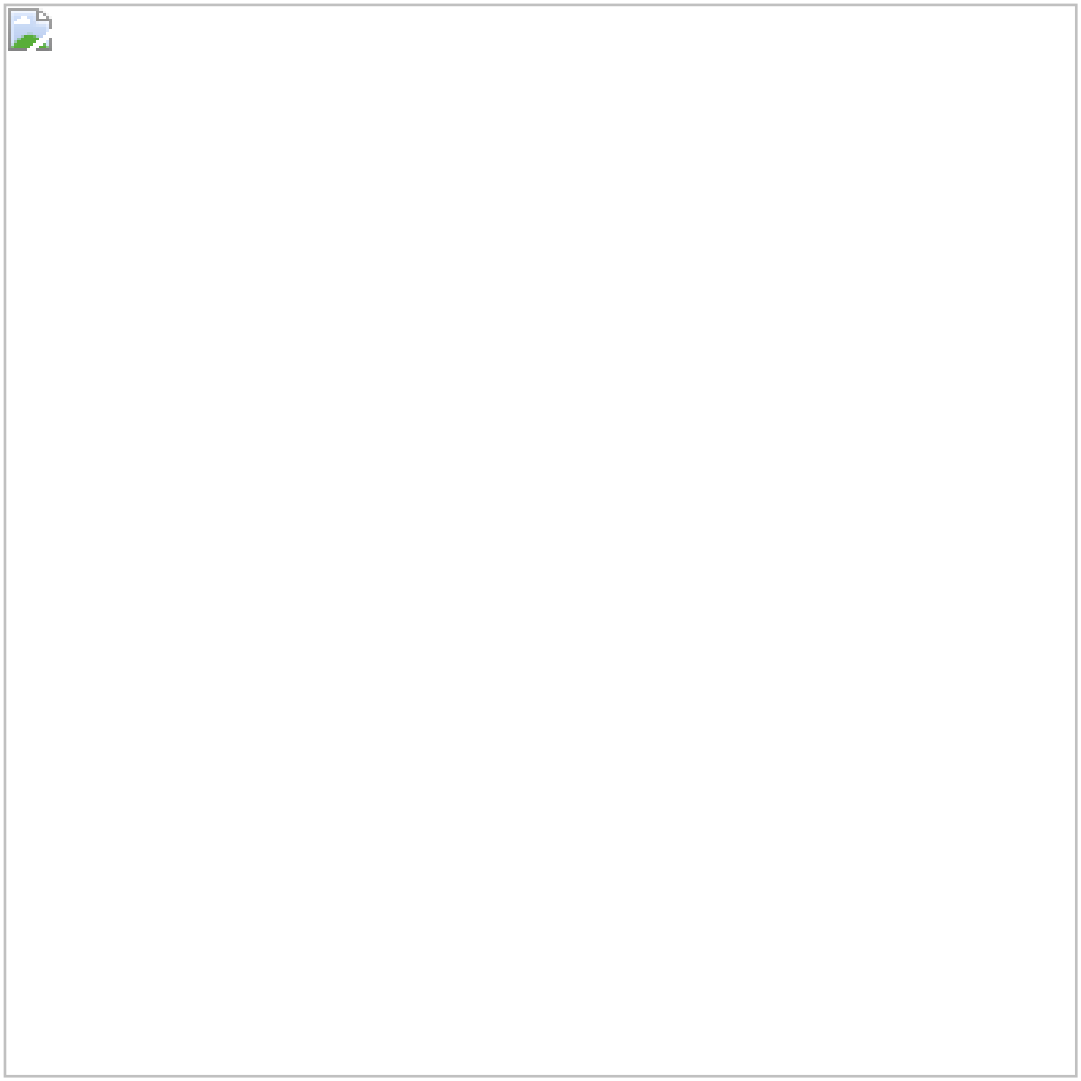

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Oomycetes Used in Arabidopsis Research

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Arabidopsis plants in their natural environment are susceptible to infection by oomycete pathogens, in particular to downy mildew and white rust diseases. These naturally occurring infectious agents have imposed evolutionary pressures on Arabidopsis populations and are therefore highly relevant for the study of host-pathogen co-evolution. In addition, the study of oomycete diseases, including infections caused by several *Phytophthora* species, has led to many scientific discoveries on Arabidopsis immunity and disease. Herein, we describe the major oomycete species used for experiments on Arabidopsis, and how these pathosystems have been used to provide significant insights into mechanistic and evolutionary aspects of plant-oomycete interactions. We also highlight understudied aspects of plant-oomycete interactions, as well as translational approaches, that can be productively addressed using the reference pathosystems described in this article.

INTRODUCTION TO OOMYCETES

Oomycetes are filamentous microorganisms that belong to the Stramenopile kingdom (Dick, 2001). Oomycetes are related to diatoms and brown algae and are proposed to have evolved from a photosynthetic ancestor (Tyler, 2006). Although oomycetes are known as “water molds”, many oomycete species are terrestrial and are pathogenic on animals and plants (Jiang and Tyler, 2012). Several oomycete species are among the most notorious plant pathogens and are known for their huge impact on agriculture and natural ecosystems (Kamoun et al., 2015). The potato late blight pathogen *Phytophthora infestans*, for instance, is the causal agent of the Irish potato famine, and *P. ramorum* is known for its devastation of oak trees and other plant species in natural ecosystems (Kamoun *et al*., 2015).

The phylogenetic relations of different genera of oomycete phytopathogens are shown in Figure 1. In the genus *Phytophthora* (Greek for plant destroyer) more than 100 species have been described that are grouped in 10 clades based on molecular and phenotypic data (Kroon *et al*., 2012). These pathogens infect a large range of plants on which they have a hemi-biotrophic life style. Initially the infection is biotrophic, during which the host is kept alive and pathogen and plant live in an intimate association. However, at a given colonization level these pathogens switch to a necrotrophic life style that includes the massive production of hydrolytic enzymes and toxins that kill host cells, leading to the typical blight and rot symptoms. In contrast, the downy mildew species have an obligate biotrophic lifestyle and cannot grow in the absence of a living host. These pathogens have often specialized on a single host plant species. More than 700 downy mildew species are known of which the phylogenetic relationships are still mostly unclear (Thines and Choi, 2016). The relatively close evolutionary relationship between downy mildews and *Phytophthora* species is reflected in them being present in a single order; the Peronosporales.

Two other orders of plant pathogenic oomycetes are outside of the Peronosporales; the Pythiales and Albuginales. Within the Pythiales the genus *Pythium* contains more than 100 species, most of which inhabit soils or aquatic environments. *Pythium* species are best known for causing damping-off of seedlings, for example caused by the necrotrophic *P. ultimum*, while many other *Pythium* species are considered opportunistic plant pathogens (Lévesque *et al*., 2010). Albuginales comprise an early diverged lineage of oomycetes that are obligate biotrophs, and contain plant pathogenic *Albugo* species causing white blister rust, like white rust of Arabidopsis caused by *A. laibachii* (Thines et al., 2009; Kemen et al., 2011).

OOMYCETES USED IN ARABIDOPSIS RESEARCH

In this section, we summarize experimental attributes of the oomycete species that are utilized for research involving Arabidopsis. We take a comparative approach that emphasizes similarities and differences in the life cycles and infection strategies of each species, along with their relative advantages and challeng-

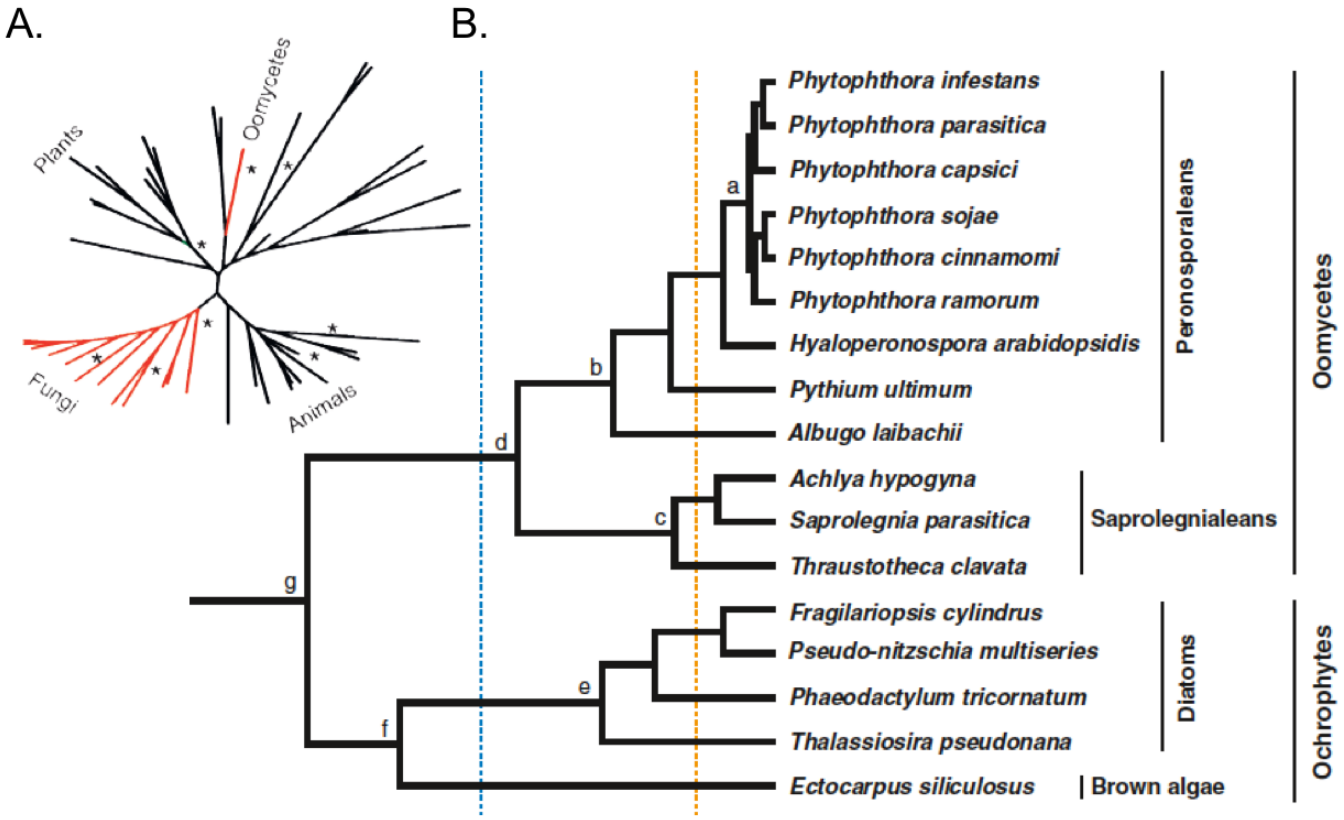

Figure 1. Evolutionary position of oomycetes used in Arabidopsis research.

(A) Phylogeny of oomycetes relative to the major kingdoms of life.
(B) Phylogeny of oomycetes used in Arabidopsis research and their relation to other Stramenopiles. Every species used for Arabidopsis research resides within the Peronosporaleans, within the orders Phytiales (e.g., *P. ultimum*), Albuginales (e.g., *A. laibacii*), or Peronosporales (*Phytophthora* spp. and *H. arabidopsidis*).

es as experimental systems. Figure 2 illustrates the life cycles of three species that encompass the diversity of oomycete infection strategies, and Table 1 summarizes key attributes of several long-used oomycete pathogens of Arabidopsis, along with others that have been adopted more recently.

Naturally occurring oomycete pathogens of Arabidopsis

In nature, *Arabidopsis thaliana* is commonly infected by two oomycete pathogen groups; the downy mildew pathogen *Hyaloperonospora arabidopsidis* (referred to as *Hpa* hereafter) and two species of the white rust pathogen *Albugo* (*A. laibachii* and *A. candida*). These naturally occurring pathogens have imposed evolutionary pressures on Arabidopsis populations that resulted in a high level of intraspecific variation in resistance (Holub and Beynon, 1996). Many of the underlying resistance genes have been cloned and their downstream signaling pathways have been characterized. Recently, several other eukaryotic microbes have been described as endophytes of Arabidopsis, including representatives of the oomycete genus *Pythium* (Durán et al., 2018; Sapp et al., 2018). Thus, it is likely that some oomycetes have a close interaction with their Arabidopsis host, but do not cause disease.

Hyaloperonospora arabidopsidis

This species was the first eukaryotic pathogen of Arabidopsis to be documented (Koch and Slusarenko, 1990). It was initially described as *Peronospora parasitica* (Koch and Slusarenko, 1990), and was later re-named as *Hyaloperonospora parasitica* (Constantinescu and Fatehi, 2002) and currently *Hyaloperonospora arabidopsidis* (Voglmayr et al., 2004; Goker et al., 2009). This species is a frequently occurring pathogen in natural Arabidopsis populations (Holub and Beynon, 1996; Holub, 2008). Moreover, the interactions are typified by abundant genetic polymorphism in the host and the pathogen (Holub et al., 1994b). For these reasons, *Hpa* was adopted as a reference pathogen during the early days of developing Arabidopsis as a system for molecular plant-microbe interactions (Dangl et al., 1992; Crute et al., 1994). These pioneering efforts substantially broadened the impact of Arabidopsis as a model system and are described in detail in several review articles (Holub and Beynon, 1996; Slusarenko and Schlaich, 2003; Coates and Beynon, 2010; McDowell, 2014).

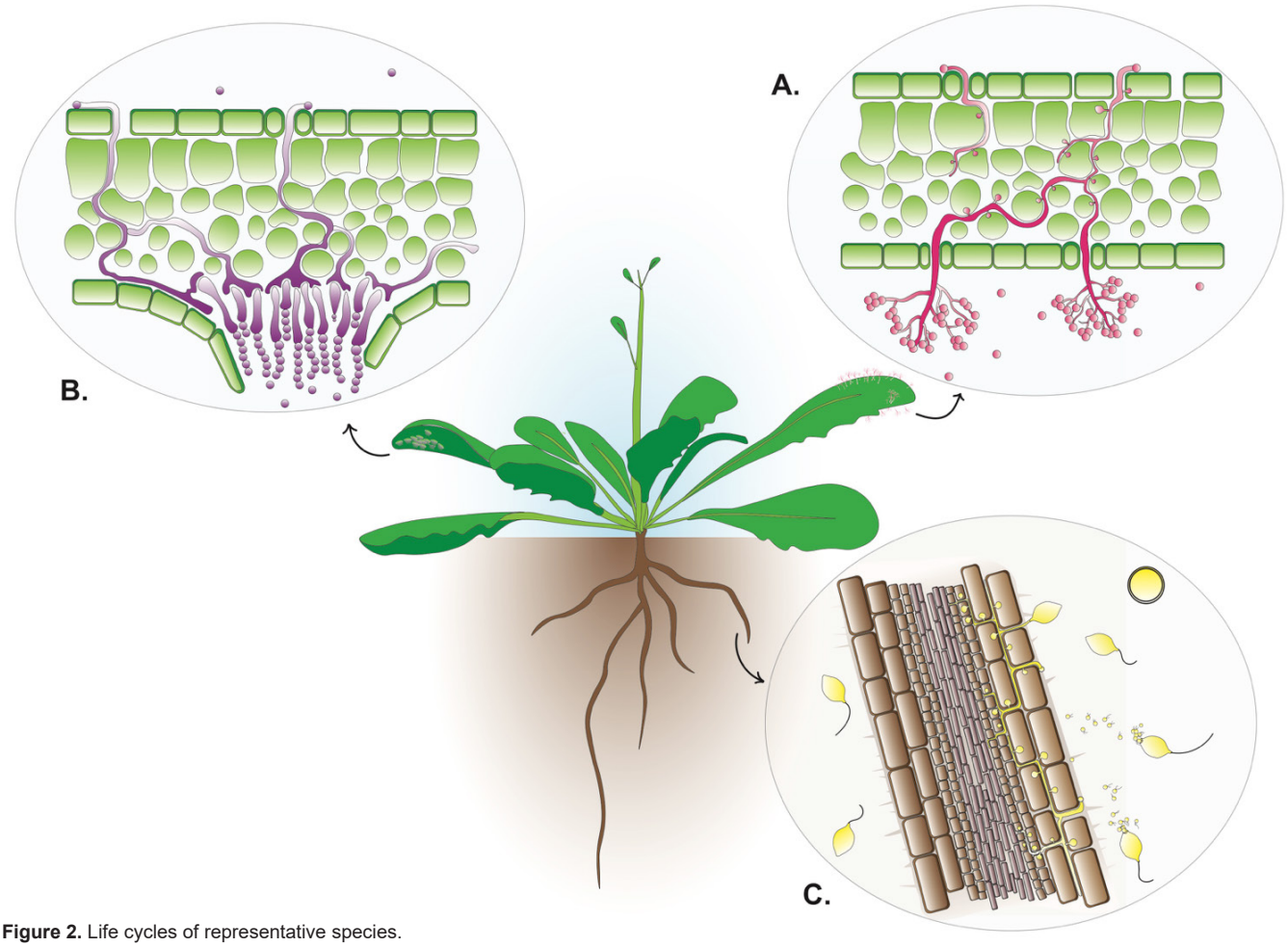

Figure 2. Life cycles of representative species.

(A) *Hyaloperonospora arabidopsidis.* **(B)** *Albugo candida* and *A. laibachii*. **(C)** *Phytophthora brassicae*, *P. capsici* and *P. parasitica.*

Table 1. Attributes of oomycetes used for *Arabidopsis* research.

Genus and Species	Natural Host	Lifestyle	Host Organ(s)	Culturable In vitro?	Transform-able?	Cross-able?	Genome status[1,2]
Hyaloperonospora arabidopsidis	*Arabidopsis thaliana*	Obligate biotroph	Leaves	No	No	Yes	1, 2
Albugo candida	Crucifers	Obligate biotroph	Leaves	No	No	Yes	1, 2
Albugo *laibachii*	*Arabidopsis thaliana*	Obligate biotroph	Leaves	No	No	Yes	1, 2
Phytophthora brassicae	Brassicaceae	Hemi-biotroph	Leaves and roots	Yes	Yes	Yes	
Phytophthora infestans	Potato, tomato	Hemi-biotroph	Leaves and roots	Yes	Yes	Yes	1, 2
Phytophthora parasitica	Broad range	Hemi-biotroph	Leaves and roots	Yes	Yes	Yes	1
Phytophthora capsici	Broad range	Hemi-biotroph	Leaves and roots	Yes	Yes	Yes	1
Pythium ultimum	Broad range	Necrotroph	Roots	Yes	Yes	Yes	1, 2

[1,2]Assembly can be accessed at [1]eumicrobedb.org or [2]fungidb.org

Hpa causes downy mildew disease of Arabidopsis (Koch and Slusarenko, 1990). The disease name comes from the downy appearance of leaves that are covered in sporangiophores (spore-producing structures, Fig. 2A) (Clark and Spencer-Phillips, 2000). Although *Hpa* does not cause disease on any crop, the closely related species *H. parasitica* is a serious problem in cabbage and other brassica crops. More distantly related downy mildew species cause important diseases on crops that include grapes, cucurbits, lettuce, spinach, sunflower, and basil (Clark and Spencer-Phillips, 2000). Like other phytopathogenic oomycetes, downy mildew species can quickly overcome host resistance genes and develop resistance to chemical control agents (Lucas et al., 1995). It has

been estimated that over 25% of the five billion dollar global fungicide market is spent to control downy mildew diseases (mostly on grape downy mildew) (Gisi, 2002). Thus, *Hpa* is a reference species for a group of pathogens with significant economic impact.

Like other downy mildew species, *Hpa* is primarily a foliar pathogen. The asexual (epidemic) phase of the life cycle begins when asexual spores are dispersed by wind or rain to a host plant organ. The spore germinates on the plant surface and produces an infection hypha that typically penetrates the anticlinal wall between epidermal cells (Fig. 2A) (Mims et al., 2004; Soylu et al., 2004). Soon afterwards, the primary hypha establishes lobed feeding structures, called haustoria, that invaginate host cells, most commonly in the mesophyll but also in epidermal cells (Mims et al., 2004; Soylu et al., 2004). Haustoria are of particular interest because they are thought to play a major role in extraction of nutrients from host cells, and in the secretion and translocation of effector proteins into host cells (Spencer-Phillips, 1997; Szabo and Bushnell, 2001; Wang et al., 2017). These aspects of the infection cycle are discussed in greater detail below. Filamentous hyphae grow in the intercellular spaces and branch frequently, forming mycelia that can occupy much of the area within a leaf in only a few days (Soylu and Soylu, 2003). The asexual cycle culminates when hyphae emerge from stomata and differentiate into asexual fruiting bodies, called sporangiophores, on which high numbers of sporangiospores are produced that each typically contain a high number of nuclei (~40/spore) (Koch and Slusarenko, 1990). *Hpa* can also produce sexual spores, called oospores, when hyphae differentiate into gametogenic antheridia and oogonia. Following meiosis gametes will fuse in the oogonium that will then develop into an oospore that contains a single nucleus (Koch and Slusarenko, 1990). Oospores are formed within the plant tissue, are durable and can persist in the soil after the host plant dies and decays. Infection cycles can then be initiated by germinating oospores through infection of an adjacent host plant root, e.g. in the next growing season when the roots of germinated Arabidopsis seeds come into contact with the soil-borne oospores (Holub and Beynon, 1996).

A major aspect of *Hpa* biology is its strict dependence on a host plant for growth, also known as obligate biotrophy (O'Connell and Panstruga, 2006; McDowell, 2011). *Hpa* cannot be cultured on synthetic media and appears capable of extracting nutrients only from viable host cells (Lucas et al., 1995). In addition, the host range of *Hpa* appears to be confined to Arabidopsis. *Hpa* isolates from Arabidopsis cannot infect related brassica species, and *vice versa* (Parker et al., 1996). However, some isolates taken from crop brassica species can infect Arabidopsis lines containing mutations that compromise immune responses, indicating that host immunity is an important determinant restricting downy mildew host range (Parker et al., 1996).

Hpa has several advantages and limitations for laboratory experiments (McDowell, 2011). The greatest limitation of this pathogen is its obligate lifestyle: *Hpa* cannot be propagated apart from the host, which impairs genetic transformation of the pathogen. However, it is relatively straightforward to achieve robust colonization of Arabidopsis by *Hpa* under laboratory conditions. Key environmental parameters include cool temperatures (~16° C), low light intensity, and high relative humidity (McDowell et al., 2011). Long-term storage of sporangia and oospores at -80 ° C is straightforward and reliable (McDowell et al., 2011). *Hpa* is homothallic and can self-fertilize, but genetic crosses between isolates can be made, albeit laboriously (Gunn et al., 2002). Genetic mapping populations have been generated in successful support of map-based cloning efforts to identify *Hpa* avirulence genes, encoding host-recognized effectors (Rehmany et al., 2003; Bailey et al., 2011; Woods-Tor et al., 2018). These and other aspects of lab experiments with *Hpa* are discussed in detail below and elsewhere (Coates and Beynon, 2010; McDowell, 2011).

A significant advantage of *Hpa* is that it is a *bona fide* pathogen of Arabidopsis in the natural world, and has been co-evolving with its host (Holub, 2001). The polymorphisms that have evolved in the host and pathogen were used to molecularly clone Arabidopsis disease resistance genes ("*R* genes") and the corresponding effector/avirulence genes from the pathogen (Table 2). Recent studies have demonstrated additional natural variation in effector gene repertoires in *Hpa*, and in the responses of different Arabidopsis accessions to *Hpa* effectors, suggesting functional variability in the Arabidopsis proteins that are targeted by *Hpa* effectors (Fabro et al., 2011; Asai et al., 2014). Thus, natural genetic variation in the *Arabidopsis*/*Hpa* interaction can be further exploited for mechanistic insight into plant-oomycete interaction and co-evolution. Furthermore, because of the experimental advantages of Arabidopsis, *Hpa* is also one of the best reference organisms for investigating obligate biotrophy (McDowell, 2011). A final advantage of *Hpa* is the availability of a reference genome sequence (Baxter et al., 2010).

Albugo candida and Albugo laibachii

White blister pathogens belonging to the genus *Albugo* are naturally occurring, obligate biotrophic pathogens of Arabidopsis, and their attributes will be summarized in comparison with *Hpa*. Like *Hpa*, *Albugo* is commonly found on Arabidopsis in the wild, and their interactions display natural variability, including gene-for-gene interactions (Holub et al., 1994a; Holub and Beynon, 1996; Borhan et al., 2001; Holub, 2008). In addition, *Albugo* species cause important crop diseases on cultivated Brassica species (Saharan et al., 2014).

Despite the superficial similarities between *Hpa* and *Albugo* species, several interesting differences are apparent: First, the infection cycle of *Albugo* is very different, as illustrated in Fig. 2. For example, *Albugo* species produce motile zoospores that deploy germ tubes to enter through the stomata (Soylu et al., 2003). The hallmark of the disease cycle is blisters that are caused by rupture of the plant epidermis to facilitate dissemination of asexual spores (Saharan et al., 2014).

Molecular phylogenies clearly illustrate that the *Albugo* and downy mildew lineages are distinct (Fig. 1) (Thines, 2014; Ascunce et al., 2017). Thus, *Albugo* and *Hpa* lineages have independently evolved to an obligate lifestyle and to compatibility with Arabidopsis (Kemen and Jones, 2012). Interestingly, *A. laibachii* and *A. candida* display different host ranges: the former, like *Hpa*, is restricted to *A. thaliana*, while host range of *A. candida* encompasses 63 genera and 241 species within the *Brassicaceae* (Thines et al., 2009). Thus, these two species provide an opportunity for comparative studies to understand the factors that control host range (McMullan et al., 2015; Jouet et al., 2018). As with *Hpa*, intraspecific variation for resistance/susceptibil-

Table 2. Features of cloned Arabidopsis resistance genes encoding NLRs mediating detection of the oomycetes *Hpa* (*RPP*) and *Albugo* (*RAC, WRR*).

Gene	Accession	Col-0 gene/allele	Remark	Reference	Recognizing	References
RPP1-WsA	Ws	At3g44480* (*rpp1*)	Original *RPP1* locus	Botella et al., 1998	unknown	
RPP1-WsB	Ws	At3g44480* (*rpp1*)	Previous *RPP10* locus	Botella et al., 1998	ATR1 alleles	Rehmany et al., 2005
RPP1-WsC	Ws	At3g44480* (*rpp1*)	Previous *RPP14* locus	Botella et al., 1998	unknown	
RPP1-NdA	Nd	At3g44480* (*rpp1*)	Similar but different specificity as *RPP-WsB*	Botella et al., 1998	ATR1 alleles	Rehmany et al., 2005
RPP1-EstA	Est	At3g44480* (*rpp1*)	Similar but different specificity as *RPP-WsB*	Goritschnig et al., 2016	WY motif 2 of ATR1 alleles	Goritschnig et al., 2016
RPP1-ZdrA	Zdr	At3g44480* (*rpp1*)	Similar but different specificity as *RPP-WsB*	Goritschnig et al., 2016	WY motif 2 of ATR1 alleles	Goritschnig et al., 2016
RPP2A	Col-0	At4g19500	Requires *RPP2B*	Sinapidou et al., 2004	unknown	
RPP2B	Col-0	At4g19510	Requires *RPP2A*	Sinapidou et al., 2002	unknown	
RPP4	Col-0	At4g16860		Van der Biezen et al., 2004	ATR4	Asai et al., 2018
RPP5	L*er*	At4g16950, paralog of *RPP4*		Parker et al., 1997	ATR5	Bailey et al., 2011
RPP7	Col-0	At1g58602		Eulgem et al., 2007	unknown	
RPP8-Ler	L*er*	At5g43470 (*rpp8*)	Alleles confer virus resistance	McDowell et al., 1998	ATR8, not cloned	Gunn et al. 2002
RPP13-Nd	Nd	At3g46530	Highly polymorphic in *A. thaliana* (Rose et al., 2004)	Bittner-Eddy & Beynon, 2001	ATR13	Allen et al., 2004
RPP39	Wei-0	At1g61180 & At1g61190	CC-NB-LRR protein signaling through NDR1	Goritschnig et al., 2012	ATR39	Goritschnig et al., 2012
RAC1	Ksk-1	At1g31540	Resistance to *Albugo laibachii* Nc14	Borhan et al., 2004	unknown	
WRR4	Col-0	At1g56510	Confers resistance to multiple *A. candida* races	Borhan et al., 2008	unknown	
WRR4B	Ws-2	At1g56540	Resistance to *A. candida* isolate Ac2V	Cevik et al., 2019	unknown	
WRR8	Sf-2	At5g46270	Resistance to *A. candida* isolate Ac2V	Cevik et al., 2019	unknown	
WRR9	Hi-0	At1g63750	Resistance to *A. candida* isolate Ac2V	Cevik et al., 2019	unknown	
WRR12	Ler	At1g17600	Resistance to *A. candida* race AcBoT	Borhan et al., 2008	unknown	

* *RPP1* orthologs or family members in Col-0 are: At3g44000, At3g44480, At3g44630, and At3g44670
# identified as a segregating locus in *Hpa* crosses

ity to *Albugo* is prevalent in Arabidopsis and has been used to clone several Arabidopsis genes for resistance to *Albugo* (Table 2). Perhaps most interestingly, *Albugo* species display a remarkable capacity to suppress defense in Arabidopsis (Cooper et al., 2008). Co-infection with *Albugo* can render Arabidopsis susceptible to otherwise incompatible pathogens, including the late blight pathogen *Phytophthora infestans* (Belhaj et al., 2017; Prince et al., 2017). The molecular mechanisms by which *Albugo* strongly suppresses plant immunity are beginning to be revealed (Prince et al., 2017). Further studies are expected to reveal *Albugo* effectors and their plant targets that are likely to be key components of the plant immune system (Kemen et al., 2011; Links et al., 2011). *Albugo*-Arabidopsis systems are also being developed to better understand how *Albugo* influences plant interactions with other microbes (Ruhe et al., 2016; Jouet et al., 2018), as well as the important phenomenon of non-host resistance (Cevik et al., 2019).

The experimental advantages and limitations of the *Albugo* species are similar to *Hpa*. Like *Hpa*, *Albugo* species cannot be cultured apart from their hosts but they are relatively straightforward to propagate on susceptible host genotypes (Crute et al.,

1993). Long-term storage is possible and genetic crosses between isolates can be performed (Adhikari et al., 2003). Importantly, draft genome sequences are available from two different strains of *A. laibachii* and one of *A. candida* (Kemen et al., 2011; Links et al., 2011). As with *Hpa*, the genome data is having a major impact on research with this organism (Kemen and Jones, 2012).

Laboratory pathosystems

Diseases of Arabidopsis caused by other oomycetes, for instance by *Phytophthora* or *Pythium* species, have not been observed under natural conditions, but have been achieved under laboratory conditions. Complete infection cycles have been obtained on Arabidopsis using the *Phytophthora* species *P. brassicae* (Roetschi et al., 2001), *P. parasitica* (Attard *et al*., 2010; Wang *et al*., 2011), *P. capsici* (Wang *et al*., 2013a), *P. palmivora* (Daniel and Guest, 2006), and *P. cinnamomi* (Robinson and Cahill, 2003). Strikingly, different immune signaling pathways seem to be involved in resistance responses to each of these *Phytophthora* species (Wang *et al*., 2013). Within Arabidopsis, different accessions show a range of infection phenotypes, from clearly compatible (resulting in disease) to fully incompatible (resistant). Other *Phytophthora* species tested, e.g., *P. infestans* (Huitema *et al*., 2003) and *P. sojae* (Takemoto et al., 2003), are not able to establish a successful infection on Arabidopsis but are effectively stopped by the plant, likely by early perception by the host immune system. These laboratory pathosystems provide valuable complements to the pathosystems based on *Hpa* and *Albugo* and are described in the following sections.

Phytophthora species

P. brassicae (formerly *P. porri*) is the first representative of the *Phytophthora* genus to be used in Arabidopsis research (Roetschi et al., 2001). This species parasitizes brassica species closely related to Arabidopsis. Given the close relationship between these hosts and Arabidopsis, it is not surprising that *P. brassicae* can grow on Arabidopsis. The initial stages of the asexual infection cycle are similar to those of *Hpa*: spores attach to the organ surface with appressoria and penetrate by growing between epidermal cells (Figure 2C). Hyphae ramify through intercellular spaces and project haustoria into plant cells. After approximately 3 dpi, a necrotrophic program commences in which the pathogen destroys host tissue. This hemi-biotrophic infection strategy is a major difference from obligate biotrophs like *Hpa* and *Albugo* species, which cannot thrive on dead plant tissue.

P. brassicae was one of the first *Phytophthora* species used to test immune responses of various Arabidopsis ecotypes and mutants (Mauch et al., 2008). Arabidopsis researchers have subsequently made use of “non-adapted” oomycete pathogens; that is, species that are incapable of causing disease on wild-type Arabidopsis even under the most permissive conditions. The most productive insights from this approach have involved the infamous species *Phytophthora infestans*, which causes late blight disease of potato and tomato and is responsible for the famine in Ireland in the 19th century (Fry, 2008). Despite its destructive effects on potato and tomato, *P. infestans* is stopped quickly in Arabidopsis by multiple, genetically redundant layers of resistance, including so-called “penetration resistance” (Stein et al., 2006). Thus, this pathogen has been used as a probe to dissect the cytological responses and genetic basis of this resistance (see below).

Two recently developed *Phytophthora* pathosystems are based on *P. capsici* and *P. parasitica*. Both of these species exhibit broad host ranges. *P. capsici* is destructive on cucurbit, legumes, and solanaceous crops (Kamoun et al., 2015; Barchenger et al., 2018). *P. parasitica* (also called *P. nicotianae*) causes a variety of diseases on field crops, fruit trees, and ornamental plants (Meng et al., 2014; Kamoun et al., 2015; Meng et al., 2015; Panabieres et al., 2016). Testing of Arabidopsis accessions with different strains of both species revealed natural genetic variation for resistance/susceptibility (Attard et al., 2010; Wang et al., 2011; Wang et al., 2013). *P. capsici* and *P. parasitica* can complete their life cycles on leaves or roots of susceptible Arabidopsis accessions. Systems for infection through Arabidopsis roots have been developed and provide a complement to foliar pathogens like *Hpa* or *Albugo* (Attard et al., 2010; Wang et al., 2011; Wang et al., 2013; Hou and Ma, 2017). Both species employ a hemi-biotrophic life cycle that is depicted in the root in Figure 2C.

Although only recently developed, these *Phytophthora* pathosystems hold considerable potential to provide complementary insights to pathosystems based on *Hpa* or *Albugo* species. Most importantly, the experimental tools on the pathogen side are relatively advanced. Both species are easy to culture and relatively straightforward to transform, enabling reverse genetics on the pathogen (Le Berre et al., 2008; Wang et al., 2018b). It is also possible to perform genetic crosses and develop segregating populations (Lamour et al., 2007; Lamour et al., 2014). Additionally, genome sequences for both species are available to delineate genes important for pathogenicity (Lamour et al., 2012) (note that the *P. parasitica* genome data has not been formally published but is publicly available at fungidb.org and eumicrobedb.org). Moreover, successful genome editing by the CRISPR/Cas9 system has now been reported for *P. capsici* (Wang et al., 2018b). Considering these experimental advantages and the practical value of research involving these emerging, broad host range pathogens, we expect that *P. capsici* and *P. parasitica* will become increasingly important for exploiting the experimental advantages of *Arabidopsis* to understand how diseases are caused by oomycetes.

Pythium species

Members of the *Pythium* genus cause a number of destructive “damping-off” diseases (Martin and Loper, 1999). Contrasting with downy mildews and *Phytophthora*, many *Pythium* species are classical necrotrophs that lack a biotrophic phase during infection (Kamoun et al., 2015). These pathogens exhibit filamentous growth but do not produce haustoria and induce host cell death soon after contact.

Pythium irregulare has been used as a model soilborne pathogen of Arabidopsis since the late 1990s. Bioassays with this pathogen have been useful in mutants with reduced immune responses, particular those in jasmonate- and ethylene-mediated defense (Browse et al., 1998; Staswick et al., 1998; Geraats et

al., 2002; Huffaker et al., 2006; Huffaker and Ryan, 2007). Contrastingly, research on *Pythium* pathogenicity, using Arabidopsis, is practically non-existent. The tools on the pathogen side are reasonably good: Genome sequences from several species have been completed, the pathogen is easy to culture and is transformable (Levesque et al., 2010; Adhikari et al., 2013; Grenville-Briggs et al., 2013). Thus, there is good opportunity to exploit Arabidopsis-*Pythium* pathosystems to better understand how necrotrophic oomycetes subjugate their hosts. In addition, recent studies of the Arabidopsis root microbiome revealed *Pythium* species as prominent endophytes, opening a new area of Arabidopsis-oomycete interactions (Durán et al., 2018; Sapp et al., 2018).

PATHOGEN PERCEPTION

Introduction to the plant immune system

Plants have evolved various ways to fend off microbes (Pieterse *et al*., 2009). A suite of surface-exposed detectors, named pattern recognition receptors (PRRs, Fig. 3), can recognize conserved microbe-associated molecular patterns (MAMPs), such as bacterial flagellin or fungal chitin. Upon detection the PRRs activate a signaling cascade that leads to pattern-triggered immunity (PTI). Most PRRs require a co-receptor to initiate signaling: for example, the receptor FLS2 interacts with the BAK1 co-receptor upon recognition of the MAMP flg22 (see (Macho and Zipfel, 2014) for details on the mechanisms of immune activation by PRRs). BAK1 also interacts with other PRRs (Macho and Zipfel, 2014). PTI signaling via mitogen-activated protein kinase (MAPK) cascades and/or calcium-dependent protein kinases (CDPKs) (Bredow and Monaghan, 2019) activates pathogen-nonspecific immune responses such as the production of reactive oxygen species and nitric oxide, cell wall reinforcement, and induction of defense genes (Hein *et al*., 2009; Nürnberger and Kemmerling, 2009). Adapted pathogens are able to suppress PTI with effectors that have evolved to interact directly with host defense-associated proteins, resulting in effector-triggered susceptibility (Jones and Dangl, 2006). For example, bacterial effectors can interfere directly with FLS2 or BAK1 (Toruno et al., 2016). Intracellular nucleotide-binding, leucine-rich repeat (NLR, Fig. 3) receptors that recognize these effectors or their activity have evolved in plants as a second layer of pathogen perception. NLR proteins are encoded by plant disease resistance genes and mediate so-called Effector-Triggered Immunity (ETI). This is the molecular basis of the genetic model of “gene-for-gene” resistance, in which pathogen “avirulence (Avr) genes” (now known to encode secreted effector proteins) are recognized inside plant cells by plant “resistance (*R*) genes” (now known to encode NLR proteins). Resistance to adapted pathogen species is often rapidly broken by loss or mutation of avirulence effector genes or through suppression of ETI by different effectors (Woods-Tor et al., 2018).

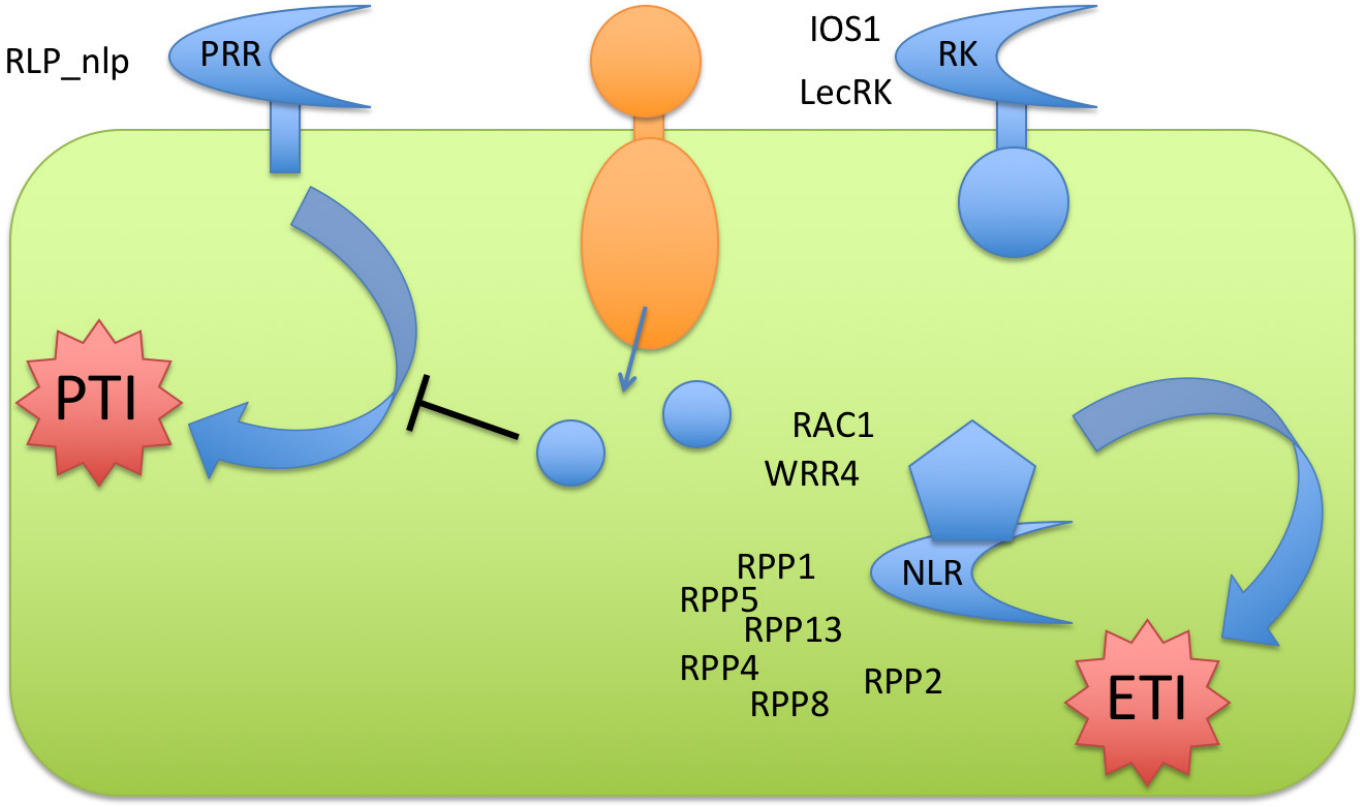

Figure 3. Schematic of PTI and ETI against oomycetes

Pattern recognition receptors

The majority of plant PRRs identified so far belongs to the family of leucine-rich repeat receptor-like kinases (LRR-RLKs), which has more than 600 members in Arabidopsis (Shiu *et al*., 2004), or receptor-like protein (LRR-RLPs) that lack a C-terminal kinase domain and interact with RLKs for transducing signals (Gust and Felix, 2014). The best-described plant PRR is FLS2 from Arabidopsis that recognizes bacterial flagellin, a major structural protein of bacterial flagella. An N-terminal 22-amino acid residue fragment of this protein (flg22) is sufficient to be recognized by FLS2 and to induce PTI (Gómez-Gómez and Boller, 2000). Naito *et al*. (2008) showed that these residues are also essential for flagellum function and motility of bacteria, which makes it difficult for bacteria to circumvent this recognition by evolving different amino acid sequences in this region of the protein. A second well-described plant PRR that seems restricted to *Brassicaceae* is EFR, which recognizes the bacterial elongation factor Tu (EF-Tu) or its shorter N-terminal fragment elf18 (Kunze, 2004). When this PRR is expressed in the solanaceous plants *Nicotiana benthamiana* and tomato (*Solanum lycopersicum*), it confers resistance to a broad spectrum of bacteria (Lacombe *et al*., 2010), illustrating the potential use of PRRs as new sources for genetic resistance to crop diseases.

Fungi can also be recognized by PRRs, e.g. through the Arabidopsis RLK CERK1 that is required for responses to fungal chitin and bacterial peptidoglycan (Miya *et al*., 2007; Willmann *et al*., 2011), and the RLP30 PRR that mediates recognition of necrotrophic fungi through a currently unknown proteinaceous MAMP (Zhang et al., 2013). As we will detail in the next section, only a few receptors have been identified so far that mediate the extracellular recognition of oomycete MAMPs.

Oomycete pattern-triggered immunity

The extracellular recognition of oomycetes triggers the first line of defense that plant cells deploy (Raaymakers and Van den Ackerveken, 2016). However, only two PRRs recognizing oomycete MAMPs have been identified to date, the receptor-like proteins ELR of potato and RLP23 of Arabidopsis. ELR mediates the recognition of elicitins, secreted oomycete proteins that have a putative function as extracellular sterol carriers (Derevnina et al., 2016). Elicitins are known to act as MAMPs in certain plant species, e.g. in *Solanum microdontum* in which it induces a cell death response. *ELR* was cloned from this wild potato species and found to encode a receptor-like protein that requires the RLK BAK1 to induce elicitin-triggered immunity. The second PRR identified against oomycetes is Arabidopsis RLP23. This protein recognizes Nep1 (Necrosis- and Ethylene-inducing Protein 1)-like proteins (NLPs), secreted proteins that are found in many plant-associated microbes that belong to three different kingdoms of life (Oome *et al*., 2014). RLP23 was found to require the co-receptors SOBIR1 and BAK1 for NLP-triggered immunity (Albert *et al*., 2015). *NLP* genes are found in oomycetes belonging to the Peronosporales and also in fungi and bacteria. In oomycetes both cytotoxic and non-cytotoxic NLPs have been found, but both types act as MAMPs in Arabidopsis (Oome *et al*., 2014; Böhm *et al*., 2014). The immunogenic fragment of NLPs could be reduced to a synthetic peptide of <20 amino acids that was shown to bind the RLP23 receptor (Albert *et al*., 2015).

Other molecules of oomycetes have been proposed to act as MAMPs (Hein *et al*., 2009): (i) glucans derived from oomycete cell wall polysaccharides that bind to a soybean glucan-binding protein that has glucanase activity, but is insufficient to trigger immunity (*Glycine max*) (Fliegmann *et al*., 2004), (ii) the 13-amino acid fragment Pep-13 derived from GP42, a calcium-dependent transglutaminase that is abundant in the cell wall of *Phytophthora sojae*, and is sufficient to elicit PTI responses in parsley (Brunner *et al*., 2002), and (iii) the *Phytophthora* cellulose-binding elicitor lectin (CBEL) that elicits a response in tobacco and Arabidopsis (Hein *et al*., 2009; Khatib *et al*., 2004). (iv) The glycoside hydrolase 12 protein XEG1 from *Phytophthora sojae*, recently shown to be recognized by a receptor complex in *N. benthamiana* that includes an extracellular LRR receptor, BAK1, and SOBIR1 (Ma et al., 2015; Wang et al., 2018a). Other groups of cell death-inducing or cytotoxic proteins from oomycetes, such as the Crinkler proteins, might also qualify as MAMPs based on their widespread occurrence among different pathogens (Thomma *et al*., 2011). We only know the tip of the iceberg with respect to oomycete MAMPs and we expect that many new patterns and their cognate receptors will be identified in the coming years. Finally, it is likely that oomycete enzymes release compounds from the host cell wall and membrane that can act as damage-associated molecular patterns (DAMPs). It was proposed that *Hpa* has evolved to circumvent the release of DAMPs by strong reduction of the number of genes encoding for hydrolytic enzymes compared to *Phytophthora* species (Baxter et al., 2010). Further exploitation of Arabidopsis to explore these aspects will undoubtedly be productive.

Intracellular perception of oomycetes in Arabidopsis

Plants have evolved intracellular receptors with nucleotide-binding, leucine-rich repeat domains (NLRs) to detect oomycete effectors, or their activity, to trigger immunity (ETI). NLR proteins typically have an N-terminal variable region, either of the coiled-coil type (CC) or Toll/Interleukin-1 receptor-like (TIR), or an RPW8-like domain. The N-terminal domains are followed by a nucleotide binding domain (NB) and C-terminal leucine-rich repeat region (LRR), further detailed in an excellent review (Cui et al., 2015).

The genes encoding these intracellular receptors were first identified in Arabidopsis through genetic mapping of resistance genes. The Arabidopsis genome (Col-0) encodes a total of around 150 NLR proteins, of which only a limited number have an assigned function. The first cloned *NLR* gene was *RPS2* that confers resistance to *Pseudomonas* bacteria expressing the effector AvrRpt2 (Mindrinos *et al*., 1994). Soon after that also *R* genes for resistance to downy mildew and *Albugo* were cloned (Table 2), based on natural variability of their functions in different accessions of Arabidopsis (Slusarenko and Schlaich, 2003; Holub, 2008; Coates and Beynon, 2010). These included the first *R* genes to be cloned against oomycetes (Parker et al., 1997; Botella et al., 1998; McDowell et al., 1998). More than 25 *R* loci conferring isolate-specific resistance to *Hpa* have been genetically identified in Arabidopsis and are named *RPP*, for RECOGNITION OF *PERONOSPORA PARASITICA*, the older name of

this pathogen (Slusarenko and Schlaich, 2003). So far, 8 *RPP* genes have been cloned: *RPP1, RPP2, RPP4, RPP5, RPP7, RPP8, RPP13*, and *RPP39* and all encode cytoplasmic NLRs. Alleles of *RPP1* confer different isolate-specific resistances (Botella *et al*., 1998), and also *RPP4* and *RPP5* can be considered allelic variants (Van der Biezen *et al*., 2002). Many of the other *RPP* loci map to the position of cloned *RPP* genes and could constitute allelic variants (Nemri *et al*., 2010). Broad resistance to downy mildew, observed in certain Arabidopsis accessions, appeared to be mediated by combinations of isolate-specific resistance loci (Lapin et al., 2012). Recently, the "helper" NLR proteins ADR1 and NRG1 were identified as signaling components for RPP proteins containing the TIR domain (Bonardi et al., 2011; Castel et al., 2019; Lapin et al., 2019; Wu et al., 2019), It is becoming clear that ETI can be underpinned by networks of NLRs, and deconvolution of these networks is an emerging area of interest (Wu et al., 2018).

At a broad scale, little is known about whether and how the activity of *RPP* genes is regulated (Lai and Eulgem, 2018). In one case, feedback control involving salicylic acid and WRKY proteins has been implicated (Mohr et al., 2010). In another, gene activity is subject to post-transcriptional control involving alternative polyadenylation, which is regulated by histone marks (Tsuchiya and Eulgem, 2013; Lai et al., 2018). This research has opened up a new avenue towards understanding the mechanisms and evolution of NLR gene regulation (McDowell and Meyers, 2013). Another recent study identified eQTLs providing resistance against *Hpa*, emphasizing the potential of this pathosystem to understand epigenetic factors that are relevant to plant-oomycete interactions (Furci et al., 2019).

Several avirulence genes from *Hpa* have also been cloned (Table 2), thanks to the development of genetic and genomic tools for the pathogen (Coates and Beynon, 2010; McDowell, 2014). In fact, the first oomycete *Avr* gene to be cloned was *ATR13* from *Hpa* (Allen et al., 2004). This gene encodes a small, secreted protein with a signal peptide followed by the conserved protein motif Arg-X-Leu-Arg, first described in (Rehmany et al., 2005). Downy mildew pathogens and *Phytophthora* species contain hundreds of putative "RXLR genes" in their genomes, and it is clear that they play an important role in pathogen virulence (discussed extensively below). RXLR proteins are secreted from pathogen haustoria and enter the interior of plant cells to reprogram plant regulatory networks and thereby promote virulence. However, RXLR proteins can also be recognized inside plants cells by NLR surveillance proteins. Indeed, every *Avr* gene cloned from a downy mildew or *Phytophthora* species to date encodes an RXLR or RXLR-like protein, recognized by the corresponding plant NLR protein. To date, *Hpa* Avr proteins recognized by *RPP1*, *RPP13*, *RPP4*, *RPP5*, and *RPP39* have been molecularly identified, and the determinants of their virulence and avirulence functions are under investigation (Allen et al., 2004; Rose et al., 2004; Rehmany et al., 2005; Bailey et al., 2011; Chou et al., 2011; Krasileva et al., 2011; Leonelli et al., 2011; Goritschnig et al., 2012; Steinbrenner et al., 2015).

Comparative and molecular studies of *RPP* genes have provided novel insights into the molecular mechanisms and selective forces that drive NLR gene evolution (McDowell and Simon, 2006). For example, *RPP5*, *RPP1*, and *RPP8* multigene families are physically linked in clusters and are subject to intra- and intergenic recombination, to produce new *NLR* gene variants in host-pathogen arms race (Botella et al., 1998; McDowell et al., 1998; Noel et al., 1999). RPP1 homologs have diversified through repeat duplication and sequence divergence, such that they can detect multiple surfaces of the corresponding effector (Goritschnig et al., 2016). ATR1 is a modular protein that can evolve to escape detection by mutations in any of several surfaces that mediate recognition by RPP1 (Chou et al., 2011). *RPP13* provides an example of a simple locus, in which a single copy gene displays substantial allelic polymorphism, driven by diversifying selection and intra-allelic recombination (Bittner-Eddy and Beynon, 2001). Interestingly, the *Hpa ATR13* locus, encoding the RXLR effector protein recognized by RPP13, displays similar attributes, suggesting that co-evolution has been a major driver for this diversity, along with balancing selection to maintain repertoires of useful alleles in the plant and pathogen populations (Allen et al., 2004). The ATR13 protein displays a novel structure and polymorphisms that mediate recognition specificity by RPP13 map to a single, surface exposed region (Leonelli et al., 2011). Much remains to be learned about plant-oomycete coevolution, and we expect a resurgence of this topic to be driven by our new capacity for cost-effective populations genomics of plants and pathogens sampled from natural populations along with sophisticated genetics (Karasov et al., 2018; Woods-Tor et al., 2018).

In the years following *RPP* gene cloning, only the RPP1 protein has been investigated in significant mechanistic detail. This protein binds directly to ATR1 via the LRRs. This interaction displays extensive allelic variability (Krasileva et al., 2010; Steinbrenner et al., 2015). In the absence of the ATR1 effector, RPP1 is likely maintained in an inactive state by intramolecular interactions between the N-terminal TIR domain, the NB domain, and the LRRs. Binding of ATR1 via the LRRs disrupts these interactions and permits oligomerization of RPP1, thereby triggering cell death and other immune responses (Krasileva et al., 2010; Steinbrenner et al., 2015). This mode of inactivation/activation appears to be a general aspect of NLR proteins, although details vary between different NLR proteins. As with other NLR proteins, the molecular events between RPP1 activation and deployment of the ultimate cellular immune response remain to be identified. This is a major knowledge gap that extends across all plant-pathogen interactions (Cui et al., 2015) and could be productively addressed by further studies of RPP proteins.

Several *Arabidopsis* genes for resistance to *Albugo* have also been cloned (Table 2). The *RAC1* gene provides isolate-specific resistance to *Albugo laibachii* Nc14 (Borhan *et al*., 2004)(Cevik et al., 2019). *WRR4* confers broad spectrum resistance to many *Albugo candida* isolates and functions as a transgene in *Brassica juncea* (Borhan *et al*., 2008, 2010). These *R* genes encode cytoplasmic NLRs that act similar to many of the RPP proteins, and helper NLRs have also been implicated for WRR-mediated resistance (Castel et al., 2019). Interestingly, the phenomenon of non-host resistance of Arabidopsis to Brassica-infecting races of *Albugo candida* is mechanistically underpinned by NLR receptors that can be identified by segregation analysis (Cevik et al., 2019). This led to the molecular cloning of WRR4B, WRR8, WRR9 and WRR12. Some of these genes were shown to provide resistance as transgenes in Brassica species (Cevik et al., 2019). Avirulence genes from *Albugo* remain to be identified, but this task is now feasible thanks to the genomic resources that

exist for this pathogen. Considering that RXLR genes do not appear prominently in the *Albugo* genome (see below) it will be very interesting to compare the structure and function of *Avr* genes from *Albugo* with those from *Hpa*.

IMMUNE SIGNALING AND RESPONSES

Following the perception of pathogens, signaling is initiated that ultimately results in the execution of a broad range of defense responses that stop the invading microorganism. Here, we summarize important signal transduction components and defense responses against oomycete infection of Arabidopsis.

In Arabidopsis, many genetic screens have been performed to identify genes that are important for defense signaling. Some of these screens were focused on signaling downstream of specific R proteins, while others were aimed to identify more general signal transduction components. It is beyond the scope of this chapter to describe the large number of genes involved in immune signaling, which are discussed in a number of excellent reviews (Pieterse *et al*., 2012; Cui *et al*., 2015). We focus on important plant proteins, EDS1 and NPR1, that play crucial roles in the interaction with oomycetes and are well studied in Arabidopsis. EDS1 and NPR1 are closely linked to the immunity-related hormone salicylic acid (SA) that is also key for resistance to oomycetes.

EDS1 (ENHANCED DISEASE SUSCEPTIBILITY 1) is a lipase-like protein that is required for the function of a subgroup of NLRs, those with a Toll–interleukin 1 receptor domain called *TIR-NLR*. In addition, EDS1 has a function in oxidative stress responses and as a positive regulator in basal resistance to virulent pathogens making the *eds1* mutant enhanced disease-susceptible (Wiermer *et al*., 2005). Two sequence-related signaling partners, PAD4 (PHYTOALEXIN-DEFICIENT 4) and SAG101 (SENESCENCE-ASSOCIATED GENE 101), form complexes with EDS1 and have signaling functions in the cytoplasm and nucleus. The NLR helper protein NRG1 also resides in this complex and plays a role in cell death signaling (Lapin et al., 2019). Interestingly, EDS1 was found to associate with AvrRps4, a *Pseudomonas* effector, and to interact with TNLs, like RPS4 that mediate recognition of AvrRps4. It was hypothesized that EDS1, being important for basal resistance, could be a target for pathogen effectors and thus serve as a molecular bridge to mediate recognition by R proteins that guard the EDS1 protein in addition to its signaling functions (Cui *et al*., 2015). This hypothesis is currently debated and under further investigation (e.g., (Huh et al., 2017; Halane et al., 2018). The *eds1* mutant was originally identified in a screen for loss of resistance to downy mildew (Parker *et al*., 1996). EDS1 was shown to be required for the function of *TIR-NLR* genes such as *RPP1*, *RPP4*, *RPP5,* and *WRR4*. Virulent strains of *Hpa* and *Albugo* are also able to cause more severe infections on *eds1* mutant plants because of the reduced level of basal resistance (Wiermer *et al*., 2005). Arabidopsis *eds1* mutants are also enhanced susceptible to a number of *Phytophthora* species, such as *P. capsici* (Wang *et al*., 2013a) and *P. parasitica* (Attard *et al*., 2010). The lowered basal immunity is associated with reduced production of the defense hormone SA, that is essential for defense against biotrophic pathogens (Vlot *et al*., 2009). EDS1 positively regulates SA accumulation, acting upstream of SA, but the *EDS1* gene is also activated at the transcriptional level by SA. EDS1 thus seems to be part of an SA-associated positive feedback loop of plant defense (Feys *et al*., 2001).

SA is not only crucial for resistance to downy mildew, but also affects basal and *R* gene-mediated resistance to many other oomycetes, such as *P. capsici* (Wang *et al*., 2013), or *P. parasitica* (Attard *et al*., 2010). Exogenous application of the hormone SA is sufficient to trigger efficient defense responses to a broad range of pathogens, including many oomycete pathogens, and in particular to downy mildew and hemi-biotrophic *Phytophthora* species. SA (2-hydroxybenzoic acid) is synthesized via two main routes, the isochorismate (IC) and phenylalanine ammonia lyase (PAL) route (Dempsey *et al*., 2011). The Arabidopsis IC route goes via two IC synthases (ICS) and downstream steps that differ from the canonical pathway defined in bacteria (Rekhter et al., 2019b; Torrens-Spence et al., 2019). The *ics1* (*sid2*) mutant has received the majority of experimental attention to date. *Ics1* mutants exhibit 90% lower levels of SA (Wildermuth *et al*., 2001), and levels are ~95% lower in the *ics1 ics2* double mutant following powdery mildew infection (Garcion *et al*., 2008). The *ICS1* gene is induced by MAMPs and by NLR-mediated pathogen recognition (Mishina and Zeier, 2007; Cui *et al*., 2015) leading to high local levels of SA that activate defense. Besides local defenses, SA plays an important role, together with pipecolic acid, in systemic acquired resistance (SAR), that enhances immunity of distant plant tissues to protect against future infections (Bernsdorff et al., 2016; Klessig et al., 2018). Recently the export protein EDS5 was implicated in synthesis of pipecolic acid, in addition to its well-known role in SA biosynthesis, revealing a surprising instance of immune convergence (Rekhter et al., 2019a).

Downstream of SA, the central NPR1 (NONEXPRESSOR of PR GENES 1) protein plays a crucial role in the transcriptional activation of SA-induced defense in Arabidopsis (Pieterse and Van Loon, 2004). Mutation of *NPR1* leads to loss of basal resistance to (hemi-)biotrophic pathogens and of SAR (Cao *et al*., 1997). NPR1 proteins form a multimeric complex in the plant cell cytoplasm. Upon triggering of the plant immune system SA accumulates and subsequently elicits the thioredoxin-mediated reduction of a cysteine residue in NPR1 (Yan and Dong, 2014). This results in the release of monomeric NPR1 that can travel into the nucleus where it interacts with TGA transcription factors to activate transcription of defense genes, like pathogenesis-related (*PR*) genes (Boyle *et al*., 2009). NPR1 and the two paralogous Arabidopsis proteins NPR3 and NPR4 have been shown to bind SA and could thus constitute the SA immune receptors (Ding et al., 2018; Innes, 2018).

Interestingly, RPP proteins vary significantly in their requirements for functionality of these signaling components. For example, resistance mediated by the TIR-NLR protein RPP5 is strongly compromised by mutations that nullify EDS1, NPR1, or SA biosynthesis (van der Biezen et al., 2002). On the other hand, the CC-NLR proteins RPP7 and RPP13 retain almost full functionality in these backgrounds (McDowell et al., 2000; Bittner-Eddy and Beynon, 2001). These differences underscore the complexity of ETI regulation.

Other defense related hormones, in particular jasmonic acid (JA) and ethylene, play important roles in immunity to *Pythium* infection. Enhanced susceptibility to necrotizing *Pythium* species was observed in Arabidopsis mutants with reduced JA levels of responses; (i) in the JA nonaccumulating *fad3-2_fad7-2_fad8*

triple mutant (Browse et al., 1998), and (ii) in the JA-insensitive *jar1-1* mutant (Staswick et al., 1998). Also, the ethylene-insensitive *ein2-1* mutant, but not the partially-ethylene insensitive mutant *etr1-1*, showed enhanced susceptibility to *Pythium* infection (Geraats et al., 2002). Interestingly, small endogenous peptides encoded by the *PROPEP1, 2*, and *3* genes, function in a feedback loop amplifying JA and ethylene defense signaling pathways initiated by *Pythium* (Huffaker and Ryan, 2007). Consequently, Arabidopsis transgenics overexpressing *PROPEP1* show enhanced resistance to *Pythium irregulare* (Huffaker et al., 2006).

It is well known that many proteins are specifically produced during plant defense (van Loon et al., 2006). In particular, many secreted and vacuolar proteins have been classified as pathogenesis-related proteins. Also in Arabidopsis many PR proteins have been identified, e.g. PR1, PR2, and PR5 that were found in apoplastic fluid of Arabidopsis treated with 2,6-dichloroisonicotinic acid (INA), which is a mimic of SA (Uknes *et al*., 1992). Several of these PR proteins have anti-microbial activities, while others contribute to resistance in unknown ways. One of the most used marker genes of SA-induced plant defense is *PR1*. Plants have multiple *PR1* genes encoding different protein variants with different activities (van Loon et al., 2006). Several tobacco PR1 proteins have anti-microbial activity on the oomycete *P. infestans*. Spore germination and pathogen growth *in planta* was effectively inhibited by PR1 protein purified from tobacco and other solanaceous plants (Niderman *et al*., 1995). Overexpression of several *PR1* genes in different plant species also increased their resistance to oomycetes (Sarowar *et al*., 2005; Broekaert *et al*., 2000), whereas the effect on other pathogen taxa is unclear. Interestingly, PR1 was recently shown to bind sterols, indicative of defensive mode of action that is based on restriction of this important nutrient for oomycetes (Gamir et al., 2017). The PR2 proteins are ß-1,3-beta-glucanases that are thought to attack the cell wall of invading fungi and oomycetes. Several cases of enhanced resistance of plants overexpressing *PR2* to oomycetes were reported (Broekaert *et al*., 2000).

Another important family of PRs are the PR5 proteins, that encompass the osmotins and thaumatin-like proteins (van Loon et al., 2006). A 24-kD protein, named osmotin, isolated from tobacco, was shown to have inhibitory activity to *P. infestans in vitro* (Woloshuk *et al*., 1991). Overexpression of osmotin in transgenic potato plants delayed the development of late blight disease caused by *P. infestans* (Liu et al., 1994). Osmotin was shown to trigger cell death in *Saccharomyces cerevisiae* through its interaction with a plasma membrane protein (Narasimhan *et al*., 2005). It is unclear whether a similar activity is also effective against oomycetes.

Other non-proteinaceous compounds that are important for resistance to oomycetes in Arabidopsis are small secondary metabolites. Important anti-microbial compounds produced by Arabidopsis are for instance indole-glucosinolates (iGS) and the phytoalexin camalexin (Glawischnig, 2007; Bednarek, 2012). Mutant plants that no longer produce either iGS or camalexin are only slightly more susceptible to *P. brassicae* (Schlaeppi et al., 2010; Schlaeppi and Mauch, 2010). The effect appeared stronger when tested with *P. capsici* (Wang *et al*., 2013). In both cases, a very strong gain in susceptibility was observed in the double mutant *cyp79b2 cyp79b3*, that is blocked in the production of indole-3-aldoxime, which is a common precursor for iGS and camalexin. Camalexin appears to contribute to resistance to Arabidopsis downy mildew, as several *phytoalexin-deficient* (*pad*) mutants are slightly more susceptible to *Hpa*. Whether iGS also contributes to downy mildew resistance is not clear, although a proposed role of iGS in innate immunity suggests it would contribute to basal resistance (Clay *et al*., 2009). Many new compounds with anti-microbial activity are still being discovered in Arabidopsis, such as the recently discovered 4-hydroxyindole-3-carbonyl nitrile (4-OH-ICN), a previously unknown Arabidopsis metabolite (Rajniak *et al*., 2015). This is not surprising as each plant species is thought to produce thousands of secondary metabolites, many of which have a presumed role in defense (Dixon, 2001). However, much remains to be learned about the exact mechanisms that inhibit oomycete growth during PTI and ETI.

GENES INVOLVED IN SUSCEPTIBILITY TO OOMYCETES

Many non-immunity related processes are thought to contribute to plant disease susceptibility. Plants often inadvertently facilitate pathogens in establishing disease. Three different levels of susceptibility-enabling processes can be distinguished: (i) attraction and attachment of pathogens to host cells, (ii) accommodation of specialized infection and feeding structures inside plant cells, and (iii) nutrient production and transport from host to pathogen (Lapin and Van den Ackerveken, 2013). One can imagine that mutation of host genes that affect any of these processes leads to plants with reduced disease susceptibility. However, such mutations could also affect basal plant processes that affect the overall physiology of the plant. In genetic terms, susceptibility (*S*) genes can be defined as genes that are involved in disease susceptibility and contribute positively to the infection process. By this definition, impairment of *S* genes leads to enhanced disease resistance.

In Arabidopsis several genes for susceptibility to oomycetes have been identified. Several of these are involved in host amino acid metabolism, e.g., the *ASPARTATE KINASE2/RAR1 SUPPRESSOR 1 (AK2/RSP1), DIHYDRODIPICOLINATE SYNTHASE2/RAR1 SUPPRESSOR 2 (DHDPS2/RSP2)* and *DOWNY MILDEW RESISTANT 1 (DMR1)*. The *rsp1* and *rsp2* mutants accumulate methionine, threonine and isoleucine, which is linked to increased resistance to *Hpa* (Stuttmann et al., 2011). Similarly, the Arabidopsis *dmr1* mutant, that is mutated in *HOMOSERINE KINASE* (*HSK*), has increased levels of homoserine, which is a common precursor of methionine, threonine, and isoleucine. L-homoserine application was sufficient to make Arabidopsis resistant to *Hpa* (van Damme et al., 2009). The resistance in *rsp1*, *rsp2* and *dmr1* mutants is independent of salicylic acid (SA)-induced defense (van Damme et al., 2009; Stuttmann et al., 2011).

A very different group of susceptibility genes are those that encode negative regulators of immunity. When impaired, for instance by mutation, these regulators are no longer able to suppress certain immune responses and therefore show an enhanced disease resistance. Two examples from Arabidopsis are the IMPAIRED OOMYCETE SUSCEPTIBILITY1 (IOS1) and the DOWNY MILDEW RESISTANT 6 (DMR6) proteins. IOS1 is a leucine-rich repeat receptor-like kinase (LRR-RLK) and is required for full susceptibility to *Hpa*. In addition, the *ios1* mutant is also

resistant to the oomycete *P. parasitica* (Hok et al., 2011; Hok et al., 2014). The loss of susceptibility of the *ios1* mutant seems linked to ABA hypersensitivity and independent of plant defense mechanisms. Impairment of negative regulation of ABA signaling in *ios1* is suggested to lead to impaired susceptibility to *Hpa* (Hok et al., 2014). Also, Arabidopsis *dmr6* mutants are resistant to *Hpa,* the oomycete *P. capsici*, and other biotrophic pathogens (van Damme et al., 2009; Zeilmaker et al., 2015). DMR6 and its close paralog DMR6-LIKE OXYGENASE 1 (DLO1/S3H) are needed for hydroxylation of SA to 2,5- and 2,3-dihydroxybenzoic acid, respectively. Overexpression of *DMR6* and *DLO1* leads to depletion of SA, which renders the plants highly susceptible to *Hpa* (Zeilmaker et al., 2015).

An important question to keep in mind when analyzing mutants with lesions in genes encoding negative regulators of immunity is whether they truly have a suppressive role in defense. This is particularly important for mutants that show autoimmune phenotypes, also referred to as lesion mimic mutants (Rodriguez *et al*., 2015). There are several examples of "negative regulator" mutants that can be suppressed by mutations in NLR genes encoding for cytoplasmic immune receptors. For instance, the Arabidopsis *mpk4* mutant exhibits constitutive activation of defense and was classified as a negative regulatory mutant. However, it appeared that the *mpk4* mutation required the presence of the NLR SUMM2 (Zhang *et al*., 2012). The idea is that the MPK4 protein, which is a target of the *Pseudomonas* effector HopAI1, is guarded by the NLR SUMM2. In the *mpk4* mutant, SUMM2 would be constitutively triggered resulting in an autoimmune phenotype.

Ongoing and future studies are likely to reveal more plant proteins that have an important role in disease susceptibility to oomycete pathogens. Besides finding additional components of the plant immune system that are targeted by oomycetes, non-immunity-related proteins that function in 'attracting', 'accommodating', and 'feeding' pathogens can be found. The identification and functional analysis of the encoding genes could uncover so far unknown molecular processes in plant disease susceptibility, that will be instrumental in the design of novel strategies for disease resistance breeding. Our understanding of such processes, in the context of Arabidopsis-oomycete interactions, is discussed in the following sections.

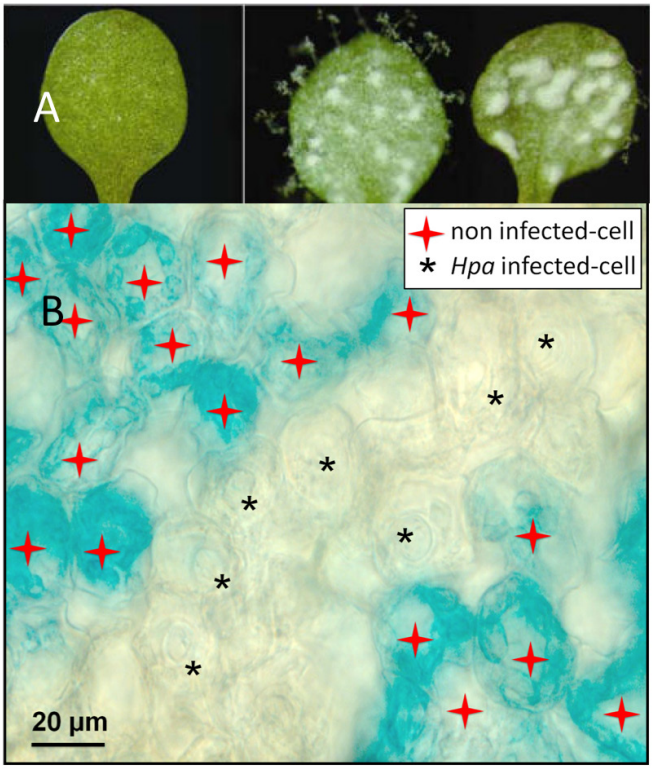

Figure 4. Suppression of host immunity by adapted oomycete pathogens.

(A) Cotyledons of *Arabidopsis thaliana* Col-5 inoculated solely with avirulent *H. Arabidopsis* Cand5 and showing no symptoms (left) or sequentially inoculated with *Albugo candida subsp. arabidopsis* isolate Acem1 and Cand5 showing downy mildew sporangiophores (center, upper surface) and white blisters (right, lower surface). Image reproduced from (Cooper et al., 2008).
(B) GUS staining of pro(*PR1*)::GUS in *Arabidopsis* leaves 6 DAI *Hpa* Waco9. No GUS was detected in *Hpa*-haustoriated mesophyll cell (black stars), while GUS staining was restricted to nonhaustoriated mesophyll cells (red stars). Image reproduced from (Caillaud et al., 2013).

SUPPRESSION OF IMMUNITY

The preceding sections of this chapter have established that plants can deploy an effective immune response against oomycete pathogens. However, it is also well-documented that phytopathogenic oomycetes are capable of potent immune suppression (Jiang and Tyler, 2012). This is illustrated in Figure 4A, in which a co-infection experiment demonstrates that *Albugo laibachii* can suppress Arabidopsis immunity and thereby enables colonization by otherwise incompatible isolate of *Hpa* (Cooper et al., 2008). As mentioned earlier, *A. laibachii* can even render Arabidopsis susceptible to *Phytophthora infestans*, which is nonpathogenic on Arabidopsis due to several layers of genetically redundant resistance mechanisms (Belhaj et al., 2017; Prince et al., 2017). An example of immune suppression at the cellular level is shown in Figure 4B, where activation of *PR1* promoter-GUS reporter gene fusion is suppressed specifically in Arabidopsis cells adjacent to *Hpa* infection structures (Caillaud et al., 2013; Asai et al., 2014). These images graphically illustrate oomycetes' capacity to tamper with plant immune systems. In the sections below, we will summarize how Arabidopsis is used to dissect the molecular mechanisms that underpin this suppression of an otherwise potent immune response.

It is becoming increasingly clear that oomycetes' most important tools for host immune suppression are effector proteins that are secreted from the pathogen to the outside (apoplast) or the interior of plant cells (Stassen and Van den Ackerveken, 2011; Fawke et al., 2015). Genomic analyses, coupled with genetic and molecular experiments, have allowed for discovery of hundreds of putative effector-encoding genes in oomycete genomes (Jiang and Tyler, 2012; Pais et al., 2013). The majority of oomycete effectors contain a classical signal peptide motif directing secretion from the pathogen. C-terminal of the signal peptide are additional known or putative functional motifs that enable classification of the secreted proteins into families (Kamoun, 2006). Such classifications provide a foundation from which to investigate the functions of the genes. Table 3 lists several families of oomycete effectors

Table 3. Predicted secreted proteins from oomycete pathogens of Arabidopsis.

Species	Genome Size	Total Secretome	RxLR	CRN	NEP	Elicitin-like	Reference
H. arabidopsidis	81.6 Mb	2157	134	20	10	15	(Baxter et al., 2010)
A. candida	45.3 Mb	929	29*	6	0	9	(Links et al., 2011)
A. laibachii	43 Mb	672	49	2	0	1	(Kemen et al., 2011)
P. capsici	64 Mb	2911	357	29	NP	NP	(Lamour et al., 2012)

NP-No prediction

and estimates of their copy numbers in genomes of oomycetes that infect Arabidopsis. These can be considered only an approximate inventory of effectors in the respective pathogens, and it is likely that these numbers will change as the quality of genome data, experimental evidence, and predictive motifs are iteratively refined (Dalio et al., 2018).

The best studied oomycete effector family is defined by the motif RXLR that is found adjacent to the N-terminal signal peptide. Hundreds of putative RXLR effector genes are present in genomes of downy mildew pathogens and species within the *Phytophthora* genus (Jiang and Tyler, 2012). The RXLR motif is proposed to mediate entry of the protein into plant cells, although this is controversial (Kale et al., 2010; Petre and Kamoun, 2014). Once inside the plant the RXLR motif does not appear to play a functional role. Rather, the function-specific region of the protein is downstream of the signal peptide and RXLR motif (Birch et al., 2006). We previously summarized how some RXLR proteins are recognized as avirulence proteins by NLR immune surveillance proteins. More relevant to this section of the chapter are lines of evidence indicating that RXLR proteins play a major role in immune suppression. These studies are reviewed in detail elsewhere (Birch et al., 2009; Schornack et al., 2009; de Jonge et al., 2011; Kale and Tyler, 2011; Koeck et al., 2011; Stassen and Van den Ackerveken, 2011; Wawra et al., 2012; Pais et al., 2013; Anderson et al., 2015; Fawke et al., 2015). Below, we highlight several examples in which the experimental resources of Arabidopsis have been used to accelerate progress towards understanding how RXLR proteins promote oomycete diseases.

RXLR effectors are thought to enter plant cells and target specific plant proteins or protein complexes for destruction or modification to benefit the pathogen. Thus, the experimental tools of Arabidopsis are well-suited to identify RXLR protein targets, to understand the functions of these targets in plant regulatory networks and define the mechanisms through which the target is engaged by the corresponding effector and the resultant effect on plant cellular functions. Arabidopsis has been used to particularly good advantage in studies aimed at high- to medium-throughput examination of effectors to provide first steps toward mechanistic understanding (Anderson et al., 2015). One such approach utilized a phytopathogenic *Pseudomonas* bacterial strain as a surrogate to deliver *Hpa* effectors to Arabidopsis cells via Type III secretion (Fabro et al., 2011). This system was based on two clever features: The first was an “effector-detector vector” in which the effector of interest is fused to a leader from a bacterial Type III effector that directs the oomycete effector through the Type III system and thus to the interior of plant cells (Sohn et al., 2007). The second feature was to engineer the bacteria with a constitutively active luciferase gene, by which *in planta* bacterial growth could be easily quantified by light emission (Fabro et al., 2011). This assay provided an estimation of whether the secreted effector promoted or retarded bacterial growth, indicative of virulence or avirulence activity, respectively. This system enabled facile examination of 64 RXLR genes, providing evidence for virulence-promoting activity for 42 effectors (Fabro et al., 2011).

Another approach is to exploit the ease of Arabidopsis transformation to generate stably transformed plants that express effector protein genes, controlled by plant regulatory sequences. This approach has been very productive for bacterial Type III effectors and is now being used to characterize RXLR proteins. For example, Pel and colleagues created transgenic lines expressing several RXLR proteins from *Hpa* and used these lines to establish virulence-promoting activities using pathogen disease phenotypes, cytological assays, and marker genes that are well-established in Arabidopsis (Pel et al., 2014). Arabidopsis transgenic plants have also been used in genetic screens to identify effectors that target specific signaling pathways/sectors, based on suppression of a promoter-reporter gene fusion that provides a readout for the pathway of interest. This approach has implicated RXLR effectors that suppress SA-mediated signaling based on reduction of SA-induced PR1-GUS activity (Asai et al., 2014). Another approach was to use Arabidopsis expressing RXLR effector-GFP protein fusions to estimate subcellular localization of 49 *Hpa* RXLR effectors (Caillaud et al., 2012). This approach revealed the nucleus and plasma membrane as predominant destinations. These clever screens represent only the beginning of what might be achieved using different Arabidopsis genetic resources. For example, it would be of great interest to exploit Arabidopsis lines that could report reprogramming of relevant host processes other than immune signaling (e.g., host cell structure, trafficking, or metabolism) (Lapin and Van den Ackerveken, 2013).

The most dramatic application of Arabidopsis tools to understand RXLR effector biology was described in two studies that exploited the Arabidopsis ORFeome to conduct a protein-protein interaction screen with effectors from *Hpa*, the bacterium *Pseudomonas syringae*, and the obligate biotrophic fungus *Golovinomyces orontii* (Mukhtar et al., 2011; Wessling et al., 2014). Together, these studies revealed nine Arabidopsis proteins that interact with effectors from all three pathogens, along with another 23 that interact with effectors from two of the three pathogens. The biological relevance of these interactions could then be tested by testing loss of function mutations, easily obtained from the Arabidopsis T-DNA knockout collection, for effects on susceptibility to patho-

gen infection. The targeting of plant proteins by pathogens from different biological kingdoms validates the prediction that pathogens might evolve convergently to target points of vulnerability in host signaling networks. This reaffirms that Arabidopsis studies will yield important knowledge of conserved immune strategies in crop species. In addition to these points of convergence, the screen also revealed proteins that bind to effectors from only one of the three species, providing leads into virulence strategies that are specific to oomycete, bacterial, or fungal lineages (Mukhtar et al., 2011; Wessling et al., 2014).

These screens provide an important basis for prioritizing hundreds of RXLR effector genes for the painstaking studies to reveal their modes of action. The experimental tools of Arabidopsis provide obvious advantages for studies aimed at deep mechanistic understanding. This is exemplified by examination of the *Hpa* effector RXLR44, through which a subunit of the mediator transcriptional complex was revealed as the target (Caillaud et al., 2013). RXLR44 targets the Med19 subunit for proteosomal degradation. Nullification of Med19 affects hormone signaling sectors that control immunity: JA-responsive genes are de-repressed, which activates antagonistic cross-talk through which SA signaling is repressed and the plant immune response is compromised (Caillaud et al., 2013). In this way, it was revealed that *Hpa* exploits JA-SA interplay to promote virulence, thereby joining bacteria and fungi in convergent exploitation of this malleable node in the immune signaling network (Gimenez-Ibanez et al., 2016; Zhang et al., 2017).

Although most of the studies discussed so far focus on effectors from the natural Arabidopsis pathogen *Hpa*, it is important to emphasize that Arabidopsis is also an informative system for effectors from *Phytophthora* species. For example, the Penetration-Specific Effector 1 (PSE1) effector from *P. parasitica* was shown to affect auxin signaling in Arabidopsis (Evangelisti et al., 2013). Arabidopsis can also be exploited to identify functions for effectors from non-pathogens of Arabidopsis that presumably target plant proteins which are broadly conserved and are targeted by diverse oomycetes. This is illustrated by a study in which *Phytophthora infestans* RXLR proteins that affect PTI were identified from screens of Arabidopsis expressing the PAMP-inducible FRK9 promoter fused to a Luciferase reporter (Zheng et al., 2014). The Arabidopsis extracellular ATP receptor kinase LeRK-I9 is targeted by the *Phytophthora* RXLR effector IPI-O, perhaps to destabilize the plasma membrane-cell wall interface and/or interfere with perception of eATP (Bouwmeester et al., 2011b; Bouwmeester et al., 2011a; Bouwmeester et al., 2014; Balague et al., 2017). Another example is the *Phytophthora* suppressor of RNA silencing (PSR) effectors from the soybean pathogen *Phytophthora sojae* that suppress plant RNA interference to manipulate immune signaling (Qiao et al., 2013; Xiong et al., 2014). In this case, Arabidopsis was used to identify one target of these proteins: an RNA-binding protein that had not been previously linked with immune system regulation (Qiao et al., 2015). These and other studies illustrate how Arabidopsis could be used to identify effector target proteins that are "conserved" in the sense that they are targeted in diverse plant species, perhaps by diverse pathogens (Anderson et al., 2012; Deb et al., 2018b; Deb et al., 2018a; Tomczynska et al., 2018). Such proteins could be attractive targets for breeding/bioengineering.

The above examples also illustrate a major benefit of effector-driven research: the effectors often "lead" us to novel insights into the composition and function of plant regulatory networks (Win et al., 2012). In this context, we draw attention back to Table 3, which illustrates that oomycetes contain several effector families with important functions in disease. The Nep1 (Necrosis and Ethylene-inducing 1)-like proteins (NLPs) were introduced previously in the section on PTI. These proteins are thought to be utilized by necrotrophic and hemi biotrophic pathogens to induce plant cell death; however, the obligate biotroph *Hpa* expresses a number of NLP proteins that are not capable of inducing necrosis (Baxter et al., 2010). Their virulence function awaits definition. The "crinkler" family of effectors was functionally defined by plant cell necrosis activity and also contains a definitive protein motif (Stam et al., 2013). Another broadly conserved class of effectors are elicitin-like proteins, which are thought to bind sterols in the apoplast (Derevnina et al., 2016). This is important because phytopathogenic oomycetes do not synthesize sterols. These and other effector families have received little attention, compared to RXLR proteins, and represent good opportunities for productive experimentation, using the Arabidopsis tools described above. It will be particularly intriguing to explore *Pythium* or *Albugo* effector families that might be analogous to RXLR effectors in these important but poorly studied oomycete lineages. The recently described CHXC family from *Albugo* is particularly intriguing and might provide a mechanistic link to the recent report that *Albugo* can alter Arabidopsis tryptophan metabolism and interfere with responses to salicylic acid (Kemen et al., 2011; Prince et al., 2017). As mentioned earlier, *Albugo* can also act as a hub to affect the microbiome of infected plants and appears highly tolerant to host immune responses (McMullan et al., 2015; Ruhe et al., 2016). These traits are theorized to provide a competitive advantage for niche colonization in the wild. Much more remains to be learned about these and other fascinating aspects of Arabidopsis-oomycete interactions.

ACCOMMODATION OF OOMYCETE INFECTION STRUCTURES

The preceding section established that secretion of effectors is a major mechanism through which oomycetes can suppress host immunity. Another critical process in which the pathogen likely manipulates the host is in accommodation of infection structures, particularly the haustorium which penetrates the plant cell wall to create intimate associations with individual host cells (Fig. 2, Fig. 5) (Lapin and Van den Ackerveken, 2013). As mentioned previously, the haustorium is a specialized feeding structure that is formed by biotrophic and hemi-biotrophic oomycetes during plant colonization (Spencer-Phillips, 1997; Szabo and Bushnell, 2001; Wang et al., 2017). Effectors are secreted from haustoria to the exterior and interior of host cells (Wang et al., 2017). Haustorium formation is initiated when oomycetes secrete hydrolases to locally degrade the plant cell wall and establish an entry point. Microscopy of infected Arabidopsis leaves has illustrated the ultrastructure of oomycete haustoria (Mims et al., 2004; Soylu et al., 2004; Lu et al., 2012) (Fig. 5). Following penetration, haustoria grow into the host cell by invagination of the plant cell membrane. The pathogen haustorial membrane is distinct from the host extrahaustorial membrane and the two membranes are separated by a glycan-rich extrahaustorial matrix. The haustorium itself contains numerous large organelles including vacuoles, mi-

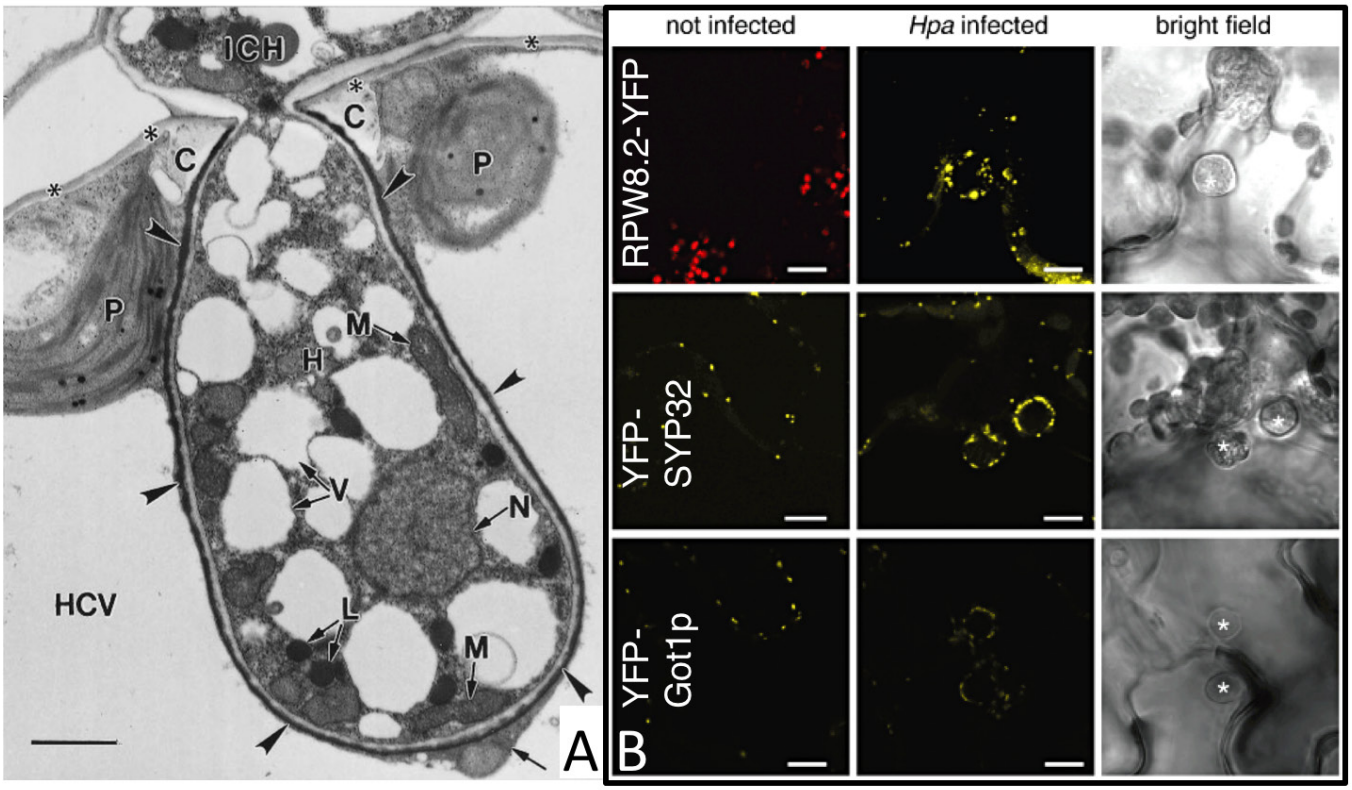

Figure 5. Manipulation of host cell structure and metabolism.

(A) Transmission electron micrograph of fixed *Hpa* haustoria in leaf cells of *At*. Near-median longitudinal section of a haustorium (H) showing its continuity with an intercellular hypha (ICH). collar of host cell wall material (C). Host-cell structures: the cell wall (asterisks), plastids (P), the central vacuole (HCV), and cytoplasm (arrow). Pathogen structures: nucleus (N), mitochondria (M), lipid bodies (L), vacuoles (V), and extrahaustorial matrix (arrowheads). Scale bar = 1 µm. Image reproduced from (Mims et al., 2004).
(B) Secretory vesicles differentially localize around *Hpa*. Confocal micrographs of *At* transgenic lines expressing the indicated fluorophore fusions show cross-sections of non-infected and *Hpa*-infected leaves at 3 dpi. *Hpa* haustoria are shown in bright field images indicated by asterisks. RPW8.2–YFP, YFP–SYP32 and YFP–Got1p are detected in vesicles around *Hpa* haustoria. Red signals seen in uninfected RPW8.2–YFP leaves are chlorophyll autofluorescence. Bar = 10 µm. Image reproduced from (Lu et al., 2012).

tochondria, and lipid bodies (Fig. 5A). These structures suggest the haustoria maintains an active metabolism and reinforces the hypothesis that it is the primary site of pathogen feeding (Mims et al., 2004). The extrahaustorial membrane displays a highly convoluted topology, perhaps resulting from vesicle traffic to and from the haustoria (Fig. 5A).

The signaling and trafficking differences that constitute host resistance versus successful haustorial formation are not well understood. It is clear that many responses originate from the plant, but the pathogen likely coopts some of these functions for its own purposes. The extrahaustorial membrane is synthesized *de novo* and lacks many integral proteins typically found in the plant PM (Lu et al., 2012). Some plant defensive proteins localize to the extrahaustorial membrane, including immune receptors such as FLS2 and trafficking proteins such as PENETRATION1 (PEN1, Fig. 5B). Studies of early *Hpa* infection reveals haustorial penetration is accompanied by reorganization of GFP-tagged cytoskeleton and organelles in *Arabidopsis* mesophyll cells (Takemoto et al., 2003; Mims et al., 2004; Lu et al., 2012). This reorganization is observed in compatible and incompatible interactions. Some of these responses are stimulated by plant immune signaling as well as mechanical disturbance (Hardham et al., 2008). However, a successful infection would likely benefit from plant organelles close to haustorium for access to plant cell trafficking and metabolism. Host cell rearrangements are accompanied by accumulation of secretory vesicles, flush with lipids for the new membrane (Takemoto et al., 2003). Other cargo contained in these vesicles remains to be identified. The purpose of these vesicles as defense mechanism or transporter of nutrients to the pathogen is unclear.

A signature of successful *Arabidopsis* resistance to *Hpa* and *Albugo* invasion is robust production of cell wall appositions, or encapsulation of haustorium in callose (Donofrio and Delaney, 2001). These callose-containing structures are hypothesized to provide physical protection against pathogen feeding structures (Allen and Friend, 1983). Arabidopsis PLASMODESMATA-LOCATED PROTEIN 1 moves to haustorial sites and stimulates callose production to impede haustorial entry. Suppression of immunity likely includes reduction in *PDLP1* expression and activity, as the gene is not expressed in fully colonized cells (Caillaud et

al., 2014). Plants deficient in salicylic acid signaling fail to produce robust callose depositions and often produce callose bands at the neck of haustorium (Donofrio and Delaney, 2001). While abundant callose appears to stymie haustorium formation, callose neck bands at the site of cell wall penetration of *Hpa* and *Albugo* haustorium may promote haustorial accommodation.

Although studies with *Arabidopsis* have provided some initial insights, the biogenesis of haustoria and cellular aspects of interaction with plant cells are poorly understood and represent a very fruitful area for inquiry that encompasses cell biology of the plant and the oomycete. Recently, Arabidopsis orthologs of symbiosis genes of legumes were shown to also play a role during downy mildew infection. Mutants were affected in haustorial maintenance, suggesting overlap in accommodation of microbes between the different plant species (Ried et al., 2018). *Arabidopsis* will be particularly useful for identifying host factors that are manipulated during haustorial accommodation.

NUTRIENT ACQUISITION

Another challenge faced by oomycetes is the need to obtain nutrients from the host (Lapin and Van den Ackerveken, 2013). This challenge is compounded in obligate oomycetes by the loss of conserved metabolic pathways (McDowell, 2011; Kemen and Jones, 2012). For example, genome analysis of the obligate biotroph *Hpa* revealed loss of the machinery to reduce inorganic nitrate and sulfite to their essential organic forms (Baxter et al., 2010). The *Albugo* genome reveals similar deficiencies along with the loss of the thymine synthetic pathway (Links et al., 2011). Thus, these pathogens must have a way to compensate for the lack of metabolic machinery in their own genomes, and it will be interesting to compare their nutrient acquisition strategies with those employed by *Phytophthora* pathogens in which these pathways are intact. Unfortunately, similar to accommodation of haustoria, nutrient acquisition by oomycetes is poorly understood and Arabidopsis has not been fully exploited. Only one publication reports physiological aspects of an Arabidopsis-oomycete interaction: Arabidopsis invertase activity is induced during *Albugo* infection, while chlorophyll production and photosynthesis are down-regulated (Chou et al., 2000). These changes occurred only in regions directly associated with the pathogen, suggestive of pathogen manipulations or a localized defense response.

Much potential exists to exploit the tools of Arabidopsis to better understand the metabolic interplay between plants and oomycetes. Vesicle traffic through the extrahaustorial matrix is hypothesized to contain effectors and virulence factors from the pathogen, and sugars, proteins, and lipids from the host. Bacterial plant pathogens are now known to induce expression of sugar transporters in their hosts at the site of infection (Chen et al., 2010), and research is ongoing to identify if this phenomenon occurs during oomycete infections. Indeed, a mutant in the Arabidopsis *SWEET2* gene reduces susceptibility to *Pythium* in roots (Chen et al., 2015). However, initial clues are evident in recent gene expression profiling studies: A survey of gene expression in *Phytophthora parasitica* during Arabidopsis root colonization suggested that uptake of host amino acids is prevalent during early infection (Attard et al., 2014). A survey of Arabidopsis gene expression during infection of leaves by *Hpa* identified an up-regulated cluster of genes involved in nitrate transport (Asai et al., 2014). Detailed experimental follow-up of these observations would likely provide important insights into the relationship between host and pathogen metabolism.

In this context, the accessible genetic tools of Arabidopsis and capacity for gene editing provide a splendid resource to identify genes related to metabolism that affect the interaction with the pathogen (Sonawala et al., 2018). If a pathogen requires specific metabolites as a carbon or nitrogen source, pathogen starvation may be caused by loss of a gene responsible for that metabolite's synthesis or transport. Such genes might fulfill the genetic definition of a susceptibility gene (S-gene) as discussed in the immune signaling section. Importantly, such genetic knockouts could be resistant because of deficiencies in nutrients critical to pathogen growth, rather than activation/priming of an immune response. One such example might be the aforementioned mutants *rsp1* and *rsp2*, which accumulate methionine, threonine and isoleucine, without induction of a defense response (Stuttmann et al., 2011). These alterations in metabolism are linked to increased resistance to *Hpa* and a biotrophic fungal pathogen (Stuttmann et al., 2011). Similarly, the Arabidopsis *dmr1* mutant, that is mutated in *HOMOSERINE KINASE* (*HSK*), has increased levels of homoserine, which is a common precursor of methionine, threonine, and isoleucine. L-homoserine application was sufficient to make Arabidopsis resistant to *Hpa* (van Damme et al., 2009). The resistance in *rsp1*, *rsp2* and *dmr1* mutants is independent of salicylic acid (SA)-induced defense (van Damme et al., 2009; Stuttmann et al., 2011). This separation of metabolic incompatibility from canonical immunity demonstrates that pathogen virulence depends on more than host immune suppression. Pathogens have adapted to use metabolites present in their host, and perturbations of these host nutrient pools apparently leads to host resistance, particularly to biotrophic species. Certain metabolic genes may be the direct or downstream targets of effectors, as is the case for bacteria. These targets might be more difficult to identify than well conserved immune hubs. Even so, Arabidopsis provides an ideal system for continued study in this area. The well-studied metabolism, and availability of T-DNA insertion mutants for most genes will allow for screens and analysis of additional S-genes and sources of metabolic incompatibility.

Although much remains to be discovered, it is clear that oomycete plant pathogens are adapted to exploit their host to their own ends. These pathogens turn their hosts' cell biology against them to achieve full virulence. Host cells respond differently in compatible and incompatible interactions giving indication of pathogen manipulations. Structures are rebuilt to physically accommodate the parasite. The pathogen metabolism is streamlined to utilize molecules found in the host. Understanding how plants accommodate pathogens has potential to establish new mechanisms of resistance. Crop species can be bred or engineered as inhospitable hosts, by removing S-genes or establishing metabolic incompatibility. This may provide novel forms of protection from dangerous and economically important plant diseases. Work in *Arabidopsis*-oomycete pathosystems will yield further insights into these interactions and open these translational opportunities.

SUMMARY AND OPPORTUNITIES FOR FUTURE RESEARCH

Much progress has been made over the three decades in which Arabidopsis has been used to understand plant-oomycete interactions. Indeed, research with Arabidopsis has been at the forefront of some of the most important insights in this area, which are summarized in Table 4.

However, there are many aspects of plant-oomycete interactions that are poorly understood. Table 5 outlines a number of general challenges and questions that remain open: For example, despite the impressive steps forward in understanding how plants perceive oomycete PAMPs and effectors, we lack a comprehensive map of downstream immune signaling pathways and we have limited direct proof about the causal mechanisms through which oomycete growth is impaired during a successful immune response. Linkages of the immune system to other plant physiological process also remain to be revealed. In addition, we have only scratched the surface of mechanistic comprehension of how oomycetes target key nodes in the immune system. The interactome studies referenced above provide an inspiring example of how these questions can be addressed productively. Furthermore, we emphasize strongly that suppression of immunity represents only one of several tasks

Table 4. Major insights into plant-oomycete interactions from *Arabidopsis* research.

Insight	Key References
Cloning of the first *R* genes against oomycetes	Parker et al., 1997; Botella et al., 1998; McDowell et al., 1998
First insights into evolutionary dynamics of *R* genes against oomycetes	Botella et al., 1998; McDowell et al., 1998; Bittner-Eddy et al., 2000
Definition of important immune system regulators	Parker et al., 1996
Identification and map-based cloning of the first mutants exhibiting gain of resistance to oomycetes	Van Damme et al., 2005; van Damme et al., 2008
Genetic definition of the complexity of the immune signaling network against oomycetes	Aarts et al., 1998; McDowell et al., 2000; Bittner-Eddy and Beynon, 2001
Definition of penetration resistance	Lipka et al., 2005
Molecular cloning of the first oomycete *Avr* gene	Allen et al., 2004
First insights into evolutionary dynamics of *Avr* gene loci	Allen et al., 2004; Armstrong et al., 2005; Rehmany et al., 2005
Identification of the RxLR motif	Rehmany et al., 2005
Test bed for new technologies to understand effector functions	Fabro et al., 2011; Caillaud et al., 2012
Discovery that oomycete, fungal, and bacterial effectors can target the same plant signaling hubs	Mukhtar et al., 2011; Wessling et al., 2014
First insights into genomic basis and evolution of obligate biotrophy	Baxter et al., 2010; Kemem et al., 2011

Table 5. Major knowledge gaps in understanding of plant-oomycete interactions that can be efficiently addressed with Arabidopsis research.

Challenge
Comprehensive map of the plant immune system, including connections to other processes
Understand how points of vulnerability in the immune network are targeted by oomycetes and other pathogens
Functional studies of unknown effector proteins
Explore how oomycetes obtain nutrients from plant hosts
Understand how oomycetes manipulate plant cell structure and physiology
Exploring the evolution and ecology of plant-oomycete interactions
Defining how the plant microbiome influences interactions with oomycetes
Leverage Arabidopsis resources to test innovative strategies for disease control

that an adapted pathogen must accomplish to complete its life cycle on the host. Very little is known about how oomycetes interpret physical and/or chemical cues to navigate through host tissues, nor how they manipulate host cell structure establish feeding conduits, nor how they manipulate host metabolism to serve their own needs (Lapin and Van den Ackerveken, 2013). All of these processes comprise major gaps in understanding of how plants and oomycetes interact. In addition, huge potential exists to exploit natural variation in Arabidopsis-oomycete interactions to consider host-microbe ecology and evolution, along with the role of the microbiome in plant-oomycete interactions and the impacts of oomycetes on plant-microbiota interactions (Holub, 2008; Kemen and Jones, 2012). In summary, there is extensive space for innovative, impactful research within Arabidopsis-oomycete pathosystems.

For these reasons, we strongly recommend that the molecular plant-microbe research community continue to exploit the well-developed experimental advantages of Arabidopsis as a reference system. In this way, we can move efficiently towards a systems-level understanding of plant-oomycete interactions, which will undoubtedly inspire new approaches towards low-input mitigation of crop diseases (Michelmore et al., 2017). Indeed, leads for translational research are already emerging directly from basic research on Arabidopsis-oomycete interactions. For example, cloned pattern-recognition receptors (e.g., RLP23) (Albert et al., 2015) and NLR proteins could be moved to crop species, as already demonstrated for WRR proteins (Borhan et al., 2010; Cevik et al., 2019). Another approach is to use the power of Arabidopsis genetics to identify plant genes in which loss-of-function mutants lead to reduced susceptibility (i.e., mutants of oomycete disease susceptibility genes or negative regulators of immunity) (Boevink et al., 2016). Such genes can then be mutated in crops (e.g., by TILLING or by editing) and tested for pathogen resistance. This approach is currently being pursued for the *DMR6* gene (Zeilmaker et al., 2015). Finally, Arabidopsis can serve as a test bed for high-risk approaches, such as host-induced gene silencing, for which a genetically facile host could speed optimization (Govindarajulu et al., 2015).

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