Translational biology of nematode effectors. Or, to put it another way, functional analysis of effectors – what's the point?

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Summary: There has been a huge amount of work put into identifying and characterising effectors from plant-parasitic nematodes in recent years. Although this work has provided insights into the mechanisms by which nematodes can infect plants, the potential translational outputs of much of this research are not always clear. This short article will summarise how developments in effector biology have allowed, or will allow, new control strategies to be developed, drawing on examples from nematology and from other pathosystems.

Keywords: effector, plant-parasitic nematode, translational biology

Biotrophic parasitism of plants is a strategy used by a wide taxonomic range of organisms, including nematodes, insects, fungi, oomycetes and bacteria, and there are considerable parallels between many aspects of the life cycles of these organisms. Each needs to overcome the physical and chemical barriers that plants use to protect themselves and many will need to overcome or suppress induced plant defence responses. All will need to extract the nutrients required for development from their chosen hosts and many, in doing so, will induce cellular or physiological modifications that improve the nutritional qualities of the host. For example, cyst nematodes exploit plant sugars as a food source (Danchin *et al.*, 2016) and sucrose transporters are upregulated during the early stages of the interaction between cyst nematodes and their hosts, allowing food to be transported into the developing syncytium (Hoffman *et al.,* 2007). Similarly, several *SWEET* genes encoding putative sugar transporters are induced in rice in response to the pathogenic bacteria *Xanthomonas oryzae* pv. *oryzae*, that can support bacterial development (Streubel *et al.*, 2013). A more direct example of a pathogen modifying the host comes from the fungal pathogen *Ustilago mays,* which introduces a chorismate mutase into the cells of its host during infection that adjusts the metabolic status of the host through metabolic priming (Djamei *et al.,* 2011).

At a molecular level, the interactions of plant pathogens with their hosts are mediated largely by effectors. There are many different definitions of "effectors" used in different branches of plant pathology. For the purposes of this article we use the relatively broad definition suggested by Bird *et al.,* (2014): *"Any pathogen molecule that suppresses host defences or manipulates the host to allow provision of food to the pathogen"*. Effectors are secreted from the pathogen into their hosts and in plant-parasitic nematodes they arise mainly from the subventral and dorsal pharyngeal gland cells, from where they are secreted into the host through the stylet. Effectors may also originate from other tissues and it has recently been shown that some effectors are secreted from the amphids into the apoplast surrounding the feeding nematode (Eves van den Akker *et al.,* 2014a). Changes in the morphology of the pharyngeal gland cells during development of cyst and root-knot nematodes suggest that the products of the subventral gland cells (which are large and active in the invasive second stage juvenile) are likely to be important at the early stages of the host-parasite interaction while those of the dorsal gland cell (which grows and becomes more active during the parasitic stages) are likely to be more important during the parasitic process (Hussey & Mimms, 1990).

Effectors play a central role in the biology of plant-parasitic nematodes and consequently a great deal of effort has been put into identifying the genes encoding these proteins. Several detailed reviews on this topic have been published recently (*e.g.* Goverse & Smant, 2014; Mitchum *et al.,* 2013; Haegeman *et al.,* 2012); here we provide only the briefest of outlines and recommend these

reviews for further details. The most productive strategy for identification of effectors has been analysis of transcriptome (or Expressed Sequence Tag – EST) sequences, either from whole nematodes or from aspirated gland cell contents (Noon *et al.*, 2015; Maier *et al.*, 2013; Gao *et al.*, 2003; Huang *et al.,* 2003). This analysis has been used for identification of effectors from sedentary endoparasites including cyst and root-knot nematodes (*e.g.* Jones *et al.,* 2009; Petitot *et al.*, 2016), *Nacobbus aberrans* (Eves van den Akker *et al.,* 2014b) and *Rotylenchulus reniformis* (Wubben *et al.,* 2010), as well as for a variety of migratory endoparasites (Kikuchi *et al.,* 2007; Haegeman *et al.,* 2009; Haegeman *et al.,* 2011). More recently, genome sequences of plant parasitic nematodes have become available and are now being analysed for the presence of effectors (Danchin *et al.*, 2013; Thorpe *et al.,* 2014, Eves van den Akker *et al.,* 2016). In all cases, effectors are first identified by a combination of bioinformatic analyses. These candidate genes are validated by functional assays such as (*i*) confirmation that the genes are expressed in secretory organs (most often the gland cells) from which they can be delivered into the host and (*ii*) evaluation of their contribution to fitness of the pathogen by silencing in the nematode. High-throughput and relatively low cost sequencing approaches mean that these types of approaches are now being applied to a far wider range of nematodes than was previously feasible; this is clearly illustrated by the development of the 959 nematode genomes initiative project (Kumar *et al.*, 2012; http://www.nematodes.org/nematodegenomes). Furthermore, new tools are being developed that allow more focused sequencing targeted specifically at effectors that will make effector identification even more accessible. For example, a method for isolation of gland cells and sequencing of amplified RNA has been described that has been used for identification of effectors in three plant-parasitic nematodes (Maier *et al.,* 2013). In addition, capture array protocols have been described that allow specific subclasses of genes to be enriched from a genome prior to sequencing, allowing sequence depth to be focused on genes of interest while ignoring other parts of the genome. This approach has allowed, for example, sequencing of the full resistance gene complement of potato, tomato and wheat (Jupe *et al.,* 2013; Andolfo *et al.*, 2014; Steuernagel *et al.*, 2016) and is currently being applied to capture and sequencing of effectors. This approach is likely to be of particular value for comparative analysis of relatively closely related populations of a nematode species for which the effector complement is known, as design of the probes for the capture array requires prior knowledge of the sequence types that are being sought.

The outputs of these projects have led to the identification of a wide range of effectors from a variety of economically important plant-parasitic nematode species. This has enabled elegant functional studies that have demonstrated the importance of effectors in several aspects of the biology of plant-parasitic nematodes. For example, effectors have been identified that suppress host

defence responses (Jaouannet *et al.,* 2012; Postma *et al.,* 2012; Lozano-Torres *et al.*, 2014; Niu *et al.*, 2016) while others have been shown to interact with auxin transporter proteins (Lee *et al.,* 2011) or exhibit transcriptional activation activity in their host (Zhang *et al.*, 2015) and these may therefore have an important role in development of the feeding site.

It is clear that functional studies of effectors provide valuable information about the molecular mechanisms by which nematodes infect plants. However, the practical outputs that are emerging from effector biology are not always understood and may not be emphasised by the authors of such work. However, the underpinning rationale for work on effectors is to provide new methods for controlling pathogens. Here we describe some of the translational outputs of effector research.

NATURAL RESISTANCE

The function and evolution of induced plant defences has been described by the zigzag model (Jones & Dangl, 2006) and is summarised in Figure 1 in the context of plant-nematode interactions. Plants are under constant attack from a wide range of potential pathogens. The majority of these pathogens are successfully repelled following detection of essential, conserved pathogen molecules (Pathogen-Associated Molecular Patterns – PAMPs) which activate the first layer of host defences – PAMP-triggered immunity (PTI) - following perception by pattern-recognition receptors (PRRs). PTI may also be induced upon detection of the breakdown products of the plant cell wall, which are termed damage-associated molecular patterns (DAMPs). Successful biotrophic pathogens deliver effectors that suppress PTI and thus induce effector-triggered susceptibility (ETS). In order to counter this, a second layer of immune receptors is present encoded by resistance (*R*) genes. Resistance proteins detect the presence of effectors and activate effector-triggered immunity (ETI). ETI is frequently accompanied by a strong, localised cell death, termed the hypersensitive reaction (HR). Effectors that are recognised by resistance proteins are termed avirulence (Avr) factors.

The HR arising from the recognition of an *Avr* gene product by its cognate R protein can be readily visualised as a patch of cell death in some plants (Figure 2). This can be achieved either by transiently expressing the *Avr* gene alone in a plant carrying the appropriate *R* gene (Figure 2A) or by transiently expressing both the *Avr* effector and the *R* genes in a plant (Figure 2B & 2E). Knowledge of an *Avr* effector therefore offers several potential practical benefits. Perhaps most importantly an *Avr* gene can be used as a tool to assist in the identification of the corresponding *R* gene, by acting as a probe that reveals the presence of the *R* gene in a given sample. The use of effectors in these resistance screening approaches has recently been reviewed (Du & Vleeshouwers, 2014), and

includes several examples of identification of *R* genes against the late blight pathogen *Phytophthora infestans*. For example, knowledge of the *R3a/Avr3a* matching pair enabled the subsequent identification of the closely linked, but functionally distinct, *R3b* resistance gene and its cognate *Avr* gene *Avr3b* (Li *et al.,* 2011). Similarly, effectors can also be used as tools to identify novel resistance sources. Vleeshouwers *et al.* (2008) screened a range of wild Solanaceous species with 54 predicted *P. infestans* effectors and were able to identify orthologues of *Rpi-blb-1* resistance gene from *Solanum stoloniferum* and *S. papita*. Similarly, Van Weymers *et al.* (2016) screened 126 wild diploid *Solanum* accessions with *P. infestans* isolates and subsequently identified functional *Rpi-vnt1.1* homologs in *S. okadae* amongst other *R* genes by screening 82 conserved effectors. Knowledge of an *Avr* gene can also be used for tracking the presence of an *R* gene in a population of plants used in a breeding programme by transient expression in leaves. The utility of integrating effector screening in a breeding program and in *R* gene cloning has been illustrated recently by the characterisation of an *R2* homolog from the Swedish potato breeding clone SW93-1015 that confers efficient broad spectrum resistance to *P. infestans* under field conditions (Lenman *et al.*, 2016). This approach offers the prospect of substantial savings of time and resource, compared to screening segregating populations with nematodes.

Knowledge of effectors/*Avr* genes can also be used to predict the durability of an *R* gene in a field situation. An *Avr* gene that accumulates mutations in response to selection pressure from the host may no longer be recognised by its cognate R protein, although there may also be constraints on the level of changes that can be accommodated while retaining function. It therefore follows that an *Avr* gene that is relatively invariant at a population level is likely to be recognised by an *R* gene product that is durable. Conversely, extensive variation in an effector sequence is likely to be characteristic of an *Avr* gene that evades recognition by what is probably a less durable *R* gene. This property can be readily observed in the one *Avr* gene (*AvrGpa2* otherwise known as *Gp-RBP-1*) that has been identified from plant-parasitic nematodes to date (Sacco *et al.,* 2009). The potato *Gpa2* resistance has been overcome by populations of *G. pallida* present in Europe and this is reflected by the presence in *G. pallida* populations of multiple alleles of the *Avr* gene that contain a point mutation in the site which determines recognition. Indeed, strong selection pressure on this amino acid residue, and on several others, has been demonstrated (Carpentier *et al.,* 2012). Similar analyses with *P. infestans* have shown that polymorphisms in a range of *Avr* genes partly explain the emergence of a virulent and aggressive strain of this pathogen in Europe (Cooke *et al.,* 2012). An understanding of effector biology can therefore inform resistance deployment and breeding strategies.

In the longer term, and given the appropriate public acceptance, knowledge of the recognition specificities of an *R*/*Avr* gene combination can be used to expand recognition in generating a modified version of the *R* gene that recognises forms of the *Avr* gene that would normally evade detection. Two forms of the *Avr3a* gene are present in *P. infestans* populations; $Avr3a^{KI}$ is recognised by *R3a* whereas $Avr3a^{EM}$, which has polymorphisms present at two positions in the Avr3a protein, evades recognition. Two studies have shown that introducing amino acid changes in R3a by point mutation or gene shuffling can generate forms of the protein that recognise both $Avr3a^{KI}$ and $Avr3a^{EM}$ (Chapman *et al.,* 2014; Segretin *et al*., 2014). Although there may be issues in converting this recognition into resistance in plants, this technology offers the clear potential to manipulate *R* genes into a more durable form where knowledge of the corresponding effector is available.

EFFECTORS AS TARGETS FOR GM APPROACHES

Functional studies on effectors that have been identified from plant-parasitic nematodes frequently include an analysis of the importance of the effector by knocking down expression using RNA interference (RNAi). Nematode effectors can be targeted using RNAi by inducing uptake of double stranded RNA (dsRNA) by second stage juveniles (*e.g* Chen *et al.,* 2005; Bakhetia *et al.,* 2008) or by producing genetically modified plants that express a dsRNA hairpin from an appropriate construct (*e.g* Eves van den Akker *et al.,* 2014a). The aim of such studies is generally to demonstrate the importance of an effector in the pathogenic process or, given sufficiently detailed phenotyping, to understand the function of the effector in the nematode life cycle. Such studies also allow effectors to be identified that might represent good targets for the development of future control methods by generating plants that express dsRNA targeting the effector sequence. In line with this idea, mining the genomes of root-knot nematodes through an evolutionary and comparative genomics approach identified 15,952 genes, including 993 effector-like proteins, that are conserved in genomes of plant-parasitic species but absent from non-target genomes of chordates, plants, annelids, insect pollinators and molluscs (Danchin *et al.*, 2013). Sixteen of these were tested in infestation assays on tomato, using siRNA-treated *M. incognita*, and 12 showed significant and reproducible reduction of nematode parasitism. Host-mediated silencing is an appealing strategy for nematode control, partly due to the exquisite specificity of the RNAi process itself, in which only genes that are very similar in sequence to the silencing dsRNA will be targeted, but also because effectors in particular are often highly restricted to specific pathogen groups, as illustrated by the fact that very little overlap is present between the effector complement of *G. pallida* and that of

root-knot nematodes (Cotton *et al.,* 2014). This suggests that an RNAi strategy targeting effector(s) has the potential to be highly specific and thus may allow off-target effects to be minimised.

The most complete example of the use of this strategy to date concerns the 16D10 effector of the root-knot nematode *M. incognita*. This effector targets a SCARECROW transcription factor in plants (Huang *et al.,* 2006a) and subsequent work showed that knocking out expression of the 16D10 effector by RNAi *in vitro* leads to a failure of parasitism (Huang *et al.,* 2006b). In addition, *Arabidopsis* expressing dsRNA hairpins targeting 16D10 were resistant to *M. incognita* and to closely related *Meloidogyne* species (Huang *et al.,* 2006b). Likewise, host-mediated RNAi silencing targeting the *M. incognita* 16D10 in transgenic grapevine hairy roots efficiently decreased nematode reproduction (Yang *et al.,* 2013). A similar approach has shown that targeting the *Meloidogyne chitwoodi* homologue of 16D10 also gives resistance in *Arabidopsis* and in potato (Dinh *et al.,* 2014). This demonstrated that RNAi of an effector can be used in a crop plant as well as in a model system. A similar strategy has been used to analyse the function of the *M. incognita* 8D05 effector gene, which is known to interact with a host protein likely to be involved in water and solute transport. Generation of transgenic *Arabidopsis* expressing a dsRNA targeting the 8D05 effector gene gave over 90% control *in vitro,* validating this effector as a potential control target (Xue *et al.,* 2013).

Although the studies described above provide, at first sight, compelling evidence that RNAi targeting effectors (or indeed any other important nematode gene) represents a promising control strategy, the sustainability of such resistance has not yet been demonstrated and a note of caution is required. The first description of RNAi in nematodes was made almost 20 years ago (Fire *et al.,* 1998) and the first publication demonstrating the applicability of this technique to plant-parasitic nematodes appeared more than 10 years ago (Urwin *et al.,* 2002). Various proof-of-concept studies, such as those described above, have subsequently appeared but there are no cultivars available that use RNAi to control nematodes. It has recently been shown that constructs generating dsRNA targeting nematode genes can become silenced in transgenic plants due to methylation of the promoter region and that this effect may become more pronounced in successive generations (Kyndt *et al.,* 2013). There may therefore be significant technical hurdles to be overcome before RNAi can be deployed as a tool against nematodes.

LONGER TERM OUTPUTS (1): TARGETING THE HOST TARGETS OF EFFECTORS

One of the ways that the function of a novel effector can be probed is to screen a yeast two-hybrid library with the effector in order to identify host proteins that interact with, and may therefore be targeted by, the effector. This approach has been used widely and has shown that a

variety of host proteins and pathways are targeted by nematode effectors. These include transcription factors (Huang *et al.,* 2006a), auxin transporters (Lee *et al.,* 2011), proteins involved in cell wall remodelling (Hewezi *et al.,* 2008) and putative *R* genes (Rehman *et al.,* 2009). This knowledge offers a further opportunity for new control strategies as it may be feasible to identify, or to generate, variants of the host proteins (also referred as susceptibility genes) that are still functional but that are no longer amenable to manipulation by the effector (van Schie & Takken, 2014). Structural information about how the interaction between the effector and its target can help in this regard and a similar approach has been used to demonstrate how changes in an effector sequence affect its ability to interact with a host target (King *et al.,* 2014). Once a target has been identified and the region that is important for interaction with the effector determined, variants of the target can be sought, or induced, that do not interact with the effector. The development of TILLING (Targeting Induced Local Lesions In Genomes) based approaches offer the hope that progress can be made in this area (McCallum *et al.,* 2000). TILLING is a method in which mutagenesis is coupled with extremely high-throughput screening techniques that identify the sites of the induced mutations. The use of TILLING as a tool for improvement of crops has recently been reviewed (Chen *et al.,* 2014). In an alternative approach – EcoTILLING – natural variants of the host species are sought that show a desired trait and the same high-throughput techniques are subsequently used to identify variation that is associated with the trait. This tool has been used, for example, to identify variants in *R* genes against powdery mildew and to seek new resistance sources against viruses (Ibiza *et al.,* 2010; Mejlhede *et al.,* 2006). Looking further ahead, new genome editing tools such as CRISPRs and TALENs (reviewed by Mahfouz *et al.,* 2014) may offer an alternative route to modifying host sequences to the disadvantage of pathogens. These approaches may not just be limited to modifying the protein targets of pathogen effectors. Some effectors may exert their activity by binding to DNA in order to modify host gene expression; the bacterial TAL effectors providing the best studied examples of these (*e.g.* Kay *et al.,* 2007). Modifying the region of the host genomic DNA that effectors need to bind to may therefore offer an alternative route to control (Wulff *et al.*, 2011).

LONGER TERM OUTPUTS (2): NON-HOST RESISTANCE

Non-host resistance (NHR) is the term used to describe the phenomenon that most plants are resistant to most pathogens. The corollary of this is that most pathogens have a very limited host range, although there are clear exceptions to this rule including some root-knot nematode species. Understanding the mechanisms underlying NHR offers the prospect of identifying resistance that is,

by definition, broad-spectrum and durable. The potential applications of NHR in crop improvement have recently been reviewed (Lee *et al.*, 2016). NHR is often multi-tiered (Thordal-Christensen, 2003; Gill *et al.*, 2015) but although structural and chemical barriers play a role in some cases of NHR, it is thought that effectors are central in the majority of cases. It has been argued (Schulze-Lefert & Panstruga, 2011) that NHR in plants distantly related to the host of a pathogen occurs as a result of a failure to suppress PTI, and that the NHR response in such species occurs as a result of PTI responses mediated by the plasma membrane localized PAMP receptors. This may occur due to co-evolution between host and pathogen leading to highly adapted effectors that are unable to interact with their targets in non-host species. NHR may be engineered by transferring PRR, that are otherwise absent, into host plants to create defence responses that pathogens are unable to circumvent due to a lack of adapted effector(s). The ground-breaking demonstration that broad-spectrum defence against bacterial pathogens can be acquired by heterologous expression of a PRR was reported by Lacombe *et al.* in 2010, where the elongation factor Tu receptor (EFR) from *Arabidopsis* was transferred into two Solanaceous plants, tomato and *Nicotiana benthamiana*. Further demonstration of the efficiency of this approach has been provided in two recent studies (Schoonbek *et al.*, 2015; Schwessinger *et al.*, 2015), showing that transfer of EFR in wheat and rice can also enhance bacterial disease resistance in cereals. This also suggests that both dicot and monocot plants contain all necessary components for EFR signalling, further revealing that immune signalling pathways may be conserved across distant phyla and that transfer of PRR across plant families may also prove to be less problematic than inter-family *R* gene transfer. In non-host species that are more closely related to the host species, NHR may occur as a result of ETI, implying the existence of highly durable *R* genes that recognise the pathogen and whose action cannot be circumvented by the pathogen (Schulze-Lefert & Panstruga, 2011). Knowledge of pathogen effectors offers the potential to identify the *R* genes that underlie NHR in non-host species. As a first step in this process one effector (HopQ1-1) has been identified from the tomato bacterial speck agent *Pseudomonas syringae* pv. *tomato* as the sole avirulence determinant responsible for the failure of the model strain DC3000 to cause disease in *N. benthamiana* (Wei *et al.*, 2007; Figure 2C). Conversely, heterologous expression of *hopQ1-1* in the tobacco wildfire pathogen *P. syringae* pv. *tabaci* 11528 rendered this strain avirulent in its susceptible host *N. benthamiana*. However, the *hopQ1-1* deletion did not extend the host range of DC3000 to tobacco *Nicotiana tabacum*, indicating that the mutation does not confer some general virulence benefit to DC3000. Effector-mediated recognition in non-host plants can also trigger cell death, as seen in R/Avr-mediated resistance (Figure 2D and 2E). As an example, *Phytophthora capsici* effector PcAvr3a1 provokes a cell death response upon transient expression in (non-host) *N. tabacum* and other related non-host species (Vega-Arreguin *et al.,* 2014). Furthermore,

host-mediated silencing of *PcAvr3a1* in the pathogen allowed infection of resistant tobacco, confirming the importance of effector recognition in NHR.

CONCLUDING REMARKS

Effector biology is currently a highly topical issue, with developments in genomics and functional genomics enabling beautifully detailed studies of these proteins. As well as revealing the tools used by pathogens to manipulate their hosts, each effector acts as a probe for the plant immune system allowing us to develop a better understanding of both sides of the host-parasite interaction (Lee *et al.*, 2013). The interactions of effectors with R proteins, and indispensable roles that they play in pathogen biology, mean that they also offer the prospect of significant practical gains. Effectors already form an integral part of the breeding process for resistance against the late blight pathogen *P. infestans* (Du & Vleeshouwers, 2014). We consider it likely that this will soon be the case for other plant pathogens, including nematodes, as information on the effector complement of various economically important pathogens is established.

Acknowledgements

The James Hutton Institute receives funding from the Scottish Government. This work benefited from interactions funded through COST FA1208.

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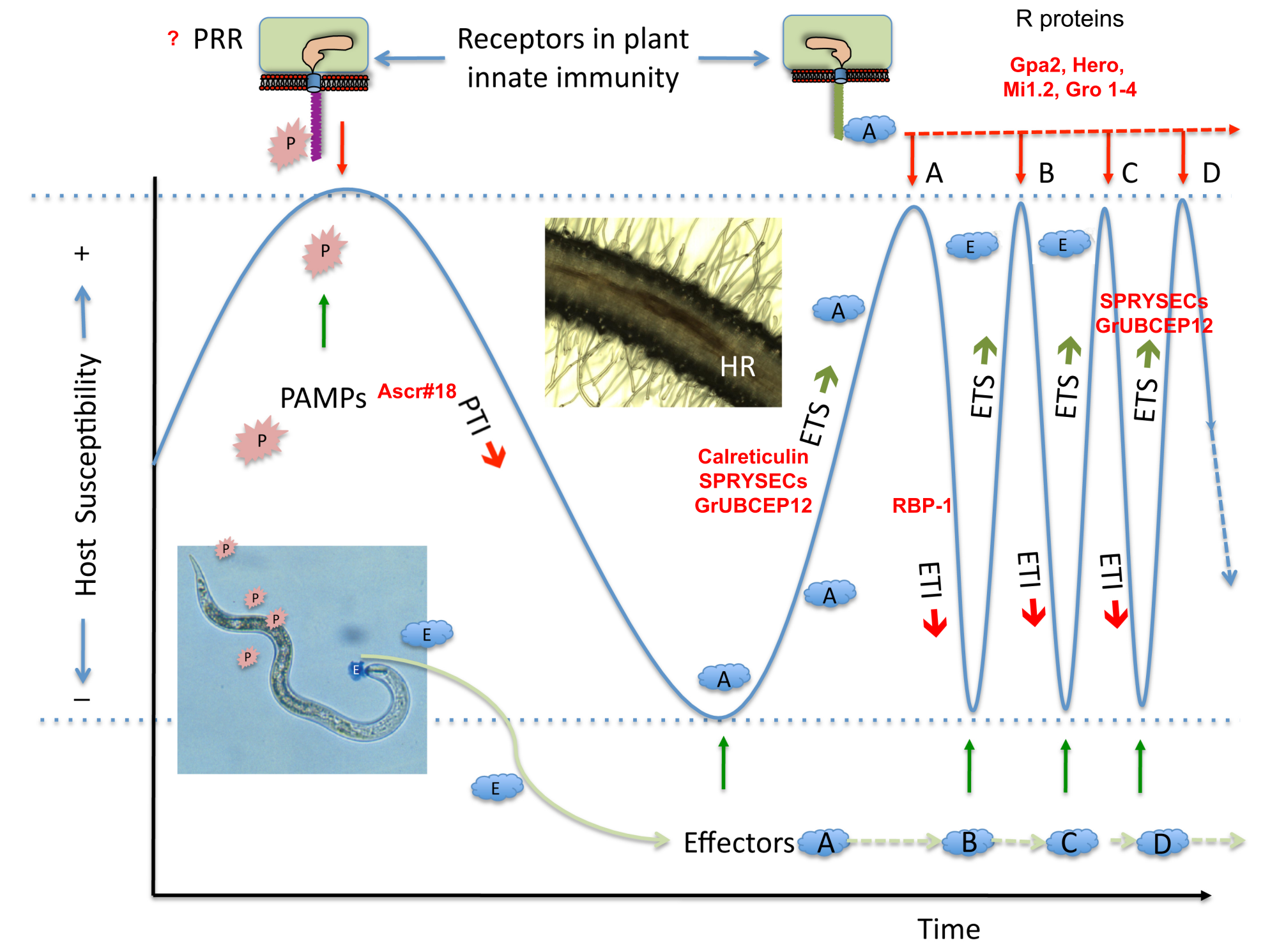
561

562 **Figure legends**

563 **Figure 1:** The zigzag model in context of plant-nematode interactions. In 2006 Jones & Dangl
564 established the zigzag model to illustrate the quantitative output of the plant immune system in
565 response to microbes but the concept has proven to be more broadly applicable to pests and
566 pathogens. Components of the zigzag model that have been identified in plant-nematode
567 interactions are shown in bold red type. The conceptual arms-race between host and pathogens can
568 be depicted in four major phases. In phase I, conserved pathogen-associated molecular patterns
569 (PAMPs; represented by the letter P in the pink forms) are recognised in plants by cell surface
570 pattern-recognition receptors (PRRs) leading to induction of PAMP-triggered immunity (PTI). The
571 only PAMP from plant-parasitic nematode identified to date is a pheromone, the ascaroside 18
572 (Ascr#18; Manosalva *et al.*, 2015), but its cognate PRR is not yet known. In phase II, adapted
573 pathogens secrete effectors into the host that interfere with PTI, leading to effector triggered
574 susceptibility (ETS). Several nematode effectors (represented by the letter E in the blue clouds) have
575 been characterisd that can supress PTI responses (see review by Mantelin *et al.*, 2015). In phase III,
576 particular effectors (represented in the blue clouds by the letter A for "Avirulence factors") are
577 detected by a second layer of plant resistance receptors (products of the *R* genes), activating
578 effector-triggered immunity (ETI) which in most cases leads to the induction of a hypersensitive
579 plant cell-death reaction (HR). Very few nematode *R* genes have been cloned (see review by Goverse
580 & Smant, 2014) and only one avirulence effector has been identified so far, the *Globodera pallida*
581 RBP-1 SPRYSEC effector AvrGpa2 (Sacco *et al.*, 2009). In phase IV, as pathogen and host coevolve
582 new effectors and *R* genes, susceptibility or resistance predominate in turn. Avirulence factors (A)
583 maybe lost or modified to avoid recognition by cognate R proteins (as is the case for RBP-1) and
584 perhaps new effectors are gained (B,C,D) that are able to suppress ETI. Such activity has been
585 demonstrated for the ubiquitin carboxyl extension protein GrUBCEP12 and many SPRYSEC effectors
586 (see review by Mantelin *et al.*, 2015).
587
588 **Figure 2:** Effector-mediated cell death in plants. **(A & B)** A typical hypersensitive reaction is elicited
589 by recognition of *Globodera pallida* effector *Gp*-RBP-1 (*St*Gpa2-cognate avirulence factor) in
590 *Agrobacterium tumefaciens*-based transient expression assay in potato accession Cara containing
591 the *StGpa2* resistance gene **(A)** or by transient co-expression of both *StGpa2* and *Gp-RBP-1* in
592 *Nicotiana benthamiana* leaf **(B)**. Conversely, *eGFP* control in potato and either *StGpa2* or *Gp-RBP-1*
593 expressed alone in *N. benthamiana* do not induce a response in plants. Transient expressions were
594 performed with untagged constructs for *StGpa2*, *Gp-RBP-1* (Sacco *et al.*, 2009) and *eGFP* control as
595 described in Mei *et al.* 2015, by infiltration of *A. tumefaciens* strains at an OD_{600nm} of 0.5. Symptoms

596 observed under white light 7 days post infiltration. Infiltrated areas are indicated by dashed circles.
597 **(C-D-E)** Effectors also participate in non-host resistance. **(C)** Wild-type *Pseudomonas syringae*
598 pathovar *tomato* (*Pst*) strain DC3000 can barely infect *Nicotiana benthamiana* while *Pst* mutant
599 strain CUCPB5460 lacking the type-III effector HopQ1-1 is able to cause disease in the non-host plant
600 (demonstrated by Wei *et al.*, 2007). Necrotic disease symptoms observed 7 days after bacteria
601 infiltration at OD_{600nm} of 1.10^{-4} in 10 mM $MgSO_4$ solution. Infiltrated areas are circled. **(D & E)** Cell
602 death is triggered specifically in non-host *Nicotiana sylvestris* **(D)** by a *Phytophthora infestans* RXLR
603 effector (*Pi*-A) while transient expression of the same effector in the host plant *N. benthamiana* **(E)**
604 does not induce symptoms in the leaf. Another effector (*Pi-B*) as well as the *Td-Tomato* construct
605 used as control do not induce symptoms. Conversely, a typical hypersensitive reaction is elicited by
606 recognition of *P. infestans* effector *Pi*-Avr3a in the presence of the potato resistance protein *St*R3a in
607 *N. benthamiana* leaf **(E)**. Effectors and controls in binary vector pGRAB were transformed in *A.*
608 *tumefaciens* GV3101 and agro-infiltrated at an OD_{600nm} of 0.1 in *Nicotiana* leaves (Mantelin and Hein,
609 personal communication). Symptoms observed under white light 7 days post inoculation. Infiltrated
610 areas are indicated by dashed circles.
611


? PRR
Receptors in plant innate immunity
R proteins
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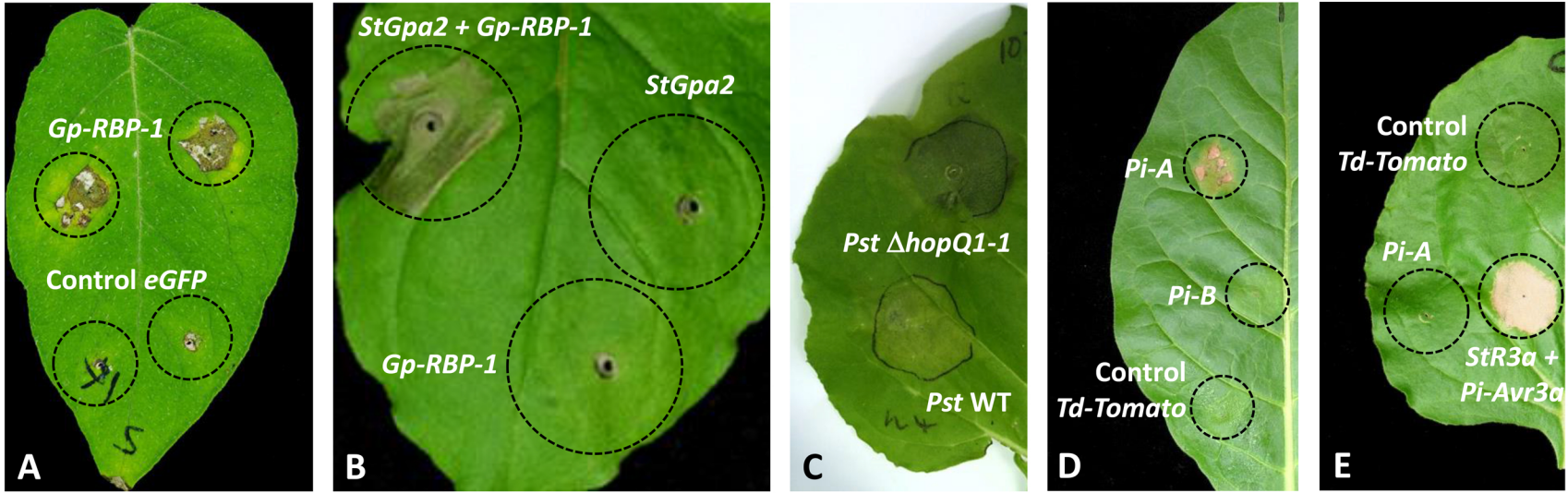
Gp-RBP-1
Control eGFP
A
StGpa2 + Gp-RBP-1
StGpa2
Gp-RBP-1
B
Pst ΔhopQ1-1
Pst WT
C
Pi-A
Pi-B
Control
Td-Tomato
D
Control
Td-Tomato
Pi-A
StR3a +
Pi-Avr3a
E