Proteinaceous necrotrophic effectors in fungal virulence

Kar-Chun Tan[1,2*], Richard P. Oliver[1,2], Peter S. Solomon[3], Caroline S. Moffat[1]

[1]Australian Centre for Necrotrophic Fungal Pathogens, Murdoch University, Murdoch, WA 6150, Australia

[2]Present address; Australian Centre for Necrotrophic Fungal Pathogens, Curtin University, Bentley, WA 6845, Australia

[3]Division of Plant Sciences, Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia

*Corresponding author; Kar-Chun.Tan@curtin.edu.au

Keywords

Host-selective toxin, lateral gene transfer, Tan spot, Stagonospora, Pyrenophora, yellow spot, septoria, glume blotch, net blotch

19 **Abstract**

20 The host-pathogen interface can be considered as a biological battlefront. Molecules
21 produced by both the pathogen and the host are critical factors determining the outcome of
22 the interaction. Recent studies have revealed that an increasing number of necrotrophic
23 fungal pathogens produce small, proteinaceous effectors which are able to function as
24 virulence factors. These molecules can cause tissue death in host plants that possess dominant
25 sensitivity genes leading to subsequent pathogen colonisation. Such effectors are only found
26 in necrotrophic fungi, yet their roles in virulence are poorly understood. However, several
27 recent key studies of necrotrophic effectors from two wheat pathogens, *Pyrenophora tritici-*
28 *repentis* and *Stagonospora nodorum*, have shed light upon how these effector proteins serve
29 to disable the host from inside-out.

30

31 **Introduction**

32 Necrotrophic fungi were traditionally considered as non-host-specific pathogens that
33 use a large array of cell wall-degrading enzymes and non-specific toxins for pathogenicity
34 (Hammond-Kosack and Rudd 2008). It is now known that some of these necrotrophs possess
35 an arsenal of effectors used to disable susceptible hosts ahead of colonisation. As with the
36 avirulence (Avr) effectors of biotrophic fungal pathogens (reviewed in this issue),
37 necrotrophic effectors share some common properties (Table 1). However, in contrast to the
38 classical gene-for-gene hypothesis, where the interaction of avirulence effectors with host R-
39 gene complexes leads to resistance, necrotrophic effectors function in an 'inverse' manner. An
40 interaction between a necrotrophic effector and the product of a host dominant sensitivity
41 gene leads instead to disease (Figure 1).

42 Necrotrophic effectors are a diverse group of molecules that induce tissue death in
43 host plants possessing the appropriate genotype. They are typically small and can be
44 proteinaceous in nature or secondary metabolites. Examples of metabolite-based effectors
45 include victorin of *Cochliobolus victoriae* and AAL-toxin of *Alternaria alternata*, and these
46 have been extensively reviewed elsewhere (Walton 1996; Wolpert *et al.* 2002). The purpose
47 of this review will focus on discussing recent discoveries of proteinaceous necrotrophic
48 effectors and their roles in virulence.

49 **Effectors of *Pyrenophora tritici* f. sp. *repentis***

50 *Pyrenophora tritici-repentis* is the causal agent of tan spot (previously called either
51 yellow spot or yellow leaf spot), a devastating disease of wheat. To date, two proteinaceous
52 effectors of this necrotroph have been identified, PtrToxA and PtrToxB encoded by the genes
53 *PtrToxA* and *PtrToxB*.

54 PtrToxA was the first effector to be isolated and is the best characterised. The
55 PtrToxA protein is the product of a single copy gene, which is present in approximately 80%
56 of a worldwide collection of isolates (Friesen *et al.* 2006). PtrToxA is a small (13.2 kDa),
57 secreted protein that causes necrosis in sensitive wheat genotypes (Ballance *et al.* 1989;
58 Tomas *et al.* 1990; Tuori *et al.* 1995). Ciuffetti and colleagues has demonstrated that the
59 *PtrToxA* gene is both necessary and sufficient for the pathogenicity of *P. tritici-repentis*,
60 since transformation of a non-pathogenic *P. tritici-repentis* isolate with the *PtrToxA* gene is

61 sufficient to render that isolate pathogenic on PtrToxA-sensitive wheat lines (Ciuffetti *et al.*
62 1997).

63 Wheat sensitivity to the PtrToxA effector is conditioned by *Tsn1*, a single gene
64 present on the long arm of chromosome 5B (Anderson *et al.* 1999; Faris *et al.* 1996). The
65 *Tsn1* gene has very recently been cloned, and found to possess nucleotide-binding site (NBS),
66 leucine-rich repeat (LRR) and serine/threonine protein kinase domains, all of which are
67 necessary for PtrToxA sensitivity (Faris *et al.* 2010). Paradoxically, these domains are
68 common features of plant disease resistance (*R*) genes which are involved in defence against
69 biotrophic pathogens (Martin *et al.* 2003).

70 Parallels can be drawn with the victorin effector of *Cochliobolus victoriae*, the causal
71 agent of victoria blight disease of oats (Meehan and Murphy 1946). The *C. victoriae*
72 susceptibility gene has been identified in *Arabidopsis thaliana* as *LOV1*, which like *Tsn1*, has
73 a NBS-LRR structure and mediates responses associated with biotrophic disease resistance
74 (Lorang *et al.* 2007). However, victorin rapidly induces resistance-like physiology in oats,
75 including a respiratory burst and apoptotic-like cell death (Wolpert *et al.* 2002). Yet how can
76 the elicitation of a "resistance" response lead to disease susceptibility? What seems at first to
77 be a contradiction in terms could in fact be accounted for by the lifestyle of the pathogen.
78 Thus an environment that would be unfavourable to biotrophic pathogens (such as
79 programmed cell death generated by the plant host) would actually be favourable to
80 pathogens with a necrotrophic lifestyle. This suggests that *R* genes could paradoxically play a
81 role in disease susceptibility, by serving as targets for necrotrophic effectors, such as
82 PtrToxA.

83 Several recent studies have helped to unravel the mode of action of PtrToxA.
84 Cytological analyses have revealed that PtrToxA is rapidly internalised into the mesophyll
85 cells of sensitive wheat cultivars (Manning and Ciuffetti 2005; Manning *et al.* 2008).
86 However, the protein or proteins with which PtrToxA interacts at the cell membrane remain
87 unidentified. Although *Tsn1* is necessary to mediate PtrToxA recognition, yeast two-hybrid
88 experiments suggest that Tsn1 does not interact directly with PtrToxA nor does it possess any
89 apparent transmembrane domains (Faris *et al.* 2010).

90 Analysis of the mature PtrToxA protein sequence has demonstrated the presence of an
91 arginyl-glycyl-aspartic (RGD) motif present at the surface of the PtrToxA protein (Zhang *et*

al. 1997), which is required for ToxA internalisation (Manning and Ciuffetti 2005; Manning *et al.* 2008). This sequence is located on a solvent-exposed loop and is easily accessible for protein-protein interactions (Sarma *et al.* 2005b). In animals, the RGD motif is involved in the binding of extracellular matrix proteins to transmembrane integrin proteins (D'Souza *et al.* 1991; Ruoslahti and Pierschbacher 1986). These integrins have been utilised by many mammalian pathogens as adhesion sites and as binding sites for effectors (Isberg and Tran Van Nhieu 1994). Thus, it is it conceivable that PtrToxA internalisation relies on recognition of the RGD motif by a plant integrin-like protein receptor. Indeed, integrin-like proteins have been identified in plants (Baluska *et al.* 2003; Faik *et al.* 1998; Laboure *et al.* 1999; Nagpal and Quatrano 1999; Sun *et al.* 2000; Swatzell *et al.* 1999) and may provide candidates for mediating PtrToxA internalisation.

Much of what occurs once PtrToxA is internalised is still unknown. However, there is evidence to suggest that the action of PtrToxA is associated with photosynthetic pathways (Manning *et al.* 2009). For example, once inside the cell, the chloroplast appears to be a target for PtrToxA. *In vitro* experiments suggest that PtrToxA is able to interact with the chloroplast-localised protein called ToxA-binding protein 1 (ToxABP1), homologs of which have been found across several plant species (Manning *et al.* 2007; Sarma *et al.* 2005a). Although the precise function of ToxABP1 is unknown, it has been suggested to play a part in photosystem function and/or thylakoid formation (Keren *et al.* 2005; Wang *et al.* 2004). Indeed, PtrToxA treatment has been demonstrated to induce changes in photosystems I and II, leading to light-dependent accumulation of reactive oxygen species (ROS) in the chloroplast (Manning *et al.* 2009). The link between PtrToxA and photosynthesis is further supported by the light-dependent nature of PtrToxA-induced necrosis and the tight regulation of *Tsn1* transcription by both the circadian clock and light (Faris *et al.* 2010; Manning and Ciuffetti 2005).

Two independent studies have examined the global transcriptional changes induced by PtrToxA on sensitive wheat cultivars (Adhikari *et al.* 2009; Pandelova *et al.* 2009). Both studies illustrate that considerable transcriptional reprogramming occurs following PtrToxA treatment. Numerous defence-related host genes were up-regulated at both early and late time points, including those associated with the phenylpropanoid pathway, lignification and ROS production, as well as genes functioning in signal transduction. Taken together, these studies suggest that PtrToxA disrupts photosynthetic electron transport, leading to ROS

124 accumulation and plant cell death upon light exposure, thus creating an environment in which
125 necrotrophic pathogens may thrive.

126 Another effector from Ptr which has been characterised is PtrToxB. Like PtrToxA,
127 PtrToxB is also a small, secreted protein (6.6 kDa) which causes chlorosis on sensitive wheat
128 genotypes, and is encoded by a multicopy gene, *PtrToxB* (Martinez *et al.* 2001; Orolaza *et al.*
129 1995; Strelkov *et al.* 1999). Although not as prevalent as PtrToxA, PtrToxB has been found
130 to be produced by a number of isolates around the world (Ali and Francl 2003; Friesen and
131 Faris 2005; Lamari *et al.* 2005). Wheat sensitivity is conditioned by the dominant *Tsc2* gene,
132 which has been mapped to the short arm of chromosome 2B (Friesen and Faris 2004;
133 Strelkov *et al.* 1999). Unlike most effectors, PtrToxB homologues have been found across a
134 broad range of plant pathogenic ascomycetes, suggesting that it may have arisen in an early
135 ancestor of the Ascomycota (Andrie *et al.* 2008). However, whether PtrToxB and its
136 homologues play a role in plant-microbe interactions is yet to be elucidated.

137 **Effectors of *Stagonospora nodorum***

138 *Stagonospora nodorum* is the causal agent of stagonospora (previously Septoria)
139 nodorum blotch (SNB) of wheat (Solomon *et al.* 2006). Evidence of necrotrophic effectors
140 produced by *S. nodorum* was first reported by Keller and colleagues (1994) using wheat
141 embryos. Genes encoding effector proteins have only been identified and characterised
142 recently (Friesen *et al.* 2006; Liu *et al.* 2009).

143 *SnToxA* was the first reported necrotrophic effector gene identified in *S. nodorum*
144 (Friesen *et al.* 2006). A BLAST search of the *S. nodorum* genome sequence with *PtrToxA*
145 identified an almost identical gene. Further genome exploration has revealed that *SnToxA* is
146 located within a highly conserved 11 kb genomic region that is present in both organisms.
147 This 'transfercon' was hypothesised to be acquired by *P. tritici-repentis* from *S. nodorum*
148 through lateral gene transfer, a biological process previously thought to be uniquely
149 prokaryotic. This hypothesis is supported by several key pieces of evidence. Firstly, SNB has
150 been known since the 1800s whilst tan spot was described as recently as 1941. Prior to this,
151 *P. tritici-repentis* was described as a saprophyte. Secondly, *ToxA* has only been found in
152 these two organisms to date. Finally, the nucleotide sequence of *SnToxA* exhibits greater
153 diversity in its polypeptide sequence than that of *PtrToxA*. Taken together, this strongly
154 suggests that *ToxA* was acquired by *P. tritici-repentis* prior to 1941 (Friesen *et al.* 2006;

155 Stukenbrock and McDonald 2007). The identification of SnToxA in *S. nodorum* highlights
156 the importance of genome sequencing in effector discovery (Hane *et al.* 2007). SnToxA and
157 PtrToxA possess the same mode of action. Both effectors cause necrosis on wheat carrying
158 *Tsn1* in a light-dependent manner (Friesen *et al.* 2006; Manning and Ciuffetti 2005).

159 The identification of *SnToxA* provided an opportunity to study the role of this gene in
160 fungal virulence, as unlike *P. tritici-repentis*, *S. nodorum* is genetically tractable. Several
161 lines of evidence have been published confirming that SnToxA interacts (directly or
162 indirectly) with *Tsn1*. Firstly, *S. nodorum* strains lacking *SnToxA* were non-pathogenic on
163 *Tsn1* wheat varieties (Friesen *et al.* 2006). Secondly, protein extracts from *SnToxA*-
164 expressing *S. nodorum* strains induced necrosis on *Tsn1* wheat whilst extracts from *SntoxA*
165 lines did not (Friesen *et al.* 2006). Lastly, transformation of an avirulent *ToxA*-deficient
166 wildtype strain of *S. nodorum* with *PtrToxA* allow the fungus to become virulent and cause
167 necrosis on wheat lines harbouring *Tsn1* (Friesen *et al.* 2006).

168 *SnTox3* was the second necrotrophic effector gene identified in *S. nodorum*. SnTox3
169 was first reported as a partially purified protein that causes necrosis on wheat carrying the
170 *Snn3* dominant sensitivity gene which is located on the short arm of chromosome 5 (Friesen
171 *et al.* 2008b; Liu *et al.* 2009). Gene knockout analysis of *SnTox3* indicated it to be a critical
172 component in *S. nodorum* virulence on *Snn3* wheat. The introduction of *SnTox3* into an
173 avirulent *Tox3*-deficient *S. nodorum* wildtype strain allowed it to infect and cause necrosis on
174 *Snn3* wheat varieties. Whilst detailed mechanistic studies have yet to be undertaken, SnTox3
175 appears to be functionally different to SnToxA. SnTox3 is cysteine-rich, a characteristic
176 typically associated with several described biotrophic avirulence effectors (Catanzariti *et al.*
177 2006; Van den Ackerveken *et al.* 1993). Also, unlike ToxA, SnTox3 does not require light to
178 induce necrosis on *Snn3* wheat. This suggests a different mode of function compared to
179 SnToxA. Gene expression analysis indicates that *SnTox3* is up-regulated during the early
180 stage of infection coinciding with host penetration (Liu *et al.* 2009). *SnToxA* also showed a
181 similar expression profile during infection (unpublished data).

182 These studies imply that these effectors function to disable host cells during the early
183 stage of infection. Thus, the invading fungus will have a readily accessible nutrient supply
184 during infection (Solomon *et al.* 2003).

185 *S. nodorum* also possesses at least three other proteinaceous necrotrophic effectors.
186 These are SnTox1, SnTox2 and SnTox4. However, genes that code for these proteins have
187 yet to be identified and therefore, the extent of their involvement in fungal virulence cannot
188 be fully gauged (Abeysekara *et al.* 2009; Friesen *et al.* 2007; Liu *et al.* 2004; Reddy *et al.*
189 2008). The wheat genes that confer sensitivity to these effectors are *Snn1*, *Snn2* and *Snn4*,
190 respectively. The use of molecular marker-based quantitative trait locus (QTL) analysis of
191 various wheat mapping populations has led to the identification of major QTLs in wheat
192 chromosome arms 1BS (*Snn1*), 2DS (*Snn2*) and 1AS (*Snn4*) that accounted for up to 58, 47
193 and 41% in disease variations, respectively (Abeysekara *et al.* 2009; Friesen *et al.* 2008a).

194 **Effectors of other necrotrophic fungi**

195 Proteinaceous effectors from other prominent necrotrophic fungi have also been
196 recently identified. *Alternaria brassicae* is a pathogen of the Brassicaceae. Evidence that this
197 pathogen produces necrotrophic effectors was reported by Parada *et al.* (2008). Semi-purified
198 protein fractions were shown to contain a 27.5 kDa protein, Abr-toxin, able to cause necrosis
199 on cabbage and oilseed. It caused no necrosis on the non-brassica, tomato. Co-inoculation of
200 the Abr-toxin and an avirulent isolate of *A. alternata* resulted in infectious symptoms on the
201 host leaf similar to *A. brassicae*. Partial protein sequencing revealed that the Abr-toxin
202 possesses amino acid sequence similarities to the protease trypsin.

203 *Pyrenophora teres* f. sp. *teres* and f. sp. *maculata* cause net-form net blotch and spot-
204 form net blotch of barley, respectively. Using protein chromatographic techniques, Sarpeleh
205 *et al.* (2007) demonstrated that both pathogens produce proteinaceous effectors that are
206 between 20 to 100 kDa. These semi-purified effectors induce strong necrosis on a barley
207 variety that is susceptible to both fungi, but caused a weak reaction in a resistant line of
208 barley. Like ToxA, these effectors require light to cause necrosis on the host plant (Sarpeleh
209 *et al.* 2008). The identity of these effectors from both *P. teres* subspecies is currently
210 unknown.

211 *Corynespora cassiicola*, a serious pathogen of rubber trees, produces a cysteine-rich
212 necrotrophic effector called cassiicolin. The effector is able to cause necrosis on detached
213 rubber tree leaves and other host plants such as tobacco and soy (de Lamotte *et al.* 2007).
214 Although the deduced effector amino acid sequence did not show significant homology with

215 other proteins, structural analysis indicates that the protein structure resembles trypsin-like
216 inhibitors (Barthe *et al.* 2007).

217 The fungus *Rhynchosporium secalis* is the causal agent of barley scald. Several small
218 cysteine-rich proteins designated as Nip1, 2 and 3 were identified in *R. secalis* and these are
219 capable of causing necrosis on a broad range of plants. Nip1 and 3 has been shown to
220 stimulate barley plasma H^+-ATPase which may be the likely cause of host tissue necrosis
221 (Wevelsiep *et al.* 1991; Wevelsiep *et al.* 1993). Nip1 has recently been shown to bind to a
222 single unidentified receptor that triggers the plant defense response (van't Slot *et al.* 2007). In
223 addition, Nip1 also functions as an avirulence effector on barley varieties that possess the
224 uncloned *Rrs1* gene (Rohe *et al.* 1995). A total of 14 Nip1 forms were identified, three of
225 which are associated with a gain in virulence on *Rrs1* barley (Schurch *et al.* 2004).

226 **Conclusion**

227 Necrotrophic fungi were, up until recently, considered as simplistic pathogens that
228 rely on a plethora of non-host-specific mechanisms to storm the host. Recent seminal
229 discoveries of host-selective necrotrophic effectors have revealed a new level of pathogenic
230 complexity. These breakthroughs highlight that necrotrophic fungi possess the ability to
231 selectively disable their host from within, before an effective defence response can be
232 mounted. The mode of action of these effectors is largely unknown, although studies on
233 ToxA clearly demonstrate that host metabolism is disabled prior to cell death. Hence, host-
234 selective effectors are paramount for these fungi to live a necrotrophic lifestyle.

235

236 **Table 1.** Properties of proteinaceous avirulence and necrotrophic effectors.

237

238 **Fig. 1.** Outcomes of fungal effector/host interactions. A to C depict the host response to a
239 fungal Avr protein. The host will mount a successful defence response against the pathogen if
240 Avr recognition occurs (A). Infection will occur if the pathogen is not recognised due to the
241 absence or altered R (resistance) receptor (B) or Avr effector (C). Necrotrophic effectors
242 function in an inverse manner (D to F). A successful infection will only occur during effector
243 recognition (D). During a non-recognition event, no disease will result due to the absence or
244 altered host sensitivity receptor (E) or fungal effector (F). For simplicity, receptors are
245 illustrated on the cell wall. We acknowledge that some host receptors are located
246 intracellularly.

247

248 **References**

249 Abeysekara NS, Friesen TL, Keller B, Faris JD (2009) Identification and characterization of a
250 novel host-toxin interaction in the wheat-*Stagonospora nodorum* pathosystem.
251 *Theoretical and Applied Genetics* **120**, 117-126.

252 Adhikari TB, Bai J, Meinhardt SW, Gurung S, Myrfield M, Patel J, Ali S, Gudmestad NC,
253 Rasmussen JB (2009) *Tsn1*-mediated host responses to ToxA from *Pyrenophora*
254 *tritici-repentis*. *Molecular Plant-Microbe Interactions* **22**, 1056-1068.

255 Ali S, Francl LJ (2003) Population race structure of *Pyrenophora tritici-repentis* prevalent on
256 wheat and noncereal grasses in the Great Plains. *Plant Disease* **87**, 418-422.

257 Anderson JA, Effertz RJ, Faris JD, Francl LJ, Meinhardt SW, Gill BS (1999) Genetic
258 analysis of sensitivity to a *Pyrenophora tritici-repentis* necrosis-inducing toxin in
259 durum and common Wheat. *Phytopathology* **89**, 293-297.

260 Andrie RM, Schoch CL, Hedges R, Spatafora JW, Ciuffetti LM (2008) Homologs of *ToxB*, a
261 host-selective toxin gene from *Pyrenophora tritici-repentis*, are present in the genome
262 of sister-species *Pyrenophora bromi* and other members of the Ascomycota. *Fungal*
263 *Genetics and Biology* **45**, 363-377.

264 Ballance GM, Lamari L, Bernier CC (1989) Purification and characterization of a host-
265 selective necrosis toxin from *Pyrenophora tritici-repentis*. *Physiological and*
266 *Molecular Plant Pathology* **35**, 203-213.

267 Baluska F, Samaj J, Wojtaszek P, Volkmann D, Menzel D (2003) Cytoskeleton-plasma
268 membrane-cell wall continuum in plants. Emerging links revisited. *Plant Physiology*
269 **133**, 482-491.

270 Barthe P, Pujade-Renaud V, Breton F, Gargani D, Thai R, Roumestand C, de Lamotte F
271 (2007) Structural analysis of cassiicolin, a host-selective protein toxin from
272 *Corynespora cassiicola*. *Journal of Molecular Biology* **367**, 89-101.

273 Catanzariti AM, Dodds PN, Lawrence GJ, Ayliffe MA, Ellis JG (2006) Haustorially
274 expressed secreted proteins from flax rust are highly enriched for avirulence elicitors.
275 *Plant Cell* **18**, 243-256.

276 Ciuffetti LM, Tuori RP, Gaventa JM (1997) A single gene encodes a selective toxin causal to
277 the development of tan spot of wheat. *Plant Cell* **9**, 135-144.

278 D'Souza SE, Ginsberg MH, Plow EF (1991) Arginyl-glycyl-aspartic acid (RGD): a cell
279 adhesion motif. *Trends in Biochemical Sciences* **16**, 246-250.

280 de Lamotte F, Duviau MP, Sanier C, Thai R, Poncet J, Bieysse D, Breton F, Pujade-Renaud
281 V (2007) Purification and characterization of cassiicolin, the toxin produced by
282 *Corynespora cassiicola*, causal agent of the leaf fall disease of rubber tree. *Journal of*
283 *Chromatography B* **849**, 357-362.
284 Faik A, Laboure AM, Gulino D, Mandaron P, Falconet D (1998) A plant surface protein
285 sharing structural properties with animal integrins. *European Journal of Biochemistry*
286 **253**, 552-559.
287 Faris JD, Anderson JA, Francl LJ, Jordahl JG (1996) Chromosomal location of a gene
288 conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from
289 *Pyrenophora tritici-repentis*. *Phytopathology* **86**, 459-463.
290 Faris JD, Zhang Z, Lu H, Lu S, Reddy L*, et al.* (2010) A unique wheat disease resistance-like
291 gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proceedings*
292 *of the National Academy of Sciences U S A*, In press.
293 Friesen TL, Faris JD (2004) Molecular mapping of resistance to *Pyrenophora tritici-repentis*
294 race 5 and sensitivity to Ptr ToxB in wheat. *Theoretical and Applied Genetics* **109**,
295 464-471.
296 Friesen TL, Faris JD (2005) Molecular mapping of resistance to *Pyrenophora tritici-repentis*
297 race 5 and sensitivity to Ptr ToxB in wheat. *Theoretical and Applied Genetics* **109**,
298 464-471.
299 Friesen TL, Faris JD, Solomon PS, Oliver RP (2008a) Host-specific toxins: effectors of
300 necrotrophic pathogenicity. *Cellular Microbiology* **10**, 1421-1428.
301 Friesen TL, Meinhardt SW, Faris JD (2007) The *Stagonospora nodorum*-wheat pathosystem
302 involves multiple proteinaceous host-selective toxins and corresponding host
303 sensitivity genes that interact in an inverse gene-for-gene manner. *Plant Journal* **51**,
304 681-692.
305 Friesen TL, Stukenbrock EH, Liu ZH, Meinhardt S, Ling H*, et al.* (2006) Emergence of a
306 new disease as a result of interspecific virulence gene transfer. *Nature Genetics* **38**,
307 953-956.
308 Friesen TL, Zhang Z, Solomon PS, Oliver RP, Faris JD (2008b) Characterization of the
309 interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat
310 susceptibility gene. *Plant Physiology* **146**, 682-693.
311 Hammond-Kosack KE, Rudd JJ (2008) Plant resistance signalling hijacked by a necrotrophic
312 fungal pathogen. *Plant Signalling and Behavior* **3**, 993-995.

313 Hane JK, Lowe RG, Solomon PS, Tan KC, Schoch CL*, et al.* (2007) Dothideomycete plant
314 interactions illuminated by genome sequencing and EST analysis of the wheat
315 pathogen *Stagonospora nodorum*. *Plant Cell* **19**, 3347-3368.
316 Isberg RR, Tran Van Nhieu G (1994) Binding and internalisation of micro-organisms by
317 integrin receptors. *Trends in Microbiology* **2**, 10-14.
318 Keller B, Winzeler H, Winzeler M, Fried PM (1994) Differential sensitivity of wheat
319 embryos against extracts containing toxins of *Septoria nodorum*: first steps towards *in*
320 *vitro* selection. *Journal of Phytopathology* **141**, 233-240.
321 Keren N, Ohkawa H, Welsh EA, Liberton M, Pakrasi HB (2005) Psb29, a conserved 22-kD
322 protein, functions in the biogenesis of Photosystem II complexes in *Synechocystis* and
323 *Arabidopsis*. *The Plant Cell* **17**, 2768-2781.
324 Laboure AM, Faik A, Mandaron P, Falconet D (1999) RGD-dependent growth of maize
325 calluses and immunodetection of an integrin-like protein. *FEBS Letters* **442**, 123-128.
326 Lamari L, Strelkov SE, Yahyaoui A, Amedov M, Saidov M, Djunusova M, Koichibayev M
327 (2005) Virulence of *Pyrenophora tritici-repentis* in the countries of the Silk Road.
328 *Canadian Journal of Plant Pathology* **27**, 383-388.
329 Liu Z, Faris JD, Oliver RP, Tan KC, Solomon PS*, et al.* (2009) SnTox3 acts in effector
330 triggered susceptibility to induce disease on wheat carrying the Snn3 gene. *PLoS*
331 *Pathogens* **5**, e1000581.
332 Liu ZH, Faris JD, Meinhardt SW, Ali S, Rasmussen JB, Friesen TL (2004) Genetic and
333 physical mapping of a gene conditioning sensitivity in wheat to a partially purified
334 host-selective toxin produced by *Stagonospora nodorum*. *Phytopathology* **94**, 1056-
335 1060.
336 Lorang JM, Sweat TA, Wolpert TJ (2007) Plant disease susceptibility conferred by a
337 "resistance" gene. *Proceedings of the National Academy of Sciences U S A* **104**,
338 14861-14866.
339 Manning VA, Chu AL, Steeves JE, Wolpert TJ, Ciuffetti LM (2009) A host-selective toxin of
340 *Pyrenophora tritici-repentis*, Ptr ToxA, induces photosystem changes and reactive
341 oxygen species accumulation in sensitive wheat. *Molecular Plant-Microbe*
342 *Interactions* **22**, 665-676.
343 Manning VA, Ciuffetti LM (2005) Localization of Ptr ToxA produced by *Pyrenophora*
344 *tritici-repentis* reveals protein import into wheat mesophyll cells. *Plant Cell* **17**, 3203-
345 3212.

346 Manning VA, Hamilton SM, Karplus PA, Ciuffetti LM (2008) The Arg-Gly-Asp-containing,
347 solvent-exposed loop of Ptr ToxA is required for internalization. *Molecular Plant-*
348 *Microbe Interactions* **21**, 315-325.
349 Manning VA, Hardison LK, Ciuffetti LM (2007) Ptr ToxA interacts with a chloroplast-
350 localized protein. *Molecular Plant-Microbe Interactions* **20**, 168-177.
351 Martin GB, Bogdanove AJ, Sessa G (2003) Understanding the functions of plant disease
352 resistance proteins. *Annual Review of Plant Biology* **54**, 23-61.
353 Martinez JP, Ottum SA, Ali S, Franci LJ, Ciuffetti LM (2001) Characterization of the *ToxB*
354 gene from *Pyrenophora tritici-repentis*. *Molecular Plant-Microbe Interactions* **14**,
355 675-677.
356 Meehan F, Murphy HC (1946) A new Helminthosporium blight of oats. *Science* **104**, 413-
357 414.
358 Nagpal P, Quatrano RS (1999) Isolation and characterization of a cDNA clone from
359 *Arabidopsis thaliana* with partial sequence similarity to integrins. *Gene* **230**, 33-40.
360 Orolaza NP, Lamari L, Ballance GM (1995) Evidence of a host-specific chlorosis toxin from
361 *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat. *Phytopathology*
362 **85**, 1282-1287.
363 Pandelova I, Betts MF, Manning VA, Wilhelm LJ, Mockler TC, Ciuffetti LM (2009)
364 Analysis of transcriptome changes induced by Ptr ToxA in wheat provides insights
365 into the mechanisms of plant susceptibility. *Molecular Plant* **2**, 1067-1083.
366 Parada RY, Sakuno E, Mori N, Oka K, Egusa M, Kodama M, Otani H (2008) *Alternaria*
367 *brassicae* produces a host-specific protein toxin from germinating spores on host
368 leaves. *Phytopathology* **98**, 458-463.
369 Reddy L, Friesen TL, Meinhardt SW, Chao S, Faris JD (2008) Genomic analysis of the *Snn1*
370 locus on wheat chromosome arm 1BS and the identification of candidate genes. *The*
371 *Plant Genome* **1**, 55-66.
372 Rohe M, Gierlich A, Hermann H, Hahn M, Schmidt B, Rosahl S, Knogge W (1995) The
373 race-specific elicitor, NIP1, from the barley pathogen, *Rhynchosporium secalis*,
374 determines avirulence on host plants of the *Rrs1* resistance genotype. *Embo J* **14**,
375 4168-4177.
376 Ruoslahti E, Pierschbacher MD (1986) Arg-Gly-Asp: a versatile cell recognition signal. *Cell*
377 **44**, 517-518.

378 Sarma GN, Manning VA, Ciuffetti LM, Karplus PA (2005a) Structure of Ptr ToxA: an RGD-
379 containing host-selective toxin from *Pyrenophora tritici-repentis*. *The Plant Cell* **17**,
380 3190-3202.
381 Sarma GN, Manning VA, Ciuffetti LM, Karplus PA (2005b) Structure of Ptr ToxA: an RGD-
382 containing host-selective toxin from *Pyrenophora tritici-repentis*. *Plant Cell* **17**,
383 3190-3202.
384 Sarpeleh A, Wallwork H, Catcheside DE, Tate ME, Able AJ (2007) Proteinaceous
385 metabolites from *Pyrenophora teres* contribute to symptom development of Barley
386 Net Blotch. *Phytopathology* **97**, 907-915.
387 Sarpeleh A, Wallwork H, Tate ME, Catcheside DEA, Able AJ (2008) Initial characterization
388 of phytotoxic proteins isolated from *Pyrenophora teres*. *Physiological and Molecular*
389 *Plant Pathology* **72**, 73-79.
390 Schurch S, Linde CC, Knogge W, Jackson LF, McDonald BA (2004) Molecular population
391 genetic analysis differentiates two virulence mechanisms of the fungal avirulence
392 gene *NIP1*. *Molecular Plant-Microbe Interactions* **17**, 1114-1125.
393 Solomon PS, Lowe RGT, Tan K-C, Waters ODC, Oliver RP (2006) *Stagonospora nodorum*:
394 cause of stagonospora nodorum blotch of wheat. *Molecular Plant Pathology* **7**, 147-
395 156.
396 Solomon PS, Tan K-C, Oliver RP (2003) The nutrient supply of pathogenic fungi; a fertile
397 field for study. *Molecular Plant Pathology* **4**, 203-210.
398 Strelkov SE, Lamari L, Ballance GM (1999) Characterization of a host-specific protein toxin
399 (Ptr ToxB) from *Pyrenophora tritici-repentis*. *Molecular Plant-Microbe Interactions*
400 **12**, 728-732.
401 Stukenbrock EH, McDonald BA (2007) Geographical variation and positive diversifying
402 selection in the host specific toxin *SnToxA*. *Molecular Plant Pathology* **18**, 321-332.
403 Sun Y, Qian H, Xu XD, Han Y, Yen LF, Sun DY (2000) Integrin-like proteins in the pollen
404 tube: detection, localization and function. *Plant and Cell Physiology* **41**, 1136-1142.
405 Swatzell LJ, Edelmann RE, Makaroff CA, Kiss JZ (1999) Integrin-like proteins are localized
406 to plasma membrane fractions, not plastids, in *Arabidopsis*. *Plant and Cell Physiology*
407 **40**, 173-183.
408 Tomas A, Feng GH, Reeck GR, Bockus WW, Leach JE (1990) Purification of a cultivar-
409 specific toxin from *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat.
410 *Molecular Plant-Microbe Interactions* **3**, 221-224.

411 Tuori RP, Wolpert TJ, Ciuffetti LM (1995) Purification and immunological characterization
412 of toxic components from cultures of *Pyrenophora tritici-repentis*. *Molecular Plant-*
413 *Microbe Interactions* **8**, 41-48.
414 van't Slot KA, Gierlich A, Knogge W (2007) A single binding site mediates resistance- and
415 disease-associated activities of the effector protein NIP1 from the barley pathogen
416 *Rhynchosporium secalis*. *Plant Physiology* **144**, 1654-1666.
417 Van den Ackerveken GF, Van Kan JA, Joosten MH, Muisers JM, Verbakel HM, De Wit PJ
418 (1993) Characterization of two putative pathogenicity genes of the fungal tomato
419 pathogen *Cladosporium fulvum*. *Molecular Plant-Microbe Interactions* **6**, 210-215.
420 Walton JD (1996) Host-selective toxins: agents of compatibility. *Plant Cell* **8**, 1723-1733.
421 Wang Q, Sullivan RW, Kight A, Henry RL, Huang J, Jones AM, Korth KL (2004) Deletion
422 of the chloroplast-localized *Thylakoid formation1* gene product in *Arabidopsis* leads
423 to deficient thylakoid formation and variegated leaves. *Plant Physiology* **136**, 3594-
424 3604.
425 Wevelsiep L, Kogel KH, Knogge W (1991) Purification and characterization of peptides
426 from *Rhynchosporium secalis* inducing necrosis in barley. *Physiological and*
427 *Molecular Plant Pathology* **39**, 471-482.
428 Wevelsiep L, Rupping E, Knogge W (1993) Stimulation of barley plasmalemma H+-ATPase
429 by phytotoxic peptides from the fungal pathogen *Rhynchosporium secalis*. *Plant*
430 *Physiology* **101**, 297-301.
431 Wolpert TJ, Dunkle LD, Ciuffetti LM (2002) Host-selective toxins and avirulence
432 determinants: what's in a name? *Annual Review of Phytopathology* **40**, 251-285.
433 Zhang HF, Francl LJ, Jordahl JG, Meinhardt SW (1997) Structural and physical properties of
434 a necrosis-inducing toxin from *Pyrenophora tritici-repentis*. *Phytopathology* **87**, 154-
435 160.
436
437

Table 1

Characteristic	Avirulence effector	Necrotrophic effector
Relative small size#	Yes	Yes
Secreted	Yes	Yes
Expression *in planta*	Yes	Yes
Receptor type^	Predominantly NBS-LRR	Unknown
Cysteine-rich*	Predominantly	Some
Compatible host response	No disease	Disease
Fungal lifestyle	Biotrophic/hemibiotrophic	Necrotrophic
Function/homology	Diverse	Unknown

#Under 30 kDa.
^NBS-LRR denotes nucleotide-binding site leucine-rich repeat protein.
*Greater than four cysteines per mature polypeptide.

Figure 1