Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2009) **10**(6), 767–775 DOI: 10.1111/J.1364-3703.2009.00587.X

Micro-Review

The evolution of *Pseudomonas syringae* host specificity and type III effector repertoires

MAGDALEN LINDEBERG, SÉBASTIEN CUNNAC AND ALAN COLLMER*

Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 14853, USA

SUMMARY

The discovery 45 years ago that many *Pseudomonas syringae* pathovars elicit the hypersensitive response in plant species other than their hosts fostered the use of these bacteria as experimental models. However, the basis for host specificity and the corresponding resistance of nonhosts remain unclear. *Pseudomonas syringae* is now known to inject into the host cytoplasm, via the type III secretion system, effector proteins that suppress basal innate immunity, but may be recognized by cognate resistance (R) proteins in a second level of defence. The identification and manipulation of complete repertoires of type III effectors have revealed the highly polymorphic nature of effector repertoires and their potential to limit the host range. However, the maintenance of compatible effector repertoires may be driven by adaptations to life in a given plant species involving many factors. Tools are now available to test several hypotheses for the nature and evolution of *P. syringae* host specificity and nonhost resistance.

INTRODUCTION

Pseudomonas syringae is a host-specific, hemibiotrophic pathogen whose interactions with plants are both archetypal and diverse. Research with a few model strains of *P. syringae*, notably *P. syringae* pv. *tomato* DC3000, has contributed significantly to the paradigm of effector-mediated pathogenesis, which holds that typical (hemi)biotrophic bacteria, fungi and oomycetes succeed as parasites mainly because the 'effector' proteins they translocate into host cells subvert defences that would otherwise be activated by microbial signatures (Abramovitch *et al*., 2006; Alfano and Collmer, 2004; Boller and Felix, 2009; Hogenhout *et al*., 2009; Nomura *et al*., 2005). Widening the study of effector-mediated pathogenesis to multiple strains of *P. syringae* has revealed a remarkable diversity in effector repertoires that scales with the diversity in hosts and disease symptoms associated with these bacteria (Cunnac *et al*., 2009; Hirano and Upper, 1990). The host specificity of the *c*. 50 pathovars of *P. syringae* for different plant species is a particularly striking aspect of this variability that has been investigated for more than 45 years but is still poorly understood.

Pseudomonas syringae and many other Proteobacteria causing diseases in plants and animals inject effector proteins into host cells via the type III secretion system (T3SS) (Cornelis and Van Gijsegem, 2000). The T3SS in plant pathogens is encoded by *hrp* genes, which are required for the bacteria to elicit the hypersensitive response (HR; a rapid, localized programmed cell death) in nonhost or resistant plants or to be pathogenic in susceptible host plants (Lindgren *et al*., 1986). The strong phenotype of *hrp* mutants highlights the collective importance of type III effectors (T3Es) in *P. syringae* interactions with plants. The host specificity of *P. syringae* is observed at two levels (with two corresponding types of resistance): pathovar–host species ('nonhost resistance') and race–host cultivar ('host resistance'). For example, at the first level of specificity, soybean is a nonhost for pv. *tomato* but a host for pv. *glycinea*; at the second level, certain cultivars of soybean are resistant to particular races of pv. *glycinea*. As first demonstrated by the avirulence conferred by heterologous expression in *P. syringae* pv. *glycinea* race 5 of the *avrA* T3E gene cloned from race 6, the presence of a single betraying T3E can entirely explain the specificity at the race–cultivar level (Staskawicz *et al*., 1984). This gain-of-function approach was subsequently applied to several other *P. syringae* pathovars and crop species, most notably *P. syringae* pv. *phaseolicola* on bean, which revealed the general role of T3Es in the control of specificity at the race–cultivar level (Keen, 1990).

The earlier discovery that many *P. syringae* pathovars elicit HR in nonhosts was seminal in the development of these bacteria as important experimental models (Klement, 1963; Klement *et al*., 1964), and the common association of HR with avirulence suggested that T3E repertoires similarly control the specificity

**Correspondence*: E-mail: arc2@cornell.edu

of interactions at the pathovar–host species level as well as the race–host cultivar level (Kobayashi *et al*., 1989). Indeed, the heterologous expression of pv. *tomato* genes in pv. *glycinea* revealed three T3Es that could collectively account for the avirulence of pv. *tomato* on all tested cultivars of soybean (Kobayashi *et al*., 1989). However, the construction of a polymutant in pv. *tomato* lacking all three of these T3E genes failed to extend the host range of the mutant to soybean (Lorang *et al*., 1994). These classic observations are based largely on qualitative differences in *P. syringae* interactions with plants. Since they were published, we have learned far more about *P. syringae* T3E repertoires and their function in defeating or evading two levels of plant defence, and the quantitative contributions that effectors and other factors can make to virulence. Despite this progress, the basis for the host specificity of *P. syringae* pathovars for different plant species and the corresponding resistance of nonhosts remains unclear. This mini-review addresses the dynamics of T3E repertoire evolution in the context of plant defences and the potential role of dysfunctional repertoires in determining pathovar–host species specificity.

TWO LEVELS OF PLANT DEFENCE AND VARIETIES OF NONHOST RESISTANCE

Successful effector-mediated pathogenesis involves the defeat of two levels of plant defence (Boller and Felix, 2009; Gohre and Robatzek, 2008; Jones and Dangl, 2006). In the first level, plants detect *P. syringae* and other microbes in the apoplast via pathogen (or microbe)-associated molecular patterns (PAMPs; e.g. flagellin, lipopolysaccharide, peptidoglycan and elongation factor TU) using surface-arrayed pattern recognition receptors (PRRs) that elicit PAMP-triggered immunity (PTI) (Boller and Felix, 2009; Jones and Dangl, 2006). *Pseudomonas syringae* defeats this defence largely by injecting T3Es that suppress PTI. The second level involves the recognition of the activity of one or more of these T3Es inside plant cells via resistance (R) proteins, which are typically nucleotide-binding leucine-rich repeat (NB-LRR) proteins. So far, it appears that R proteins recognize all of their cognate *P. syringae* T3Es indirectly by detecting effector activity on other plant proteins that are 'guarded' by NB-LRR proteins. According to an emerging model, 'guardees', such as the Pto kinase, are decoys for primary effector targets, such as PRR kinases (van der Hoorn and Kamoun, 2008; Zhou and Chai, 2008).

Resistance (*R*) gene-dependent effector-triggered immunity (ETI) confers qualitative resistance that has been exploited by plant breeders and pathologists because of the relative ease with which single *R* genes can be introduced into elite cultivars of many crop species (Poland *et al*., 2009). However, such resistance is typically not durable in the field because of the multiple mechanisms by which pathogens inactivate a betraying T3E gene, thus producing a new race that is virulent (Jones and Dangl, 2006; Ma and Guttman, 2008). This dynamic works for the pathogen because the co-evolutionary battle between plant *R* gene surveillance systems and pathogen T3Es has produced effector repertoires with internal redundancy (discussed further below), and consequently individual T3Es are dispensable. Another mechanism for evading ETI is through disruption of ETI itself. For example, many of the *P. syringae* pv. *tomato* DC3000 T3Es are capable of suppressing the defence response elicited by the T3E HopA1 (Guo *et al*., 2009) and, remarkably, AvrPtoB can suppress its own detection using its C-terminal E3 ligase domain to facilitate the degradation of Fen kinase, a host protein capable of activating ETI in response to the AvrPtoB N-terminal region (Rosebrock *et al*., 2007). The dispensability of individual effectors in a repertoire has been observed repeatedly through studies involving bacterial mutants in the laboratory or through *R* gene 'breakdown' in the field, and similar observations suggest that this property of repertoires is widespread among bacterial, fungal and oomycete pathogens of plants (Hogenhout *et al*., 2009; Jones and Dangl, 2006; Kvitko *et al*., 2009). Thus, the internal redundancy of *P. syringae* T3E repertoires appears to be archetypal for a wide collection of pathogens that defeat plants through effector-mediated pathogenesis.

In contrast with host resistance, nonhost resistance appears to be highly stable in the field (Mysore and Ryu, 2004). Nonhost resistance is canonically shown by all members of a plant species against a pathogen species or pathotype (Heath, 2000), and experience with various crops suggests that a given plant species is durably resistant in the field to all but a few of the *P. syringae* pathovars. For example, common bean is susceptible to pv. *phaseolicola* (halo blight) and *syringae* (brown spot), but not to pv. *tomato* or *glycinea* (Schwartz *et al*., 2005). However, many *P. syringae* strains can cause disease in more than one plant species (or family), and some plants can show nonhost resistance to nonadapted strains that have been nominally assigned to a pathovar that also contains adapted strains. For example, pv. *tomato* strain DC3000 can cause disease in tomato and Arabidopsis, whereas pv. *tomato* strain T1 causes disease only in tomato; Arabidopsis is a nonhost for this strain (Whalen *et al*., 1991). Importantly, multilocus sequence typing of close relatives of DC3000, which occurs in pv. *tomato*, *maculicola* and *antirrhini* (Hendson *et al*., 1992), reveals that strains in the T1 clade, but not the DC3000 clade, are consistently isolated from tomato plants with bacterial speck in the field (Yan *et al*., 2008). As discussed below, T1 is demonstrably nonadapted for Arabidopsis. Why DC3000-like strains are virulent on tomato in the laboratory but rarely isolated from tomato in the field is unknown.

Two types of nonhost resistance have previously been distinguished on the basis of interaction phenotypes: type I occurs without apparent host cell death, whereas type II is accompanied by HR (Mysore and Ryu, 2004). Arabidopsis displays type I

nonhost resistance against *P. syringae* pv. *phaseolicola* and type II nonhost resistance against *P. syringae* pv. *tomato* T1. Although ETI has typically been distinguished from PTI by the elicitation of HR, it now appears that some PAMPs may elicit plant cell death and some T3E/R protein combinations, such as AvrB/TAO1 and HopA1$_{Psy61}$/RPS6, may contribute to ETI without HR (Eitas *et al*., 2008; Kim *et al*., 2009; Naito *et al*., 2008; Shimizu *et al*., 2003), which suggests that there is overlap in the threshold for the elicitation of HR (Jones and Dangl, 2006). Similarly, there is overlap in the profiles of plant genes expressed in response to elicitors of PTI and ETI (Navarro *et al*., 2004; Tao *et al*., 2003; Thilmony *et al*., 2006). Thus, the threshold model for HR elicitation raises the possibility that there is no fundamental difference between type I and type II nonhost resistance, and both could result from T3E repertoires that are incompatible with nonhost ETI systems.

POLYMORPHISMS IN THE MOLECULAR INTERACTIONS OF *P. SYRINGAE* WITH PLANT DEFENCES

Polymorphisms in the repertoires of T3Es and *R* genes underlie race–cultivar specificity, but polymorphisms in multiple systems could underlie pathovar–host specificity, as summarized in Fig. 1. The model depicted postulates that all *P. syringae* strains share a basic ability to grow in the apoplast and that, despite potential adaptations to differences in nutrients and antimicrobials associated with different plant species, the polymorphisms that qualitatively limit host range involve defence recognition. The model further postulates that polymorphisms involving the recognition of PAMPs or the suppression of PTI by T3Es are not qualitative limiters of host range, although such polymorphisms do exist. For example, FLS2, the prototypical PRR, is lacking in

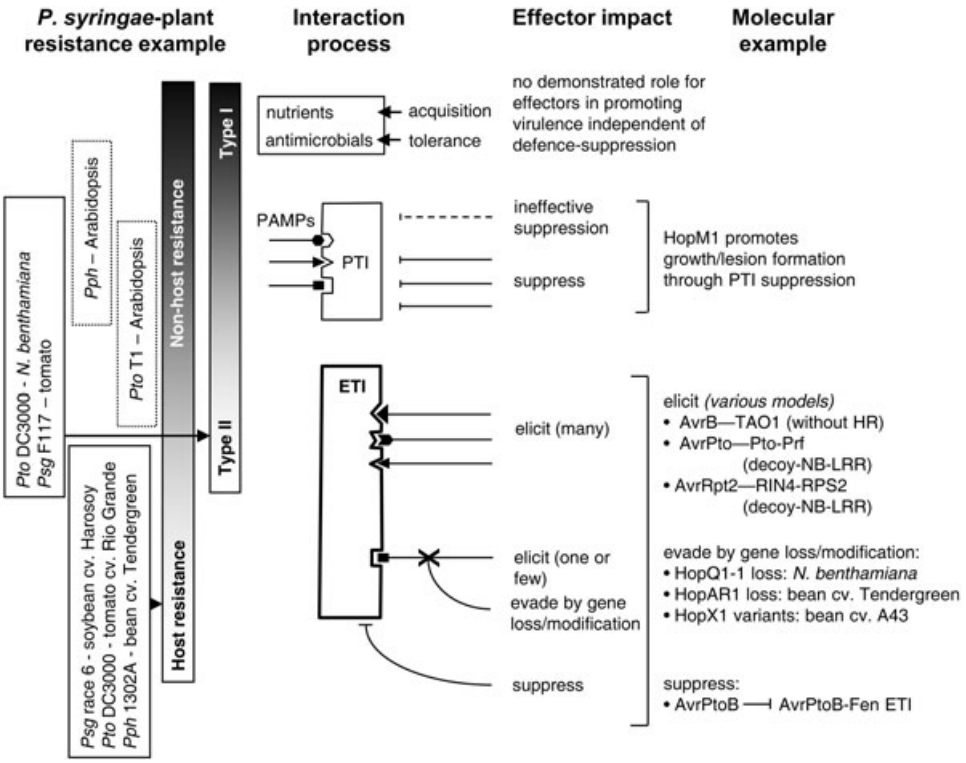

Fig. 1 Model for the role of type III effector repertoires as highly polymorphic, qualitative limiters of *Pseudomonas syringae* host range at the pathovar–species level (nonhost resistance) and race–cultivar level (host resistance). The model presents several examples of *P. syringae* strains interacting with resistant plants. Host vs. nonhost resistance and type I vs. type II nonhost resistance are represented as outcomes of a continuum of processes, shown in the next column, contributing to these interaction types. The model postulates that host specialization may evolve through adaptation to quantitative effects of polymorphisms among plant species involving nutrients, antimicrobials, pathogen-associated molecular pattern (PAMP) perception and PAMP-triggered immunity (PTI) suppression. However, effector repertoires co-evolving to evade effector-triggered immunity (ETI)-mediated host resistance will accumulate many incompatibilities with the ETI systems of other plant species. The ETI system is presented in bold because the recognition of a single effector by highly polymorphic resistance (R) protein repertoires can produce qualitative resistance. Because of the apparent conservation of several components of the PTI system and potential redundancies in the effectors targeting them, we postulate that nonadapted strains will be only quantitatively penalized at the PTI suppression step. *Pseudomonas syringae* pathovar abbreviations: *Pph*, *phaseolicola*; *Psg*, *glycinea*; *Pto*, *tomato*.

some Arabidopsis ecotypes, and EFR, which mediates the recognition of the elongation factor Tu PAMP, appears to be unique to the Brassicaceae (Boller and Felix, 2009). However, FLS2 is found in all plant groups, plants appear to recognize multiple PAMPs and there has been no report of a *P. syringae* strain that defeats PTI by simply evading detection by the PAMP perception system of its host species (Boller and Felix, 2009), although calcium chelation by bacterial polysaccharides may partially reduce PAMP signalling for all strains (Aslam *et al*., 2008). Similarly, it appears less likely that polymorphisms in plant factors involved in defence signalling or antimicrobial deployment systems are qualitative limiters of host range. *Pseudomonas syringae* T3Es that suppress PTI and have been well characterized, such as AvrPto and HopM1, have so far been found to target apparently universal plant proteins (Cunnac *et al*., 2009; Nomura *et al*., 2006; Shan *et al*., 2008; Xiang *et al*., 2008). Furthermore, an analysis of 10 Arabidopsis genes encoding defence signal transduction proteins revealed that most of them lacked the signatures of positive selection that would be a hallmark of co-evolution with pathogen effectors (Caldwell and Michelmore, 2009). In contrast, a single-nucleotide analysis of 20 Arabidopsis accessions revealed that, among all protein families, the NB-LRR gene family was the most polymorphic (Clark *et al*., 2007).

The current data continue to support the originally proposed central role in *P. syringae* pathogenesis of T3SS and the effectors it delivers into the host cytoplasm (Alfano and Collmer, 1996), and highlight the importance of two aspects of relative timing in *P. syringae*–plant interactions and their evolution. First, the *P. syringae* T3SS appears to be capable of delivering effectors rapidly on inoculation (Huynh *et al*., 1989; Puri *et al*., 1997), and this enables PTI suppression to trump PAMP detection. Second, the highly dynamic genetics of T3E repertoires suggest that *P. syringae* can rapidly and repeatedly trump ETI in game-like interactions of populations of bacteria and plants, which underpins the perpetual importance of co-evolving T3E and *R* gene repertoires in determining the host range at all levels. Thus, the molecular dialogue between *P. syringae* and plants can involve a variety of factors, but ETI will always have the last word.

THE DYNAMIC GENETICS UNDERLYING T3E REPERTOIRES

The evolution of T3E repertoires has been addressed in recent reviews (Ma and Guttman, 2008; Stavrinides *et al*., 2008), but it is useful to highlight a few key points and examples here. The acquisition of new effector capabilities occurs through a combination of horizontal transfer and recombination, the latter process facilitating the re-assortment of domains for the creation of T3Es having novel functions (Stavrinides *et al*., 2006; Yan *et al*., 2008). Genome sequencing has provided extensive support for the horizontal transfer origins of many T3Es, revealing the atypical sequence profiles and proximity to mobile genetic elements that are hallmarks of this process (Lindeberg *et al*., 2008). For example, *avrPto* and *hopW1* are found to be associated with phage genes in multiple *P. syringae* genomes and diverse sets of T3E genes are embedded within integrative and conjugative elements (ICElands) (Lindeberg *et al*., 2008; Pitman *et al*., 2005). Intra-genome recombination also plays a role in the generation of new T3Es, most importantly in uniting new functional domains with the regulatory elements and translocation motifs necessary for their co-expression with and translocation by T3SS.

Pseudomonas syringae genomes are also littered with effector pseudogenes with coding sequences or regulatory regions disrupted by frame shifts, internal stop codons or mobile elements (Lindeberg *et al*., 2006). *Pseudomonas syringae* genomes have an unusually high proportion of mobile elements (Buell *et al*., 2003), and efficient inactivation of betraying T3E genes is expected to be important for the evasion of ETI. Indeed, such inactivation was observed with the *P. syringae* pv. *phaseolicola hopAR1* T3E gene during exposure to HR in a resistant bean plant (Pitman *et al*., 2005). In addition to simple loss of T3E gene function, *P. syringae* possesses other mechanisms for evading ETI, for example through the generation of allelic variations. Members of the AvrB family (formerly AvrB and AvrC) originate from a common ancestor, but trigger a resistance response in different soybean cultivars (Tamaki *et al*., 1991). Similar differences in host recognition are observed among alleles of the HopX1 and HopZ1 subfamilies, in some cases with only a single residue change accounting for the difference (Ma *et al*., 2006; Stevens *et al*., 1998). In general, *P. syringae* strains appear to be capable of rapid adaptation of T3E repertoires in response to plant ETI surveillance.

THE DIVERSITY OF EFFECTOR REPERTOIRES

Comprehensive T3E repertoires have been identified for model strains that represent different phylogenetic groups within *P. syringae* and that have different host specificities. Phylogenetic analyses show that the *P. syringae* pathovars fall into three major clades, plus a fourth that is enriched in pathogens of monocots (Sarkar and Guttman, 2004; Sawada *et al*., 1999). Complete genome sequences are available for strains in each of the first three clades: *P. syringae* pv. *tomato* DC3000 (Buell *et al*., 2003), *P. syringae* pv. *syringae* B728a (Feil *et al*., 2005) and *P. syringae* pv. *phaseolicola* 1448A (Joardar *et al*., 2005). Genome-enabled approaches exploiting promoter and T3SS targeting patterns associated with T3E genes, coupled with functional screens and extensive experimental validation, have produced comprehensive inventories of the T3E repertoires for these strains (Chang *et al*., 2005; Cunnac *et al*., 2009; Lindeberg *et al*., 2006; Vinatzer and Greenberg, 2007). These approaches have also

been used to identify T3E genes in a draft genome of *P. syringae* pv. *tomato* T1 (Almeida *et al*., 2009). Although B728a and 1448A are both pathogens of bean, and DC3000 and T1 are both pathogens of tomato, the T3E repertoires of these strains are remarkably different, beyond a small core of apparently universal T3Es (Cunnac *et al*., 2009). Despite the high overall similarity of DC3000 and T1, only 14 of the T3Es are shared between them, whereas as many as 15 T3E genes are present only in the DC3000 genome and 11 only in the T1 genome (Almeida *et al*., 2009). Thus, there appears to be no pattern in the composition of these four repertoires that would predict their hosts, and there are multiple combinations of effectors that can confer *P. syringae* virulence in the same plant.

Combinatorial deletions involving 20 of the active T3E genes in *P. syringae* pv. *tomato* DC3000 have revealed a redundancy-based structure in the effector repertoire, such that some deletions diminish growth *in planta* only in combination with other deletions (Kvitko *et al*., 2009). It was found that two redundant effector groups are particularly important in promoting DC3000 growth *in planta* and, based on the known activities of some of the members, these internally redundant groups have been proposed to target different high-level processes in PTI: perception of PAMPs and vesicle trafficking of antimicrobial factors. These observations suggest that a few defence processes are particularly important for DC3000 to block, and each process is targeted redundantly. Two of these effectors, AvrPto and HopM1, as mentioned above, have potentially universal plant targets, an observation that further supports the hypothesis that T3E repertoires may productively suppress PTI in a wide range of plants, although they may be incompatible with the ETI systems of all but a few plant species.

TESTING THE ROLE OF T3Es IN NONHOST RESISTANCE

Experiments that address the role of T3Es and plant defences in nonhost resistance have provided new insights but no comprehensive model. A microarray analysis of Arabidopsis genes expressed at different levels in response to bacterial challenge revealed similar patterns for *P. syringae* pv. *phaseolicola* and *P. syringae* pv. *tomato* DC3000 heterologously expressing T3Es with HR-eliciting avirulence activity, although the amplitude of the expression changes was lower for pv. *phaseolicola* (Tao *et al*., 2003). There is additional evidence that *P. syringae* pv. *phaseolicola* elicits weak ETI in Arabidopsis. Wild-type bacteria elicit the formation of larger callose deposits and stronger defence gene expression than do T3SS$^-$ mutants (Ham *et al*., 2007; Mishina and Zeier, 2007). Importantly, only small callose deposits are elicited by the wild-type in an ETI-compromised *rar1* mutant. Any T3Es in the *P. syringae* pv. *phaseolicola* repertoire that elicit weak ETI in Arabidopsis await identification, but the type I nonhost resistance of Arabidopsis to pv. *phaseolicola* appears to be ETI based.

There is now evidence from solanaceous plants that the presence of betraying T3Es can indeed limit the host range at the pathovar–species level and that interference with the recognition of these T3Es can overcome type II nonhost resistance. For example, HopQ1-1 acts as a typical avirulence determinant for *P. syringae* pv. *tomato* DC3000 in *Nicotiana benthamiana*, and DC3000 mutants lacking this one T3E gain the ability to grow from low levels of inoculum to high population levels and to produce typical bacterial speck lesions in this apparent nonhost species (Wei *et al*., 2007). Another, broadly important example of this phenomenon involves tomato Pto/Prf-mediated recognition of AvrPto and AvrPtoB (Lin and Martin, 2007). Both of these T3Es are widespread among nonadapted pathovars, which cause disease in plants other than tomato. However, 10 of these nonadapted pathovars, e.g. *P. syringae* pv. *glycinea* F117, were able to cause pathovar-unique symptoms in tomato lacking *Pto* or *Prf*. Although bacterial growth and symptom severity were reduced relative to those observed with *P. syringae* pv. *tomato* DC3000, this finding highlights the potential importance of Pto and Prf in the nonhost resistance of tomato against multiple *P. syringae* pathovars, as well as the importance of pathogen factors in the production of pathovar-unique symptoms (Lin and Martin, 2007). The role of ETI in the nonhost resistance of solanaceous plants is also supported by the observation that compromising ETI in *N. benthamiana* plants through virus-induced gene silencing of *NbSGT1* results in a 100-fold higher growth of nonadapted *P. syringae* pv. *maculicola* (Peart *et al*., 2002). Similarly, multiple plant species in different families produce necrotic responses suggestive of ETI during *Agrobacterium*-mediated transient expression of T3Es from *P. syringae* strains nonadapted for the test plant more frequently than with T3Es from adapted strains (Wroblewski *et al*., 2009).

However, a simple model of nonhost resistance based on 'correctable' incompatibilities at the ETI level may not be universally applicable. The nonhost resistance of Arabidopsis to *P. syringae* pv. *tomato* T1 was not defeated despite the use of Arabidopsis mutants compromised in ETI. Among the T3Es present in T1, but not DC3000, is AvrRpt2, which elicits an RPS2-dependent HR in Arabidopsis (Almeida *et al*., 2009). Strain T1 no longer elicited HR in an *rps2-101c* mutant line of Arabidopsis, but it also did not grow, thus revealing an underlying capacity for type I nonhost resistance against T1. Interestingly, Arabidopsis lines with mutations in *RAR1* and *SGT1*, which are needed for ETI mediated by many T3E–*R* gene combinations, also retained resistance to T1. This raises the possibility that Arabidopsis possesses a novel mechanism for type I nonhost resistance against T1 that is independent of ETI.

The multifactorial nature of nonhost resistance is particularly evident with the type I resistance of Arabidopsis to *P. syringae*

pv. *phaseolicola*, because growth of the bacteria can be partially promoted by a variety of manipulations. These include the mutation of a single plant defence gene, such as *NHO1* (Lu *et al*., 2001), or inoculation of *P. syringae* pv. *phaseolicola* strains heterologously expressing HopM1 (a known suppressor of PTI normally not produced by this pathovar) into Arabidopsis mutants with multiple defence gene mutations, such as *pmr4*/*pad4* or *pmr4*/*npr1* (Ham *et al*., 2007). Importantly, co-inoculation of *P. syringae* pv. *tomato* DC3000 suppressed defences elicited by *P. syringae* pv. *phaseolicola* and promoted strong growth of the latter strain (Ham *et al*., 2007). The simplest explanation for this observation is that T3Es in the DC3000 repertoire suppress the weak ETI elicited by the *P. syringae* pv. *phaseolicola* repertoire. The availability of a growing collection of Arabidopsis defence mutants and comprehensive T3E repertoires for DC3000 and two contrasting nonadapted pathogens, *P. syringae* pv. *phaseolicola* 1448A and *P. syringae* pv. *tomato* T1, provides an excellent toolkit for exploring the basis for nonhost resistance in a model plant. Similarly, strains closely related to *P. syringae* pv. *tomato* DC3000 but differing in host specificity and performance in the field provide tools for exploring host specialization in the field using a model pathogen (Yan *et al*., 2008).

NONHOST RESISTANCE EVOLUTION AND DURABLE DISEASE RESISTANCE

It is paradoxical that host specificity at the pathovar–species level appears so stable given the flexible genetics of *P. syringae* T3E repertoires and the demonstration that the loss of a single effector can enable a model laboratory strain to cause disease in a new plant species (Wei *et al*., 2007). The explanation for this paradox may reside in the ubiquity of *P. syringae* in the environment and the diverse interactions of *P. syringae* with plants in the field (Hirano and Upper, 2000; Morris *et al*., 2008). Because of these ecological factors, clonal groups within *P. syringae* are likely to have ongoing opportunities to interact with many plants. Importantly, competition among such groups may drive host specialization through subtle adaptations for life in different plant species. Strains adapted to a particular species would then be under ongoing selection pressure to maintain T3E repertoires that are compatible with their host *R* gene surveillance systems, whereas less adapted (and therefore less competitive) strains could accumulate incompatibilities in their T3E repertoires without relevant penalty. As discussed above, this model suggests that the defeat of PTI is ancient and that any given T3E repertoire will have a basic ability to suppress PTI in a variety of plants. Therefore, host specialization may be driven primarily by the accumulation of many factors that contribute quantitatively to fitness, but a consequence of this process will be the evolution of T3E repertoires that may trigger qualitative nonhost resistance in a myriad of other species.

The quantitative factors driving host adaptation could involve T3E-mediated PTI suppression and/or factors unrelated to T3Es. Importantly, no single T3SS-delivered *P. syringae* effector has been shown to be sufficient to confer substantial growth and symptom production to a nonpathogen (Huang *et al*., 1988; Mohr *et al*., 2008), and neither the minimal set of T3Es needed for pathogenesis nor the basis for needing multiple T3Es has been determined. One key to the exploration of quantitative factors underlying host adaptation is to eliminate qualitative ETI from the experimental system, which otherwise masks weaker interaction phenotypes. One promising approach involves the exploitation of natural variation in the susceptibility of Arabidopsis ecotypes to *P. syringae* pv. *tomato* DC3000 to identify quantitative trait loci underlying basal defence (Fan *et al*., 2008). An understanding of these factors may have practical benefits. Quantitative disease resistance in crops shares with nonhost resistance the properties of being multifactorial and usefully durable, but the underlying basis for quantitative resistance is poorly understood (Poland *et al*., 2009). Our increasing capacity to deconvolute the diverse interactions of *P. syringae* with model plants and crops may provide new tools for improving quantitative resistance and for breeding crops with durable resistance.

ACKNOWLEDGEMENTS

Research in our programmes is supported by National Science Foundation grant DBI-0605059.

REFERENCES

Abramovitch, R.B., Anderson, J.C. and Martin, G.B. (2006) Bacterial elicitation and evasion of plant innate immunity. *Nat. Rev. Mol. Cell Biol.* **7**, 601–611.

Alfano, J.R. and Collmer, A. (1996) Bacterial pathogens in plants: life up against the wall. *Plant Cell*, **8**, 1683–1698.

Alfano, J.R. and Collmer, A. (2004) Type III secretion system effector proteins: double agents in bacterial disease and plant defense. *Annu. Rev. Phytopathol.* **42**, 385–414.

Almeida, N.F., Yan, S., Lindeberg, M., Studholme, D.J., Schneider, D.J., Condon, B., Liu, H., Viana, C.J., Warren, A., Evans, C., Kemen, E., MacLean, D., Angot, A., Martin, G.B., Jones, J.D., Collmer, A., Setubal, J.C. and Vinatzer, B.A. (2009) A draft genome sequence of *Pseudomonas syringae* pv. *tomato* strain T1 reveals a repertoire of Type III related genes significantly divergent from that of *Pseudomonas syringae* pv. *tomato* strain DC3000. *Mol. Plant–Microbe Interact.* **22**, 52–62.

Aslam, S.N., Newman, M.A., Erbs, G., Morrissey, K.L., Chinchilla, D., Boller, T., Jensen, T.T., De Castro, C., Ierano, T., Molinaro, A., Jackson, R.W., Knight, M.R. and Cooper, R.M. (2008) Bacterial polysaccharides suppress induced innate immunity by calcium chelation. *Curr. Biol.* **18**, 1078–1083.

Boller, T. and Felix, G. (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* **60**, 379–406.

Buell, C.R., Joardar, V., Lindeberg, M., Selengut, J., Paulsen, I.T.,

Gwinn, M.L., Dodson, R.J., Deboy, R.T., Durkin, A.S., Kolonay, J.F., Madupu, R., Daugherty, S., Brinkac, L., Beanan, M.J., Haft, D.H., Nelson, W.C., Davidsen, T., Liu, J., Yuan, Q., Khouri, H., Fedorova, N., Tran, B., Russell, D., Berry, K., Utterback, T., Vanaken, S.E., Feldblyum, T.V., D'Ascenzo, M., Deng, W.-L., Ramos, A.R., Alfano, J.R., Cartinhour, S., Chatterjee, A.K., Delaney, T.P., Lazarowitz, S.G., Martin, G.B., Schneider, D.J., Tang, X., Bender, C.L., White, O., Fraser, C.M. and Collmer, A. (2003) The complete sequence of the Arabidopsis and tomato pathogen *Pseudomonas syringae* pv. *tomato* DC3000. *Proc. Natl. Acad. Sci. USA*, **100**, 10 181–10 186.

Caldwell, K.S. and Michelmore, R.W. (2009) *Arabidopsis thaliana* genes encoding defense signaling and recognition proteins exhibit contrasting evolutionary dynamics. *Genetics*, **181**, 671–684.

Chang, J.H., Urbach, J.M., Law, T.F., Arnold, L.W., Hu, A., Gombar, S., Grant, S.R., Ausubel, F.M. and Dangl, J.L. (2005) A high-throughput, near-saturating screen for type III effector genes from *Pseudomonas syringae*. *Proc. Natl. Acad. Sci. USA*, **102**, 2549–2554.

Clark, R.M., Schweikert, G., Toomajian, C., Ossowski, S., Zeller, G., Shinn, P., Warthmann, N., Hu, T.T., Fu, G., Hinds, D.A., Chen, H., Frazer, K.A., Huson, D.H., Scholkopf, B., Nordborg, M., Ratsch, G., Ecker, J.R. and Weigel, D. (2007) Common sequence polymorphisms shaping genetic diversity in *Arabidopsis thaliana*. *Science*, **317**, 338–342.

Cornelis, G.R. and Van Gijsegem, F. (2000) Assembly and function of type III secretory systems. *Annu. Rev. Microbiol.* **54**, 735–774.

Cunnac, S., Lindeberg, M. and Collmer, A. (2009) *Pseudomonas syringae* type III secretion system effectors: repertoires in search of functions. *Curr. Opin. Microbiol.* **12**, 53–60.

Eitas, T.K., Nimchuk, Z.L. and Dangl, J.L. (2008) *Arabidopsis* TAO1 is a TIR-NB-LRR protein that contributes to disease resistance induced by the *Pseudomonas syringae* effector AvrB. *Proc. Natl. Acad. Sci. USA*, **105**, 6475–6480.

Fan, J., Crooks, C. and Lamb, C. (2008) High-throughput quantitative luminescence assay of the growth in planta of *Pseudomonas syringae* chromosomally tagged with *Photorhabdus luminescens* luxCDABE. *Plant J.* **53**, 393–399.

Feil, H., Feil, W.S., Chain, P., Larimer, F., Dibartolo, G., Copeland, A., Lykidis, A., Trong, S., Nolan, M., Goltsman, E., Thiel, J., Malfatti, S., Loper, J.E., Lapidus, A., Detter, J.C., Land, M., Richardson, P.M., Kyrpides, N.C., Ivanova, N. and Lindow, S.E. (2005) Comparison of the complete genome sequences of *Pseudomonas syringae* pv. *syringae* B728a and pv. *tomato* DC3000. *Proc. Natl. Acad. Sci. USA*, **102**, 11 064–11 069.

Gohre, V. and Robatzek, S. (2008) Breaking the barriers: microbial effector molecules subvert plant immunity. *Annu. Rev. Phytopathol.* **46**, 189–215.

Guo, M., Tian, F., Wamboldt, Y. and Alfano, J.R. (2009) The majority of the type III effector inventory of *Pseudomonas syringae* pv. *tomato* DC3000 can suppress plant immunity. *Mol. Plant–Microbe Interact.* **22**, 1069–1080.

Ham, J.H., Kim, M.G., Lee, S.Y. and Mackey, D. (2007) Layered basal defenses underlie non-host resistance of Arabidopsis to *Pseudomonas syringae* pv. *phaseolicola*. *Plant J.* **51**, 604–616.

Heath, M.C. (2000) Nonhost resistance and nonspecific plant defenses. *Curr. Opin. Plant Biol.* **3**, 315–319.

Hendson, M., Hildebrand, D.C. and Schroth, M.N. (1992) Relatedness of *Pseudomonas syringae* pv. *tomato, Pseudomonas syringae* pv. *maculicola* and *Pseudomonas syringae* pv. *antirrhini*. *J. Appl. Bacteriol.* **73**, 455–464.

Hirano, S.S. and Upper, C.D. (1990) Population biology and epidemiology of *Pseudomonas syringae*. *Annu. Rev. Phytopathol.* **28**, 155–177.

Hirano, S.S. and Upper, C.D. (2000) Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*—a pathogen, ice nucleus, and epiphyte. *Microbiol. Mol. Biol. Rev.* **64**, 624–653.

Hogenhout, S.A., Van der Hoorn, R.A., Terauchi, R. and Kamoun, S. (2009) Emerging concepts in effector biology of plant-associated organisms. *Mol. Plant–Microbe Interact.* **22**, 115–122.

van der Hoorn, R.A. and Kamoun, S. (2008) From Guard to Decoy: a new model for perception of plant pathogen effectors. *Plant Cell*, **20**, 2009–2017.

Huang, H.-C., Schuurink, R., Denny, T.P., Atkinson, M.M., Baker, C.J., Yucel, I., Hutcheson, S.W. and Collmer, A. (1988) Molecular cloning of a *Pseudomonas syringae* pv. *syringae* gene cluster that enables *Pseudomonas fluorescens* to elicit the hypersensitive response in tobacco plants. *J. Bacteriol.* **170**, 4748–4756.

Huynh, T.V., Dahlbeck, D. and Staskawicz, B.J. (1989) Bacterial blight of soybean: regulation of a pathogen gene determining host cultivar specificity. *Science*, **245**, 1374–1377.

Joardar, V., Lindeberg, M., Jackson, R.W., Selengut, J., Dodson, R., Brinkac, L.M., Daugherty, S.C., DeBoy, R., Durkin, A.S., Giglio, M.G., Madupu, R., Nelson, W.C., Rosovitz, M.J., Sullivan, S., Haft, D.H., Creasy, T., Davidsen, T., Zafar, N., Zhou, L., Halpin, R., Holley, T., Khouri, H., Feldblyum, T., White, O., Fraser, C.M., Chatterjee, A.K., Cartinhour, S., Schneider, D.J., Mansfield, J., Collmer, A. and Buell, C.R. (2005) Whole genome sequence analysis of *Pseudomonas syringae* pv. *phaseolicola* 1448A reveals sequence divergence among pathovars in genes involved in virulence and mobile genetic elements. *J. Bacteriol.* **187**, 6488–6498.

Jones, J.D. and Dangl, J.L. (2006) The plant immune system. *Nature*, **444**, 323–329.

Keen, N.T. (1990) Gene-for-gene complementarity in plant–pathogen interactions. *Annu. Rev. Genet.* **24**, 447–463.

Kim, S.H., Kwon, S.I., Saha, D., Anyanwu, N.C. and Gassmann, W. (2009) Resistance to the *Pseudomonas syringae* effector HopA1 is governed by the TIR-NBS-LRR protein RPS6 and is enhanced by mutations in SRFR1. *Plant Physiol.* **150**, 1723–1732.

Klement, Z. (1963) Rapid detection of pathogenicity of phytopathogenic pseudomonads. *Nature*, **199**, 299–300.

Klement, Z., Farkas, G.L. and Lovrekovich, L. (1964) Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. *Phytopathology*, **54**, 474–477.

Kobayashi, D.Y., Tamaki, S.J. and Keen, N.T. (1989) Cloned avirulence genes from the *tomato* pathogen *Pseudomonas syringae* pv. *tomato* confer cultivar specificity on soybean. *Proc. Natl. Acad. Sci. USA*, **86**, 157–161.

Kvitko, B.H., Park, D.H., Velásquez, A.C., Wei, C.-F., Russell, A.B., Martin, G.B., Schneider, D.J. and Collmer, A. (2009) Deletions in the repertoire of *Pseudomonas syringae* pv. *tomato* DC3000 type III secretion effector genes reveal functional overlap among effectors. *PLoS Pathog.* **5**, e1000388.

Lin, N.-C. and Martin, G.B. (2007) Pto/Prf-mediated recognition of AvrPto and AvrPtoB restricts the ability of diverse *Pseudomonas syringae* pathovars to infect tomato. *Mol. Plant–Microbe Interact.* **20**, 806–815.

Lindeberg, M., Cartinhour, S., Myers, C.R., Schechter, L.M.,

Schneider, D.J. and Collmer, A. (2006) Closing the circle on the discovery of genes encoding Hrp regulon members and type III secretion system effectors in the genomes of three model *Pseudomonas syringae* strains. *Mol. Plant–Microbe Interact.* **19**, 1151–1158.

Lindeberg, M., Myers, C., Collmer, A. and Schneider, D. (2008) Roadmap to new virulence determinants in *Pseudomonas syringae*: insights from comparative genomics and genome organization. *Mol. Plant–Microbe Interact.* **21**, 685–700.

Lindgren, P.B., Peet, R.C. and Panopoulos, N.J. (1986) Gene cluster of *Pseudomonas syringae* pv. '*phaseolicola*' controls pathogenicity of bean plants and hypersensitivity on nonhost plants. *J. Bacteriol.* **168**, 512–522.

Lorang, J.M., Shen, H., Kobayashi, D., Cooksey, D. and Keen, N.T. (1994) *avrA* and *avrE* in *Pseudomonas syringae* pv. *tomato* PT23 play a role in virulence on tomato plants. *Mol. Plant–Microbe Interact.* **7**, 508–515.

Lu, M., Tang, X. and Zhou, J.M. (2001) Arabidopsis *NHO1* is required for general resistance against *Pseudomonas* bacteria. *Plant Cell*, **13**, 437–447.

Ma, W. and Guttman, D.S. (2008) Evolution of prokaryotic and eukaryotic virulence effectors. *Curr. Opin. Plant Biol.* **11**, 412–419.

Ma, W., Dong, F.F., Stavrinides, J. and Guttman, D.S. (2006) Type III effector diversification via both pathoadaptation and horizontal transfer in response to a coevolutionary arms race. *PLoS Genet.* **2**, e209.

Mishina, T.E. and Zeier, J. (2007) Bacterial non-host resistance: interactions of Arabidopsis with non-adapted *Pseudomonas syringae* strains. *Physiol. Plant.* **131**, 448–461.

Mohr, T.J., Liu, H., Yan, S., Morris, C.E., Castillo, J.A., Jelenska, J. and Vinatzer, B.A. (2008) Naturally occurring non-pathogenic isolates of the plant pathogen species *Pseudomonas syringae* lack a type III secretion system and effector gene orthologues. *J. Bacteriol.* **190**, 2858–2870.

Morris, C.E., Sands, D.C., Vinatzer, B.A., Glaux, C., Guilbaud, C., Buffiere, A., Yan, S., Dominguez, H. and Thompson, B.M. (2008) The life history of the plant pathogen *Pseudomonas syringae* is linked to the water cycle. *ISME J.* **2**, 321–334.

Mysore, K.S. and Ryu, C.-M. (2004) Nonhost resistance: how much do we know? *Trends Plant Sci.* **9**, 97–104.

Naito, K., Taguchi, F., Suzuki, T., Inagaki, Y., Toyoda, K., Shiraishi, T. and Ichinose, Y. (2008) Amino acid sequence of bacterial microbe-associated molecular pattern flg22 is required for virulence. *Mol. Plant–Microbe Interact.* **21**, 1165–1174.

Navarro, L., Zipfel, C., Rowland, O., Keller, I., Robatzek, S., Boller, T. and Jones, J.D. (2004) The transcriptional innate immune response to flg22. Interplay and overlap with Avr gene-dependent defense responses and bacterial pathogenesis. *Plant Physiol.* **135**, 1113–1128.

Nomura, K., Debroy, S., Lee, Y.H., Pumplin, N., Jones, J. and He, S.Y. (2006) A bacterial virulence protein suppresses host innate immunity to cause plant disease. *Science*, **313**, 220–223.

Nomura, K., Melotto, M. and He, S.Y. (2005) Suppression of host defense in compatible plant–*Pseudomonas syringae* interactions. *Curr. Opin. Plant Biol.* **8**, 361–368.

Peart, J.R., Lu, R., Sadanandom, A., Malcuit, I., Moffett, P., Brice, D.C., Schauser, L., Jaggard, D.A., Xiao, S., Coleman, M.J., Dow, M., Jones, J.D., Shirasu, K. and Baulcombe, D.C. (2002) Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. *Proc. Natl. Acad. Sci. USA*, **99**, 10 865–10 869.

Pitman, A.R., Jackson, R.W., Mansfield, J.W., Kaitell, V., Thwaites, R. and Arnold, D.L. (2005) Exposure to host resistance mechanisms drives evolution of bacterial virulence in plants. *Curr. Biol.* **15**, 2230–2235.

Poland, J.A., Balint-Kurti, P.J., Wisser, R.J., Pratt, R.C. and Nelson, R.J. (2009) Shades of gray: the world of quantitative disease resistance. *Trends Plant Sci.* **14**, 21–29.

Puri, N., Jenner, C., Bennet, M., Stewart, R., Mansfield, J., Lyons, N. and Taylor, J. (1997) Expression of *avrPphB*, an avirulence gene from *Pseudomonas syringae* pv. *phaseolicola*, and the delivery of signals causing the hypersensitive reaction in bean. *Mol. Plant–Microbe Interact.* **10**, 247–256.

Rosebrock, T.R., Zeng, L., Brady, J.J., Abramovitch, R.B., Xiao, F. and Martin, G.B. (2007) A bacterial E3 ubiquitin ligase targets a host protein kinase to disrupt plant immunity. *Nature*, **448**, 370–374.

Sarkar, S.F. and Guttman, D.S. (2004) Evolution of the core genome of *Pseudomonas syringae*, a highly clonal, endemic plant pathogen. *Appl. Environ. Microbiol.* **70**, 1999–2012.

Sawada, H., Suzuki, F., Matsuda, I. and Saitou, N. (1999) Phylogenetic analysis of *Pseudomonas syringae* pathovars suggests the horizontal gene transfer of *argK* and the evolutionary stability of *hrp* gene cluster. *J. Mol. Evol.* **49**, 627–644.

Schwartz, H.F., Steadman, J.R., Hall, R. and Forster, R.L. (2005) *Compendium of Bean Diseases*, 2nd edn. St. Paul, MN: APS Press.

Shan, L., He, P., Li, J., Heese, A., Peck, S.C., Nurnberger, T., Martin, G.B. and Sheen, J. (2008) Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. *Cell Host. Microbe.* **4**, 17–27.

Shimizu, R., Taguchi, F., Marutani, M., Mukaihara, T., Inagaki, Y., Toyoda, K., Shiraishi, T. and Ichinose, Y. (2003) The D*fliD* mutant of *Pseudomonas syringae* pv. *tabaci*, which secretes flagellin monomers, induces a strong hypersensitive reaction (HR) in non-host tomato cells. *Mol. Genet. Genomics*, **269**, 21–30.

Staskawicz, B.J., Dahlbeck, D. and Keen, N.T. (1984) Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race specific incompatibility on *Glycine max* (L.) Merr. *Proc. Natl. Acad. Sci. USA*, **81**, 6024–6028.

Stavrinides, J., Ma, W. and Guttman, D.S. (2006) Terminal reassortment drives the quantum evolution of type III effectors in bacterial pathogens. *PLoS Pathog.* **2**, e104.

Stavrinides, J., McCann, H.C. and Guttman, D.S. (2008) Host–pathogen interplay and the evolution of bacterial effectors. *Cell Microbiol.* **10**, 285–292.

Stevens, C., Bennett, M.A., Athanassopoulos, E., Tsiamis, G., Taylor, J.D. and Mansfield, J.W. (1998) Sequence variations in alleles of the avirulence gene *avrPphE.R2* from *Pseudomonas syringae* pv. *phaseolicola* lead to loss of recognition of the AvrPphE protein within bean cells and a gain in cultivar-specific virulence. *Mol. Microbiol.* **29**, 165–1677.

Tamaki, S.J., Kobayashi, D.Y. and Keen, N.T. (1991) Sequence domains required for the activity of avirulence genes *avrB* and *avrC* from *Pseudomonas syringae* pv. *glycinea*. *J. Bacteriol.* **173**, 301–307.

Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H.S., Han, B., Zhu, T., Zou, G. and Katagiri, F. (2003) Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. *Plant Cell*, **15**, 317–330.

Thilmony, R., Underwood, W. and He, S.Y. (2006) Genome-wide transcriptional analysis of the *Arabidopsis thaliana* interaction with the

plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000 and the human pathogen *Escherichia coli* O157:H7. *Plant J.* **46**, 34–53.

Vinatzer, B.A. and Greenberg, J.T. (2007) Whole-genome analysis to identify type III-secreted effectors. *Methods Mol. Biol.* **354**, 19–34.

Wei, C.-F., Kvitko, B.H., Shimizu, R., Crabill, E., Alfano, J.R., Lin, N.-C., Martin, G.B., Huang, H.-C. and Collmer, A. (2007) A *Pseudomonas syringae* pv. *tomato* DC3000 mutant lacking the type III effector HopQ1-1 is able to cause disease in the model plant *Nicotiana benthamiana*. *Plant J.* **51**, 32–46.

Whalen, M.C., Innes, R.W., Bent, A.F. and Staskawicz, B.J. (1991) Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both Arabidopsis and soybean. *Plant Cell*, **3**, 49–59.

Wroblewski, T., Caldwell, K.S., Piskurewicz, U., Cavanaugh, K.A., Xu, H., Kozik, A., Ochoa, O., McHale, L.K., Lahre, K., Jelenska, J., Castillo, J.A., Blumenthal, D., Vinatzer, B.A., Greenberg, J.T. and Michelmore, R.W. (2009) Comparative large-scale analysis of interactions between several crop species and the effector repertoires from multiple pathovars of *Pseudomonas* and *Ralstonia*. *Plant Physiol.* **150**, 1733–1749.

Xiang, T., Zong, N., Zou, Y., Wu, Y., Zhang, J., Xing, W., Li, Y., Tang, X., Zhu, L., Chai, J. and Zhou, J.M. (2008) *Pseudomonas syringae* effector AvrPto blocks innate immunity by targeting receptor kinases. *Curr. Biol.* **18**, 74–80.

Yan, S., Liu, H., Mohr, T.J., Jenrette, J., Chiodini, R., Zaccardelli, M., Setubal, J.C. and Vinatzer, B.A. (2008) Role of recombination in the evolution of the model plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000, a very atypical tomato strain. *Appl. Environ. Microbiol.* **74**, 3171–3181.

Zhou, J.M. and Chai, J. (2008) Plant pathogenic bacterial type III effectors subdue host responses. *Curr. Opin. Microbiol.* **11**, 179–185.

