

## Commentary

# Effector-mediated partial and nonhost disease resistance in wheat

Plants are considered to have three major forms of resistance against pathogen infection. The first is ‘nonhost resistance’, which operates broadly at the species level, meaning all members of a plant species generally resist all members of the would-be pathogen species. The other two systems operate at the ‘host’ level. ‘Qualitative host resistance’ is seen when an individual plant cultivar (or genotype) completely resists attempted attack by an individual pathogen strain, whilst ‘partial resistance’ is when a plant cannot completely defend itself. Each of these systems have some underlying molecular principles which will be outlined later. In this issue of *New Phytologist*, Meile *et al.* (2023; pp. 1562–1577) describe a novel mechanism by which both partial and nonhost resistance can be achieved in wheat facing the fungal pathogen *Zymoseptoria tritici* and its closely related species.

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*‘This suggests that the stomatal penetration step is key to mediating various forms of disease resistance to Zymoseptoria species.’*

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Plant-infecting fungal pathogens like *Z. tritici* release hundreds of small secreted effector proteins to aid infection (Stergiopoulos & de Wit, 2009). This fungus is typical of many other *Mycosphaerella* fungi, in that it penetrates the leaves of host plants almost exclusively through stomata. It then colonises leaf apoplastic spaces, growing as intercellular hyphae for extended periods, before eventually triggering host cell death, which coincides with pathogen reproduction (sporulation). Transcriptomic studies have shown that many of the *Z. tritici* genes encoding putative effector proteins are upregulated very soon after spores alight the leaf surface, often preceding stomatal penetration events (Rudd *et al.*, 2015).

Returning to the generic molecular principles of plant disease resistance, nonhost resistance is generally thought to be genetically conferred as a multigenic trait, which can also involve preformed chemical and physical barriers. It has, however, also been suggested to be supported by plant recognition of pathogen effectors in a few cases (Schulze-Lefert & Panstruga, 2011). The classic molecular mechanism for qualitative resistance involves the recognition of

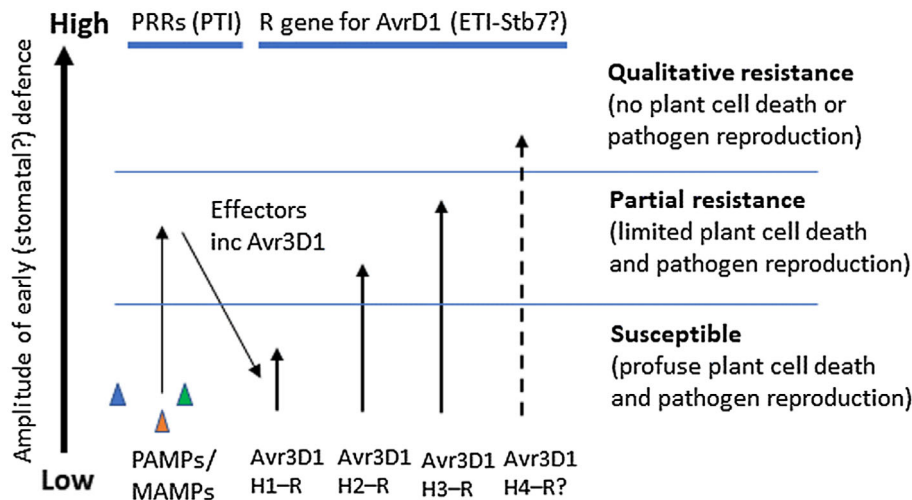
effectors by corresponding disease resistance genes (gene-for-gene interactions – Flor, 1971), which is one event in the classical ‘zig-zag’ model for the regulation of plant immunity (Jones & Dangl, 2006). The original function of the effectors themselves is generally considered to be suppression of innate immune responses triggered following recognition of pathogen/microbial-associated molecular patterns (PAMPs/MAMPs) by plant plasma membrane receptors (PRRs, pattern recognition receptors). An effector that is subsequently recognised by a disease-resistance gene, which then restores immunity, is considered to be an avirulence (Avr) factor. Although there are notable exceptions, to date, the majority of plant disease resistance proteins that recognise pathogen effectors are intracellular proteins possessing nucleotide-binding and leucine-rich repeat regions (NBS–LRRs). Historically, the zig-zag scheme has proved to be very useful for describing the regulation of immunity, particularly against biotrophic pathogens. The scheme proposed various thresholds for achieving qualitative resistance, with the highest threshold ultimately stimulating plant cell death, a ‘hypersensitive response’ (Jones & Dangl, 2006). Qualitative resistance was thus considered complete, allowing for no pathogen reproduction and defined as an ‘incompatible’ interaction (Flor, 1971). By contrast, partial resistance, like nonhost resistance, was considered to be a polygenic trait involving a diversity of different mechanisms. Effector protein recognition was not perceived to be a common means by which nonhost or partial host resistance could be achieved. However, the article by Meile *et al.* (2023) demonstrates that it can form a significant component of both (Meile *et al.*, 2023).

The story began in an earlier study by the same authors (Meile *et al.*, 2018). *Zymoseptoria tritici* vs wheat interactions also fit the gene-for-gene model for plant disease resistance in that sequence polymorphic secreted Avr proteins are often ‘recognised’ by single plant disease resistance proteins (Brading *et al.*, 2002). However, there are some interesting differences in the generic features of the qualitative model. First, the few cloned *R* genes to date encode plasma membrane receptors, which are more likely to monitor the extracellular environment, and second, qualitative disease resistance occurs with no plant cell death (Saintenac *et al.*, 2018, 2021). The previous study (Meile *et al.*, 2018) used a genetic approach to identify the fungal gene(s) responsible for an apparently strong, but incomplete (thus partial), yet cultivar-specific, resistance. The authors found that it was determined by a small secreted protein with the structural features of effector proteins. Thus, they identified what appeared to be an Avr protein, Avr3D1 (named after the original isolate possessing the avirulent protein form) that did not confer complete qualitative resistance when recognised. This suggested that this recognition may instead confer partial resistance.

The current study confirmed this. The authors used genome sequences of global *Z. tritici* isolate collections to identify a range of

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This article is a Commentary on Meile *et al.* (2023), 238: 1562–1577.



**Fig. 1** Adaptation of the zig-zag model for plant immunity to integrate an effector-mediated partial resistance of wheat towards the fungal pathogen *Zymoseptoria tritici*. Whilst the early function of effectors in suppressing pattern-triggered immunity (PTI) is still anticipated, the magnitude of R gene-mediated effector-triggered immunity (ETI) can vary with effector haplotypes (H1–H4). This supports effector-mediated partial resistance responses. The dashed line leading to full qualitative resistance through an as yet undetected haplotype remains speculative. Note that the role of plant cell death in this scheme is the opposite to what was proposed by the original zig-zag model (Jones & Dangl, 2006). MAMPs, microbial-associated molecular patterns; PAMPs, pathogen-associated molecular patterns.

sequence polymorphic versions (haplotypes) of Avr3D1. To test for any differential recognition of these haplotypes, the authors expressed them all in an identical genetic background. They used an isolate in which the native *Avr3D1* gene had been deleted, so the haplotypes were able to fully infect and reproduce on specific resistant cultivars. These assays demonstrated that various haplotypes conferred different levels of resistance in the same cultivar(s). Intriguingly, whilst some haplotypes were more weakly recognised than the original *Avr3D1* sequence, allowing for more disease, other haplotypes were more strongly recognised, permitting less disease but still not at qualitative levels. Thus, the authors demonstrated that an apparent partial host resistance of variable magnitudes can be conferred through the recognition of different haplotypes of a single Avr protein. The overall scheme supported by the data is summarised in Fig. 1 as an adaptation of the early phases of the classic zig-zag model.

However, the story did not end there. Closely related *Zymoseptoria* species exist that are adapted to particular wild grasses, but which cannot cause disease in wheat. Hence, wheat is a nonhost to these species. Due to their relative recent divergence, the genomes of these *Zymoseptoria* species are also very similar (Feurtey *et al.*, 2020), and the authors were able to identify Avr3D1 haplotypes in two of the related species (*Z. pseudotritici* and *Z. ardabiliae*). Remarkably, expression of these by an otherwise virulent *Z. tritici* isolate (as above) also resulted in strong partial resistance on wheat cultivars recognising ZtAvr3D1. These data suggest that recognition of a single Avr protein may be sufficient in some cases to confer (or contribute significantly to) nonhost resistance. The biological relevance of the observation was also supported by the fact that the *Avr3D1* orthologues were strongly upregulated during the early phase of the nonhost interaction.

The current study also began to characterise the mechanisms of the observed effector-mediated partial host and nonhost

resistances. Using fluorescent protein-expressing strains, the authors examined initial hyphal growth on the leaf surface and the frequency of successful stomatal penetration events. Whilst no changes were seen for any of the interactions in early surface hyphal growth, successful stomatal penetration events were much less frequent during both partial host resistance and nonhost resistance responses. In fact, there was a strong correlation observed between disease levels and the frequency of successful stomatal penetrations. These data suggest that the Avr3D1 recognition event appears around the point of stomatal penetration resulting in a reaction associated with these cells. These findings agree with results recently reported for other *Z. tritici* vs wheat Avr-R interactions. For example, *Z. tritici* strains harbouring the avirulence genes *AvrStb16q* and *AvrStb6* were also shown to be mostly arrested at the stomatal penetration step on *Stb16q* and *Stb6* wheat cultivars, respectively (Battache *et al.*, 2022). This suggests that the stomatal penetration step is key to mediating various forms of disease resistance to *Zymoseptoria* species. The fact that both the *Stb6* and *Stb16q* R genes encode plasma membrane localised receptor-like proteins, may also be notable. Are these proteins present on, or in the vicinity of, the guard cell plasma membranes one wonders?

This study, and those previously published, are beginning to define some common features and components of disease resistance reactions of wheat to *Zymoseptoria* (and perhaps more broadly *Mycosphaerella*) species. Near qualitative, partial and nonhost resistances all occur without plant cell death. Instead, the recognised fungal hyphae appear to arrest (or be arrested) at the key stomatal penetration step. This correlates with reduced levels of subsequent disease. Changes in stomatal aperture (transient closure) in resistant wheat cultivars following contact with avirulent fungal hyphae may be a key feature (Battache *et al.*, 2022). Overall, many of these characteristics are somewhat more reminiscent of

events typical of PAMP/MAMP-triggered plant immunity (Zhang *et al.*, 2008). Also, whilst the *R* gene, which recognises *Avr3D1*, remains to be cloned, the fact that *Stb6* and *Stb16q* both encode plasma membrane-anchored receptor-like proteins, agrees well with the structure of almost all plant PRRs recognising PAMP/MAMPs and other pathogen elicitors (Zipfel, 2014). This provides further support for the concept that *R* gene-mediated and PAMP/MAMP-triggered immunity in plants are often not clearly distinct. It instead supports the existence of conceptual and functional overlaps, constituting a 'blurring' of the two systems (Thomma *et al.*, 2011). From the data presented in this paper and others, along with the stomatal-based mechanisms emerging, it may well be that *R* gene-mediated recognition event(s) of wheat to *Z. tritici* underpin all three types of conceptual disease resistances, through effectively co-opting elements of a PAMP/MAMP-based form of immunity. These ideas, and many others arising from this article, merit further testing.

## Acknowledgements

Jason Rudd acknowledges funding from the Biotechnology and Biological Scientific Research Council (BBSRC) of the United Kingdom through the institute strategic grant 'Designing Future Wheat' (BBS/E/C00010250).

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**Key words:** diversifying selection, dothideomycetes, effector-triggered immunity, fungal effectors, hypersensitive response, PAMP-triggered immunity, plant disease resistance, stomatal guard cells.