten, M.H.A.J., Van den Ackerveken, G.F.J.M., Van den Broek, H.W.J., De Wit, P.J.G.M., 1997. The in planta-produced extracellular proteins ECP1 and ECP2 of *Cladosporium fulvum* are virulence factors. *Mol. Plant-Microbe Interact.* 10, 725–734. <https://doi.org/10.1094/MPMI.1997.10.6.725>.
- Lawrence, G.J., Dodds, P.N., Ellis, J.G., 2007. Rust of flax and linseed caused by *Melampsora lini*. *Mol. Plant Pathol.* 8, 349–364. <https://doi.org/10.1111/j.1364-3703.2007.00405.x>.
- Li, J.-M., Fokkens, L., van Dam, P., Rep, M., 2020. Related mobile pathogenicity chromosomes in *Fusarium oxysporum* determine host range on cucurbits. *Mol. Plant Pathol.* (in press).
- Li, W., Wang, B., Wu, J., Lu, G., Hu, Y., Zhang, X., Zhang, Z., Zhao, Q., Feng, Q., Zhang, H., Wang, Z., Wang, G., Han, B., Wang, Z., Zhou, B., 2009. The Magnaporthe oryzae avirulence gene AvrPiz-t encodes a predicted secreted protein that triggers the immunity in rice mediated by the blast resistance gene Piz-t. *Mol. Plant-Microbe Interact.* 22, 411–420. <https://doi.org/10.1094/MPMI-22-4-0411>.
- Liu, Z., Farris, J.D., Oliver, R.P., Tan, K.-C., Solomon, P.S., McDonald, M.C., McDonald, B.A., Nunez, A., Lu, S., Rasmussen, J.B., Friesen, T.L., 2009. SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the Sn3 gene. *PLoS Pathog.* 5, e1000581. <https://doi.org/10.1371/journal.ppat.1000581>.
- Liu, Z., Friesen, T.L., Ling, H., Meinhardt, S.W., Oliver, R.P., Rasmussen, J.B., Farris, J.D., 2006. The Tsn1–ToxA interaction in the wheat–Stagonospora nodorum pathosystem parallels that of the wheat–tan spot system. *Genome* 49, 1265–1273. <https://doi.org/10.1139/g06-088>.
- Liu, Z., Gao, Y., Kim, Y.M., Farris, J.D., Shelver, W.L., Wit, P.J.G.M., Xu, S.S., Friesen, T.L., 2016. SnTox1, a Parastagonospora nodorum necrotrophic effector, is a dual-function protein that facilitates infection while protecting from wheat-produced chitinases. *New Phytol.* 211, 1052–1064. <https://doi.org/10.1111/nph.13959>.
- Liu, Z., Zhang, Z., Farris, J.D., Oliver, R.P., Syme, R., McDonald, M.C., McDonald, B.A., Solomon, P.S., Lu, S., Shelver, W.L., Xu, S., Friesen, T.L., 2012. The cysteine rich necrotrophic effector SnTox1 produced by *Stagonospora nodorum* triggers susceptibility of wheat lines harboring Sn1. *PLoS Pathog.* 8, e1002467. <https://doi.org/10.1371/journal.ppat.1002467>.
- Liu, Z.H., Farris, J.D., Meinhardt, S.W., Ali, S., Rasmussen, J.B., Friesen, T.L., 2004. Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially purified host-selective toxin produced by *Stagonospora nodorum*. *Phytopathology* 94, 1056–1060. <https://doi.org/10.1094/PHYTO.2004.94.10.1056>.
- Lo Presti, L., Lanver, D., Schweizer, G., Tanaka, S., Liang, L., Tollot, M., Zuccaro, A., Reissmann, S., Kahmann, R., 2015. Fungal Effectors and Plant Susceptibility. *Annu. Rev. Plant Biol.* 66, 513–545. <https://doi.org/10.1146/annurev-arplant-043014-114623>.
- Lorrain, C., Gonçalves dos Santos, K.C., Germain, H., Hecker, A., Duplessis, S., 2019. Advances in understanding obligate biotrophy in rust fungi. *New Phytol.* 222, 1190–1206. <https://doi.org/10.1111/nph.15641>.
- Lu, X., Kracher, B., Saur, I.M.L., Bauer, S., Ellwood, S.R., Wise, R., Yaeno, T., Maekawa, T., Schulze-Lefert, P., 2016. Allelic barley MLA immune receptors recognize sequence-unrelated avirulence effectors of the powdery mildew pathogen. *Proc. Natl. Acad. Sci.* 113, E6486–E6495. <https://doi.org/10.1073/pnas.1612947113>.
- Luderer, R., Takken, F.L.W., de Wit, P.J.G.M., Joosten, M.H.A.J., 2002. *Cladosporium fulvum* overcomes Cf-2-mediated resistance by producing truncated AVR2 elicitor proteins. *Mol. Microbiol.* 45, 875–884. <https://doi.org/10.1046/j.1365-2958.2002.03060.x>.
- Ma, L.-J., van der Does, H.C., Borkovich, K.A., Coleman, J.J., Daboussi, M.-J., Di Pietro, A., Dufresne, M., Freitag, M., Grabherr, M., Henrissat, B., Houterman, P.M., Kang, S., Shim, W.-B., Woloshuk, C., Xie, X., Xu, J.-R., Antoniw, J., Baker, S.E., Bluhm, B.H., Breakspear, A., Brown, D.W., Butchko, R.A.E., Chapman, S., Coulson, R., Coutinho, P.M., Danchin, E.G.J., Diener, A., Gale, L.R., Gardiner, D.M., Goff, S., Hammond-Kosack, K.E., Hilburn, K., Hua-Van, A., Jonkers, W., Kazan, K., Kodira, C.D., Koehrsen, M., Kumar, L., Lee, Y.-H., Li, L., Manners, J.M., Miranda-Saavedra, D., Mukherjee, M., Park, G., Park, J., Park, S.-Y., Proctor, R.H., Regev, A., Ruiz-Roldan, M.C., Sain, D., Sakhthikumar, S., Sykes, S., Schwartz, D.C., Turgeon, B.G., Wapinski, I., Yoder, O., Young, S., Zeng, Q., Zhou, S., Galagan, J., Cuomo, C.A., Kistler, H.C., Rep, M., 2010. Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* 464, 367–373. <https://doi.org/10.1038/nature08850>.
- Ma, L., Houterman, P.M., Gawehns, F., Cao, L., Sillo, F., Richter, H., Clavijo-Ortiz, M.J., Schmidt, S.M., Boeren, S., Vervoort, J., Cornelissen, B.J.C., Rep, M., Takken, F.L.W., 2015. The AVR2-SIX5 gene pair is required to activate I-2-mediated immunity in tomato. *New Phytol.* 208, 507–518. <https://doi.org/10.1111/nph.13455>.
- Martinez, J.P., Oesch, N.W., Ciuffetti, L.M., 2004. Characterization of the multiple-copy host-selective toxin gene, ToxB, in pathogenic and nonpathogenic isolates of *Pyrenophora tritici-repentis*. *Mol. Plant-Microbe Interact.* 17, 467–474. <https://doi.org/10.1094/MPMI.2004.17.5.467>.
- Martinez, J.P., Ottum, S.A., Ali, S., Francel, L.J., Ciuffetti, L.M., 2001. Characterization of the ToxB gene from *Pyrenophora tritici-repentis*. *Mol. Plant-Microbe Interact.* 14, 675–677. <https://doi.org/10.1094/MPMI.2001.14.5.675>.
- Meena, M., Gupta, S.K., Swapnil, P., Zehra, A., Dubey, M.K., Upadhyay, R.S., 2017. Alternaria toxins: potential virulence factors and genes related to pathogenesis. *Front. Microbiol.* 8. <https://doi.org/10.3389/fmicb.2017.01451>.
- Mehrabi, R., Bahkali, A.H., Abd-El Salam, K.A., Moslem, M., Ben M'Barek, S., Gohari, A.M., Jashni, M.K., Stergiopoulos, I., Kema, G.H.J., de Wit, P.J.G.M., 2011. Horizontal gene and chromosome transfer in plant pathogenic fungi affecting host range. *FEMS Microbiol. Rev.* 35, 542–554. <https://doi.org/10.1111/j.1574-6976.2010.00263.x>.
- Mesarich, C.H., Griffiths, S.A., van der Burg, A., Ökmen, B., Beenen, H.G., Etalo, D.W., Joosten, M.H.A.J., de Wit, P.J.G.M., 2014. Transcriptome sequencing uncovers the Avr5 avirulence gene of the tomato leaf mold pathogen *Cladosporium fulvum*. *Mol. Plant-Microbe Interact.* 27, 846–857. <https://doi.org/10.1094/MPMI-02-14-0050-R>.
- Mesarich, C.H., Ökmen, B., Rovenich, H., Griffiths, S.A., Wang, C., Karimi Jashni, M., Mihajlovski, A., Collemare, J., Hunziker, L., Deng, C.H., van der Burg, A., Beenen, H.G., Templeton, M.D., Bradshaw, R.E., de Wit, P.J.G.M., 2018. Specific hypersensitive response-associated recognition of new apoplastic effectors from *Cladosporium fulvum* in wild tomato. *Mol. Plant-Microbe Interact.* 31, 145–162. <https://doi.org/10.1094/MPMI-05-17-0114-FI>.
- Milgroom, M.G., del Mar Jiménez-Gasco, M., Olivares-García, C., Jiménez-Díaz, R.M., 2016. Clonal expansion and migration of a highly virulent, defoliating lineage of *Verticillium dahliae*. *Phytopathology* 106, 1038–1046. <https://doi.org/10.1094/PHYTO-11-15-0300-R>.
- Mohd-Assaad, N., McDonald, B.A., Croll, D., 2019. The emergence of the multi-species NIP1 effector in *Rhynchosporium* was accompanied by high rates of gene duplications and losses. *Environ. Microbiol.* 21, 2677–2695. <https://doi.org/10.1111/1462-2920.14583>.
- Möller, M., Stukenbrock, E.H., 2017. Evolution and genome architecture in fungal plant pathogens. *Nat. Rev. Microbiol.* 15, 756–771. <https://doi.org/10.1038/nrmicro.2017.76>.
- Niu, X., Zhao, X., Ling, K.-S., Levi, A., Sun, Y., Fan, M., 2016. The FonSIX6 gene acts as an avirulence effector in the *Fusarium oxysporum* f. sp. niveum – watermelon pathosystem. *Sci. Rep.* 6, 28146. <https://doi.org/10.1038/srep28146>.
- Nyarko, A., Singarapu, K.K., Figueroa, M., Manning, V.A., Pandelova, I., Wolpert, T.J., Ciuffetti, L.M., Barbar, E., 2014. Solution NMR Structures of *Pyrenophora tritici-repentis* ToxB and Its Inactive Homolog Reveal Potential Determinants of Toxin Activity. *J. Biol. Chem.* 289, 25946–25956. <https://doi.org/10.1074/jbc.M114.569103>.
- Okuyama, Y., Kanzaki, H., Abe, A., Yoshida, K., Tamiru, M., Saitoh, H., Fujibe, T., Matsumura, H., Shenton, M., Galam, D.C., Undan, J., Ito, A., Sone, T., Terauchi, R., 2011. A multifaceted genomics approach allows the isolation of the rice Pta-blast resistance gene consisting of two adjacent NBS-LRR protein genes. *Plant J.* 66, 467–479. <https://doi.org/10.1111/j.1365-313X.2011.04502.x>.
- Oliver, R.P., Friesen, T.L., Farris, J.D., Solomon, P.S., 2012. *Stagonospora nodorum*: from pathology to genomics and host resistance. *Annu. Rev. Phytopathol.* 50, 23–43.

- <https://doi.org/10.1146/annurev-phyto-081211-173019>.
- Orbach, M.J., Farrall, L., Sweigard, J.A., Chumley, F.G., Valent, B., 2000. A telomeric avirulence gene determines efficacy for the rice blast resistance gene Pi-ta. *Plant Cell* 12, 2019–2032. <https://doi.org/10.1105/tpc.12.11.2019>.
- Panaccione, D.G., Scott-Craig, J.S., Pocard, J.A., Walton, J.D., 1992. A cyclic peptide synthetase gene required for pathogenicity of the fungus *Cochliobolus carbonum* on maize. *Proc. Natl. Acad. Sci.* 89, 6590–6594. <https://doi.org/10.1073/pnas.89.14.6590>.
- Parlange, F., Daverdin, G., Fudal, I., Kuhn, M.-L., Balesdent, M.-H., Blaise, F., Grezes-Besset, B., Rouxel, T., 2009. Leptosphaeria maculans avirulence gene AvrLm4-7 confers a dual recognition specificity by the Rlm4 and Rlm7 resistance genes of oil-seed rape, and circumvents Rlm4-mediated recognition through a single amino acid change. *Mol. Microbiol.* 71, 851–863. <https://doi.org/10.1111/j.1365-2958.2008.06547.x>.
- Pennisi, E., 2010. *Armed and Dangerous*. *Science* (80- ). 327, 804–805.
- Petit-Houdenot, Y., Fudal, I., 2017. Complex interactions between fungal avirulence genes and their corresponding plant resistance genes and consequences for disease resistance management. *Front. Plant Sci.* 8. <https://doi.org/10.3389/fpls.2017.01072>.
- Petit-Houdenot, Y., Degrave, A., Meyer, M., Blaise, F., Ollivier, B., Marais, C., Jauneau, A., Audran, C., Rivas, S., Veneault-Fourrey, C., Brun, H., Rouxel, T., Fudal, I., Balesdent, M., 2019. A two genes – for – one gene interaction between *Leptosphaeria maculans* and *Brassica napus*. *New Phytol.* 223, 397–411. <https://doi.org/10.1111/nph.15762>.
- Petre, B., Joly, D.L., Duplessis, S., 2014. Effector proteins of rust fungi. *Front. Plant Sci.* 5. <https://doi.org/10.3389/fpls.2014.00416>.
- Pietro, A.D., Madrid, M.P., Caracul, Z., Delgado-Jarana, J., Roncero, M.I.G., 2003. *Fusarium oxysporum*: exploring the molecular arsenal of a vascular wilt fungus. *Mol. Plant Pathol.* 4, 315–325. <https://doi.org/10.1046/j.1364-3703.2003.00180.x>.
- Pitkin, J.W., Panaccione, D.G., Walton, J.D., 1996. A putative cyclic peptide efflux pump encoded by the TOXA gene of the plant-pathogenic fungus *Cochliobolus carbonum*. *Microbiology* 142, 1557–1565. <https://doi.org/10.1099/13500872-142-6-1557>.
- Plissonneau, C., Daverdin, G., Ollivier, B., Blaise, F., Degrave, A., Fudal, I., Rouxel, T., Balesdent, M.-H., 2016. A game of hide and seek between avirulence genes AvrLm4-7 and AvrLm3 in *Leptosphaeria maculans*. *New Phytol.* 209, 1613–1624. <https://doi.org/10.1111/nph.13736>.
- Plissonneau, C., Rouxel, T., Chèvre, A.-M., Van De Wouw, A.P., Balesdent, M.-H., 2018. One gene-one name: the AvrLmJ1 avirulence gene of *Leptosphaeria maculans* is AvrLm5. *Mol. Plant Pathol.* 19, 1012–1016. <https://doi.org/10.1111/mps.12574>.
- Prasad, P., Savadi, S., Bhardwaj, S.C., Gangwar, O.P., Kumar, S., 2019. Rust pathogen effectors: perspectives in resistance breeding. *Planta* 250, 1–22. <https://doi.org/10.1007/s00425-019-03167-6>.
- Praz, C.R., Bourras, S., Zeng, F., Sánchez-Martín, J., Menardo, F., Xue, M., Yang, L., Roffler, S., Böni, R., Herren, G., McNally, K.E., Ben-David, R., Parlange, F., Oberhaensli, S., Flückiger, S., Schäfer, L.K., Wicker, T., Yu, D., Keller, B., 2017. AvrPm2 encodes an RNase-like avirulence effector which is conserved in the two different specialized forms of wheat and rye powdery mildew fungus. *New Phytol.* 213, 1301–1314. <https://doi.org/10.1111/nph.14372>.
- Raffaele, S., Kamoun, S., 2012. Genome evolution in filamentous plant pathogens: why bigger can be better. *Nat. Rev. Microbiol.* 10, 417–430. <https://doi.org/10.1038/nrmicro2790>.
- Ransom, R.F., Walton, J.D., 1997. Histone hyperacetylation in maize in response to treatment with HC-toxin or infection by the filamentous fungus *Cochliobolus carbonum*. *Plant Physiol.* 115, 1021–1027. <https://doi.org/10.1104/pp.115.3.1021>.
- Ray, S., Singh, P.K., Gupta, D.K., Mahato, A.K., Sarkar, C., Rathour, R., Singh, N.K., Sharma, T.R., 2016. Analysis of Magnaporthe oryzae genome reveals a fungal effector, which is able to induce resistance response in transgenic rice line containing resistance gene, Pi54. *Front. Plant Sci.* 7. <https://doi.org/10.3389/fpls.2016.01140>.
- Rep, M., Van Der Does, H.C., Meijer, M., Van Wijk, R., Houterman, P.M., Dekker, H.L., De Koster, C.G., Cornelissen, B.J.C., 2004. A small, cysteine-rich protein secreted by *Fusarium oxysporum* during colonization of xylem vessels is required for I-3-mediated resistance in tomato. *Mol. Microbiol.* 53, 1373–1383. <https://doi.org/10.1111/j.1365-2958.2004.04177.x>.
- Ridout, C.J., Skamnioti, P., Porritt, O., Sacristan, S., Jones, J.D.G., Brown, J.K.M., 2006. Multiple Avirulence Paralogs in Cereal Powdery Mildew Fungi May Contribute to Parasite Fitness and Defeat of Plant Resistance. *Plant Cell* 18, 2402–2414. <https://doi.org/10.1105/tpc.106.043307>.
- Rivas, S., Thomas, C.M., 2005. Molecular interactions between tomato and the leaf mold pathogen *Cladosporium fulvum*. *Annu. Rev. Phytopathol.* 43, 395–436. <https://doi.org/10.1146/annurev.phyto.43.040204.140224>.
- Rohe, M., Gierlich, A., Hermann, H., Hahn, M., Schmidt, B., Rosahl, S., Knogge, W., 1995. The race-specific elicitor, NIP1, from the barley pathogen, *Rhynchosporium secalis*, determines avirulence on host plants of the Rrs1 resistance gene. *EMBO J.* 14, 4168–4177. <https://doi.org/10.1002/j.1460-2075.1995.tb00090.x>.
- Rose, M.S., Yun, S.-H., Asvarak, T., Lu, S.-W., Yoder, O.C., Turgeon, B.G., 2002. A Decarboxylase encoded at the *Cochliobolus heterostrophus* translocation-associated Tox1B locus is required for polyketide (T-toxin) biosynthesis and high virulence on T-cytoplasm maize. *Mol. Plant-Microbe Interact.* 15, 883–893. <https://doi.org/10.1094/MPMI.2002.15.9.883>.
- Rouxel, T., Balesdent, M.-H., 2017. Life, death and rebirth of avirulence effectors in a fungal pathogen of Brassica crops. *Leptosphaeria maculans*. *New Phytol.* 214, 526–532. <https://doi.org/10.1111/nph.14411>.
- Rouxel, T., Grandaubert, J., Hane, J.K., Hoede, C., van de Wouw, A.P., Couloux, A., Dominguez, V., Anthouard, V., Bally, P., Bourras, S., Cozijnsen, A.J., Ciuffetti, L.M., Degrave, A., Dilmaghani, A., Duret, L., Fudal, I., Goodwin, S.B., Gout, L., Glaser, N., Linglin, J., Kema, G.H.J., Lalalu, N., Lawrence, C.B., May, K., Meyer, M., Ollivier, B., Poulain, J., Schoch, C.L., Simon, A., Spatafora, J.W., Stachowiak, A., Turgeon, B.G., Tyler, B.M., Vincent, D., Weissenbach, J., Amselem, J., Quesneville, H., Oliver, R.P., Wincker, P., Balesdent, M.-H., Howlett, B.J., 2011. Effector diversification within compartments of the *Leptosphaeria maculans* genome affected by Repeat-Induced Point mutations. *Nat. Commun.* 2, 202. <https://doi.org/10.1038/ncomms1189>.
- Salcedo, A., Rutter, W., Wang, S., Akhunova, A., Bolus, S., Chao, S., Anderson, N., De Soto, M.F., Rouse, M., Szabo, L., Bowden, R.L., Dubcovsky, J., Akhunov, E., 2017. Variation in the AvrSr35 gene determines Sr35 resistance against wheat stem rust race Ug99. *Science* (80-) 358, 1604–1606. <https://doi.org/10.1126/science.aao7294>.
- Sarma, G.N., Manning, V.A., Ciuffetti, L.M., Karplus, P.A., 2005. Structure of Ptr ToxA: an RGD-containing host-selective toxin from *Pyrenophora tritici-repentis*. *Plant Cell* 17, 3190–3202. <https://doi.org/10.1105/tpc.105.034918>.
- Saur, I.M.L., Bauer, S., Kracher, B., Lu, X., Franzesekakis, L., Müller, M.C., Sabelleck, B., Kümme, F., Panstruga, R., Maekawa, T., Schulze-Lefert, P., 2019. Multiple pairs of allelic MLA immune receptor-powdery mildew AVRA effectors argue for a direct recognition mechanism. *Elife* 8, e44471. <https://doi.org/10.7554/eLife.44471>.
- Schmidt, S.M., Houterman, P.M., Schreiber, I., Ma, L., Amyotte, S., Chellappan, B., Boeren, S., Takken, F.L.W., Rep, M., 2013. MITES in the promoters of effector genes allow prediction of novel virulence genes in *Fusarium oxysporum*. *BMC Genomics* 14, 119. <https://doi.org/10.1186/1471-2164-14-119>.
- Schmidt, S.M., Lukaszewicz, J., Farrer, R., van Dam, P., Bertoldo, C., Rep, M., 2016. Comparative genomics of *Fusarium oxysporum* f. sp. melonis reveals the secreted protein recognized by the Fom-2 resistance gene in melon. *New Phytol.* 209, 307–318. <https://doi.org/10.1111/nph.13584>.
- Schulze-Lefert, P., Panstruga, R., 2011. A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. *Trends Plant Sci.* 16, 117–125. <https://doi.org/10.1016/j.tplants.2011.01.001>.
- Schürch, S., Linde, C.C., Knogge, W., Jackson, L.F., McDonald, B.A., 2004. Molecular population genetic analysis differentiates two virulence mechanisms of the fungal avirulence gene NIP1. *Mol. Plant-Microbe Interact.* 17, 1114–1125. <https://doi.org/10.1094/MPMI.2004.17.10.1114>.
- Selin, C., de Kievit, T.R., Belmonte, M.F., Fernando, W.G.D., 2016. Elucidating the role of effectors in plant-fungal interactions: progress and challenges. *Front. Microbiol.* 7. <https://doi.org/10.3389/fmicb.2016.00600>.
- Singh, R.P., Hodson, D.P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., Herrera-Foessel, S., Singh, P.K., Singh, S., Govindan, V., 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. In: Van Alfen, N.K., Bruening, G., Leach, J.E. (Eds.), *Annual Review of Phytopathology*, vol 49, pp. 465–481. <https://doi.org/10.1146/annurev-phyto-072910-095423>.
- Snelders, N.C., Rovenich, H., Petti, G.C., Rocafort, M., Vorholt, J.A., Mesters, J.R., Seidl, M.F., Nijland, R., Thomma, B.P.H.J., 2020. A plant pathogen utilizes effector proteins for microbiome manipulation. *bioRxiv* 2020.01.30.926725. <https://doi.org/10.1101/2020.01.30.926725>.
- Spasieva, S.D., Markham, J.E., Hille, J., 2002. The plant disease resistance gene Asc-1 prevents disruption of sphingolipid metabolism during AAL-toxin-induced programmed cell death. *Plant J.* 32, 561–572. <https://doi.org/10.1046/j.1365-313X.2002.01444.x>.
- Stergiopoulos, I., de Wit, P.J.G.M., 2009. Fungal effector proteins. *Annu. Rev. Phytopathol.* 47, 233–263. <https://doi.org/10.1146/annurev.phyto.112408.132637>.
- Stotz, H.U., Mitrousis, G.K., de Wit, P.J.G.M., Fitt, B.D.L., 2014. Effector-triggered defence against apoplastic fungal pathogens. *Trends Plant Sci.* 19, 491–500. <https://doi.org/10.1016/j.tplants.2014.04.009>.
- Strelkov, S.E., Lamari, L., Ballance, G.M., 1999. Characterization of a host-specific protein toxin (Ptr-ToxB) from *Pyrenophora tritici-repentis*. *Mol. Plant-Microbe Interact.* 12, 728–732. <https://doi.org/10.1094/MPMI.1999.12.8.728>.
- Sweigard, J.A., Carroll, A.M., Kang, S., Farrall, L., Chumley, F.G., Valent, B., 1995. Identification, cloning, and characterization of PWL2, a gene for host species specificity in the rice blast fungus. *Plant Cell* 7, 1221–1233. <https://doi.org/10.1105/tpc.7.8.1221>.
- Takabayashi, N., Tosa, Y., Oh, H.S., Mayama, S., 2002. A Gene-for-gene relationship underlying the species-specific parasitism of Avena/Triticum Isolates of Magnaporthe grisea on wheat cultivars. *Phytopathology* 92, 1182–1188. <https://doi.org/10.1094/PHYTO.2002.92.11.1182>.
- Takken, F., Rep, M., 2010. The arms race between tomato and *Fusarium oxysporum*. *Mol. Plant Pathol.* 11, 309–314. <https://doi.org/10.1111/j.1364-3703.2009.00605.x>.
- Toruño, T.Y., Stergiopoulos, I., Coaker, G., 2016. Plant-pathogen effectors: cellular probes interfering with plant defenses in spatial and temporal manners. *Annu. Rev. Phytopathol.* 54, 419–441. <https://doi.org/10.1146/annurev-phyto-080615-100204>.
- Tsuge, T., Harimoto, Y., Akimitsu, K., Ohtani, K., Kodama, M., Akagi, Y., Egusa, M., Yamamoto, M., Otani, H., 2013. Host-selective toxins produced by the plant pathogenic fungus *Alternaria alternata*. *FEMS Microbiol. Rev.* 37, 44–66. <https://doi.org/10.1111/j.1574-6976.2012.00350.x>.
- Upadhyaya, N.M., Mago, R., Staskawicz, B.J., Ayliffe, M.A., Ellis, J.G., Dodds, P.N., 2014. A Bacterial Type III Secretion Assay for Delivery of Fungal Effector Proteins into Wheat. *Mol. Plant-Microbe Interact.* 27, 255–264. <https://doi.org/10.1094/MPMI-07-13-0187-FI>.
- van Dam, P., Fokkens, L., Ayukawa, Y., van der Gragt, M., ter Horst, A., Brankovics, B., Houterman, P.M., Arie, T., Rep, M., 2017. A mobile pathogenicity chromosome in *Fusarium oxysporum* for infection of multiple cucurbit species. *Sci. Rep.* 7, 9042. <https://doi.org/10.1038/s41598-017-07995-y>.
- Van de Wouw, A.P., Lowe, R.G.T., Elliott, C.E., Dubois, D.J., Howlett, B.J., 2014. An avirulence gene, AvrLmJ1, from the blackleg fungus, *Leptosphaeria maculans*, confers avirulence to Brassica juncea cultivars. *Mol. Plant Pathol.* 15, 523–530. <https://doi.org/10.1111/mps.12105>.
- van Dijkman, A., Kaars Sijpesteijn, A., 1973. Leakage of pre-absorbed 32P from tomato leaf disks infiltrated with high molecular weight products of incompatible races of

- Cladosporium fulvum. *Physiol. Plant Pathol.* 3, 57–67. [https://doi.org/10.1016/0048-4059\(73\)90022-2](https://doi.org/10.1016/0048-4059(73)90022-2).
- van Esse, H.P., Bolton, M.D., Stergiopoulos, I., de Wit, P.J.G.M., Thomma, B.P.H.J., 2007. The chitin-binding cladosporium fulvum effector protein Avr4 is a virulence factor. *Mol. Plant-Microbe Interact.* 20, 1092–1101. <https://doi.org/10.1094/MPMI-20-9-1092>.
- van Kan, J.A.L., 1991. Cloning and characterization of cDNA of avirulence gene avr9 of the fungal pathogen *Cladosporium fulvum*, causal agent of tomato leaf mold. *Mol. Plant-Microbe Interact.* 4, 52. <https://doi.org/10.1094/MPMI-4-052>.
- van Schie, C.C.N., Takken, F.L.W., 2014. Susceptibility genes 101: how to be a good host. *Annu. Rev. Phytopathol.* 52, 551–581. <https://doi.org/10.1146/annurev-phyto-102313-045854>.
- Ve, T., Williams, S.J., Catanzariti, A.-M., Rafiqi, M., Rahman, M., Ellis, J.G., Hardham, A.R., Jones, D.A., Anderson, P.A., Dodds, P.N., Kobe, B., 2013. Structures of the flax rust effector AvrM reveal insights into the molecular basis of plant-cell entry and effector-triggered immunity. *Proc. Natl. Acad. Sci.* 110, 17594–17599. <https://doi.org/10.1073/pnas.1307614110>.
- Vlaardingerbroek, I., Beerens, B., Rose, L., Fokkens, L., Cornelissen, B.J.C., Rep, M., 2016. Exchange of core chromosomes and horizontal transfer of lineage-specific chromosomes in *Fusarium oxysporum*. *Environ. Microbiol.* 18, 3702–3713. <https://doi.org/10.1111/1462-2920.13281>.
- von Felten, A., Zaffarano, P.L., McDonald, B.A., 2011. Electrophoretic karyotypes of *Rhynchosporium commune*, *R. secalis* and *R. agropyri*. *Eur. J. Plant Pathol.* 129, 529–537. <https://doi.org/10.1007/s10658-010-9717-7>.
- Walton, J.D., 2006. HC-toxin. *Phytochemistry* 67, 1406–1413. <https://doi.org/10.1016/j.phytochem.2006.05.033>.
- Wang, B. hua, Ebbole, D.J., Wang, Z. hua, 2017. The arms race between *Magnaporthe oryzae* and rice: diversity and interaction of Avr and R genes. *J. Integr. Agric.* 16, 2746–2760. [https://doi.org/10.1016/S2095-3119\(17\)61746-5](https://doi.org/10.1016/S2095-3119(17)61746-5).
- Wang, C.-I.A., Gunčar, G., Forwood, J.K., Teh, T., Catanzariti, A.-M., Lawrence, G.J., Loughlin, F.E., Mackay, J.P., Schirra, H.J., Anderson, P.A., Ellis, J.G., Dodds, P.N., Kobe, B., 2007. Crystal structures of flax rust avirulence proteins AvrL567-A and -D reveal details of the structural basis for flax disease resistance specificity. *Plant Cell* 19, 2898–2912. <https://doi.org/10.1105/tpc.107.053611>.
- Wang, Yan, Wang, Yuanchao, 2018. Trick or treat: microbial pathogens evolved apoplast effectors modulating plant susceptibility to infection. *Mol. Plant-Microbe Interact.* 31, 6–12. <https://doi.org/10.1094/MPMI-07-17-0177-FI>.
- Westerink, N., Brandwagt, B.F., De Wit, P.J.G.M., Joosten, M.H.A.J., 2004. *Cladosporium fulvum* circumvents the second functional resistance gene homologue at the Cf-4 locus (Hcr9-4E) by secretion of a stable avr4E isoform. *Mol. Microbiol.* 54, 533–545. <https://doi.org/10.1111/j.1365-2958.2004.04288.x>.
- Wevelsiep, L., Kogel, K.-H., Knogge, W., 1991. Purification and characterization of peptides from *Rhynchosporium secalis* inducing necrosis in barley. *Physiol. Mol. Plant Pathol.* 39, 471–482. [https://doi.org/10.1016/0885-5765\(91\)90013-8](https://doi.org/10.1016/0885-5765(91)90013-8).
- Wu, J., Kou, Y., Bao, J., Li, Y., Tang, M., Zhu, X., Ponaya, A., Xiao, G., Li, J., Li, C., Song, M.-Y., Cumagun, C.J.R., Deng, Q., Lu, G., Jeon, J.-S., Naqvi, N.I., Zhou, B., 2015. Comparative genomics identifies the *Magnaporthe oryzae* avirulence effector AvrPi9 that triggers Pi9-mediated blast resistance in rice. *New Phytol.* 206, 1463–1475. <https://doi.org/10.1111/nph.13310>.
- Yan, X., Talbot, N.J., 2016. Investigating the cell biology of plant infection by the rice blast fungus *Magnaporthe oryzae*. *Curr. Opin. Microbiol.* 34, 147–153. <https://doi.org/10.1016/j.mib.2016.10.001>.
- Yang, G., Rose, M.S., Turgeon, B.G., Yoder, O.C., 1996. A polyketide synthase is required for fungal virulence and production of the polyketide T-toxin. *Plant Cell* 8, 2139–2150. <https://doi.org/10.1105/tpc.8.11.2139>.
- Zhang, D., Wang, Jie, Wang, D., Kong, Z., Zhou, L., Zhang, G., Gui, Y., Li, J., Huang, J., Wang, B., Liu, C., Yin, C., Li, R., Li, T., Wang, Jin-Long, Short, D.P.G., Klosterman, S.J., Bostock, R.M., Subbarao, K.V., Chen, J., Dai, X., 2019. Population genomics demystifies the defoliation phenotype in the plant pathogen *Verticillium dahliae*. *New Phytol.* 222, 1012–1029. <https://doi.org/10.1111/nph.15672>.
- Zhang, S., Wang, L., Wu, W., He, L., Yang, X., Pan, Q., 2015. Function and evolution of *Magnaporthe oryzae* avirulence gene AvrPib responding to the rice blast resistance gene Pib. *Sci. Rep.* 5, 11642. <https://doi.org/10.1038/srep11642>.
- Zhang, X., Farah, N., Rolston, L., Ericsson, D.J., Catanzariti, A.-M., Bernoux, M., Ve, T., Bendak, K., Chen, C., Mackay, J.P., Lawrence, G.J., Hardham, A., Ellis, J.G., Williams, S.J., Dodds, P.N., Jones, D.A., Kobe, B., 2018. Crystal structure of the *Melampsora lini* effector AvrP reveals insights into a possible nuclear function and recognition by the flax disease resistance protein P. *Mol. Plant Pathol.* 19, 1196–1209. <https://doi.org/10.1111/mpp.12597>.
- Zhang, Z., Henderson, C., Perfect, E., Carver, T.L.W., Thomas, B.J., Skamnioti, P., Gurr, S.J., 2005. Of genes and genomes, needles and haystacks: *Blumeria graminis* and functionality. *Mol. Plant Pathol.* 6, 561–575. <https://doi.org/10.1111/j.1364-3703.2005.00303.x>.