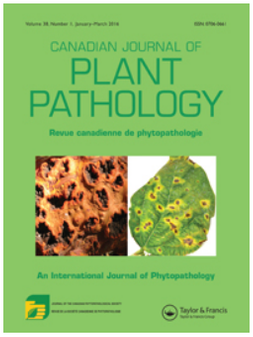

Canadian Journal of Plant Pathology

ISSN: 0706-0661 (Print) 1715-2992 (Online) Journal homepage: http://www.tandfonline.com/loi/tcjp20

Plant pathogenic oomycetes: counterbalancing resistance, susceptibility and adaptation

Alyssa Burkhardt & Brad Day

To cite this article: Alyssa Burkhardt & Brad Day (2016) Plant pathogenic oomycetes: counterbalancing resistance, susceptibility and adaptation, Canadian Journal of Plant Pathology, 38:1, 31-40, DOI: 10.1080/07060661.2016.1149519

To link to this article: http://dx.doi.org/10.1080/07060661.2016.1149519

Accepted author version posted online: 22 Feb 2016.
Published online: 02 Mar 2016.

Can. J. Plant Pathol., 2016
Vol. 38, No. 1, 31–40, http://dx.doi.org/10.1080/07060661.2016.1149519

Taylor & Francis
Taylor & Francis Group

Symposium contribution/Contribution à un symposium

Plant pathogenic oomycetes: counterbalancing resistance, susceptibility and adaptation

ALYSSA BURKHARDT[1,] AND BRAD DAY[1,2,3]

[1,]*Graduate Program in Cell and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA*
[2]*Graduate Program in Genetics, Michigan State University, East Lansing, MI 48824, USA*
[3]*Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI48824, USA*

(Accepted 29 January 2016)

Abstract: The genetic basis for the interaction(s) between plants and pathogens has been classically illustrated as a gene-for-gene relationship, through which a single gene product from the pathogen interacts within a single gene product found within the plant. In the simplest terms, it is the outcome of these interactions that underpin resistance and/or susceptibility signalling processes. While this basic concept shapes our understanding of many of the molecular-genetic mechanisms controlling immune signalling in plants, current research has revealed that the timing and ultimate outcome of these interactions are far more complex, and are typically regulated in a genome-by-genome manner. As a central theme for this review, we will focus on recent discoveries in the field of plant-oomycete interactions, primarily on the downy mildews, an important group of obligate oomycete pathogens of plants. Recent transcriptome-based studies have shown that survival, adaptation and virulence rely on complex, bi-directional interactions between the host and pathogen genomes. Taking advantage of the obligate nature of the downy mildews, insight into the transcriptional plasticity of these genomes has revealed a remarkable ability to adapt to host and environmental stressors. Herein, we will highlight a recent body of research using the *Pseudoperonospora cubensis – Cucumis sativus* interaction, which has identified a suite of alternative splicing and sRNA-based regulatory signals that are induced in a temporal and host-specific manner. In combination with recent studies in other plant-oomycete pathosystems, a comprehensive transcriptional profile of resistance and susceptibility within the host and pathogen illustrates the remarkable ability of this group of pathogens to adapt to host and environment to infect and cause disease in a diverse array of agriculturally important crops.

Keywords: cucumber, downy mildew, genomics, host–pathogen interactions, oomycetes, RNA-Seq, transcriptome

Résumé: La base génétique des interactions qui existent entre les plantes et les agents pathogènes a été généralement illustrée par la relation gène pour gène au cours de laquelle un seul produit génique de l'agent pathogène interagit avec un seul produit génique de la plante. En termes simples, ce sont les résultats de ces interactions qui sont à la base de la résistance ou des processus de la signalisation de la sensibilité. Tandis que ce concept fondamental contribue à façonner notre compréhension de nombreux mécanismes moléculaires et génétiques régulant la signalisation de l'immunité chez les plantes, des recherches récentes ont démontré que le déroulement de ces interactions et le résultat final sont beaucoup plus complexes et qu'ils sont généralement régulés, en quelque sorte, de génome à génome. En tant que thème central de ce compte rendu, nous mettrons l'accent sur les récentes découvertes dans le domaine des interactions plantes-oomycètes, principalement des mildious, un groupe important d'oomycètes pathogènes obligatoires des plantes. Des études récentes basées sur le transcriptome ont montré que la survie, l'adaptation et la virulence dépendent d'interactions bidirectionnelles complexes entre les génomes de l'hôte et de l'agent pathogène. Tirant parti de la nature obligatoire des mildious, un aperçu de la plasticité transcriptionnelle de ces génomes a révélé chez eux une capacité remarquable à s'adapter à l'hôte et aux stresseurs environnementaux. En cela, nous soulignerons plusieurs recherches qui ont porté sur l'interaction *Pseudoperonospora cubensis-Cucumis sativus*, ce qui a permis de caractériser une suite d'épissages alternatifs et de signaux de

Correspondence to: B. Day. E-mail: bday@msu.edu
This paper was a contribution to the symposium entitled 'Basic and applied approaches to improve disease resistance in plants' held during the Canadian Phytopathological Society Annual Meeting in Edmonton, Alberta, July 2015.

régulation basés sur l'ARN soluble qui sont induits temporellement et spécifiquement. Parallèlement aux études récentes menées sur d'autres pathosystèmes plantes-oomycètes, un profil transcriptionnel détaillé de la résistance et de la sensibilité chez l'hôte et l'agent pathogène illustre la remarquable capacité de ce groupe d'agents à s'adapter à l'hôte et à l'environnement dans le but d'infecter une grande variété de cultures importantes sur le plan agricole et de causer des maladies chez ces dernières.

Mots clés: concombre, génomique, interactions hôte-agent pathogène, mildiou, oomycètes, transcriptome, séquençage à ARN

Introduction

From gene-for-gene, to genome-for-genome

Over the past decade, the application of whole genome-based sequencing approaches has facilitated numerous advances towards a fundamental understanding of the biochemical, cellular and genetic mechanisms that underpin the interaction between plants, pathogens and the environment. As a foundation for much of the research in this area, early research typically focused on the identification and characterization of single genes, or in some cases, single gene pair interactions, between the host and microbe. Importantly, these initial studies provided – and still serve as – a point of reference to guide current, ongoing, whole-genome interaction studies. Historically, and in what is viewed as the foundation for much of the current research in the field of molecular plant pathology, Flor (1955) defined the genetic basis underpinning pathogen recognition and the elicitation of resistance and susceptibility through what is known as the 'Gene-for-Gene' hypothesis. In brief, this interaction posits that resistance (*R*) genes in the plant and avirulence genes (*Avr*) genes in the pathogen interact – directly and/or indirectly – in a highly specific pairwise manner to initiate immune signalling. Since this early discovery, subsequent research has expanded our understanding of these processes to yield the definition of multiple additional 'layers' of resistance signalling, including the convergent function and regulation of each of these pathways (Macho & Zipfel 2015). From these studies, our understanding of immune signalling in plants has further expanded to include the description of a highly specific pathogen associated molecular pattern (PAMP)-triggered immune (PTI) node, as well an extension of Flor's early research to yield what is now known as the 'Guard Hypothesis', broadly referred to as effector-triggered immunity (ETI) (Dangl & Jones 2001; Chisholm et al. 2006; Jones & Dangl 2006; Dangl et al. 2013).

As an extension of these initial studies, recent research has taken a broader approach to identify quantitative mechanisms of resistance, which has defined broad quantitative trait loci (QTL) that specify resistance to pathogens in a non-gene-for-gene associated manner (i.e. non-*R*-mediated; Lapin et al. 2012; Boer den et al. 2013; Simko et al. 2013; Zhang et al. 2013; Yoshioka et al. 2014). Using this concept to highlight the importance for future research in this area, we will use the 'genome-for-genome hypothesis' (Krasileva et al. 2011) to guide our discussion of the regulation and specificity underpinning the interactions between host, pathogen and environment. In brief, this concept has been used to describe the molecular-genetic interactions between a fast-evolving, highly destructive class of plant pathogens and their hosts, and herein, we will further illustrate how genome-wide approaches are used to define how pathogens evolve mechanisms to regulate gene expression and how plants resist pathogens. Herein, we will primarily focus on recent advances using obligate parasitism of plants as a paradigm to define the complex interactions between host and pathogen. Our aim is to provide an overview of genome-scale methods that have enabled the discovery of the processes required for plant resistance and pathogen adaptation.

Sequencing advances enable genome-scale studies

Over the last several years, sequencing technology has greatly improved; the quality and quantity of reads has increased, while in parallel, the cost of sequencing has decreased (van Dijk et al. 2014). During this time, the standard for sequencing has shifted from Sanger sequencing to next-generation technologies such as Roche 454 or Illumina, which incorporate sequencing by synthesis with the use of pyrosequencing and immersed beads (454) or reversible terminator, fluorescently tagged nucleotides and bridge amplification (Illumina) (van Dijk et al. 2014). For genome assembly projects in which long reads are preferred, new technologies – third generation sequencing platforms like Pacific Biosciences – now allow for the sequencing of a single molecule in real time with > 10 kb read length (Buermans & Dunnen den 2014). In parallel, the methods used to analyse the next-generation sequencing data for this purpose are also rapidly advancing (Wang et al. 2009; Buermans & Dunnen den 2014; Love et al. 2014), and in total, these technologies have enabled rapid, whole genome, transcriptome, and small RNA (sRNA) sequencing to become accessible to researchers working on any

organism. As a result, it is now possible to examine the genomes and transcriptomes of non-model systems and to use corresponding advances in bioinformatics to analyse systems-level data, including the parallel analysis of complex plant, microbial and environmental data.

Not surprisingly, the application of advanced genome sequencing technologies has enabled broader, deeper investigations towards defining the biotic and abiotic interactions and associations of plants with a multitude of beneficial and pathogenic microorganisms (reviewed in Guttman et al. 2014). In total, this body of research has revealed how plants respond to changes in environment and associated microbial populations; in parallel, they have also shed light on how plant pathogens adapt to changes in host and environment. At a fundamental level, a basic understanding of many of the processes governing the associations between plants, microbes, and the environment was gleaned through early metagenomic studies, through which genome profiling provided clues into the structure of microbial communities, and moreover, the composition and function of plant-associated community structure on basic plant physiology (i.e. the phytobiome; Hale et al. 2014). In total, further studies in this area have the potential to not only broaden our understanding of the phytobiome, but will also likely serve as a key area of focus to define the mechanisms at the intersection of ecology, microbiology and plant pathology.

The host–pathogen interaction as a paradigm for genome-scale analysis of coordinated plant and pathogen gene regulation during infection

Currently, draft genome sequences have been assembled for more than 20 plant pathogenic oomycetes (Judelson 2012), illustrating the significance of this important class of plant pathogens that are fungus-like in their nutrition and hyphal growth, yet differ in their evolutionary history and structural composition. While many of these studies have focused on the characterization of highly conserved pathogenic features (i.e. effector proteins), a number of these have revealed additional significant features of plant pathogenic oomycetes; these include, for example, the relationship(s) between genome size and host range (Pais et al. 2013), pathogen life cycle and the inheritance of virulence (Raffaele et al. 2010), and the relationship(s) amongst host range, virulence and geographic distribution (Ojiambo et al. 2015). Among these studies, many have focused on an important group of obligate plant pathogens, the downy mildews (DM). As a function of genome architecture, obligate parasites like DMs have also been shown to possess fewer genes associated with the induction of host cell death, as compared with other oomycetes, a function attributed to its requirement for living host tissue for survival (Baxter et al. 2010).

As a group, DMs represent some of the most agriculturally important pathogens of plants, capable not only of causing significant crop losses in a relatively short period of time, but also in terms of their expansive host range(s) and broad geographic distribution(s) (Ojiambo et al. 2015). As a foundation for much of the advances in the study of the DM pathogens of crop species, the molecular-genetic basis of resistance and virulence has been best characterized through the study of *Arabidopsis thaliana* and *Hyaloperonospora arabidopsidis*, an obligate pathosystem through which the genetic diversity of host and pathogen has revealed a complex interaction of several host resistance (*R*) genes and race-specific effectors from *H. arabidopsidis* (Coates & Beynon 2010; Mohr et al. 2010; Nemri et al. 2010; Krasileva et al. 2011). In addition, parallel research has characterized the contribution of broad-spectrum resistance, collectively defining the relationship(s) and genetic interactions between quantitative and qualitative trait loci within the host (Lapin et al. 2012; Gao et al. 2014). For example, research in this area has identified specific classes of transcription factors – notably, *Vv*WRKY33 and *Vv*WKRY1 – which are required for resistance to grapevine downy mildew (i.e. *Plasmopara*) (Marchive et al. 2013; Merz et al. 2014). Further examples of transcription-based resistance have also been identified against *Pseudoperonospora cubensis* in cucurbits, by which constitutive overexpression of glyoxylate aminotransferases (*At1* and *At2*) results in resistant melons (Taler et al. 2004). In addition to host genes that contribute to resistance signalling, additional genes have been associated with susceptibility, such as those from Arabidopsis mutant screens that have been shown to be required for response to *H. arabidopsidis*. Among these are 2-oxoglutarate (2OG)-Fe(II) oxygenase; DMR6 (van Damme et al. 2008, 2009), the malectin-like receptor kinase IOS1 (Hok et al. 2011, 2014), and the negative regulator of plant defence response GSL5/PMR4 (Wawrzynska et al. 2010). Collectively, these studies have demonstrated the importance of transcriptional regulation of defence and susceptibility during host–oomycete interactions.

As illustrated above, resistance signalling in plants in response to DM infection shows marked variability from host to host, and recent research suggests that the classical gene-for-gene signalling model may not fully explain, nor account for, host resistance to this important class of pathogens. For example, research using *Bremia lactucae*, the casual agent of lettuce downy mildew, has shown that resistance is mediated by a combination of *R*-gene

mediated pathways and the activity of host QTLs (Wroblewski et al. 2007; Boer den et al. 2013, 2014). Similarly, research using the *P. viticola*–grape pathosystem has shown that host resistance to infection involves a multitude of regulatory steps and complex associations, including the activity of WRKY transcription factors (Marchive et al. 2013; Merz et al. 2014) and the production of phenolic compounds (Boubakri et al. 2013), both of which point to the coordinated expression of multiple genes and processes to yield full resistance.

The Pseudoperonospora cubensis–cucumis sativus *pathosystem*

Pseudoperonospora cubensis is an obligate biotrophic oomycete pathogen, which causes cucurbit downy mildew, a foliar disease that is characterized by the development of angular lesions on the leaf surface, and by the production of sporangiophores on the abaxial surface of the leaf (Savory et al. 2010; Lebeda & Cohen 2011). First identified by Berkeley & Curtis (1868) from herbarium specimens originating in Cuba (hence, *cubensis*), *P. cubensis* was not identified on live plants until the early 1900s in Moscow by Rostovzev (Lebeda & Cohen 2011). While the taxonomy of this pathogen has varied, it is currently classified in the Kingdom Stramenopila, Phylum Oomycota, Class Oomycetes, Order Peronosporales, Family Peronosporaceae, Genus *Pseudoperonospora*, Species *cubensis* (Göker et al. 2007; Savory et al. 2010). Like other oomycetes, *P. cubensis* forms haustoria and requires a living host for survival (obligate biotrophy) (Göker et al. 2007).

In 2004, *P. cubensis* overcame host resistance in the USA (Holmes et al. 2006) and since its re-emergence, a substantial effort has been placed on the development of genetic and genomic resources (Tian et al. 2011; Adhikari et al. 2012; Savory et al. 2012a, 2012b; Burkhardt et al. 2015). Coupled with breeding and cultivar screening to define new sources of resistance (Call et al. 2012a, 2012b), significant progress has been made towards understanding the geographic and genetic diversity of this pathogen. However, while resistant cucumber lines have been identified, a full understanding of inheritance patterns still remains unclear, and the specific genes and pathways involved in cucumber resistance to *P. cubensis* have not been identified. Aiding in this research is the recent advent of next-generation sequencing, which has enabled genome, transcriptome and small RNA sequencing of several non-model hosts and pathogens, including the genomes and transcriptomes *Cucumis sativus* (cucumber) and *P. cubensis*.

In support of defining the basis of DM resistance in cucumber, two advances have significantly enhanced our understanding of the complex traits associated with cucumber DM resistance/susceptibility: the sequencing of the cucumber genome in 2009 (Huang et al. 2009), and the sequencing of the *P. cubensis* genome (Tian et al. 2011; Savory et al. 2012a). In the case of the former, the availability of plant genetic resources has enabled numerous transcriptome and SNP-based discoveries, including an analysis of the relationship between genetic variation and resistance (Huang et al. 2009). As a function of pathogen virulence and adaptation, recent studies have used a variety of genome-enabled technologies to define how the pathogen survives, and spreads, over diverse geographic regions (reviewed in Ojiambo et al. 2015). In total, these studies suggest that the pathogen spreads via wind currents from warm areas in the southern USA to northern areas during the summer months (Cohen et al. 2015), and that this movement is influenced by a suite of abiotic (e.g. solar radiation; Kanetis et al. 2010), and host factors (e.g. leaf temperature and wetness), both of which affect *P. cubensis* sporangia germination and symptom development (Neufeld & Ojiambo 2012).

The plant–pathogen transcriptome

Studies examining the induced changes in gene transcription during pathogen infection have provided a wealth of information towards defining the cellular processes associated with resistance and susceptibility. Indeed, the recent explosion in the availability of genome technologies has led to an exponential growth in the availability of plant and pathogen genome sequences, which has effectively reshaped the landscape of molecular plant pathology. In short, these advances have transformed previously inaccessible non-model systems into genetically tractable paradigms for the analysis of host–pathogen studies. In this vein, one of the next major hurdles is to define how transcriptomes are regulated, and moreover, how this regulation influences pathogen virulence, as well as host resistance and susceptibility. Numerous recent studies have investigated transcriptional changes during oomycete pathogen infection of plants using RNA-Seq-based approaches (Kunjeti et al. 2012; Chen et al. 2013; Burkhardt et al. 2015; Derevnina et al. 2015; Burkhardt & Day 2016).

Transcriptome profiling during resistance and susceptibility

As noted above, the elucidation of the temporal changes in gene expression from both plant and pathogen is an

effective first step in assigning function to the multitude of cellular processes associated with host resistance and pathogen virulence. In parallel, transcriptome-based studies have provided insight into the expressed genome of an organism, not only highlighting the precise changes in gene expression as a function of virulence, susceptibility and disease, but also assisting in the annotation of the genes themselves. Initial studies investigating transcriptome-wide changes in gene expression during pathogen infection utilized a variety of 'first generation' approaches, including large-scale sequencing of expressed sequence tags (ESTs), as was the case with the analysis of extracellular effectors from *Phytophthora infestans* (Mont. de Bary) (Torto et al. 2003). Similarly, the use of serial analysis of gene expression (SAGE)-based approaches has shed light on the expression of the potato transcriptome during *P. infestans* infection (Gyetvai et al. 2012). Likewise, the *H. arabidopsidis* transcriptome has been sequenced using a combination of whole-genome-based approaches, an early analysis of ESTs expression during Arabidopsis infection (Cabral et al. 2011), and more recently, a RNA-Seq transcriptome analysis aimed at defining the transcriptional changes during compatible or incompatible infection of an Arabidopsis host (Asai et al. 2014). In the case of the latter, the authors were able to identify a mechanism by which *H. arabidopsidis* suppresses SA-induced gene expression through the expression of a highly induced effector. This finding is exciting, as it illustrates the power of whole-transcriptome guided approaches to address fundamental questions related to the mechanisms that drive pathogen compatibility, and moreover, how resistance/susceptibility is governed in the absence of an *R*-gene mediated defence response (Asai et al. 2014).

In 2012, the first comprehensive studies to investigate the transcriptome-wide changes during an oomycete infection of a plant were published (Adhikari et al. 2012; Savory et al. 2012b). Taking advantage of the obligate nature of the host–pathogen interaction, an analysis of the simultaneous changes in cucumber tissues and *P. cubensis* described the stepwise expression of host and pathogen genes over a time-course of infection, beginning with sporangia and continuing through the onset of disease symptoms in a susceptible host. Because the sequenced samples represented a mixed population (i.e. plant + pathogen), this study permitted a stepwise evaluation of the relationship between differentially expressed genes not only at a given point in time during the infection process, but with respect to changes in the host transcriptome relative to simultaneous changes in the pathogen transcriptome. From these studies, it was demonstrated that pathogen gene expression at discrete stages of infection correlated with the expression of orthologous genes in another previously characterized plant-oomycete pathosystem (i.e. potato and *P. infestans*; Haas et al. 2009). For example, the expression of genes related to virulence and pathogenicity (e.g. proteases and lipases) in *P. cubensis* correlated with previously described expression patterns in other host–pathogen interactions (Zuluaga et al. 2016). In addition, the expression of effector genes in *P. cubensis* were shown to have a similar expression pattern in cucumber tissues (i.e. peak *c*. 4 days post inoculation (dpi)) as those previously described from *P. infestans* (Baxter et al. 2010; Stassen & Van den Ackerveken 2011).

As a follow-up to the initial transcriptome analysis of the *P. cubensis*–cucumber interaction, a recent comparative analysis of a time-course of infection between a susceptible cultivar (i.e. 'Vlaspik') and resistant genotype (i.e. plant introduction (PI) 197088) interaction was conducted to identify genome-wide patterns of coordinately regulated host genes during resistance and susceptibility (Burkhardt & Day 2016). In brief, using DESeq and weighted genome correlation network analysis (WGCNA), it was shown that a resistant cucumber line had an earlier response to the pathogen, with initial transcriptional changes in signalling defence hormone responses (e.g. salicylic acid and jasmonic acid) being the most identifiable. Additionally, using a comparative approach to previous studies from other plant–oomycete interactions, a suite of differentially expressed transcription factors were identified; interestingly, at least one of these was located within a QTL that is involved in downy mildew resistance (Gao et al. 2014; Yoshioka et al. 2014).

Genome plasticity: alternative splicing

Given that many sequenced transcriptomes now exist for oomycete associations with their host plants, one of the next steps forward in this area is to elucidate how changes in the transcriptome are regulated, and moreover, what are the implications of these changes on pathogen adaptation and virulence. One example of a regulatory mechanism controlling gene expression that can now be analysed on a genome-wide scale is that of alternative splicing, a largely understudied process in host–pathogen interactions, especially in non-model systems. As a key regulatory process underpinning both genome plasticity and the regulation of gene expression, alternative splicing is a process in which pre-mRNA transcripts, typically transcribed from DNA by RNA polymerase II, are processed into multiple isoforms to include different combinations of introns and exons via a mechanism mediated by a complex of proteins and RNAs called the spliceosome

(Nilsen & Graveley 2010; Kelemen et al. 2013). In short, this form of transcript processing can lead to an expansion in diversity in the transcriptome, and potentially the proteome, using four main splicing mechanisms: (1) intron retention, (2) exon skipping, (3) alternative 3′ splice site selection, and (4) alternative 5′ splice site selection (Nilsen & Graveley 2010). Best characterized in humans, alternative splicing has been observed in transcripts across all kingdoms, including animals, fungi, protists and plants (McGuire et al. 2008). In general, fungi and protists (including the oomycetes *P. infestans* and *P. sojae*) were far more likely to use intron retention as an alternative splicing mechanism compared with other types of splicing mechanisms (McGuire et al. 2008).

To date, few comprehensive surveys have investigated the breadth, type and/or impact of alternative splicing on the secretome of plant pathogenic oomycetes. Additionally, and as a function of pathogen virulence, none have specifically investigated the activity and consequences of alternative splicing of pathogen effector genes, nor the role of this process on the pathogen association with its host. The first discovery of alternative splicing in a plant pathogenic oomycete was reported for a family of 5-endoglucanases in the genus *Phytophthora* (Costanzo et al. 2007). Subsequent research, using a genome-wide survey of alternative splicing based on EST data from *P. infestans* and *P. sojae*, identified 4762 and 2125 spliced transcripts, respectively, with a majority of the splice variants being retained introns (McGuire et al. 2008). A subsequent paper by Shen and colleagues, focusing on *P. sojae* transcription via ESTs, identified 4013 introns in the genome sequence, identifying 122 genes that were alternatively spliced (Shen et al. 2011). As a mechanism for regulating gene expression in downy mildews, alternative splicing was first fortuitously identified in *P. cubensis* following sequencing of its transcriptome (Savory et al. 2012b). As an initial investigation to survey the pathogen secretome, including putative effector genes, this research classified the constellation of secreted effector proteins based on their temporal patterns of expression during discrete stages of infection. Of particular interest was the observation that a full-length gene predicted to be a non-effector multi-drug transporter could be alternatively spliced (e.g. intron retention) to yield a functional effector whose expression pattern correlated with pathogen infection and the onset of disease in the host. Using this as a foundation, more recent research has examined alternative splicing in *P. cubensis* using a transcriptome-wide approach, ultimately defining how splicing within the pathogen genome changes over the course of infection (Burkhardt et al. 2015). Through this analysis, the *P. cubensis* genome was also reannotated, and approximately 4000 additional genes were predicted. Alternative splicing on a transcriptome-wide scale was shown to be more prevalent than previously thought. In total, approximately 24% of the expressed genome and 55% of intron-containing genes had evidence for alternative splicing, which is in contrast to previous research in oomycetes, yet is consistent with more recent research using RNA-Seq (McGuire et al. 2008; Shen et al. 2011; Burkhardt et al. 2015). In addition, the most frequent mechanism of alternative splicing was intron retention, which is consistent with previous research in oomycetes and plants (McGuire et al. 2008; Burkhardt et al. 2015), with the mechanisms and frequency of alternative splicing in *P. cubensis* being more similar to that found in plants. This is consistent with the placement of oomycetes closer to plants than to fungi on the evolutionary tree (Simpson & Roger 2004; Burki et al. 2012; Burkhardt et al. 2015).

The role of miRNAs during oomycete–plant interactions

As a final illustration of the regulatory changes in the host and pathogen transcriptomes during infection, two recent studies have re-shaped our understanding of how plant pathogens manipulate their host to cause disease, effectively raising the bar on mainstream transcriptome-based approaches aimed at defining how plants and pathogens interact. In short, these studies have demonstrated that small RNAs (sRNAs) play a key role in simultaneously regulating the host and pathogen transcriptomes during infection (Qiao et al. 2013; Weiberg et al. 2013), and moreover, serve as an example of a novel virulence mechanism. At a fundamental level, these studies have demonstrated that the sRNA regulatory network represents an essential component of plant defence signalling – one that is actively targeted by pathogens during infection. Weiberg *et al*. showed that the plant fungal pathogen *Botrytis cinerea* delivers sRNAs into the host, where they function to silence host defence-associated transcripts during infection (Weiberg et al. 2013). In a second study, it was demonstrated that oomycete pathogens – specifically, *P. infestans* – have evolved secreted effectors that are delivered into the host where they silence sRNA biogenesis, resulting in the onset of increased susceptibility and disease (Qiao et al. 2013). As a catalyst for further research in this area, a number of questions remain: How are sRNAs moved between the host and pathogen? What is the role of host sRNAs in modulating the timing and amplitude of the defence response?

To specifically address these questions, and moreover, to define the role of sRNA signalling during plant–oomycete interactions, there have been several recent studies describing the identification of sRNAs during oomycete infection of plants. In crop species, several studies have identified and characterized sRNAs, yet none have done so in the presence of a pathogen during a resistant interaction. Among these studies, Martínez et al. (2011) identified 19 conserved families of micro RNAs (miRNAs) in cucumber and identified seven new miRNAs. In a novel approach, Mao et al. (2012) combined small RNA sequencing with degradome sequencing in cucumber tissues to identify a total of 64 miRNA and to validate 21 of their targets, which included genes involved in plant growth and development, signal transduction, and transcriptional regulation. In a study among five different species of cucurbit, Hu et al. (2014) identified 220 predicted miRNA among all the Cucurbitaceae species studied and identified 41 within *C. sativus*. A grafting-based study identified 48 new miRNA in pumpkin (*Cucurbita moschata*) and cucumber and identified their targets (Li et al. 2014). Interestingly, they also determined that a miRNA could silence genes in tissue that was grafted onto the tissue expressing the miRNA, which could lead to potential applications in silencing cucumber genes for genetic studies.

An additional layer of transcript expression/availability regulation during the interaction between plants and pathogens occurs at the miRNA level, through which miRNAs target mRNAs within the same, or perhaps opposing, organism (Axtell 2013; Weiberg et al. 2013). Within cucumber tissues, several studies have previously identified miRNA and their corresponding targets when plants were not under biotic stress conditions (Martínez et al. 2011; Mao et al. 2012; Li et al. 2014). In a recent study, miRNAs expressed during *P. cubensis* infection were identified, with a few targets confirmed that are uniquely regulated during biotic stress (Jin & Wu 2015). In parallel to the recent RNA-Seq analysis of a resistant and susceptible interaction between cucumber and *P. cubensis* (Burkhardt & Day 2016), inoculated samples from susceptible ('Vlaspik') and resistant (PI 197088) cucumber leaves were sequenced for the identification and prediction of novel cucumber and *P. cubensis* miRNAs. In total, this recent study represents the deepest sequencing of cucumber and/or *P. cubensis* sRNAs to date, and has led to the prediction that both cucumber and *P. cubensis* miRNAs have targets within the host genome.

Beyond the genome: new frontiers employing proteomics and metabolomics

The next decade of research in the field of plant–pathogen interactions offers much, not only in terms of further defining the processes underpinning resistance and susceptibility, but also in terms of the potential to harness these processes to generate crops that are resistant to a suite of biotic and abiotic pressures. In addition to the wealth of existing transcriptome data describing the transcriptional changes occurring during pathogen infection of plants, a relatively untapped area is defining the relationship(s) between these changes and the function and activity of the plant–pathogen proteome and metabolome (Feussner & Polle 2015). We posit that insight into these relationships will likely indicate the outcome of transcriptional changes, providing an understanding of the cellular environment within which plants and pathogens interact. Indeed, recent research using a compatible and an incompatible interaction between *P. infestans* and potato showed that approximately half of the differentially abundant apoplastic proteins had a corresponding change in transcript levels, providing supporting evidence for the hypothesis that additional factors, other than transcript levels, affect protein accumulation (Ali et al. 2014). In addition, proteomics-based approaches will also shed light on the functionality of the proteome through an analysis of the role, and activity, of post-translational modifications (Melo-Braga et al. 2012; Hemsley et al. 2013). Additionally, proteomics-based approaches will also enable an understanding of the spatial dynamics during host–pathogen interactions, such as by identification and quantification of proteins from unique locations within the plant, like those in the apoplast, an interaction interface between fungal and oomycete effectors and the plant defence system (Delaunois et al. 2014).

In parallel to proteome-based approaches, an analysis of the plant metabolome will also serve to define the complexity and regulation of the processes underlying plant–pathogen interactions. For example, early research in this area has led to an understanding of the metabolic processes that are uniquely or differentially induced during host resistance, as well as those produced by pathogens to facilitate nutrient acquisition during susceptibility (Sumner et al. 2003). More recent research, coupling transcriptomic and metabolomic approaches has illuminated our understanding of the mechanisms shaping the immune response in wheat to the hemibiotrophic fungal pathogen *Zymoseptoria tritici* (Rudd et al. 2015). Similar

approaches have also been employed to identify possible mechanisms of resistance in wheat during *Fusarium graminearum* infection (Gunnaiah & Kushalappa 2014), and resistance signalling in response to *Verticillium longisporum* infection in Arabidopsis (König et al. 2014). In the future, metabolomics could focus on identifying changes in phytoalexins and uncovering a more precise role of these secondary metabolites during pathogen infection (reviewed in Hammerschmidt 1999).

Prospects for the future: from lab to field

Over the past decade, much progress has been made towards defining the regulation and function of the host and pathogen processes that determine how plants and pathogens interact. In large part, these advances have been made through the application of next-generation sequencing technologies, which have effectively transformed most, if not all, pathosystems into model systems. As an example of this, the re-emergence of cucumber DM in the USA has been met with significant progress, focused both on identifying sources of host resistance through breeding, as well on the development of genomic and transcriptomic datasets that can be used to identify potential mechanisms of resistance and pathogen virulence. Additional studies on existing and re-emerging diseases of plants – worldwide – will likely serve to not only address the biological processes underpinning these interactions, but will also drive new discoveries in genome-related areas, including technology and data analysis development. These can lead to a better understanding and development of pathogen-resistant cultivars to major diseases of economic importance.

References

Adhikari BN, Savory EA, Vaillancourt B, Childs KL, Hamilton JP, Day B, Buell CR. 2012. Expression profiling of *Cucumis sativus* in response to infection by *Pseudoperonospora cubensis*. PLoS ONE. 7:e34954.

Ali A, Alexandersson E, Sandin M, Resj S, Lenman M, Hedley P, Levander F, Andreasson E. 2014. Quantitative proteomics and transcriptomics of potato in response to *Phytophthora infestans* incompatible and incompatible interactions. BMC Gen. 15:1–18.

Asai S, Rallapalli G, Piquerez SJM, Caillaud M-C, Furzer OJ, Ishaque N, Wirthmueller L, Fabro G, Shirasu K, Jones JDG. 2014. Expression profiling during Arabidopsis/downy mildew interaction reveals a highly-expressed effector that attenuates responses to salicylic acid. PLoS Pathog. 10:e1004443.

Axtell MJ. 2013. Classification and comparison of small RNAs from plants. Annu Rev Plant Biol. 64:137–159.

Baxter L, Tripathy S, Ishaque N, Boot N, Cabral A, Kemen E, Thines M, Ah-Fong A, Anderson R, Badejoko W, et al. 2010. Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsidis* genome. Science. 330:1549–1551.

Berkeley MJ, Curtis MA. 1868. Peronospora cubensis. Bot J Linn Soc. 10:363.

Boer den E, Pelgrom KTB, Zhang NW, Visser RGF, Niks RE, Jeuken MJW. 2014. Effects of stacked quantitative resistances to downy mildew in lettuce do not simply add up. Theor Appl Genet. 127:1805–1816.

Boer den E, Zhang NW, Pelgrom K, Visser RGF, Niks RE, Jeuken MJW. 2013. Fine mapping quantitative resistances to downy mildew in lettuce revealed multiple sub-QTLs with plant stage dependent effects reducing or even promoting the infection. Theor Appl Genet. 126:2995–3007.

Boubakri H, Poutaraud A, Wahab MA, Clayeux C, Baltenweck-Guyot R, Steyer D, Marcic C, Mliki A, Soustre-Gacougnolle I. 2013. Thiamine modulates metabolism of the phenylpropanoid pathway leading to enhanced resistance to *Plasmopara viticola* in grapevine. BMC Plant Biol. 13:31.

Buermans HPJ, Dunnen den JT. 2014. Biochimica et Biophysica Acta. BBA - Mol Basis Disease. 1842:1932–1941.

Burkhardt A, Buchanan A, Cumbie JS, Savory EA, Chang JH, Day B. 2015. Alternative splicing in the obligate biotrophic oomycete pathogen *Pseudoperonospora cubensis*. Mol Plant Microbe Interact. 28:298–309.

Burkhardt A, Day B. 2016. Transcriptome and Small RNAome Dynamics during a Resistant and Susceptible Interaction between Cucumber and Downy Mildew. Plant Genome. 9:1–19.

Burki F, Okamoto N, Pombert J-F, Keeling PJ. 2012. The evolutionary history of haptophytes and cryptophytes: phylogenomic evidence for separate origins. Proc Biol Sci. 279:2246–2254.

Cabral A, Stassen JHM, Seidl MF, Bautor J, Parker JE, Van den Ackerveken G. 2011. Identification of *Hyaloperonospora arabidopsidis* transcript sequences expressed during infection reveals isolate-specific effectors. PLoS ONE. 6:e19328.

Call AD, Criswell AD, Wehner TC, Ando K, Grumet R. 2012a. Resistance of cucumber cultivars to a new strain of cucurbit downy mildew. HortScience. 47:171–178.

Call AD, Criswell AD, Wehner TC, Klosinska U, Kozik EU. 2012b. Screening cucumber for resistance to downy mildew caused by (Berk. and Curt.). Rostov Crop Sci. 0:1.

Chen XR, Xing YP, Li YP, Tong YH, Xu JY. 2013. RNA-Seq reveals infection-related gene expression changes in *Phytophthora capsici*. PLoS ONE. 8:e74588.

Chisholm S, Coaker G, Day B, Staskawicz BJ. 2006. Host-microbe interactions: shaping the evolution of the plant immune response. Cell. 124:803–814.

Coates ME, Beynon JL. 2010. *Hyaloperonospora arabidopsidis* as a pathogen model. Annu Rev Phytopathol. 48:329–345.

Cohen Y, Van den Langenberg KM, Wehner TC, Ojiambo PS, Hausbeck M, Quesada-Ocampo LM, Lebeda A, Sierotzki H, Gisi U. 2015. Resurgence of *Pseudoperonospora cubensis*: the causal agent of cucurbit downy mildew. Phytopathology. 105:998–1012.

Costanzo S, Ospina-Giraldo MD, Deahl KL, Baker CJ, Jones RW. 2007. Alternate intron processing of family 5 endoglucanase transcripts from the genus *Phytophthora*. Curr Genet. 52:115–123.

Dangl JL, Horvath DM, Staskawicz BJ. 2013. Pivoting the plant immune system from dissection to deployment. Science. 341:746–751.

Dangl JL, Jones JDG. 2001. Plant pathogens and integrated defence responses to infection. Nature. 411:826–833.

Delaunois B, Jeandet P, Clément C, Baillieul F, Dorey S, Cordelier S. 2014. Uncovering plant-pathogen crosstalk through apoplastic proteomic studies. Front Plant Sci. 5:1–18.

Derevnina L, Reyes-Chin Wo S, Martin F, Wood K, Froenicke L, Spring O, Michelmore RW. 2015. Genome sequence and architecture of the tobacco downy mildew pathogen, *Peronospora tabacina*. Mol Plant Microbe Interact. 28:1198–1215.

Feussner I, Polle A. 2015. What the transcriptome does not tell - proteomics and metabolomics are closer to the plants' patho-phenotype. Curr Opin Plant Biol. 26:26–31.

Flor HH. 1955. Host-parasite interaction in flax rust - its genetics and other implications. Phytopathology. 45:680–685.

Gao D, Appiano M, Huibers RP, Chen X, Loonen AE, Visser RG, Wolters AM, Bai Y. 2014. Activation tagging of ATHB13 in *Arabidopsis thaliana* confers broad-spectrum disease resistance. Plant Mol Biol. 86:641–653.

Göker M, Voglmayr H, Riethmüller A, Oberwinkler F. 2007. How do obligate parasites evolve? A multi-gene phylogenetic analysis of downy mildews. Fun Gen Biol. 44:105–122.

Gunnaiah R, Kushalappa AC. 2014. Metabolomics deciphers the host resistance mechanisms in wheat cultivar Sumai-3, against trichothecene producing and non-producing isolates of *Fusarium graminearum*. Plant Physiol Biochem. 83:40–50.

Guttman DS, McHardy AC, Schulze-Lefert P. 2014. Microbial genome-enabled insights into plant-microorganism interactions. Nat Rev Genet. 15:797–813.

Gyetvai G, Sonderkaer M, Gobel U, Basekow R, Ballvora A, Imhoff M, Kersetn B, Nielsen K-L, Gebhardt C. 2012. The transcriptome of compatible and incompatible interactions of potato (*Solanum tuberosum*) with *Phytophthora infestans* revealed by DeepSAGE analysis. PLoS ONE. doi:10.1371/journal.pone.0031526

Haas BJ, Kamoun S, Zody MC, Jiang RH, Handsaker RE, Cano LM, Grabherr M, Kodira CD, Raffaele S, Torto-Alalibo T, et al. 2009. Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. Nature. 461:393–398.

Hale IL, Broders K, Iriarte G. 2014. A Vavilovian approach to discovering crop-associated microbes with potential to enhance plant immunity. Front Plant Sci. 5:492.

Hammerschmidt R. 1999. Phytoalexins: what have we learned after 60 years? Annu Rev Phytopathol. 37:285–306.

Hemsley PA, Weimar T, Lilley KS, Dupree P, Grierson CS. 2013. A proteomic approach identifies many novel palmitoylated proteins in Arabidopsis. New Phytol. 197:805–814.

Hok S, Allasia V, Andrio E, Naessens E, Ribes E, Panabieres F, Attard A, Ris N, Clement M, Barlet X, et al. 2014. The receptor kinase IMPAIRED OOMYCETE SUSCEPTIBILITY1 attenuates abscisic acid responses in Arabidopsis. Plant Physiol. 166:1506–1518.

Hok S, Danchin EG, Allasia V, Panabieres F, Attard A, Keller H. 2011. An Arabidopsis (malectin-like) leucine-rich repeat receptor-like kinase contributes to downy mildew disease. Plant Cell Environ. 34:1944–1957.

Holmes GJ, Wehner TC, Thornton A. 2006. An old enemy re-emerges. Disease Manag. 2:14–15.

Hu J, Sun L, Zhu Z, Zheng Y, Xiong W, Ding Y. 2014. Characterization of conserved microRNAs from five different cucurbit species using computational and experimental analysis. Biochimie. 102:137–144.

Huang S, Li R, Zhang Z, Li L, Gu X, Fan W, Lucas WJ, Wang X, Xie B, Ni Y, et al. 2009. The genome of the cucumber, *Cucumis sativus* L. Nature. 41:1275–1281.

Jin W, Wu F. 2015. Identification and characterization of cucumber microRNAs in response to *Pseudoperonospora cubensis* infection. Gene. 569:225–232.

Jones J, Dangl JL. 2006. The plant immune system. Nature. 444:323–329.

Judelson HS. 2012. Dynamics and innovations within oomycete genomes: insights into biology, pathology, and evolution. Eukaryot Cell. 11:1304–1312.

Kanetis L, Holmes GJ, Ojiambo PS. 2010. Survival of *Pseudoperonospora cubensis* sporangia exposed to solar radiation. Plant Pathol. 59:313–323.

Kelemen O, Convertini P, Zhang Z, Wen Y, Shen M, Falaleeva M, Stamm S. 2013. Function of alternative splicing. Gene. 514:1–30.

König S, Feussner K, Kaever A, Landesfeind M, Thurow C, Karlovsky P, Gatz C, Polle A, Feussner I. 2014. Soluble phenylpropanoids are involved in the defense response of Arabidopsis against *Verticillium longisporum*. New Phytol. 202:823–837.

Krasileva KV, Zheng C, Leonelli L, Goritschnig S, Dahlbeck D, Staskawicz BJ. 2011. Global analysis of Arabidopsis/downy mildew interactions reveals prevalence of incomplete resistance and rapid evolution of pathogen recognition. PLoS ONE. 6:e28765.

Kunjeti SG, Evans TA, Marsh AG, Gregory NF, Kunjeti S, Meyers BC, Kalavacharia VS, Donofrio NM. 2012. RNA-Seq reveals infection-related global gene changes in *Phytophthora phaseoli*, the causal agent of lima bean downy mildew. Mol Plant Pathol. 13:454–466.

Lapin D, Meyer RC, Takahashi H, Bechtold U, Van den Ackerveken G. 2012. Broad-spectrum resistance of Arabidopsis C24 to downy mildew is mediated by different combinations of isolate-specific loci. New Phytol. 196:1171–1181.

Lebeda A, Cohen Y. 2011. Cucurbit downy mildew (*Pseudoperonospora cubensis*)—biology, ecology, epidemiology, host-pathogen interaction and control. Eur J Plant Pathol. 129:157–192.

Li C, Li Y, Bai L, Zhang T, He C, Yan Y, Yu X. 2014. Grafting-responsive miRNAs in cucumber and pumpkin seedlings identified by high-throughput sequencing at whole genome level. Physiol Plant. 151:406–422.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. Genome Biol. 15:550.

Macho AP, Zipfel C. 2015. Targeting of plant pattern recognition receptor-triggered immunity by bacterial type-III secretion system effectors. Curr Opin Microbiol. 23:14–22.

Mao W, Li Z, Xia X, Li Y, Yu J. 2012. A combined approach of high-throughput sequencing and degradome analysis reveals tissue specific expression of microRNAs and their targets in cucumber. PLoS ONE. 7:e33040.

Marchive C, Léon C, Kappel C, Coutos-Thévenot P, Corio-Costet M-F, Delrot S, Lauvergeat V. 2013. Over-expression of *Vv*WRKY1 in grapevines induces expression of jasmonic acid pathway-related genes and confers higher tolerance to the downy mildew. PLoS ONE. 8:e54185.

Martínez G, Forment J, Llave C, Pallás V, Gómez G. 2011. High-throughput sequencing, characterization and detection of new and conserved cucumber miRNAs. PLoS ONE. 6:e19523.

McGuire AM, Pearson MD, Neafsey DE, Galagan JE. 2008. Cross-kingdom patterns of alternative splicing and splice recognition. Genome Biol. 9:R50.

Melo-Braga MN, Verano-Braga T, León IR, Antonacci D, Nogueira FCS, Thelen JJ, Larsen MR, Palmisano G. 2012. Modulation of protein phosphorylation, N-glycosylation and Lys-acetylation in grape (*Vitis vinifera*) mesocarp and exocarp owing to *Lobesia botrana* infection. Mol Cell Proteom. 11:945–956.

Merz PR, Moser T, Höll J, Kortekamp A, Buchholz G, Zyprian E, Bogs J. 2014. The transcription factor VvWRKY33 is involved in the regulation of grapevine (*Vitis vinifera*) defense against the oomycete pathogen *Plasmopara viticola*. Physiol Plant. 153:365–380.

Mohr TJ, Mammarella ND, Hoff T, Woffenden BJ, Jelesko JG, McDowell JM. 2010. The Arabidopsis downy mildew resistance gene *RPP8* is induced by pathogens and salicylic acid and is regulated by W box cis elements. Mol Plant-Microbe Interact. 23:1303–1315.

Nemri A, Atwell S, Tarone AM, Huang YS, Zhao K, Studholme DJ, Nordborg M, Jones JDG. 2010. Genome-wide survey of Arabidopsis natural variation in downy mildew resistance using combined association and linkage mapping. Proc Nat Acad Sci. USA. 107:10302–10307.

Neufeld KN, Ojiambo PS. 2012. Interactive effects of temperature and leaf wetness duration on sporangia germination and infection of cucurbit hosts by *Pseudoperonospora cubensis*. Plant Dis. 96:345–353.

Nilsen TW, Graveley BR. 2010. Expansion of the eukaryotic proteome by alternative splicing. Nature. 463:457–463.

Ojiambo PS, Gent DH, Quesada-Ocampo LM, Hausbeck MK, Holmes GJ. 2015. Epidemiology and population biology of *Pseudoperonospora cubensis*: a model system for management of downy mildews. Annu Rev Phytopathol. 53:223–246.

Pais M, Win J, Yoshida K, Etherington GJ, Cano LM, Raffaele S, Banfield MJ, Jones A, Kamoun S, Saunders DG. 2013. From pathogen genomes to host plant processes: the power of plant parasitic oomycetes. Genome Biol. 14:211.

Qiao Y, Liu L, Xiong Q, Flores C, Wong J, Shi J, Wang X, Liu X, Xiang Q, Jiang S, et al. 2013. Oomycete pathogens encode RNA silencing suppressors. Nature. 45:330–333.

Raffaele S, Win J, Cano LM, Kamoun S. 2010. Analyses of genome architecture and gene expression reveal novel candidate virulence factors in the secretome of *Phytophthora infestans*. BMC Genom. 11:637.

Rudd JJ, Kanyuka K, Hassani-Pak K, Derbyshire M, Andongabo A, Devonshire J, Lysenko A, Saqi M, Desai NM, Powers SJ, et al. 2015. Transcriptome and metabolite profiling of the infection cycle of *Zymoseptoria tritici*on wheat reveals a biphasic interaction with plant immunity involving differential pathogen chromosomal contributions and a variation on the hemibiotrophic lifestyle definition. Plant Physiol. 167:1158–1185.

Savory EA, Adhikari BN, Hamilton JP, Vaillancourt B, Buell CR, Day B. 2012a. mRNA-Seq analysis of the *Pseudoperonospora cubensis* transcriptome during cucumber (*Cucumis sativus* L.) infection. PLoS ONE. 7:e35796.

Savory EA, Granke LL, Quesada-Ocampo LM, Varbanova M, Hausbeck MK, Day B. 2010. The cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Mol Plant Pathol. 12:217–226.

Savory EA, Zou C, Adhikari BN, Hamilton JP, Buell CR, Shiu S-H, Day B. 2012b. Alternative splicing of a multi-drug transporter from *Pseudoperonospora cubensis* generates an RXLR effector protein that elicits a rapid cell death. PLoS ONE. 7:e34701.

Shen D, Ye W, Dong S, Wang Y, Dou D. 2011. Characterization of intronic structures and alternative splicing in *Phytophthora sojae* by comparative analysis of expressed sequence tags and genomic sequences. Can J Microbiol. 57:84–90.

Simko I, Atallah AJ, Ochoa OE, Antonise R, Galeano CH, Truco MJ, Michelmore RW. 2013. Identification of QTLs conferring resistance to downy mildew in legacy cultivars of lettuce. Sci Rep. 3:2875.

Simpson AGB, Roger AJ. 2004. The real "kingdoms" of eukaryotes. Curr Biol. 14:R693–R696.

Stassen JHM, Van den Ackerveken G. 2011. How do oomycete effectors interfere with plant life? Curr Opin Plant Biol. 14:407–414.

Sumner LW, Mendes P, Dixon RA. 2003. Plant metabolomics: Large-scale phytochemistry in the functional genomics era. Phytochemistry. 62:817–836.

Taler D, Galperin M, Benjamin I, Cohen Y, Kenigsbuch D. 2004. Plant eR genes that encode photorespiratory enzymes confer resistance against disease. Plant Cell. 16:172–184.

Tian M, Win J, Savory E, Burkhardt A, Held M, Brandizzi F, Day B. 2011. 454 Genome sequencing of *Pseudoperonospora cubensis* reveals effector proteins with a QXLR translocation motif. Mol Plant-Microbe Interact. 24:543–553.

Torto TA, Li S, Styer A, Huitema E, Testa A, Gow NAR, van West P, Kamoun S. 2003. EST mining and functional expression assays identiy extracellular effector proteins from the plant pathogen *Phytophthora*. Gen Res. 13:1675–1685.

van Damme M, Huibers RP, Elberse J, Van den Ackerveken G. 2008. Arabidopsis DMR6 encodes a putative 2OG-Fe(II) oxygenase that is defense-associated but required for susceptibility to downy mildew. Plant J. 54:785–793.

van Damme M, Zeilmaker T, Elberse J, Andel A, de Sain-van der Velden M, Van den Ackerveken G. 2009. Downy mildew resistance in Arabidopsis by mutation of homoserine kinase. Plant Cell. 21:2179–2189.

van Dijk EL, Auger H, Jaszczyszyn Y, Thermes C. 2014. Ten years of next-generation sequencing technology. Trends Gen. 30:418–426.

Wang Z, Gerstein M, Snyder M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 10:57–63.

Wawrzynska A, Rodibaugh NL, Innes RW. 2010. Synergistic activation of defense responses in *Arabidopsis* by simultaneous loss of the GSL5 callose synthase and the EDR1 protein kinase. Mol Plant-Microbe Interact. 23:578–584.

Weiberg A, Wang M, Lin F-M, Zhao H, Zhang Z, Kaloshian I, Huang H-D, Jin H. 2013. Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science. 342:118–123.

Wroblewski T, Piskurewicz U, Tomczak A, Ochoa O, Michelmore RW. 2007. Silencing of the major family of NBS-LRR-encoding genes in lettuce results in the loss of multiple resistance specificities. Plant J. 51:803–818.

Yoshioka Y, Sakata Y, Sugiyama M, Fukino N. 2014. Identification of quantitative trait loci for downy mildew resistance in cucumber (*Cucumis sativus* L.). Euphytica. 198:265–276.

Zhang SP, Liu MM, Miao H, Zhang SQ, Yang YH, Xie BY, Wehner TC, Gu XF. 2013. Chromosomal mapping and QTL analysis of resistance to downy mildew in *Cucumis sativus*. Plant Dis. 97:245–251.

Zuluaga AP, Vega-Arreguin JC, Fei Z, Ponnala L, Lee SJ, Matas AJ, Patev S, Fry WE, Rose JK. 2016. Transcriptional dynamics of *Phytophthora infestans* during sequential stages of hemibiotrophic infection of tomato. Mol Plant Pathol. 17:29–41.