# Nonhost resistance to *Phytophthora*: novel prospects for a classical problem

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Members of the oomycete genus *Phytophthora* are the most devastating pathogens of dicot plants. Recent developments in the study of these organisms have led to improved understanding of their phylogenetic relationships and trends in their evolution. Molecular analyses of nonhost (species-level) resistance offer exciting prospects for disease management. A model that evokes a complex interplay of several layers of specific resistance, mediated by a set of ancient broad-spectrum *R*-gene loci, is sufficient to explain existing cellular and molecular data on nonhost resistance to *Phytophthora*.

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**Abbreviations**

| | |
|---|---|
| ***Avr*** | avirulence |
| **HR** | hypersensitive response |
| ***R*** | resistance |

## Introduction

Oomycetes, such as *Phytophthora*, downy-mildews and *Pythium*, form a unique branch of eukaryotic plant pathogens with an independent evolutionary history [1•]. Among the oomycetes, *Phytophthora* species cause some of the most destructive plant diseases in the world [2,3]. For example, *Phytophthora infestans*, the cause of the Irish potato famine, remains a destructive pathogen that is responsible for multibillion-dollar losses in potato and tomato production [4,5]. Other economically important *Phytophthora* diseases include root and stem rot, caused by *Phytophthora sojae*, which hampers soybean production in several continents; black pod of cocoa, caused by *Phytophthora palmivora* and *Phytophthora megakarya*, a recurring threat to chocolate production; and sudden oak death, caused by a recently discovered *Phytophthora* species, which is decimating oak trees along the Pacific coast of the United States. Typically, these diseases are difficult to manage and sources of sustainable genetic resistance are limited.

Several *Phytophthora* species exhibit high degrees of specialization and can only infect a limited number of species. For example, most plants are resistant to the strains of *P. infestans* that infect potato and tomato [1•,6]. This phenomenon of nonhost resistance, the ability of a pathogen to cause a disease in particular plant species but not in others, has always intrigued plant pathologists but remains poorly understood [1•,7–9]. In recent years, there has been renewed interest in examining interactions between nonhost plants and oomycetes. In this review, we examine recent developments in understanding the basic pathogenicity and host specialization of *Phytophthora* and discuss three nonhost pathosystems: parsley, *Nicotiana*, and *Arabidopsis*.

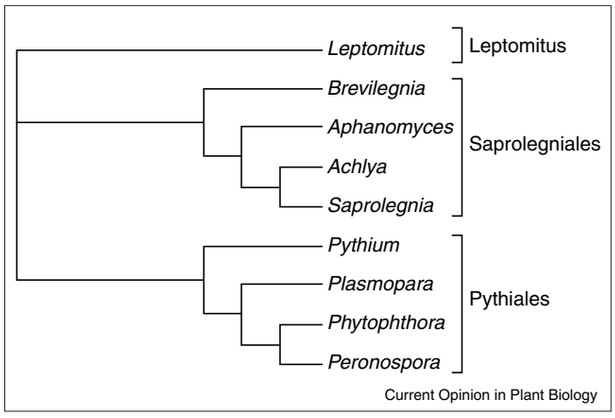
## Evolutionary trends in *Phytophthora*

The oomycetes represent a diverse group of organisms that includes pathogens of plants and animals, as well as saprophytic species (i.e. water molds) [10]. The position of the oomycetes as a unique lineage of stramenopile eukaryotes, unrelated to true fungi but closely related to heterokont (i.e. brown) algae, has been well established using molecular phylogenies that are based on ribosomal RNA (rRNA) sequences [11–13]. Recently, these findings received additional support from phylogenetic analyses of compiled amino-acid data for mitochondrial proteins [14] and of four protein-encoding chromosomal genes [15]. From these analyses, it is evident that oomycetes evolved the ability to infect plants independently of other eukaryotic plant pathogens and are likely to have unique mechanisms for doing so.

Several recent studies have examined phylogenetic relationships within the oomycetes and contributed to our understanding of their host specialization and pathogenicity [16•,17,18]. The ability to infect plants has evolved at least twice in the oomycete lineage (Figure 1). Acquisition of plant pathogenicity probably occurred early in the ancient monophyletic group Pythiales, which is comprised of the majority of plant-pathogenic genera of oomycetes including *Phytophthora*. More recently, a group of plant pathogens emerged within the genus *Aphanomyces*. These species are distantly related to the Pythiales and closely related to animal pathogens. Therefore, members of the Pythiales appear to have acquired the ability to infect plants early in the evolution of oomycetes and subsequently radiated into the diverse modern forms, whereas *Aphanomyces* species appear to have emerged as plant pathogens more recently and independently. In the future, genomic analyses comparing Pythiales species and *Apahanomyces*, as well as plant and animal pathogenic oomycetes, should help to define the basic set of pathogenicity genes that allow oomycetes to infect dicot plants.

Cooke and collaborators [19••] examined the phylogenetic relationships among 50 species of *Phytophthora* and related oomycetes using internal transcribed spacer (ITS) sequences of genomic ribosomal DNA (rDNA). These analyses revealed that *Phytophthora* form a highly diverse but monophyletic group. Interestingly, the biotrophic and

**Figure 1**




Phylogenetic tree illustrating the evolutionary relationships among the major genera of oomycetes. Lineages that include plant pathogenic species are indicated with a thick line. Note that plant pathogenesis has probably emerged at least twice during the evolution of oomycetes. First, in an ancient lineage that diverged into the Pythiales (including *Pythium*, *Phytophthora* and the downy mildews). Second, in a lineage that includes *Aphanomyces* species.

host-specific downy mildew pathogens of the genus *Peronospora* appear to have evolved from a *Phytophthora* lineage, suggesting that biotrophy is a derived character in the *Phytophthora*/*Peronospora* clade, and that the evolutionary trend is towards host specialization. In *Phytophthora*, however, there seems to be significant flexibility in adaptation to unrelated hosts. For example, *P. infestans*, a pathogen of solanaceous plants, forms a monophyletic group with two other foliar pathogens, *Phytophthora mirabilis* and *Phytophthora phaseoli*, which infect *Mirabilis jalapa* (Nyctaginaceae) and lima beans (Leguminae), respectively [19••,20]. In addition, there are a number of recent reports of the infection of diverse solanaceous plants, such as wild *Solanum* species, petunia and *Nicotiana benthamiana*, by wild-type isolates of *P. infestans* [21,22]. This information, and older reports of *P. infestans* infection on plants from various botanical families [2], raises questions about the traditional view that this pathogen is strictly host-specific.

## Nonhost resistance to *Phytophthora*

The molecular basis of nonhost resistance remains one of the major unknowns in the study of plant–microbe interactions. Preformed barriers and compounds such as saponins are ubiquitous in plants and play important roles in nonhost resistance to filamentous fungi [23,24]. However, there is little evidence for a role for preformed barriers in resistance to oomycetes. Oomycete plant pathogens contain little or no membrane sterols, the target for toxic saponins [23], suggesting that they must have alternative distinct genetic and biochemical mechanisms for interacting with nonhost plants.

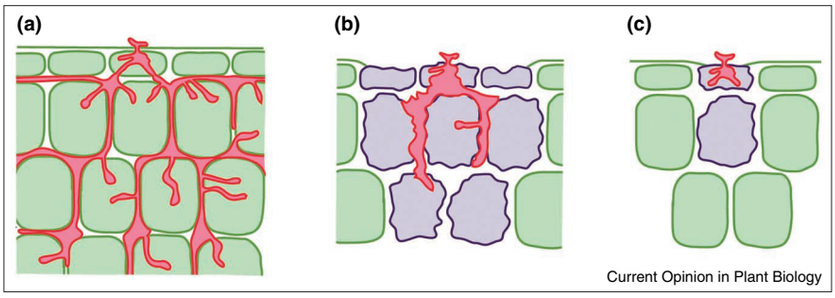
An important insight into the basis of nonhost resistance to *Phytophthora* has come from detailed cytological analyses that revealed penetration of epidermal cells by *P. infestans* in all examined interactions, including those with plant species unrelated to the solanaceous hosts [1•,25–28,29••] (Figure 2). Fully resistant plants, such as the nonhosts *Solanum nigrum*, parsley, tobacco, and *Arabidopsis thaliana*, display a typical localized hypersensitive response (HR), that is, a programmed cell death, at all infection sites [1•,6,26–28,29••]. The HR can be localized to a single epidermal cell or a group of cells surrounding the penetrating hyphae [1•,28,29••]. The view that has emerged from these studies is that the HR is associated with all known forms of genetic resistance to *P. infestans*, including nonhost resistance [1•]. Arguably, several *Phytophthora* species can be viewed as analogous to bacterial plant pathogens in that they systematically cause the HR in nonhost plants. Perhaps, phenotypic analogs of bacterial *hrp* (*hypersensitive response and pathogenicity*) mutants (which are deficient in both the induction of the HR on nonhost plants and pathogenicity on host plants) will be identified in oomycetes.

Contemporary models of nonhost resistance generally evoke several layers of specific resistance and nonspecific defense responses [1•,9,30•]. Specific resistance has been extensively studied in host pathosystems and typically follows Flor's gene-for-gene model [8], in which resistance is determined by the simultaneous expression of a pathogen avirulence (*Avr*) gene and the corresponding plant resistance (*R*) gene. The extent to which the gene-for-gene model applies to nonhost interactions remains unclear. A classical genetic approach to this problem is hampered by the absence of variation in plant resistance and pathogen virulence, as well as by sexual incompatibility between host and nonhost plants. In *Phytophthora*, a mechanism that involves gene-for-gene interactions is consistent with the ubiquitous association of the HR with nonhost resistance and the occurrence of species-specific elicitors, which can be viewed as *Avr* genes that function at the nonhost level. Nonhost resistance to *Phytophthora* could, therefore, be explained simply by the occurrence of an arsenal of *R* genes that recognize multiple or essential *Avr* genes [1•].

### The parsley–*Phytophthora* pathosystem

Parsley is a nonhost of *P. sojae* and *P. infestans*. Following inoculation with *Phytophthora*, parsley cells exhibit a complex and coordinated series of morphological and biochemical defense responses that culminate in HR cell death [27,31–33]. An extracellular 42-kiloDalton (kDa) glycoprotein elicitor from *P. sojae* or a 13-amino-acid oligopeptide (Pep-13) derived from this protein are sufficient to induce changes in plasma membrane permeability, an oxidative burst, activation of defense genes, and the accumulation of defense compounds [34]. Recently, the 42-kDa glycoprotein was shown to possess a calcium-dependent transglutaminase activity and to be highly conserved among ten *Phytophthora* species (T Nürnberger, personal communication). In addition, mutational analyses have revealed residues that are

**Figure 2**




Schematic view of early infection events during susceptible and resistant interactions between *Phytophthora infestans* and plants. Penetration of plant tissue is observed on all plants. **(a)** In susceptible plants, no visible defense responses occur. Secondary hyphae grow into the intercellular space, form haustoria (digit-like feeding structures) inside mesophyll cells, and rapidly colonize the mesophyll tissue. In resistant plants, cells display the HR. The dying hyphae of the pathogen are contained **(b)** within a group of dead plant cells or **(c)** within the penetrated epidermal cell, depending on the genotypes of the interacting plant and pathogen. Macroscopically, the HR lesions can be visible as brownish-black spots on leaves or may not be visible. In several nonhost plants, the HR is induced extremely quickly and is usually localized to one or two plant cells.

essential for both the elicitor and enzymatic activity, suggesting that mutations in the glycoprotein gene may result in loss of enzymatic activity and perhaps a fitness penalty for the pathogen (T Nürnberger, personal communication). Thus, nonhost resistance in parsley may be mediated by the recognition of an essential and conserved surface 'epitope' in *Phytophthora*.

The signal(s) that lead to HR cell death in parsley are now being unraveled. A 24-kDa elicitor protein that triggers phytoalexin production and cell death in parsley cells, as well as HR-like formation of lesions in parsley leaves, was recently identified in several *Phytophthora* species and may elicit nonhost resistance in this plant (T Nürnberger, personal communication). However, whether this protein functions as a species-specific elicitor remains unclear. The 24-kDa protein shares significant sequence similarity to a nonspecific necrosis-inducing protein from the fungal pathogen *Fusarium oxysporum* [35]. It has also been found to induce HR-like symptoms in several other plant species including *Nicotiana* and *Arabidopsis* (D Qutob, M Gijzen, S Kamoun, abstract 474, 21st Fungal Genetics Conference, Asilomar, California, March 2001; T Nürnberger, personal communication).

### The *Nicotiana–Phytophthora* pathosystem

In tobacco and other species of the genus *Nicotiana*, resistance to *P. infestans* is diverse and the HR varies in intensity depending on the plant species examined [28]. *P. infestans*, as well as other *Phytophthora* species, produces 10-kDa extracellular proteins known as elicitins, which induce the HR and other biochemical changes associated with defense responses specifically in *Nicotiana* [36–40], presumably after binding to a plasma-membrane receptor [41,42••]. *P. infestans* strains that are engineered to be deficient in the elicitin INF1 induce disease lesions on *Nicotiana benthamiana*, suggesting that INF1 functions as an *Avr* factor that conditions resistance in this species [28]. *N. benthamiana* does not fulfill the definition of a nonhost, however, as it is susceptible to a number of wild-type isolates of *P. infestans* that produce normal levels of INF1 (F Govers, personal communication; C Smart, WE Fry, personal communication). This suggests that other factors, in addition to the recognition of INF1, can alter the fine balance between disease and resistance in this interaction. Nevertheless, because *N. benthamiana* is highly amenable to high-throughput functional assays using virus-induced gene silencing (VIGS) [43], this pathosystem holds great promise for dissecting elicitor response and resistance to *Phytophthora* (I Malcuit, D Baulcombe, personal communication).

The genetic basis of *Nicotiana* resistance to *P. infestans*, like the phenotypic expression of this resistance, could be diverse. *P. infestans* strains that are deficient in INF1 remain unable to infect most *Nicotiana* species, such as tobacco. In this case, tobacco may react to additional elicitors, such as other members of the complex family of elicitin-like proteins of *P. infestans* [44,45•]. Indeed, the elicitin-like proteins INF2, INF4, INF5, and INF6 induce various degrees of HR symptoms in *Nicotiana* (E Huitema, S Kamoun, unpublished data). In addition, several novel *P. infestans* genes, which encode proteins that are unrelated to elicitins, have been shown to trigger HR-like symptoms in *Nicotiana* (T Torto, A Testa, S Kamoun, unpublished data). Overall, these results suggest that in *Nicotiana*, a complex genetic control, perhaps involving arrays of *R* genes with different specificities, could mediate nonhost resistance to *Phytophthora*.

### The *Arabidopsis–Phytophthora* pathosystem

Several biotrophic oomycetes, such as *Peronospora parasitica* and *Albugo candida*, are known to infect the model plant *Arabidopsis* [46–49]. However, the only *Phytophthora* known to infect *Arabidopsis* are the cabbage isolates of *Phytophthora porri* (A Si-Ammour, A Roetschi, B Mauch-Mani, F Mauch, abstract 183, 11th International Conference on *Arabidopsis* Research, Madison, Wisconsin,

June 2000). Most *Phytophthora* species, such as *P. infestans* and *P. sojae*, cannot infect *Arabidopsis* [1•,29••], and induce active defense responses involving a typical HR and upregulation of defense genes in this plant species (E Huitema, S Kamoun, unpublished data). With the completion of the *Arabidopsis* genome project, nonhost (*Phytophthora*) resistance genes have now been cloned and sequenced, and the challenge now is to identify them from the 26,000 or so genes present in the *Arabidopsis* genome. Considering the impressive set of functional genomics tools that are available, the *Arabidopsis* system offers good prospects for dissecting the complex interactions that take place between a nonhost plant and an oomycete pathogen.

## Conclusions

Nonhost resistance remains an unresolved issue in the study of plant–microbe interactions. Recent developments suggest, however, that multiple layers of gene-for-gene interactions form the initial defense barrier to *Phytophthora* in nonhost plants. This model raises the question of how nonhost plants evolved specific resistance to a wide range of pathogens. This issue is often perceived as puzzling because of the prevalence of the 'arms-race' hypothesis in most evolutionary interpretations of gene-for-gene interactions [50]. In this hypothesis, resistance alleles are proposed to evolve rapidly in response to adapting pathogens, a model that is unrealistic for most interactions between nonhosts and pathogens.

There is, however, increasing support for a 'trench-warfare' model, in which ancient resistance alleles evolve slowly and go through cycles of advances and retreats, sometimes described as a 'birth-and-death' process [51,52•,53]. Perhaps, nonhost resistance is mediated by a set of ancient broad-spectrum *R* gene loci that coevolved with pathogen populations following the trench-warfare model. In this scenario, a nonhost interaction would take place when a high frequency of *R*-gene alleles in a plant species is combined with a high frequency of the matching *Avr*-gene alleles in the pathogen. For example, most species of the genus *Nicotiana* recognize the ubiquitous elicitin proteins of *Phytophthora*, presumably through a family of ancient broad-spectrum *R* genes (E Huitema, S Kamoun, unpublished data).

Recently, *Arabidopsis* genes that confer broad-spectrum resistance to all tested strains of powdery mildew fungi were identified [54••]. In the future, the identification of similar broad-spectrum *R* genes that function against multiple *Phytophthora* isolates and species will have a tremendous impact on our fundamental understanding of nonhost resistance and on disease management. In addition, detailed dissection of the multiple layers of specific responses that form nonhost resistance to *Phytophthora* will be achieved through the identification of *Avr* genes that function at the nonhost level, the identification of susceptible or elicitor-insensitive mutants, and the characterization of signal transduction components that are involved in nonhost resistance.

## Acknowledgements

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- •• of outstanding interest

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