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Just the surface: advances in the discovery and characterization of necrotrophic wheat effectors

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For many years pathogens of wheat have remained poorly understood. Hindered by an inaccessible host and the obligate nature of many of the pathogens, our understanding of these interactions has been limited compared to other more amenable pathosystems. However, breakthroughs over recent years have shed new light on diseases of wheat, particularly those caused by the genetically tractable necrotrophic pathogens. We now understand that many of the necrotrophic fungal pathogens do interact with wheat in a strict gene-for-gene relationship, and that pathogen and host partners in these interactions have now been identified. This improved understanding of necrotrophic effector biology has fundamentally changed the way we consider these important wheat diseases.

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Introduction

In 2017 the world is set to produce over 700 million tons of wheat (http://www.fao.org/). Intensive production has led to the evolution of highly sophisticated pathogens, with specialized molecular tools to exploit one of our most widely grown crops. For this article, we will leave biotrophic wheat pathogens aside, as they will be discussed in detail by others in this special issue (Bourras *et al.*, this issue; Lorrain *et al.*, this issue). The pathogens discussed herein are generally classified as necrotrophs or latent necrotrophs of wheat [1,2]. Although the discovery of necrotrophic effector proteins in some species dates back to the early 1990s, detailed functional and interaction studies are hindered by three major factors: (1) wheat's complex genome, (2) wheat's immunity to *Agrobacterium* infection, (3) and the chaotic environment that necrotrophic effectors induce in susceptible cultivars. Despite these hurdles significant progress has been made in the last three to five years in both the understanding and identification of wheat necrotrophic effectors.

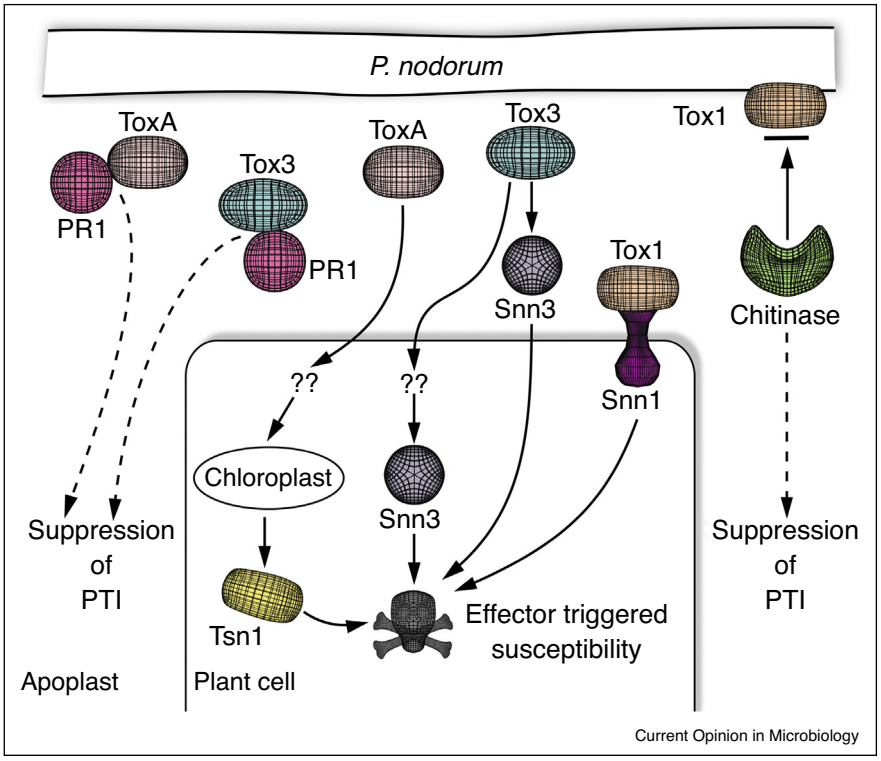
Arguably, the greatest progress has come from the foliar pathogens *Parastagonospora nodorum* and *Pyrenophora tritici-repentis*, the causal agents of Septoria nodorum blotch and tan spot respectively [3]. In *P. tritici-repentis*, the genes encoding the effector proteins ToxA and ToxB have been cloned and characterized [4,5]. Neither protein has a known function other than the induction of necrosis/chlorosis in susceptible cultivars but localization studies suggest that ToxA is internalized in host cells and targeted to the chloroplast (Figure 1) [6–8]. ToxA interacts with the dominant wheat susceptibility gene *Tsn1* in a gene-for-gene relationship resulting in cell death whilst ToxB interacts with *TscB* to cause chlorosis in susceptible wheat lines. In *P. nodorum*, three effectors have been cloned to date, SnToxA, SnTox1 and SnTox3 [9–11]. SnToxA is functionally identical to ToxA from *P. tritici-repentis* and is reported to be the subject of a horizontal gene transfer event between the two pathogens [10,11]. Like ToxA, SnToxA interacts with *Tsn1* resulting in cell death. Tox1 and Tox3 also induce cell death upon interaction with *Snn1* and *Snn3*, respectively [12,13]. Of these host genes, both *Tsn1* and *Snn1* have been cloned and encode a serine/threonine protein kinase, nucleotide binding site leucine rich repeat protein (NB-LRR) and a wall-associated kinase protein (WAK) [14,15]. Both proteins are typically associated with resistance (to biotrophic and hemibiotrophic pathogens) and provide a glimpse of the mechanistic similarities between these inverse-gene-for-gene effectors and the more commonly described avirulence genes. It should though be noted that the identification of these effectors have been the subject of recent reviews and would not be discussed below (please see [16–18]).

In this review, we discuss the most recent advances in necrotrophic effector biology in wheat pathogens. This includes the progress on discovery of additional functions for effectors described above and the impact of genomics on effector discovery in other wheat pathogens.

Gene-for-gene but quantitative

Effector triggered susceptibility (ETS) in the context of necrotrophic effectors, refers to the widespread necrotic response induced when the effector is expressed in the presence of the corresponding wheat susceptibility

Figure 1



An illustrative summary of the known and postulated interactions between the proteinaceous effectors ToxA, Tox3 and Tox1 and their host counterparts. Each effector is drawn linked to its corresponding wheat susceptibility gene leading to Effector triggered susceptibility and host cell death. The other roles of all three effectors are illustrated as needed. These interactions are hypothesized to lead to suppression of PAMP Triggered Immunity (PTI).

gene [19]. These gene-for-gene interactions often express themselves as quantitative traits, due to the number of simultaneous interactions in both the host and pathogen genetic backgrounds [13,19–23]. In some cases, the necrotrophic effectors appear to contribute additively to lesion development [19–21]. In other cases however, the presence of one necrotrophic effector masks all measurable symptoms of another [13,22,23]. Attempts to link effector gene expression to host genotype have also come to conflicting results, with specific isolates showing statistically significant differences in effector gene expression and other isolates showing no significant change [20,22]. A much broader screen of isolates comparing effector gene expression on a broad range of host genotypes would be required to understand these differences. Similarly, it is widely accepted that the known effectors *SnToxA*, *SnTox3* and *SnTox1* are highly expressed *in planta* peaking between 24 and 72 hours post-infection, well before visible symptoms develop (typically around 72 hours post-infection) [20,24•,25]. This expression pattern is difficult to mimic outside of the plant host, indicating that there are yet unknown cues from pathogenicity-related development or from the environment inside the host that trigger effector gene expression.

Recent studies have attempted to tease apart the contribution of individual effectors and reveal new interactions by knocking-out known effector genes and examining symptom development and disease expression in these mutants [22,23,26–29]. Tan *et al.* [28] observed necrosis on three cultivars from culture filtrate of a triple knockout of *SnToxA*, *SnTox3* and *SnTox1* in the isolate SN15. These knock-out strains have also revealed subtler epistatic interactions between the effectors, where-by knockout *SnTox1* led to a threefold increase in expression of *SnTox3* [22]. How the fungus can detect or sense the absence of a specific effector in knock-out strains and in-turn increase the expression of other genes remains unknown.

Genome sequencing combined with genetics is a powerful approach for identifying effectors

To date there is no recognized motif or sequence homology that enables *in silico* identification of wheat necrotrophic effectors from genome sequences, though a new machine learning approach is making in-roads in this area [30]. Genome sequencing has however, enabled discovery of effectors/avirulence genes through two techniques traditionally used only on the host, quantitative trait locus