1 **Septoria nodorum blotch of wheat: disease management and resistance breeding in the**
2 **face of shifting disease dynamics and a changing environment**
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20 **Key words**
21 Wheat disease resistance, quantitative trait loci (QTL), Septoria nodorum blotch (SNB),
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23
24

25 **Abstract**

26 The fungus *Parastagonospora nodorum* is a narrow host range necrotrophic fungal pathogen
27 that causes Septoria nodorum blotch (SNB) of cereals, most notably wheat (*Triticum aestivum*
28 L.). Although commonly observed on wheat seedlings, *P. nodorum* infection has the greatest
29 effect on the adult crop. It results in leaf blotch, which limits photosynthesis and thus crop
30 growth and yield. It can also affect the wheat ear, resulting in glume blotch which directly
31 affects grain quality. Reports of *P. nodorum* fungicide resistance, the increasing use of reduced
32 tillage agronomic practices and high evolutionary potential of the pathogen, combined with
33 changes in climate and agricultural environments, mean that genetic resistance to SNB remains
34 a high priority in many regions of wheat cultivation. In this review, we summarise current
35 information on *P. nodorum* population structure and its implication for improved SNB
36 management. We then review recent advances in the genetics of host resistance to *P. nodorum*
37 and the necrotrophic effectors it secretes during infection, integrating the genomic positions of
38 these genetic loci using the recently released wheat reference genome assembly. Finally, we
39 discuss the genetic and genomic tools now available for SNB resistance breeding and consider
40 future opportunities and challenges in crop health management using the wheat-*P. nodorum*
41 interaction as a model.

42 **Septoria nodorum blotch of wheat: a disease of shifting global importance**

43 Septoria nodorum blotch (SNB) is a fungal disease of wheat (*Triticum aestivum*), a key crop
44 underpinning global food security. SNB is caused by the necrotrophic fungal pathogen
45 *Parastagonospora nodorum* (syn. *Phaeosphaeria nodorum* (E. Müll.), syn. *Leptosphaeria*
46 *nodorum* (E. Müll.), syn. *Stagonospora nodorum* (Berk.), syn. *Septoria nodorum* (Berk.)) and
47 is prevalent in wheat growing environments with relatively high, or periodically high, rainfall
48 such as regions within Australia, Canada, Scandinavia, central and eastern Europe, eastern
49 USA, and South America. Compared to biotrophic pathogens which require living host tissue,
50 necrotrophs actively kill host tissue during colonisation, subsequently living on the contents of
51 the dead or dying host cells (Laluk & Mengiste, 2010). The visual symptoms of SNB are
52 chlorosis and necrosis of wheat leaf tissue (often in the form of necrotic lesions surrounded by
53 chlorosis, later developing into irregular dark brown lesions), as well as discoloration and
54 necrosis of the glumes, referred to as leaf blotch and glume blotch, respectively (Figure 1)
55 (Solomon et al. 2006). Leaf blotch reduces the plant surface area capable of photosynthesis,
56 therefore limiting overall crop growth and yield, while glume blotch directly affects grain
57 quality. Due to such damage, SNB is known to cause yield losses of up to ~30 % (Bhathal et
58 al. 2003). In practice, SNB disease often occurs in combination with other necrotrophic fungal
59 diseases such as septoria tritici blotch (STB, caused by *Zymoseptoria tritici*) and tan spot (TS,
60 caused by *Pyrenophora tritici-repentis*). When such disease complexes occur, it can often be
61 difficult to visually determine which necrotrophic diseases are present. However, quantitative
62 polymerase chain reaction (qPCR) molecular assays for *P. nodorum* (Oliver et al. 2008), *Z.*
63 *tritici* (Bearchell et al. 2005) and *P. tritici-repentis* (Antoni et al. 2010) are now available,
64 helping to distinguish the contributors to co-infections of wheat. Additionally, an ITS-RFLP
65 test has been developed that distinguishes between necrotrophic pathogens including *P.*
66 *nodorum* and *P. tritici-repentis* (Hafez et al. 2020). Before the 1980s, *P. nodorum* was the

67 dominant pathogen of the leaf blotch complex in Europe (Bearchell et al. 2005). However,
68 SNB has undergone changes in its regional prevalence in recent decades. For example, over
69 the last thirty years there has been a focal shift in much of North Western European countries
70 from *P. nodorum* to *Z. tritici* (Bearchell et al. 2005; Shaw et al. 2008). The underlying reasons
71 for this change are not fully understood and have been attributed to increased levels of *Z. tritici*
72 host susceptibility, changes in climate, higher use of fertilisers use and increased SO_2 emissions
73 (West et al. 2012; Shaw et al. 2008). It is notable that in Norway, *P. nodorum* is still the major
74 necrotrophic fungal pathogen of wheat and that sulphur pollution has not been reported to be
75 higher in Norway than in any other European countries in which *Z. tritici* dominates the wheat
76 leaf blotch complex (Lin et al. 2020a). One possibility is that the overall SNB to STB shift is
77 due to *Z. tritici* being better at adapting to fungicides, although this hypothesis would need
78 further investigation. Nevertheless, *P. nodorum* remains an important pathogen of wheat
79 worldwide, and appears to be moving into new niches. For example, in 2017 it was observed
80 for the first time on emmer wheat (*T. dicoccoides*) in Turkey, and due to changing climatic
81 conditions, SNB has now become a major problem in Himachal Pradesh, India (Cat et al. 2018;
82 Katoch et al. 2019).
83

84 ***Parastagonospora nodorum*** **lifecycle, infection process and epidemics**

85 *P. nodorum* is a fungal pathogen belonging to the Ascomycota as a member of the class
86 Dothideomycetes. As the first of the Dothiodeomycete class of fungal pathogens to have its
87 genome sequenced (37 Mbp; Hane et al. 2007), *P. nodorum* became established as a model for
88 the narrow host range necrotrophic pathogen lifecycle. It is known mostly as a wheat pathogen,
89 but has also been reported to occasionally infect the related cereal crop barley (*Hordeum*
90 *vulgare*) but with less damage (reviewed by Cunfer 2000), as well as wild grasses (Zhang &
91 Nan, 2018). *P. nodorum* is a necrotrophic fungal pathogen that assimilates nutrients released

92 after host cell death (De Wit et al. 2009). A recent reclassification of fungal and oomycete
93 pathogens (Hane et al. 2020) differentiated a new grouping described as narrow host range
94 polymertrophs to which *P. nodorum* belongs. This group has a narrow host range (unlike
95 *Botrytis cinera*) and induces immediate cell death so that polymeric plant substances become
96 available for assimilation. This group typically produces proteinaceous effectors to fuel disease
97 progression and triggering the plant's receptors to promote sensitivity and tissue death (De Wit
98 et al. 2009). *P. nodorum* has both asexual and sexual cycles (Figure 2). As part of the asexual
99 cycle, fruiting bodies, called pycnidia, form in lesions on the leaf to promote spore development
100 for local dispersal. In contrast, the sexual life cycle produces ascospores, derived from
101 pseudothecia, that allow long distance aerial dispersion. The presence of both sexual and
102 asexual reproduction mechanisms is hypothesised to provide *P. nodorum* with a high
103 evolutionary potential, resulting in increased diversity and fast clonal reproduction of
104 favourable genotypes (Ruud & Lillemo, 2018). The primary inoculum of SNB is mostly
105 forcibly discharged ascospores originating from wheat debris, although it is also seed-
106 transmitted. Reduced tillage (the practice of minimising disturbance of the soil by allowing
107 crop stubble to remain on the ground rather than being incorporated into the soil or discarded)
108 is advocated to reduce soil erosion and limit water evaporation. However, this practice leads to
109 higher amounts of infected wheat straw on the soil surface, which can serve as primary
110 inoculum (Ficke et al. 2018). Once the pathogen has established the initial infection on a plant,
111 large amounts of pycnidiospores can be produced and subsequently spread by rain-splash.
112 Indeed, the high density of wheat fields makes it easier for pycnidiospores to spread to
113 neighbouring plants. Semi-dwarf varieties of wheat may have a higher risk of secondary *P.*
114 *nodorum* infection due to the close vertical spacing of the leaves, as conidia, produced by
115 pycnidia, are sent on an upward trajectory by water droplets (Bahat et al. 1980). This is

116 particularly relevant as the majority of modern wheat varieties have a short 'semi-dwarf'
117 stature.
118
119 **Genetic structure of the *P. nodorum* pathogen population**
120 As *P. nodorum* undergoes frequent sexual reproduction, the resulting genetic recombination
121 results in high genetic diversity in the pathogen population (McDonald & Linde, 2002). Isolates
122 from the Middle East have been found to possess the highest genetic diversity globally.
123 Indicating it is highly probable that the Fertile Crescent serves as the *P. nodorum* centre of
124 origin (Ghaderi et al. 2020). Over the years, studies of *P. nodorum* population structure have
125 been undertaken using a variety of different molecular marker types. Of the various populations
126 investigated to date, sourced from a wide range of geographic locations, studies have typically
127 found little population substructure and high genetic diversity (Blixt et al. 2008; Keller et al.
128 1997; Lin et al. 2000a; McDonald et al. 2012; Murphy et al. 2000; Stukenbrock et al. 2006).
129 For example, genetic studies carried out on *P. nodorum* populations collected from Europe and
130 the USA found evidence of high gene flow but little evidence of genetic differentiation between
131 populations (Keller et al. 1997), with similar results observed for populations from Australia
132 (Murphy et al. 2000) and Norway (Lin et al. 2020a). Indeed, high levels of genetic diversity
133 have even been found among isolates collected from the same lesion (McDonald et al. 1994).
134 The most notable investigation to go against this general trend was an analysis of an
135 international *P. nodorum* population sourced from five continents, where moderate
136 differentiation was observed between geographically divided populations (Stukenbrock et al.
137 2006). More recently, Richards et al. (2019) carried out a comprehensive analysis of the
138 population structure and genome evolution of 197 *P. nodorum* isolates collected across the
139 United States from durum, spring and winter wheat varieties, finding evidence of two *P.*
140 *nodorum* populations that corresponded to the Upper Midwest and South-Eastern US.

141 Interestingly, most isolates in the South-Eastern US population lacked the effector *SnToxA*.
142 This correlated with the lack of the ToxA effector sensitivity gene *Tsn1* in winter wheat
143 varieties that were widely planted in the region thus suggesting that host genotype is a strong
144 driver on the maintenance of effector genes.

145

146 Notably, most regional *P. nodorum* population genetic studies have been carried out using
147 isolates sampled across a narrow timeframe, and thus offer limited insight into potential
148 changes in the population structure over time. However, recently Phan et al. (2020) have
149 examined the population structure of 155 *P. nodorum* isolates collected over a 44 year period
150 across the South-Western Australian wheat growing region. Analysis of genetic
151 polymorphisms using 28 simple sequence repeat (SSR) markers revealed that the population
152 consisted of genetically distinct groups. Most isolates sampled were attributed to 'core groups'
153 that possessed the highest level of genetic diversity in the Australian population, and these
154 groups were found throughout locations and times. Isolates belonging to 'non-core groups'
155 possessed a much lower level of genetic diversity, with limited distribution across locations
156 and time. It was also observed that changes in group genotypes occurred during periods that
157 coincided with major changes in the mass adoption of popular wheat cultivars across large
158 areas of the Australian wheat cultivation zone. It was hypothesised that core groups maintain
159 genetic variability whilst non-core groups emerge in response to large-scale changes in cultivar
160 near-monocultures. Finally, work investigating the genetic diversity of *P. nodorum* and the
161 closely related pathogen species *P. avenaria* f. sp. *tritici* 1 (*Pat*1) shows evidence of
162 hybridisation at a frequency of ~4%, indicating that such gene transfer could be an additional
163 source of genetic diversity in those regions in which the range of the two species overlap
164 (McDonald et al. 2012).

165

166 **SNB disease management**

167 Disease management of SNB includes cultivar resistance (considered in more detail in the next
168 section), fungicide treatment, seed cleaning and stubble management. Despite decades of
169 breeding effort, all current wheat cultivars retain a significant level of susceptibility (Aguilar
170 et al. 2005). Reduced tillage practices are becoming increasingly common all around the world,
171 and significant correlations have been observed between the amount of residue and SNB
172 disease severity in the field (Mehra et al. 2015). Residue management can effectively decrease
173 the amount of primary inoculum and reduce disease severity (Solomon et al. 2006). SNB
174 transmission via seed is regularly reported in some parts of the world such as the eastern USA
175 but rarely elsewhere (Bennett et al. 2007). Seed fungicide treatment, directed primarily to
176 control bunts and smuts, seems to be an efficient way to eradicate SNB from seed stocks.
177 However, fungicidal control of foliar and glume SNB is more problematic. SNB typically
178 occurs in combination with other diseases (tan spot, STB, yellow rust and powdery mildew),
179 and is not normally the most predominant disease. The conditions where SNB is dominant are
180 currently limited to particular geographic locations where yield is typically under 3-4 tonnes
181 per hectare, as well as on lower value feed cereals such as triticale where fungicidal applications
182 are limited in number and dose.

183

184 Before its relative decline in much of north-western Europe at or around the year 2000, *P.*
185 *nodorum* was considered a model pathogen for fungicide discovery (Dancer et al. 1999). All
186 the current major fungicide classes are efficient at controlling SNB: sterol demethylation
187 inhibitors (DMIs), Qo inhibitors (QoIs) and Succinate Dehydrogenase Inhibitors (SDHIs).
188 Reports of fungicide resistance in SNB are relatively rare. Resistance to QoI fungicides in
189 Sweden was reported in isolates collected between 2003 and 2005 (Blixt et al. 2009) and
190 resistance to DMI fungicides has been reported in isolates collected before 2000 in Denmark,

191 Sweden and Switzerland (Pereira et al. 2017, see also
192 https://www.biorxiv.org/content/10.1101/2020.03.26.010199v1.full). To our knowledge, no
193 reports of resistance to SDHI fungicides have been made.
194
195 Fungicide resistance management focusses on reducing the selection pressure for resistance,
196 by minimising dose and number of applications and using mixtures and alternations (Jørgensen
197 et al. 2017). The primary foci of foliar fungicide application in wheat are normally yellow rust,
198 STB and powdery mildew. The latter two diseases are particularly adept at evolving resistance
199 (Oliver & Hewitt, 2014). QoI resistance was detected in both pathogens within two years of
200 QoI application in 2001 (Bartlett et al. 2002). Control of STB by DMIs was substantially
201 compromised by about the year 2010 (Cools et al. 2013). In the last decade, SDHIs became the
202 main weapon against STB but resistance was well developed by 2016 in the UK and Ireland
203 (Dooley et al. 2016). As SNB is not typically the only, or most dominant, pathogen amongst
204 the disease complexes present in most geographic regions, it is possible that SNB has been
205 inadvertently protected against resistance evolution by the development of resistance in the
206 more damaging pathogen forcing a change in fungicide regime. New fungicides were
207 introduced, lower doses applied and either mixtures or rotations carried out. As a result, SNB
208 is not commonly subject to sustained pressure from a single mode of action class and has
209 therefore likely only relatively rarely developed resistance.
210
211 **Genetics of wheat sensitivity to *P. nodorum*: necrotrophic effectors and host response**
212 While chemical control represents an important part of SNB disease management, the use of
213 cultivars with increased genetic resistance helps to underpin more economically and
214 environmentally sustainable wheat production. Resistance to both SNB leaf blotch and glume
215 blotch are quantitatively inherited, but are reported to be controlled by different genetic

216 mechanisms (Chu et al. 2010; Wicki et al. 1999). Increased disease severity is also associated
217 with shorter plant height and later plant maturation. However, residual resistance that is not
218 associated with these traits is identifiable. It is this residual genetic resistance, along with the
219 identification of host-specific gene-for-gene interactions determining the *P. nodorum*-wheat
220 pathosystem (Liu et al. 2004), that provide immediate opportunities to further explore host
221 genetic resistance in wheat breeding (Ruud & Lillemo, 2018).
222
223 Necrotrophic fungal pathogens are known to secrete effectors (typically proteins, but also low-
224 molecular weight phytotoxic metabolites) during host infection which act as virulence factors
225 facilitating disease development. The presence of effectors, also known as 'host selective
226 toxins', was first described in 1933 through the study of the host-pathogen interaction between
227 *Alternaria alternata* and Japanese pear, *Pirus serotine* (Tanaka, 1933). Since then, effectors
228 and their corresponding host sensitivity loci have been identified in numerous necrotrophic
229 fungal and bacterial plant pathogens (reviewed by Laluk & Mengiste, 2010). The necrotic
230 response in a sensitive host plant is hypothesised to help pathogen colonisation, promoting
231 infection and ultimately providing a rich nutrient source (Oliver & Solomon, 2010). This is
232 known as effector-triggered susceptibility (ETS) and is genetically induced via an `inverse gene
233 for gene system' (Friesen et al. 2007). Understanding the genetics of host sensitivity to such
234 effectors provides the opportunity to break down at least some components of the genetics of
235 field resistance into their constitutive parts. *P. nodorum* is thought to derive nutrients from
236 dying plant tissue, utilizing secreted effectors. These effectors induce a hypersensitive response
237 in the host, which takes the form of programmed cell death (Friesen et al. 2007; Liu et al. 2009;
238 Oliver et al. 2012). Evidence of eight *P. nodorum* effectors have been described to date, and
239 designated SnToxA, SnTox1, SnTox2, SnTox3, SnTox4, SnTox5, SnTox6 and SnTox7, along
240 with nine corresponding major wheat sensitivity loci *Tsn1* (Faris et al. 2010), *Snn1* (Shi et al.

241 2016), *Snn2* (Friesen et al. 2007), *Snn3-B1*/*Snn3-D1* (Friesen et al. 2008; Zhang et al. 2011),
242 *Snn4* (Abeysekara et al. 2012), *Snn5* (Friesen et al. 2012), *Snn6* (Gao et al. 2015) and *Snn7*
243 (Shi et al. 2015), respectively. Of these, only three effectors (SnToxA, SnTox1, SnTox3) and
244 two host sensitivity loci (*Tsn1* and *Snn1*) have been identified at the gene level, discussed in
245 more detail below. In addition to these major host loci, several minor effector sensitivity QTLs
246 have been identified in wheat (Supplementary Table 1) (Cockram et al. 2015; Downie et al.
247 2018; Lin et al. 2020b; Phan et al. 2016).
248
249 *ToxA-Tsn1 interaction*: ToxA was first discovered to be secreted *by P. nodorum* in 2006
250 (Friesen et al. 2006) and found to have 99.7 % DNA sequence similarity to the previously
251 identified ToxA gene from *P. tritici-repentis* (subsequently termed here, PtrToxA). Due to the
252 monomorphism of *PtrToxA* compared to the high levels of *ToxA* diversity, it is thought *ToxA*
253 was introduced into the *P. tritici-repentis* genome through interspecific gene transfer from *P.*
254 *nodorum* (Friesen et al. 2006). The corresponding host sensitivity locus, *Tsn1*, was first
255 discovered in 1996 as conferring sensitivity to PtrToxA (Faris et al. 1996), and later confirmed
256 as the corresponding host sensitivity locus for *P. nodorum* ToxA (Liu et al. 2006). This
257 interaction was found to significantly contribute to disease incidence, accounting for up to 62%
258 of disease severity at the seedling stage (Liu et al. 2006) and up to 20% at adult plant stage
259 (Friesen et al. 2009). *Tsn1* is typically present at relatively high frequencies in wheat
260 germplasm, e.g. 59% of Canadian varieties representing wheat development over that last
261 century (Hafez et al. 2020). *Tsn1* encodes a predicted protein containing three predicted
262 domains: a serine/threonine protein kinase (S/TPK) (with ATP binding, substrate binding site
263 and activation loop), a nucleotide binding site (NBS) and 24 leucine-rich repeats (LRRs) (Faris
264 et al. 2010). NBS-LRRs form the largest class of plant resistance (*R*) genes, and are well
265 documented as controlling race-specific resistance to biotrophic fungal pathogens (Dubey &

266 Singh, 2018). Tsn1 is localised to the chloroplast and does not directly interact with ToxA
267 (Faris et al. 2010). However, ToxA has been shown to interact with the dimeric PR-1-type
268 pathogenesis-related protein, TaPR-1-5, to activate Tsn1-controlled cell death pathways (Breen
269 et al. 2016). *Tsn1* expression is subjected to regulation by light and the circadian clock,
270 providing a possible explanation for the light dependent nature of the ToxA-*Tsn1* interaction
271 (Faris et al. 2010; Manning & Ciuffetti, 2005). Recently, it was shown that another wheat and
272 barley pathogen *Bipolaris sorokiniana*, the cause of spot blotch, also possesses a *ToxA* gene
273 that likely originated from *P. nodorum*, pointing to a selective advantage of carrying the
274 virulence factor ToxA (Friesen et al. 2018).

275

276 Tox1-*Snn1 interaction*: Tox1 was first characterized as a host selective effector produced in *P.*
277 *nodorum* culture filtrates interacting with the wheat sensitivity locus *Snn1* on chromosome 1B
278 (Liu et al. 2004). *Tox1* encodes a cysteine rich protein with 117 amino acids which is light
279 dependent and critical for fungal penetration (Liu et al. 2012) and serves a dual function:
280 binding host chitinases to protect fungal infection and causing host tissue death to promote
281 infection (Liu et al. 2016). The Tox1-*Snn1* interaction was found to contribute up to 58 % and
282 19 % of SNB at juvenile and mature plant stages, respectively (Liu et al. 2004; Phan et al.
283 2016). The recent map-based cloning of *Snn1* found it to encode a galacturonic acid binding
284 (GUB) wall associated kinase (WAK), and to possess calcium binding epidermal growth factor
285 (EGF CA) and serine/threonine kinase (S/TPK) domains (Shi et al. 2016). WAK proteins are
286 known to be members of pattern recognition receptors (PRRs) which directly interact with
287 pathogen-associated molecular patterns (PAMPs), such as oligogalacturonides (OGs), which
288 trigger programmed cell death and are involved in plant defence mechanisms against biotrophic
289 pathogens (Brutus et al. 2010).

290

291 SnTox3-*Snn3-B1 interaction*: The *P. nodorum* effector SnTox3 was first identified by Friesen
292 et al. (2008), and the protein sequence later characterised as a 25.8 kDa immature protein, with
293 the first 20 residues of the 230 aa chain forming a signal peptide for secretion (Liu et al. 2009).
294 Tox3 has six cysteine residues that form disulphide bonds, at least one of these bonds is
295 essential for biological function. Recent work has shown that an avirulent *P. nodorum* strain
296 could become virulent with just the addition of the 693 bp intron-free *Tox3* (Liu et al. 2009;
297 Waters et al. 2011). Discovery of SnTox3 led to the identification of the corresponding wheat
298 sensitivity locus, *Snn3* (more recently termed *Snn3-B1*), on the short arm of chromosome 5B.
299 This interaction has been shown to explain 24 % of the phenotypic variation in field SNB
300 resistance/susceptibility, and more than 51 % of the variation in seedling inoculation (Ruud et
301 al. 2017). Culture filtrate containing SnTox3 was first produced using a wild-type *P. nodorum*
302 isolate, SN15, and host sensitivity was genetically mapped using the BR34 x Grandin wheat
303 population (Friesen et al. 2008) and later confirmed in subsequent studies (e.g. Downie et al.
304 2018; Phan et al. 2016; Shi et al. 2016; Lin et al. 2020b). While a *Snn3-B1* homoeologue was
305 found on chromosome 5D in the diploid wild wheat relative *Aegilops tauschii* (*Snn3-D1*)
306 (Zhang et al. 2011), a corresponding locus on the D sub-genome of hexaploid wheat has not
307 been reported. As was the case for ToxA, yeast-two-hybrid studies have shown that the Tox3
308 protein interacts with PR-1 proteins (Breen et al. 2016)

309

310 ***P. nodorum effectors hijack pathways involved in biotrophic pathogen host defence***
311 ***signalling***

312 Given *Tsn1* and *Snn1* both encode classes of proteins that are well known to control disease
313 resistance in biotrophic pathogens, it is hypothesised that *P. nodorum* has evolved to hijack
314 existing pathways in order to become a susceptibility pathway for necrotrophs (Faris et al.
315 2010; Shi et al. 2016; Faris & Friesen, 2020). Specifically, it is thought that host recognition

316 of SnTox1 activates pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI)
317 and that ToxA/PtrToxA recognition activates effector-triggered immunity (ETI). The finding
318 that Tox1 does not enter the plant cell (Liu et al. 2016) indicates that its recognition is mediated
319 via host membrane-bound proteins. This fits both with the prediction that Snn1 spans the host
320 cell membrane and contains extracellular binding domains (Liu et al. 2016; Shi et al. 2016),
321 and with the interaction of Snn1 with Tox1 *in vitro* (Shi et al. 2016). As noted by Shi et al.
322 (2016), while the expression patterns of PTI and ETI pathways overlap, the expression patterns
323 of certain classes of genes commonly differ. Activation of mitogen-activated protein kinase
324 (*MAPK*) genes have been shown to be transient in PTI responses, whilst their expression is
325 more prolonged during ETI (Tsuda & Katagiri, 2010). Notably, the rapid and transient
326 upregulation of *TaMAPK3* in a compatible *Snn1*-Tox1 interaction within 15 minutes of Tox1
327 infiltration further implicates the PTI pathway (Shi et al. 2016). Finally, it has been noted that
328 wheat varieties carrying both *Tsn1* and *Snn1* have higher levels of necrosis than varieties
329 carrying either *Tsn1* or *Snn1* alone, indicating that simultaneous hijacking of both the PTI and
330 ETI pathways for necrotrophic effector (NE) triggered susceptibility enhances pathogen
331 survival and reproduction (Chu et al. 2010; Shi et al. 2016).

332

333 ***Epistatic interactions between P. nodorum effectors and between host sensitivity loci***

334 The NE–*Snn* model supports additive contributions to disease from each compatible interaction
335 (Friesen et al. 2007; Tan et al. 2012). However, epistatic interactions are also evident. For
336 example, SnTox5-*Snn5* and SnTox6-*Snn6* are epistatic to *Snn3-B1* (et al Friesen. 2012; Gao et
337 al. 2015). Similarly, Friesen et al. (2008) showed that the SnToxA-*Tsn1* interaction is epistatic
338 to SnTox3-*Snn3-B1*, and that the SnTox3-*Snn3-B1* interaction is only evident in the absence
339 of a compatible SnTox2-*Snn2* interaction (Friesen et al. 2008). The epistatic effects on SnTox3-
340 *Snn3-B1* were further explored in subsequent work using a series of effector gene deletion

341 mutants generated in the *P. nodorum* strain SN15. While the SnTox1-*Snn1* interaction
342 dominated seedling sensitivity using the wild-type SN15 strain, deletion of the *SnTox1* gene in
343 SN15 led to an increase in *SnTox3* expression in the pathogen and the identification of *Snn3-*
344 *B1* as contributing to host sensitivity at the seedling stage (Phan et al. 2016). Furthermore, a
345 modified strain of SN15 in which *SnToxA, SnTox1* and *SnTox3* were deleted unmasked a
346 sensitivity QTL in the region of the *Snn2* locus which was not identified using the wild-type or
347 *SnTox1* mutant strain, indicating that *SnToxA* and/or *SnTox3* could be epistatic to *Snn2* (Phan
348 et al. 2016). Unlike ToxA, it was found that Tox3 interacts with a broad range of PR-1 proteins
349 and it has been hypothesised that interactions with TaPR-1 proteins facilitate host infection
350 (Breen et al. 2016). As more effectors and host sensitivity loci are cloned and their allelic
351 diversity characterised, it is likely that the identification of new alleles at these loci will further
352 increase the complexity of the NE-*Snn* network. Thus, the epistatic and allelic interactions
353 occurring between effectors in the pathogen, and between sensitivity loci in the host, take what
354 are largely relatively simple gene-for-gene interactions to create a more complex set of possible
355 interactions. As the effect of a NE–host receptor interaction can vary depending on the presence
356 or absence of other effectors and receptors present at the time of infection makes this disease
357 typically quantitative and difficult to predict.
358
359 **Genetics of wheat sensitivity to *P. nodorum* at the juvenile and adult plant stages**
360 In order to characterise the *P. nodorum*-wheat pathosystem and use this information to improve
361 SNB resistance, knowledge of host resistance to target pathogens at the juvenile and adult
362 stages are commonly investigated. Resistance to SNB at both of these plant stages is polygenic
363 and large genotype-by-environment interactions are observed (Fried & Meister, 1987; Wicki
364 et al. 1999). Correlation between seedling and adult plant resistance is generally reported to be
365 low (e.g. Francki 2013; Fried & Meister, 1987; Rosielle & Brown, 1980; Ruud & Lillemo

366 2018; Shankar et al. 2008; Tommasini et al. 2007). This has been suggested to be due to the
367 use of different isolates in greenhouse seedling testing compared to those used in adult plant
368 field trials (Ruud & Lillemo 2018; Ruud et al. 2017). Additionally, as the natural *P. nodorum*
369 population is usually genetically diverse, it is difficult to identify representative isolates for
370 greenhouse assays, and field testing can be affected by cross-infection with the natural *P.*
371 *nodorum* population. Such complications mean that even where the same isolate mixtures are
372 used for greenhouse and field trials, correlation between seedling and flag leaf disease scores
373 can be low (0.31) or even not significant between seedling and glume blotch severity (Shankar
374 et al. 2008). Despite this, there are examples of relatively high correlations when the same
375 isolate is used for both seedling and field testing (Jönsson, 1985). Genetic mapping of seedling
376 SNB resistance has identified genetic loci on all 21 wheat chromosomes except for
377 chromosomes 1D and 3D (Abeysekara et al. 2009; Adhikari et al. 2011; Arseniuk et al. 2004;
378 Czembor et al. 2003; Friesen et al. 2006, 2007, 2012; Gao et al. 2015; Gonzalez-Hernandez et
379 al. 2009; Gurung et al. 2014; Hu et al. 2019; Jighly et al. 2016; Lin et al. 2020b; Liu et al. 2004,
380 2015; Phan et al. 2016; Ruud et al. 2017, 2019; Rybak et al. 2017). Similarly, numerous adult
381 plant QTLs have been identified: across 16 chromosomes for leaf blotch (1A, 1B, 2A, 2B, 2D,
382 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6B, 7A, 7B and 7D (Aguilar et al., 2005; Czembor et al. 2019;
383 Francki et al. 2011, 2018, 2020; Friesen et al. 2009; Lin et al. 2020b, 2020c; Lu & Lillemo,
384 2014; Phan et al. 2016; Ruud et al. 2017, 2019; Shankar et al. 2008), and 12 chromosomes for
385 glume blotch (2A, 2B, 2D, 3A, 3B, 4A, 4B, 5A, 5D, 6A, 6B and 7D (Aguilar et al. 2005;
386 Czembor et al. 2019; Francki et al. 2018; Jighly et al. 2016; Lin et al. 2020b; Schnurbusch et
387 al. 2003, Shankar et al. 2008; Shatalina et al. 2014; Tommasini et al. 2007; Uphaus et al. 2007).
388 All QTLs are listed in Supplementary Table 1.
389

390 While it has been clear from the outset that NE-*Snn* interactions are relevant to seedling
391 resistance, discussion of their importance on SNB resistance in the field is ongoing (Francki,
392 2013). However, there is now mounting evidence that at least some NE-*Snn* interactions also
393 contribute to susceptibility to SNB in the field. Friesen et al. (2009) used an isolate producing
394 both ToxA and Tox2 for spray inoculation in the field on a mapping population segregating for
395 *Tsn1*, *Snn2* and *Snn3-B1*, finding *Tsn1* and *Snn2* to explain 18 % and 15 % of the phenotypic
396 variation for SNB resistance, respectively. Significant correlation between ToxA sensitivity
397 and SNB disease severity have been observed in an association mapping panel under
398 Norwegian field conditions (Ruud et al. 2019). Another study applied artificial inoculation of
399 an isolate producing all three known NEs, showing *Snn1* explained 19 % of the phenotypic
400 variation for adult plant disease severity (Phan et al. 2016). Similarly, studies in Norway have
401 found *Snn3-B1* to affect field SNB disease susceptibility using a bi-parental population (Ruud
402 et al. 2017).
403
404 Further cross-comparison of juvenile plant and adult plant sensitivity with major and minor
405 effector and culture filtrate sensitivity loci have historically been problematic due to factors
406 such as the relatively large genetic intervals identified and the use of different genetic mapping
407 populations and genetic marker systems. Genetic mapping of response to *P. nodorum* infection
408 has mainly relied on different bi-parental wheat populations. However, more recently
409 association mapping (Cockram et al. 2015; Downie et al. 2018; Ruud et al. 2019; Tommasini
410 et al. 2007) and multi-founder (e.g. Lin et al. 2020b, 2020c) populations have also been used.
411 While each type of population comes with its own advantages and disadvantages (reviewed by
412 Cockram & Mackay, 2018), one benefit of association mapping and multi-founder populations
413 is that allelic variation at the genomic locations controlling the target traits are more likely to
414 be sampled than might be the case in bi-parental populations, and the effects of these alleles

415 are assessed in a wider range of genetic backgrounds. This allows straightforward cross-
416 comparison of QTLs for numerous related traits within a single genetic mapping population.
417 Furthermore, the availability of high-density genotyping platforms and a wheat reference
418 genome assembly (IWGSC, 2018) means that cross-comparison of previously published SNB
419 QTLs identified using different genetic mapping populations is much more straightforward to
420 do. Here, we have used these resources to anchor previously published QTLs controlling host
421 response to *P. nodorum* infection, as well as infiltration using culture filtrates and necrotrophic
422 effectors, to the wheat physical map (Figure 3; Supplementary Table 1). The results help
423 highlight several interesting observations. For example, recent studies using multiparent
424 advanced generation inter-cross (MAGIC) populations constructed using wheat varieties
425 grown in UK (Mackay et al. 2014) and German (Stadlmeier et al. 2018) agronomic
426 environments have allowed genetic control of resistance to *P.* nodorum, as well as sensitivity
427 to known effectors, to be assessed in experimental populations that capture relatively high
428 amounts of genetic variation (Lin et al. 2020b, 2020c). Field testing of SNB resistance
429 identified robust co-localising QTLs on the long arm of chromosome 2A controlling leaf blotch
430 in the UK MAGIC (*QSnb.niab-2A.3*; Lin et al. 2020b) and German MAGIC (*QSnb.nmbu-2A.1*;
431 Lin et al. 2020c) populations, as well as culture filtrate sensitivity QTL that co-locate to the
432 same locus in the UK MAGIC population (Lin et al. 2020b). This chromosome 2A QTL is
433 located within the confidence interval for the seedling resistance QTL *QSnb.fcu-2A*
434 (Abeysekara et al. 2009) and the SNB resistance QTL *Qsnb.cur-2AS.1* (Phan et al. 2016).
435 However, whether these QTL represent the same underlying locus is not currently known, and
436 the *Qsnb.cur-2AS.1* physical interval is notably large. Nevertheless, collectively these results
437 suggest that an as-yet uncharacterised necrotrophic effector present in *P. nodorum* culture
438 filtrate used by Lin et al. (2020b) interacts with the *QSnb.niab-2A.3* locus and is implicated in
439 the control of SNB resistance in adult plants. While Lin et al. (2020b) also found a QTL

440 controlling glume blotch to colocalise to the same genetic locus on chromosome 2A, the allelic
441 effects at the QTL were predicted to be opposite to those for glume blotch, suggesting that a
442 different mechanism may be involved. This supports previous reports that resistance to leaf
443 blotch and glume blotch are thought to predominantly be controlled by different genetic
444 mechanisms (Aguilar et al. 2005; Chu et al. 2010; Francki et al. 2018; Schnurbusch et al. 2003;
445 Shankar et al. 2008).

446

447 Analysis of additional culture filtrate sensitivity QTL and minor-effect effector sensitivity QTL
448 finds several to co-locate with genetic loci controlling adult plant SNB resistance (Figure 3,
449 Supplementary Table 1), further supporting the presence of additional effector sensitivity loci
450 relevant to field resistance. For example, *QTox3.niab-2A.1* controlling Tox3 sensitivity
451 (Downie et al. 2018) co-locates with a QTL for adult plant leaf blotch (*QSnb.niab-2A.4*, also
452 controlling seeding resistance, Lin et al. 2020b), all in the same eight-founder MAGIC
453 population. Additionally, SNB resistance QTL *QSnb.niab-3A* and *QSnb.niab-6A.2* identified
454 in the MAGIC population collocated with a culture filtrate sensitivity QTL (Lin et al. 2020b)
455 and the previously reported effector sensitivity locus *Snn6* (Gao et al. 2015; Arseniuk et al.
456 2004), respectively. The co-location of culture filtrate/effector sensitivity loci with SNB QTL
457 indicates that natural variation at genetic loci controlling additional components of effector
458 sensitivity pathways may play a role in modulating adult plant resistance phenotype. Whether
459 there are additional NE-*Snn* interactions playing roles in adult plant susceptibilities is still yet
460 to be determined.

461

462 **Common QTL between SNB and tan spot diseases of wheat**

463 Increasing numbers of publications on QTL mapping of both SNB and tan spot has revealed a
464 number of common QTL between the two diseases. That *Tsn1* confers sensitivity to both ToxA

465 and PtrToxA is a well-known example (Friesen et al. 2006), although investigation of
466 resistance to *P. tritici-repentis* and *P. nodorum* using a bi-parental tetraploid wheat (*T. durum*)
467 population indicated while the *Tsn1*-ToxA interaction was important for *P. nodorum* infection,
468 it did not play a significant role in *P. tritici-repentis* interaction in the tetraploid wheat *T. durum*
469 system, and that this was likely due to low *PtrToxA* expression in *P. tritici-repentis* (Virdi et
470 al. 2016).

471

472 *P. nodorum* resistance/sensitivity QTL *Qsnb.cur-2AS.1* (Phan et al. 2016) which was detected
473 at the seedling and adult plant stage has also been found to be a major contributor to tan spot
474 resistance in seedlings and mature plants (Manisha et al. 2017; Phan et al. 2016). A QTL
475 identified on the long arm of chromosome 5A is another instance of shared common genomic
476 regions significantly associated with both diseases (Hu et al. 2019). This phenomenon may
477 indicate that the two diseases possibly share common susceptibility/resistance mechanisms. It
478 would be interesting to find out if they have more effectors in common. The mutual interactions
479 could be promising targets for wheat breeders, as they could introduce resistance to both
480 diseases - especially for those QTL with relatively large effect and at both the seedling and
481 adult plant stages.

482

483 **Roles of new technology-based and breeding approaches in delivering genetic gains in**
484 **SNB resistance**

485 Advances in the understanding of SNB resistance have been applied in breeding programmes
486 since 2005. For example, sequencing the *P. nodorum* genome revealed the presence of *ToxA*
487 and that it was the source of the related gene previously identified in *P. tritici-repentis*. It was
488 a simple matter to express the gene in microbial hosts, infiltrate the protein into wheat seedling
489 leaves and determine whether plants were sensitive or not. An important factor was that these

490 assays could be carried out with equipment as simple as a refrigerator and a needleless syringe;
491 even a greenhouse was not essential. As such, crop breeders found this assay practical and
492 accurate. Armed with expressed ToxA since 2005, Tox3 since 2011 and Tox1 since 2012,
493 researchers and breeders could determine the relationship between effector sensitivity and
494 cultivar susceptibility. For *P. tritici-repentis* in Australia, a very simple picture emerged; all
495 isolates of the pathogen carried *PtrToxA*, and sensitivity to this effector in wheat was strongly
496 correlated with tan spot disease susceptibility. Large numbers of ToxA doses were distributed
497 to breeders over the next few years and the use of ToxA sensitive wheat grown was reduced
498 by half in three years. Considering these changes in more detail in more recent periods, the
499 total area sown to *tsn1* wheat varieties in Western Australia increased from 69.9% in 2009–
500 2010 to well over 85% in 2018 (Oliver et al. 2014; Western Australia Crop Growing Guide
501 2020; https://www.cbh.com.au/en/customers) and no detectable yield penalty is associated
502 with insensitivity to ToxA (Oliver et al. 2014; Vleeshouwers & Oliver, 2014). The application
503 of "effector-assisted breeding" to SNB was more complicated. In Australia, effectively all *P.*
504 *nodorum* isolates carried all three effectors, but the relationship between effector insensitivity
505 and cultivar resistance was not as clear cut. As noted above, epistasis between NE genes was
506 apparent. Nonetheless the elimination of effector sensitivity genes has never been shown to
507 decrease SNB resistance or to have any other deleterious effect. It either has no effect or a
508 positive effect on resistance. Analysis of the *ToxA* sequence in a diverse *P. nodorum* isolate
509 collection indicates that the gene is positively selected (Stuckenbrock & McDonald, 2007). It
510 is likely that *ToxA* will continually evolve into forms that are more potent in host cell death
511 induction unless *Tsn1* is bred out from widely planted wheat germplasms (Tan et al. 2012). In
512 the case of Tox1 sensitivity, while the gene underlying the sensitivity locus *Snn1* has been
513 cloned, the natural genetic variants determining insensitivity have not been formally identified.
514 For Tox3 sensitivity, while highly significant markers closely linked to *Snn3-B1* have been

515 identified in experimental mapping populations, the observation that these markers provide
516 surprisingly low prediction of Tox3 sensitivity in screens of wider germplasm collections (eg
517 Downie et al. 2018) indicates that multiple sensitivity alleles may be present. Similarly, while
518 the *WAK* gene underlying the Tox1 sensitivity locus *Tsn1* has been cloned using a bi-parental
519 population, the natural variant(s) controlling insensitivity have not yet been determined, and so
520 screening with the Tox1 protein remains likely the most pragmatic approach for robustly
521 determining sensitivity, at least until the causative variant(s) controlling insensitivity are
522 identified.
523
524 In the coming years, the use of other emerging technologies will help speed up the identification
525 and functional characterisation of SNB/effector resistance genes and provide efficient routes
526 to use these in breeding programmes. Here we briefly summarise a subset of these resources
527 and approaches, ending with an example of how a combination of these could be applied to
528 future SNB resistance research and breeding.
529
530 Access to the wheat gene space within a target genetic interval is a key resource to help identify
531 causative genes and variants. While a wheat reference genome is now available (IWGSC,
532 2018), it has been constructed using an Asian landrace called 'Chinese Spring', genetically
533 distant to the wheat grown in most of the world. This may be particularly relevant to effector
534 sensitivity, as of the two cloned effector sensitivity loci in wheat, allelic variation at the *Tsn1*
535 locus conferring ToxA sensitivity is due to the presence or absence of the underlying gene
536 (Faris et al. 2010). As 'Chinese Spring' is insensitive to ToxA, the wheat reference genome
537 assembly lacks the *Tsn1* gene. To help address such issues, the construction of genome
538 assemblies for several additional bread wheat varieties are underway. This includes 14 cultivars
539 via the 10+ Wheat Genomes Project (www.10wheatgenomes.com) and the founders of the UK

540 MAGIC population (https://gtr.ukri.org/projects?ref=BB%2FP010741%2F1). To help
541 annotate the genes in any new wheat assembly, and to provide information on where and when
542 a gene within your genomic region of interest is expressed, high-throughput RNA sequencing
543 using next-generation sequencing platforms can be undertaken. This can be done using
544 relatively short read technologies (e.g. RNA-seq using Illumina platforms), or long-read
545 technologies to sequence full-length transcripts (e.g. Isoform Sequencing using PacBio
546 platforms or Nanopore technology). By combining genomic and RNA sequence datasets,
547 candidate genes and polymorphisms within a target genomic region can be identified.
548 Candidate genes can then be explored using reverse genetic approaches. Currently, a TILLING
549 (Targeting Local Lesions in Genomes) population with an associated exome capture-based
550 genomic sequence databased is available for the wheat variety 'Cadenza' (Krasileva et al.
551 2017), allowing lines with putative deleterious mutations to be identified *in silico* and ordered.
552 Alternatively, transgenic approaches such as RNA interference (RNAi), CRISPR/Cas9 gene
553 editing and virus-induced gene silencing (VIGS) are all now used in wheat (e.g. Travella et al.
554 2006; Shan et al. 2013; Scofield et al. 2005). For further reading on the routes for wheat gene
555 functional annotation, see the recent review by Adamski et al. (2020).
556
557 Next, we outline a case study for SNB improvement based on the environmentally stable adult
558 plant resistance QTL *QSnb.niab-2A.3* identified in the UK MAGIC population by Lin et al.
559 (2020b). First, to rapidly generate suitable germplasm to further the investigation of this locus,
560 the residual genetic variation present in MAGIC RILs could be exploited to generate a pair of
561 nearly isogenic lines (NILs) for a given QTL in a single generation (as described in more detail
562 by Scott et al. 2020). This NIL pair could be inter-crossed to generate F_1 seed, and the F_1s
563 selfed to produce large numbers of F_2 seed. As the culture filtrate from *P. nodorum* isolate
564 203649 was found to identify a QTL at the *QSnb.niab-2A.3* locus, F_2 individuals could be

565 screened for genetic recombination within the target interval, and their F_3 progeny phenotyped
566 at the seedling stage for sensitivity to culture filtrate. This subset of recombinant lines, and
567 their progenies, would be used to further refine the genetic interval. Once sufficient genetic
568 mapping resolution is obtained, the gene content in the interval could be determined by
569 projecting the genomic sequence and gene annotations of the relevant MAGIC founders onto
570 the interval, and RNA-seq and IsoSeq gene expression data from leaf tissues harvested from
571 the NIL germplasm pre- and post- culture filtrate infiltration overlaid. Collectively, these
572 datasets would allow candidate genes within the genetic interval to be identified and accurately
573 annotated via bioinformatic analysis of the DNA variants, gene expression and splice variant
574 data generated. Subsequently, VIGS could be used to transiently silence candidate genes at the
575 seedling stage, and any effect on sensitivity to culture filtrate infiltration determined. Further
576 functional validation of the candidates prioritised/validated by VIGS could then be assessed at
577 the adult plant stage using stable gene silencing methods such as CRISPR/Cas9. Diagnostic
578 markers for the natural causative polymorphisms underlying the functionally validated gene
579 would be developed for marker assisted selection, preferably using genotyping systems
580 commonly used by wheat breeding companies, such as Kompetitive Allele-Specific PCR
581 (KASP) assays (LGC Biosearch Technologies).
582
583 It is important to mention that application of the marker-informed breeding methodology
584 ‘genomic selection’ is now feasible in large genome crop species such as wheat (reviewed by
585 Sun et al. 2019). Rather than relying on explicit identification of the QTL/genes underlying the
586 target trait, genomic selection exploits the ability to cheaply generate high-density genetic
587 marker datasets across the genome, and use this alongside phenotypic data generated in a
588 ‘training set’ lines to use the markers to predict the performance of their progeny across
589 multiple subsequent generations. This allows selection to be applied based on genetic marker

590 data and phenotypic data on the training set alone, without the need for field-based phenotypic
591 selection in multiple subsequent rounds of population advancement. This potentially reduces
592 breeding cycle time, increases selection accuracy and increases selection intensity. Genomic
593 selection is likely to be a major source of improvement in plant breeding practice over the next
594 decades, and the methodologies can also likely be modulated to incorporate additional datasets
595 such as diagnostic markers in order to help improve prediction accuracy (Mackay et al. 2020).
596 Numerous studies have followed on from the first report of genomic selection in wheat (De los
597 Campos et al. 2009) and include studies of diseases such as yellow rust (Ornella et al. 2012),
598 Fusarium head blight (Herter et al. 2019) and STB (Herter et al. 2019). Of these, the study
599 conducted by Herter et al. (2019) using 1120 lines derived from 14 bi-parental families found
600 that while genomic selection provided a selection advantage of ~10 % for fusarium head blight,
601 no significant advantage was observed for STB resistance (Herter et al. 2019). This suggests
602 that for phenotypes with strong genotype × environment interaction, genomic selection appears
603 to be challenging (Herter et al. 2019). Based on the published literature, genomic selection has
604 not been explicitly applied to SNB improvement, indicating a possible as yet untested route for
605 genetic improvement. We also noted that genome editing approaches such as CRISPR/Cas9
606 would be well suited for host-pathogen interactions that follow the inverse gene-for-gene
607 model, whereby host effector sensitivity loci could be edited to make them insensitive. In the
608 future, we might see application of genomic selection methodology that combine targeted
609 selection against NE sensitivity alleles and/or selection for gene edited NE insensitivity alleles
610 along with the use of genome-wide markers to capture all small-effect loci in a cost-effective
611 manner for plant breeding programs.
612
613 **General conclusions**

614 Ultimately, the most efficient control of SNB will involve a combined approach based on
615 agricultural and agronomic practices, disease monitoring and genetic improvement. The
616 widespread adoption of conservation agriculture including limited tillage methods means that
617 SNB is likely to increase in prevalence in areas where ploughing has previously been the norm.
618 Methods to improve the genetic resistance of cultivars will surely remain the most important
619 method of control. So far, no full genetic resistance to SNB has been identified. It is becoming
620 increasingly apparent that SNB is found not only in the presence of easily distinguished
621 diseases like yellow rust and powdery mildew, but also with the symptomatically similar
622 diseases such as STB, tan spot and possibly spot blotch as well. Selection for resistance to
623 diseases occupies a substantial amount of time and resources available to breeders, particularly
624 as yield and quality will always be prioritised. Furthermore, we know very little about how
625 diseases interact. This is a particular area of fascination given that three of these pathogens
626 share effectors.
627
628 Breeding for resistance to SNB has always been challenging because full evaluation of a new
629 cultivar requires the use of adult plants under field conditions. Inoculation with a representative
630 set of isolates adds to the difficulties. One clear recommendation to emerge from recent studies
631 is to make large annual isolate collections especially from the current most resistant cultivar.
632 These new isolates can be assessed phenotypically for new effectors and virulence
633 characteristics as well as genotypically to track for selected chromosomal regions. Any new
634 effectors can be expressed and assessed for their role in virulence. The main value of the isolate
635 collections is that they allow the rational selection of the minimum set that represents the total
636 phenotypic variance of the pathogen to which resistance should be sought. Finally, based on
637 our current understanding of *P. nodorum* epidemiology and host resistance, we provide the
638 following recommendations for SNB management:

639

640 1. Establish annual *P. nodorum* isolate collections and disease outbreak monitoring
641 programmes.
642 2. Use these contemporary *P. nodorum* isolates to test for cultivar resistance and assess
643 for the presence of new effectors.
644 3. Where genetic structure is observed in a regional pathogen population, undertake rapid
645 genotypic analysis to monitor the population.
646 4. Grow wheat cultivars with differing genetic background to avoid a build-up of a
647 specialised pathogen population, especially in areas where minimum tillage practices
648 are common.
649 5. Where local pathogen populations contain known effector genes, grow wheat varieties
650 with insensitive alleles at the corresponding host loci.
651 6. Continue wheat research and development activities to identify and deploy additional
652 sources of SNB genetic resistance.

653

654

655

656

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666

667 **Author contributions**

668 RD, MinL and JC wrote the manuscript. MinL and JC undertook bioinformatic analysis. All
669 other authors edited the manuscript and contributed to scientific supervision and/or discussions.

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1139 **Supplementary Table legends**

1140

1141 **Supplementary Table 1.** Summary of QTLs for *P. nodorum* infection, culture filtrate
1142 infiltration and effector sensitivity. QTLs, and relevant cloned genes from wheat, are anchored
1143 to the reference wheat genome assembly (cultivar Chinese Spring, RefSeq v1.0. IWGSC,
1144 2018). Anchoring was undertaken using marker DNA sequences as queries for BLASTn
1145 searches of the wheat reference genome. Where BLASTn hits were identified on a non-target
1146 homoeologous location, and the e-values for the homoeologues were comparable, physical
1147 location is reported for the homoeologue on the chromosome identified by the relevant genetic
1148 map. Genetic markers for which no associated DNA sequences could be found for BLASTn
1149 analysis are highlighted in red.

1150 **Figure legends**

1151

1152 **Figure 1.** Septoria nodorum botch (SNB) symptoms in bread wheat. (A) On leaves. (B) On the
1153 spikelets of a wheat inflorescence (ear).

1154

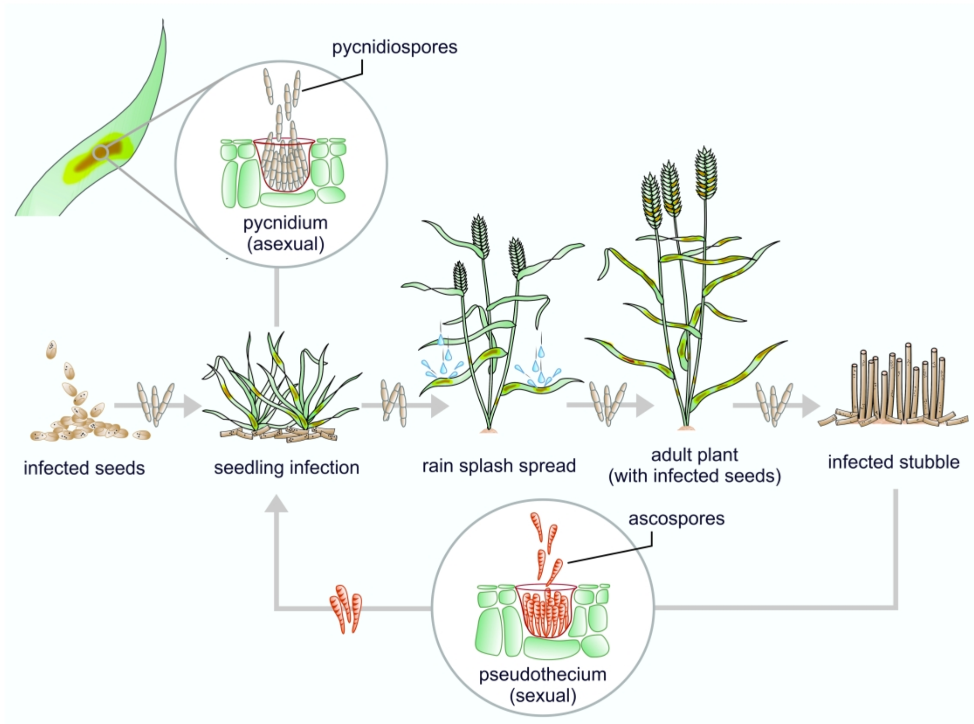
1155 **Figure 2.** Illustration of the *Parastagonospora nodorum* infection cycle on wheat. Initial
1156 infection of wheat seedlings is via *P. nodorum* ascospores present in infected stubble, or via
1157 seeds infected with *P. nodorum* mycelium which produce pycnidiospores under wet or humid
1158 conditions. Pycnidiospores produced as a result of this initial infection can then be spread via
1159 rain splash or wind, causing secondary infection further up the wheat canopy as the crop
1160 matures, and can result in infection of the wheat ears.

1161

1162 **Figure 3.** Projection of published QTLs for SNB leaf blotch (black), glume blotch (blue),
1163 culture filtrate/effector infiltration sensitivity at the seedling stage (brown) and seedling *P.*
1164 *nodorum* resistance (green) onto the wheat reference genome assembly (RefSeq v1.0; IWGSC,
1165 2018). The locations of relevant cloned wheat genes are shown in red. QTL are named
1166 according to their publication, and full details for all QTL are listed in Supplementary Table 1.

A

B



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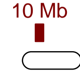
205x153mm (600 x 600 DPI)

scale



1A

(Abeysekara et al. 2009) B /SnTox4-Snn4
(Ruud et al. 2019) G*
(Ruud et al. 2017) B*
(Francki et al. 2020) G*
(Ruud et al. 2017) B*

1B

(Cockram et al. 2015) M /SnTox1-Snn1
(Downie et al. 2018) G /SnTox1-Snn1
(Ruud et al. 2019) G*
(Francki et al. 2018) B
(Francki et al. 2011) B
Snn1
(Francki et al. 2018) B
(Phan et al. 2016) B /SnTox1-Snn1
(Phan et al. 2016) B
(Phan et al. 2016) B
(Liu et al. 2004b) B /SnTox1-Snn1
(Ruud et al. 2017) B*
(Francki et al. 2020) G*
(Lu and Lillemo 2014) B*
(Friesen et al. 2009) B*
(Francki et al. 2020) G
(Czembor et al. 2019) B
(Hu et al. 2019) B
(Francki et al. 2020) G*
(Francki et al. 2020) G*

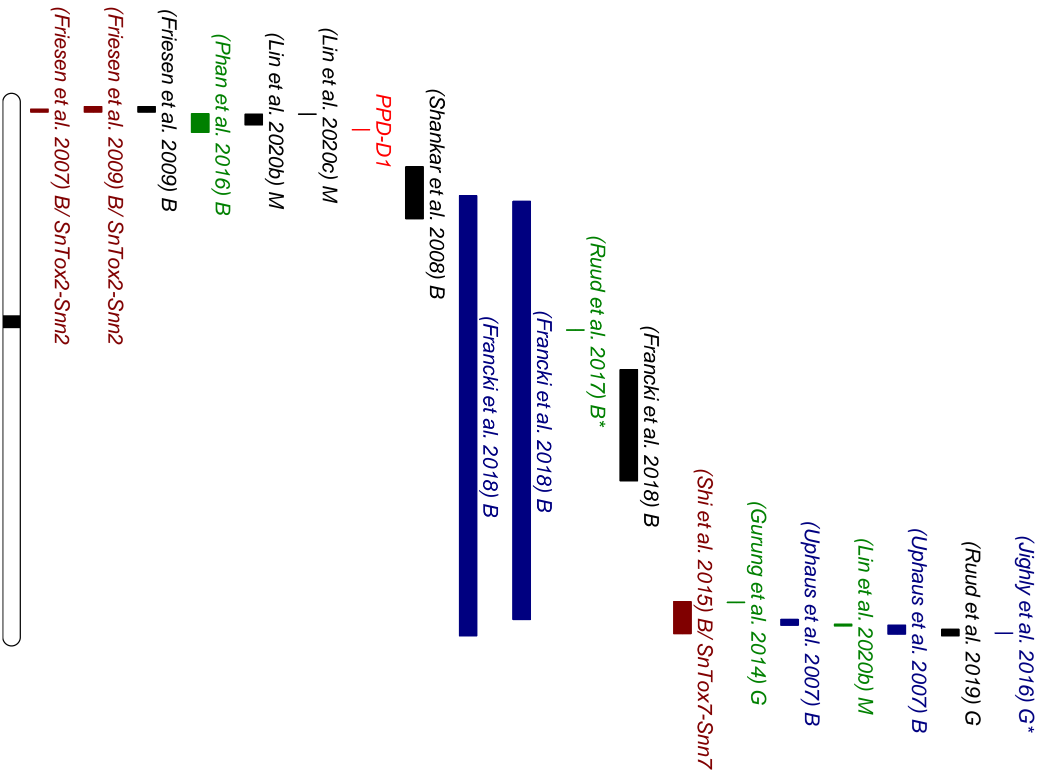
2A

(Phan et al. 2016) B /CF
(Lin et al. 2020b) M
(Francki et al. 2018) B
(Francki et al. 2018) B
PPD-A1
(Jighly et al. 2016) G*
(Phan et al. 2016) B
(Lin et al. 2020b) M
(Francki et al. 2011) B*
(Lin et al. 2020b) M
(Lin et al. 2020c) M
(Lin et al. 2020b) M
(Lin et al. 2020b) M
(Abeysekara et al. 2009) B*
(Hu et al. 2019) B
(Lin et al. 2020b) M
(Downie et al. 2018) M /SnTox3
(Lin et al. 2020b) M

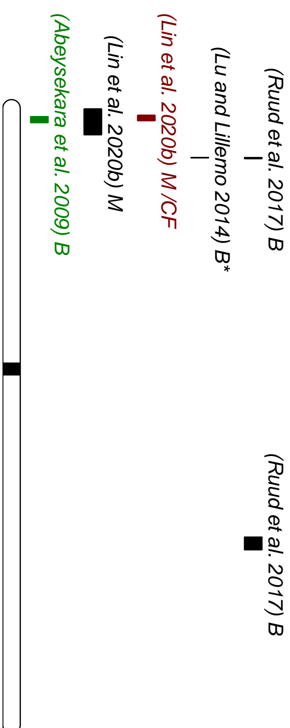
2B

(Hu et al. 2019) M
(Phan et al. 2016) B* /CF
(Ruud et al. 2019) G*
(Czembor et al. 2003) B
(Hu et al. 2019) B
(Lin et al. 2020c) M
(Downie et al. 2018) M* /SnTox3

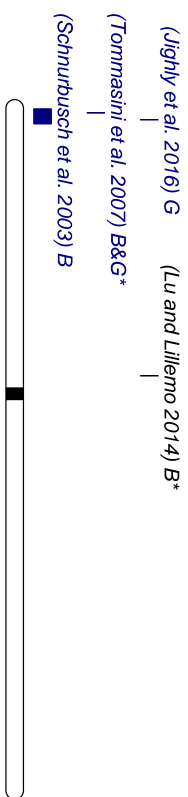
2D



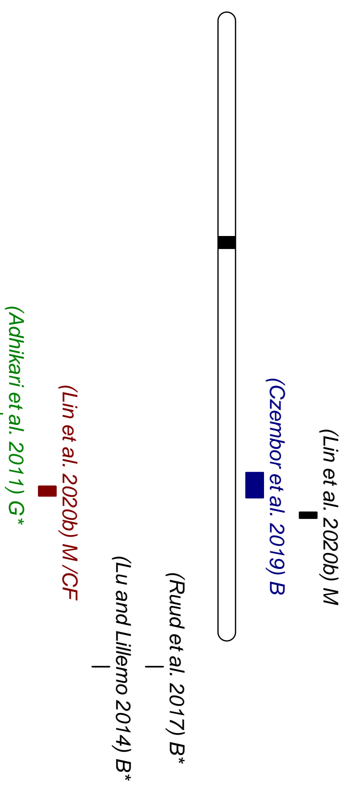
3A

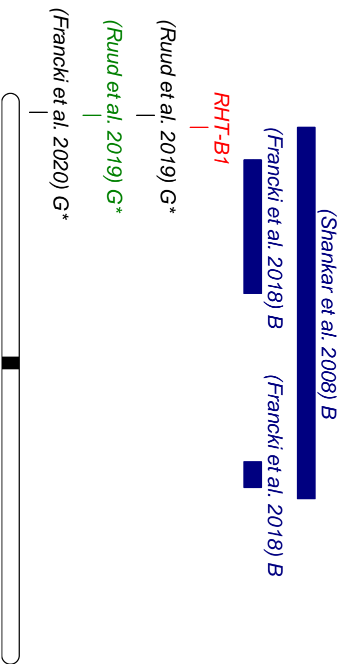


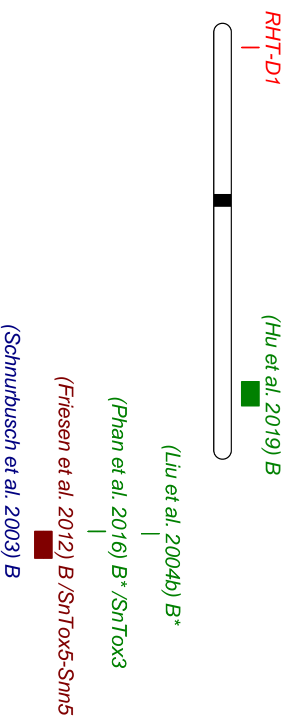
3B

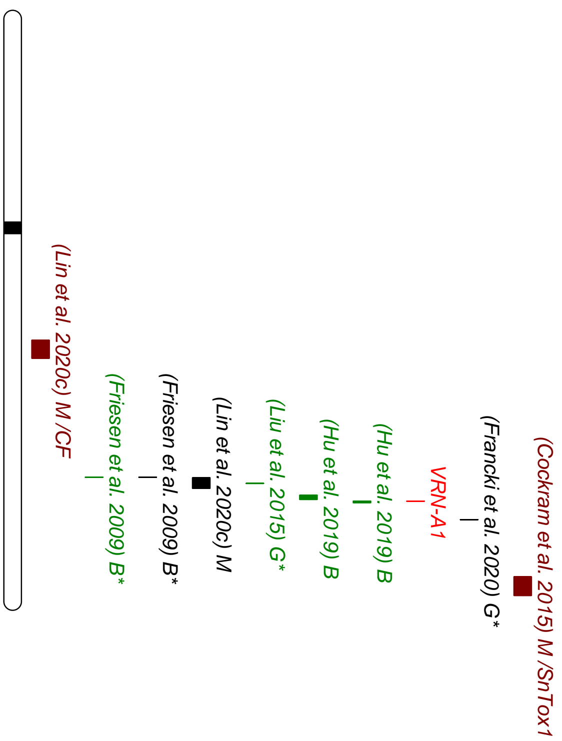


4A



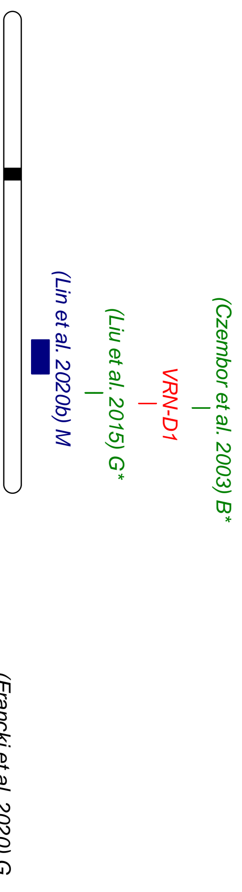




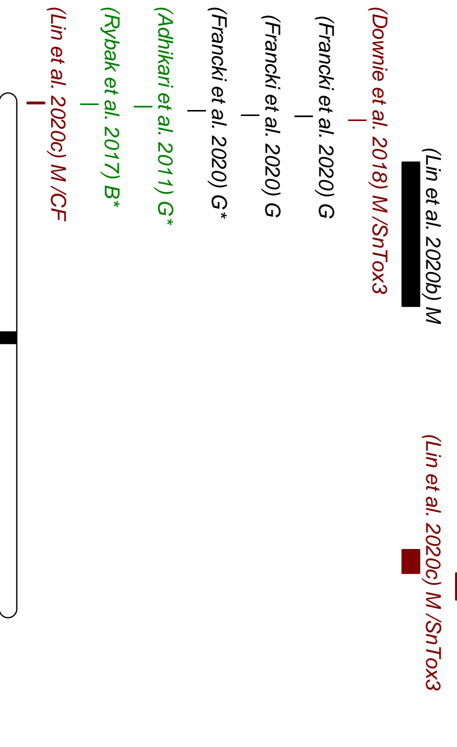


5BS

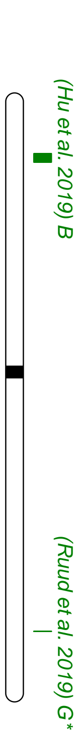
5BL



6A

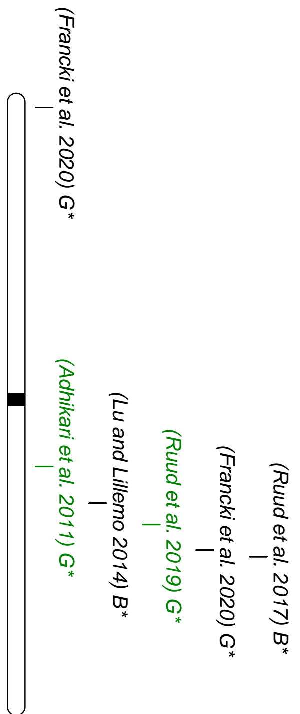


6B

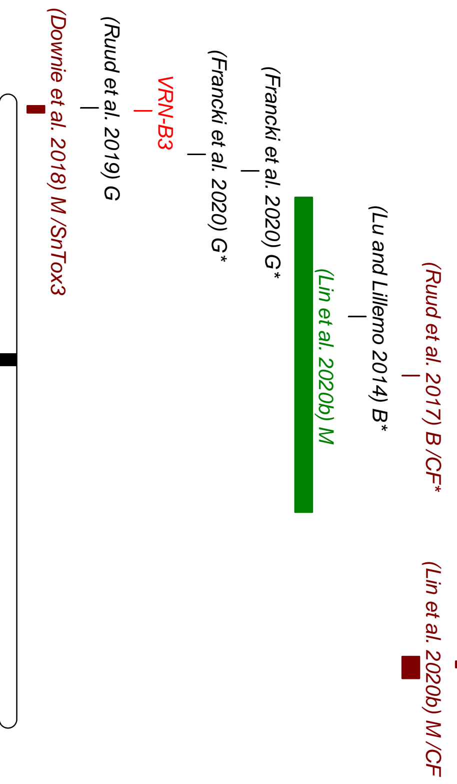


6D

7A



7B



7D