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The *Fusarium graminearum* transporters Abc1 and Abc6 are important for xenobiotic resistance, trichothecene accumulation, and virulence to wheat.

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8 **Abstract**

9 The plant pathogenic fungus *Fusarium graminearum* is the causal agent of Fusarium Head Blight
10 (FHB) disease on small grain cereals. *F. graminearum* produces trichothecene mycotoxins such as
11 deoxynivalenol (DON) that are required for full virulence. DON must be exported outside the cell to
12 cause FHB disease, a process that may require the involvement of membrane-bound transporters. In
13 this study we how the deletion of membrane-bound transporters results in reduced DON accumulation
14 as well as reduced FHB symptoms on wheat. Deletion of the ATP-Binding Cassette (ABC) transporter
15 *Abc1* results in the most severe reduction in DON accumulation and virulence. Deletion of another ABC
16 transporter, Abc6, also reduces FHB symptoms to a lesser degree. Combining deletions fails to reduce
17 DON accumulation or virulence in an additive fashion, even when including an *Δabc1* deletion.
18 Heterologous expression of *F. graminearum* transporters in a DON-sensitive strain of yeast confirms
19 Abc1 as a major DON resistance mechanism. Yeast expression further indicates that multiple
20 transporters, including Abc1 play an important role in resistance to the wheat phytoalexin 2-
21 benzoxazolinone (BOA) and other xenobiotics. Thus, Abc1 may contribute to wheat virulence both by
22 allowing export of DON and by providing resistance to the wheat phytoalexin BOA. This research
23 provides useful information which may aid in designing novel management techniques of FHB or other
24 destructive plant diseases.

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25 **Introduction**

26 Fusarium is a cosmopolitan genus comprised of not only soil-borne and endophytic fungi which
27 live asymptomatically within hosts, but also fungal species that are economically and agronomically
28 important plant pathogens (Imazaki and Kadota 2015; Lofgren et al. 2018; Wachowska et al. 2017;
29 Waweru et al. 2014). *Fusarium graminearum* is one of the causal agents of Fusarium Head Blight (FHB)
30 disease of small grain cereals and has received considerable attention due to its ability to act as a plant
31 pathogen and to produce mycotoxins that impact animal and human health. While occurring world-
32 wide, FHB has been a persistent problem in the United States for many years, especially since a large
33 outbreak in the 1990's. The disease management costs and direct impact of FHB outbreaks have
34 resulted in annual losses exceeding $1.4B in the United States (Wilson et al. 2018).

35 *F. graminearum* produces several secondary metabolites which act as virulence factors during
36 plant host infection (Bahadoor et al. 2018; Proctor 1995; Wipfle et al. 2019), including sesquiterpenoid
37 trichothecenes. Trichothecenes inhibit protein synthesis by binding to the peptidyl-transferase domain
38 of eukaryotic ribosomes (Fried and Warner 1981; Garreau de Loubresse et al. 2014; Harris and Gleddie
39 2001), an effect which poses significant risk to plants and animals. The major trichothecene produced by
40 many strains of *F. graminearum* is deoxynivalenol (DON), which is essential for full virulence on wheat
41 (Desjardins et al. 1996) and may persist as a contaminant in affected grains. Exposure to DON can lead
42 to many cytological effects in eukaryotes, including DNA/RNA synthesis disruption, ribotoxic stress,
43 induction of apoptosis, and membrane cytotoxicity (Pestka 2007; Rocha et al. 2005). Export of
44 trichothecences by *F. graminearum*, whether by vesicular traffic or by membrane-bound transporters,
45 may be essential for full virulence to plants (Abou Ammar et al. 2013; Gardiner et al. 2013; Menke et al.
46 2012; O'Mara et al. 2020) Additionally, due to potential trichothecene toxicity, *F. graminearum* must
47 either sequester or export this secondary metabolite or risk self-inhibition (Menke et al. 2012; Wang et
48 al. 2018).

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49 One of the primary sources of resistance to toxic secondary metabolites in fungi is through the
50 actions of membrane-bound transporters, such as ATP-binding cassette (ABC) and Major-facilitator
51 superfamily (MFS) transporters (Gulshan and Moye-Rowley 2007; Perlin et al. 2014). The typical
52 structure of an ABC transporter includes two core domains, a nucleotide-binding domain (NBD) and a
53 transmembrane domain consisting of six transmembrane-spanning helices (TMD) (Kovalchuk and
54 Driessen 2010). MFS transporters are generally smaller than ABC transporters and consist of 12 or 14
55 transmembrane spanning helices without any NBD domains. ABC transporters utilize adenosine
56 triphosphate (ATP) to move molecules across membranes while MFS transporters utilize electrochemical
57 membrane potential (often due to a pH gradient) to accomplish the same functionality (Coleman and
58 Mylonakis 2009).

59 Both ABC and MFS transporters have many crucial functions in fungi including nutrient
60 uptake/acquisition, waste removal, cellular signaling, self-defense, and other developmental processes.
61 In pathogenic fungi, transporters are important for fungal infection, colonization, and disease
62 progression (Perlin et al. 2014). The first identified pleiotropic drug resistance protein, the ABC-G
63 transporter *PDR5*, was described from baker's yeast *Saccharomyces cerevisiae* (Gulshan and Moye-
64 Rowley 2007). *PDR5* has orthologs in most other ascomycetes including Fusarium where it has been
65 associated with virulence by conferring resistance to plant phytoalexins (Coleman et al. 2011; Fleissner
66 et al. 2002).

67 *F. graminearum* has 62 predicted ABC proteins (Kovalchuk and Driessen 2010), four of which
68 appear to be involved in virulence (Yin et al. 2018). The ABC transporter FgAbc9 (FGSG_07325) plays a
69 crucial role in the export of the plant defense hormone salicylic acid, and is also involved in growth,
70 resistance to the anti-fungal compound tebuconazole, and accumulation of DON (Qi et al. 2018).
71 Perhaps the most studied membrane-bound transporter of *F. graminearum*, the ABC transporter Abc1
72 (FGSG_04580) also is important for resistance to fungicides and other xenobiotics. Deletion mutants of

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73 *Abc1* show reduced virulence during wheat crown and root rot infections (Gardiner et al. 2013).
74 Similarly, other *F. graminearum* strains with the FGSG_04580 gene deleted (called *Abc3* in this
75 reference), show reduced virulence on wheat, barley, and maize, as well as altered mycotoxin
76 production (Abou Ammar et al. 2013). Abc1 is also involved in zearalenone (an estrogenic metabolite of
77 *F. graminearum*) transport and tolerance to antifungal compounds (Abou Ammar et al. 2013; Gardiner
78 et al. 2013; Lee et al. 2011).

79 In addition to ABC transporters, a number of *F. graminearum* MFS transporters have been
80 suggested to be involved in toxin export and resistance. Wang and colleagues (2018) identified 33
81 transporters, 15 of which were MFS transporters, which were specifically induced by externally added
82 DON. Furthermore, a number of the identified MFS transporters had homologs known to be involved in
83 resistance to toxic compounds and secondary metabolite export in other fungi (Wang et al. 2018).
84 Within the core trichothecene biosynthetic gene cluster of *F. graminearum* is the gene *Tri12*
85 (FGSG_03541) which encodes an MFS transporter (Proctor et al. 2009). Disruption of the *Tri12* gene of *F.*
86 *sporotrichioides*, a close relative of *F. graminearum*, results in highly reduced trichothecene
87 accumulation in culture and reduced resistance to exogenously applied trichothecenes (Alexander et al.
88 1999). However, disruption of *Tri12* in *F. graminearum* results in only slightly reduced DON
89 accumulation (Menke et al. 2012).

90 The goal of this research was to test other ABC and MFS transporters or combinations of
91 transporters, for their role in trichothecene export and xenobiotic resistance in *F. graminearum*. In
92 addition to further characterization of the transporters Tri12 and Abc1, we included other ABC and MFS
93 transporters previously identified and suspected to have a role in DON export. The ABC transporter *Abc6*
94 (FGSG_11028) and an MFS transporter *Mfs1* (FGSG_07802) are either regulated by or otherwise co-
95 expressed with genes under the control of the trichothecene regulatory protein Tri6 (Seong et al. 2009).
96 During *F. graminearum* infection of wheat, *Abc6* and *Mfs1* are significantly up-regulated

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97 contemporaneously with high levels of DON production (Zhang et al. 2012) and in a *Tri12* deletion
98 mutant background, these two genes are significantly upregulated under toxin producing conditions
99 (Nakajima et al. 2015). We hypothesize that their gene products may play a redundant role in DON
100 transport, and “fill in” when Tri12 is not available.

101 We hypothesize that multiple, functionally redundant mechanisms for DON transport may exist
102 within *F. graminearum* cells. If this is the case, deletion of only a single mechanism for transport may not
103 lead to a discernable phenotype with respect to fungal growth, DON accumulation, or pathogenic
104 aggressiveness. However, disruption of two or more mechanisms for DON transport might be expected
105 to have greater impact. To test this, we combined deletion mutations for different membrane bound
106 transporters to assess the effect of disrupting multiple export pathways. These deletion mutants were
107 analyzed for growth and DON accumulation *in planta*. Additionally, the protein coding regions of
108 individual genes were expressed in a DON sensitive strain of *S. cerevisiae* to verify their direct
109 involvement in DON export and resistance to inhibition by a number of xenobiotic compounds.

110 **Materials and Methods**

111 *Generating F. graminearum deletion mutants*

112 Genetic deletion mutants of *F. graminearum* strain PH-1 (NRRL 31084) were generated using the
113 split marker homologous recombination technique (Goswami 2012) with modifications previously
114 described (O’Mara et al. 2020). A previously generated *Δtri12* disruption mutant was used (Menke et al.
115 2012). Primers utilized in the transformation and confirmation process are listed in Table S1. Target
116 genes were replaced with the neomycin phosphotransferase (NPT) or nourseothricin acyltransferase
117 (NAT) resistance genes as selectable markers (Fuchs et al. 2004; Menke et al. 2013). Transformations
118 and single-spore isolations were completed as previously described (O’Mara et al. 2020). Up to 10
119 transformant colonies were picked for further single-spore isolation and genetic confirmation. Mutants

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120 of *F. graminearum* were crossed to generate double- and triple knockout mutants. Mutant parents
121 contained different antibiotic-resistance selectable markers. Sexual crosses and ascospore collection
122 and screening were performed as previously described (Pasquali and Kistler 2006; O'Mara et al. 2020).
123 Single spore isolation of each transformant was performed as for single knockout mutants, except that
124 media contained two antibiotics.

125 *Confirmation of F. graminearum transformants*

126 Site-directed deletion of native genes in *F. graminearum* mutants was confirmed by PCR as
127 previously described (O'Mara et al. 2020) using the CTAB genomic DNA extraction technique (Gale et al.
128 2011). Site-directed gene deletion and replacement with an antibiotic-resistance selectable marker was
129 confirmed by amplifying the targeted locus using a primer pair which annealed upstream and
130 downstream of the gene. Changes in amplicon size corresponding to the replacement of the native gene
131 with the antibiotic-resistance marker indicated site-directed gene replacement. In cases where the
132 amplicon sizes were too similar to distinguish using this method, an either/or amplification technique
133 was employed. In the latter case, a forward primer which annealed upstream of the targeted locus was
134 paired with either a reverse primer which annealed to the end of the native gene or a reverse primer
135 which annealed to the end of the antibiotic-resistance gene. This amplification pair would indicate
136 whether the full native gene remained in its resident locus, or if the full antibiotic-resistance gene was
137 inserted into the locus.

138 *Growth of F. graminearum in culture*

139 Conidial suspensions of each *F. graminearum* mutant were made by growing in 50 mL of
140 carboxy-methyl cellulose (CMC) medium (Cappellini and Peterson 1965) for ~5 days. Conidia were
141 collected and enumerated as previously described (O'Mara et al. 2020) then suspended in water at 2 x
142 10^4 conidia/mL for *in vitro* analyses or 1 x 10^6 conidia/mL for plant inoculations.

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143 To assess the ability of *F. graminearum* deletion mutants to utilize different laboratory media,
144 deletion mutants were grown on carrot, ½ PDA, Czapek-Dox, complete, minimal, and V8 agar media
145 (Klittich and Leslie 1988; Puhalla and Spieth 1983; Rodriguez Estrada et al. 2011). A 3 mm diameter plug
146 of mycelium was placed at the center of each and grown in triplicate at 25°C with 12 h light, 12 h dark
147 for 3 days before determining the area (mm^2) of hyphal growth using a Carestream 4000MM Pro Image
148 Station, with Carestream Molecular Imaging Software v.5.2.2.15761 (Carestream Health, Inc., Rochester,
149 NY, USA).

150 DON accumulation and pathogenicity of F. graminearum transporter deletion mutants

151 The ability of *F. graminearum* mutants to cause FHB symptoms and accumulate DON was
152 evaluated in wheat cultivar Norm, as previously described (O'Mara et al. 2020). Two weeks after point-
153 inoculation of the fifth fully formed spikelet, heads were scored for FHB disease symptoms (bleached,
154 shriveled, necrotic grains) by counting the number of diseased spikelets, up to 10, surrounding the point
155 of inoculation. The inoculated spikelet was then removed from the wheat head and weighed in a tared
156 1-dram screw-cap vial, frozen at -80°C overnight and analyzed for DON, 3-ADON, and 15-ADON (total
157 DON) by GC-MS using methods previously described (Goswami and Kistler 2005).

158 *Generation of F. graminearum transporter containing yeast transformants*

159 *F. graminearum* transporters were expressed in the DON-sensitive *Saccharomyces cerevisiae*
160 strain YZGA515 (Poppenberger et al. 2003), generously provided by Dr. Gerhard Adam. Expression
161 plasmids were generated using Gateway cloning technology (Invitrogen, Thermo-Fisher Scientific,
162 Waltham, MA, USA). A cDNA of the *F. graminearum Abc1* gene codon-optimized for yeast was
163 synthesized by Invitrogen GeneArt gene synthesis and inserted onto the pENTR221 vector. Codon-
164 optimized cDNAs for *Tri12, Mfs1,* and *Abc6* were synthesized by Integrated DNA Technologies (IDT,
165 Coralville, IA, USA) and inserted on pUC-IDT vectors. *E. coli* strains containing the empty expression

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166 vector ZM552 or the yeast pleiotropic drug resistance transporter Pdr5 on the ZM552 plasmid were
167 purchased from the DNASU plasmid repository (Arizona State University, Tempe, AZ, USA). ZM552 was
168 used as an empty vector control for all tests, and Pdr5 was used as a positive control complementation.

169 To begin the Gateway cloning process, forward primers containing the attB1 site and reverse
170 primers containing the attB2 site (Table S1) were purchased from Invitrogen. These primers were used
171 to amplify the *F. graminearum* transporter coding regions (*Tri12, Mfs1,* and *Abc6*) from the pUC-IDT
172 plasmids. Once purified, the attB site flanked amplicons were cloned onto the Gateway donor vector
173 pDONR221 using the Gateway BP Clonase II enzyme mix kit, generating pENTR221 vectors. Expression
174 plasmids were generated by cloning the *F. graminearum* transporter coding regions from the pENTR221
175 vectors (now *Tri12, Abc1, Mfs1,* and *Abc6*) onto the ZM552 empty vector using the Gateway LR Clonase
176 II enzyme mix kit. Following the Gateway LR cloning step, all the transporter genes from *F. graminearum*
177 and *S. cerevisiae* were located on the ZM552 vector backbone and named pEXP552_*Gene Name* (e.g.
178 pEXP552_FgTri12 or pEXP552_ScPdr5). All pENTR221 and pEXP552 plasmids were cloned into Invitrogen
179 OmniMAX 2-T1 or New England Biolabs (Ipswich, MA, USA) 10-Beta *E. coli* for propagation. The
180 pDONR221 plasmid was cloned into Invitrogen ccdB Survival 2-T1 *E. coli* for propagation. Confirmations
181 of all cloning steps were performed by extracting plasmid DNA from *E. coli* using the Qiagen Miniprep kit
182 (Hilden, Germany) and performing double restriction enzyme digests using EcoRI and HindIII purchased
183 from New England Biosciences (NEB, Ipswich, MA, USA), using the manufacturer's recommended
184 procedures.

185 All transformation plasmids were transformed into *S. cerevisiae* YZGA515 using the Sigma-
186 Aldrich (St. Louis, MO, USA) YEAST1 yeast transformation kit, using manufacturer's recommended
187 procedures. Transformed YZGA515 strains were streaked and maintained on Synthetic Complete
188 Medium (Dunham et al. 2015) supplemented with leucine drop out powder (Sigma Aldrich). To confirm
189 proper transformation of yeast strains, plasmid DNA was extracted from transformed yeast using the

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190 Qiagen Miniprep kit and re-transformed into *E. coli* for propagation. Plasmid DNA from re-transformed
191 *E. coli* was extracted again using the Qiagen Miniprep kit and digested using EcoRI and HindIII as before.
192 Restriction digests from original transformed *E. coli* strains were compared to digests from re-
193 transformed *E. coli* to confirm identical plasmid composition.

194 *Expression of F. graminearum transporters in a susceptible yeast line*

195 Transformed *S. cerevisiae* YZGA515 strains were analyzed for their sensitivity to DON and its
196 acetylated derivatives 3-acetyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON). Pre-
197 cultures of YZGA515 transformants were grown in liquid SCM-leu medium for 4-5 days at 30˚C and 300
198 rpm. Pre-cultures were diluted with 2x SCM-leu to an optical density (600 nm absorbance) of 0.1 for
199 inoculation. In a 96-well microtiter plate, 100 µL of YZGA515 inoculum was added to 100 µL ddH_2O
200 supplemented with a concentration gradient of DON, 3-ADON, or 15-ADON. Concentration series
201 included 0, 20, 30, 60, 120, and 250 ppm DON or 3-ADON, and 0, 5, 10, 15, 20, and 30 ppm 15-ADON. All
202 six YZGA515 transformants were inoculated onto the same 96-well microtiter plate containing six
203 DON/ADON concentrations, with an extra well containing 1x SCM-leu without yeast as a medium
204 control. Plates were sealed with parafilm and incubated in the dark for 5 days at 30˚C and 300 rpm.
205 After incubation, well contents were homogenized by gentle pipetting and the optical density of each
206 well was taken using a Thermo-Fisher Scientific Varioskan Flash with SkanIt RE software. DON/ADON
207 plates were run in triplicate and sensitivity was analyzed as a percent change in optical density
208 compared to 0 ppm control.

209 To test whether low pH would better facilitate DON export in *S. cerevisiae* YZGA515 lines
210 expressing *F. graminearum* MFS transporters (Gardiner et al. 2009), the plate assay was repeated using
211 glycine-HCl buffered SCM-leu. To make buffered medium, 1.875 g glycine was added to 200 mL 2x SCM-
212 leu. Mixture was then titrated with 5M HCl to pH 2.5. The buffered medium was then brought up to 250

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213 mL using ddH_2O and sterilized by filtering through a Corning 0.22 µm filter unit (Corning Life Sciences,
214 Corning, NY, USA). Assay setup and optical density measurements were performed as previously
215 described above.

216 To understand how the *F. graminearum* transporters may provide resistance to xenobiotic
217 compounds, the transformed yeast lines were tested against a concentration series of the plant
218 phytoalexin 2-benzoxazolinone (BOA). Transformant strains were grown in 5 mL SCM-leu medium for
219 4~5 days at 30˚C and 300 rpm. Cultures were centrifuged at 1,200 x g for 5 minutes and resuspended in
220 sterile water to an optical density of OD=0.38. BOA was dissolved in DMSO to concentration of 500
221 mg/mL. In a 96-well plate, 4 µL of YZGA515 transformant cell suspensions were inoculated into 200 µL
222 SCM-leu medium containing 0, 100, 200, 300, 400, or 500 ppm BOA, with a final concentration of 0.1%
223 DMSO. After inoculation, 96-well plates were incubated at 30˚C and 300 rpm for 3 days. Optical density
224 of each well was determined using a Thermo-Fisher Scientific Varioskan Flash with SkanIt RE software.
225 Plates were run in triplicate and sensitivity was analyzed as a percent change in optical density
226 compared to 0 ppm control.

227 The yeast transformants were also tested against 24 xenobiotic compounds at 4 concentrations
228 using Biolog plate PM24C (Biolog Inc, Hayward, CA, USA). Transformants were grown and resuspended
229 as for BOA plates then 630 µL of the cell suspension was added to 29.38 mL of Biolog PM inoculating
230 fluids using the manufacturer's recommended protocol and mixed thoroughly. Using an 8-channel
231 pipette, 100 µL of the inoculation fluid was transferred into each well of the 96-well PM24C plate. Plates
232 were incubated at 30˚C for 3 days before recording the OD value of each well. Plates were conducted in
233 triplicate for each yeast transformant. Raw OD values for each transformant were subtracted from the
234 OD values of the ZM552 empty vector control for each replicate. Afterwards, the relative OD values for
235 each transformant were averaged across the three replicates for a final growth value.

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236 *Data analysis*

237 *F. graminearum* growth rates, FHB disease symptoms, and DON concentrations were analyzed in
238 R Statistical Software version 3.5.1 (R Core Team 2018). Data were analyzed by a one-way ANOVA with a
239 Tukey's post-hoc test to compare all pair-wise interactions. ANOVAs for *in vitro* and *in planta*
240 inoculations were blocked for inoculation date, to better address inter-genotype differences. Sensitivity
241 of S. *cerevisiae* to exogenous DON and BOA was analyzed by a one-way ANOVA with a Dunnett's post-
242 hoc test (Hothorn et al. 2008) to compare differences from the control group. ANOVAs were conducted
243 on raw optical density values (600nm) while figures show percent growth of genotype compared to the
244 0 ppm concentrations.

245 **Results**

246 *Confirmation of F. graminearum mutants*

247 Genes for *F. graminearum* membrane-bound transporters potentially involved in DON export
248 and FHB virulence were knocked out to assess their function in these processes. All knockouts were
249 performed by replacing the native gene with a neomycin (neoR) or nourseothricin (ntcR) resistance
250 gene. Mutant genotypes were confirmed by PCR, using changes in amplicon size or by selectively
251 amplifying either the wild-type locus or its resistance-construct replacement (Figure S1). All
252 transformations yielded at least one mutant which grew on antibiotic selection and amplified a PCR
253 product indicative of a successful site-directed, gene replacement deletion; most yielded multiple
254 deletion candidates and a single mutant was selected for further analysis. Deletion phenotypes were
255 further confirmed for DON accumulation by comparing neoR or ntcR deletion mutants with an
256 independently generated hygromycin (hygR) deletion mutant. All deletion mutant pairs (neoR vs. hygR
257 or ntcR vs. hygR) accumulated the same levels of DON *in vitro* (Figure S2).

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258 *F. graminearum transporter deletion mutants are not altered in growth or morphology*

259 Deletion mutants of *F. graminearum* were assessed for their growth rates and phenotype on six
260 different laboratory media. Growth of all mutant genotypes at three days post inoculation was not
261 significantly different from the wildtype (p>0.05; Table S2). Additionally, deletion of membrane-bound
262 transporters in *F. graminearum* did not manifest any overt phenotypes when grown on laboratory
263 media, even when combining multiple deletions (Figure S3).

264 *Multiple transporter mutants show reduced DON accumulation and virulence in wheat*

265 *F. graminearum* knockout mutants were tested for their ability to accumulate DON plus its
266 acetylated derivatives (total DON) and cause FHB symptoms *in planta*. Numerous combinations of
267 transporter deletion mutants were significantly reduced in the ability to accumulate DON (F=35.76,
268 df=346, p<2.2e-16). Two levels of effect were seen (Figure 1A). Deletion of the *Tri12* gene reduced DON
269 accumulation in wheat approximately 35% while deletion of the *Abc1* transporter gene reduced DON
270 accumulation in wheat by 65%. Combining the *Δabc1* and the *Δtri12* alleles did not reduce DON
271 accumulation beyond that seen for the *Δabc1* alone. Likewise, combining the *Δabc6* or *Δmfs1* alleles
272 with *Δtri12*, as double or triple mutants, didn't reduce DON accumulation beyond the reduction seen in
273 the *Δtri12* mutant alone.

274 Infected spikes were assessed 14 days post inoculation for the spread of FHB disease symptoms
275 and significant differences were detected between the genotypes (F=25.96, df=346, $p<2.2e^{-16}$). FHB
276 symptoms followed a somewhat similar trend as seen for DON accumulation *in* planta (Figure 1B).
277 However, deletion of the Abc6 gene reduced FHB spread by ~30% while deletion of A*bc1* reduced FHB
278 spread by ~78%. Again, combining multiple mutations was not able to significantly reduce FHB
279 symptoms below those of the parent genotypes. These results fail to indicate an additive effect of

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280 multiple transporter deletions but do suggest that Abc1 plays a substantial role in DON accumulation
281 and FHB virulence, while other transporters play a more minor role.

282 *F. graminearum transporters alter DON and BOA sensitivity in a susceptible yeast line*

283 Four *F. graminearum* membrane-bound transporters were expressed in a DON-sensitive yeast
284 strain YZGA515 and exposed to a concentration series of DON and acetylated derivatives 15-ADON and
285 3-ADON to assess the function of these transporters. The yeast multidrug resistance transporter *Pdr5*
286 expressed in YZGA515 was used as a positive control, and YZGA515 without an insert was used as a
287 negative control. The YZGA515 *FgAbc1* strain showed a substantial increase in resistance to DON, 15-
288 ADON, and 3-ADON (Figure 2), showing up to six-fold increased resistance to the toxins as compared to
289 the vector control. The native *ScPdr5* strain also showed a substantial increase in resistance to the
290 toxins. The others, including the *FgTri12*, *FgMfs1*, and *FgAbc6* strains, showed little to no increase in
291 resistance to DON and 15-ADON but *FgAbc6* may allow for some resistance to higher levels of 3ADON.
292 Interestingly, the two MFS transporter strains, *FgTri12* and *FgMfs1*, were actually more sensitive to DON
293 and 15-ADON at lower concentrations than the empty vector control. To determine if these MFS
294 transporters, which are predicted proton antiporters, would provide resistance to DON at low pH, all
295 transformants were subsequently tested against DON in acidified medium.

296 When the assays were repeated in medium buffered to pH 2.5 with glycine-HCl, the *FgTri12*
297 strain was increased in resistance and was no more sensitive to DON than the empty vector control at
298 20 ppm, the lowest concentration of DON tested (Figure 3). However, at the next higher concentration
299 (30 ppm) the *FgTri12* strain again showed greater DON sensitivity than the control as did *FgMfs1* and
300 *FgAbc6*. This suggests that the hydrogen ion concentration can impact the activity of *FgTri12* but only at
301 lower DON concentrations. This is of note because *F. graminearum* establishes an acidic extracellular
302 environment under toxin-inducing conditions (Gardiner et al. 2009) that may provide the proton-motive

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303 force to affect Tri12-mediated transport. The *FgAbc1* and *ScPdr5* strains continued to show increased
304 resistance to DON as compared to the empty vector control (Figure 3).

305 To assess the ability of the *F. graminearum* transporters to provide resistance to xenobiotics,
306 YZGA515 strains were exposed to a concentration series of the wheat phytoalexin 2-benzoxazolinone
307 (BOA) for 3 days. The empty vector control strain ZM552 showed moderate sensitivity to BOA, with an
308 estimated EC50 value of approximately 250 ppm (Figure 4). The *FgMfs1* strain showed increased
309 sensitivity to BOA, with an estimated EC50 value of 160 ppm. Surprisingly, the remaining strains
310 (*FgTri12*, *FgAbc1*, *FgAbc6*, and *ScPdr5*) all showed increased resistance to BOA, with all having an
311 estimated EC50 value of 350 ppm.

312 Chemical sensitivity was further analyzed by exposing the yeast strains to 24 xenobiotics
313 included in the Biolog PM24C plate (Biolog Inc, Hayward, CA, USA) (Figure S4). With the exception of a
314 marked increased resistance to azole fungicides in the *ScPdr5* positive control and the *FgAbc1* strain,
315 there was only moderate resistance to xenobiotics for the other transformants. Other than *FgMfs1*, all
316 strains showed moderately increased resistance to antibiotics and metal salts with the *FgAbc1* and
317 *ScPdr5* strains additionally having increased resistance to anticancer and other antifungal compounds.
318 This finding fits into a larger pattern in which the *F. graminearum* Abc1 transporter plays a key role in
319 the export of endogenous trichothecenes and a broad range of exogenous xenobiotics, including azole
320 fungicides (Abou Ammar et al. 2013; Gardiner et al. 2013; Lee et al. 2011), providing both increased
321 virulence and defense for *F. graminearum.*

322 **Discussion**

323 While ABC- and MFS transporters play multiple roles in the normal physiology of fungi, for
324 pathogenic species, they may also allow resistance to stresses related to host infection and can be
325 essential for full expression of animal and plant pathogenesis (Cavalheiro et al. 2018; Coleman and

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326 Mylonakis 2009). Additionally, transporters are known to confer resistance to man-made antifungal
327 compounds with consequences in clinical and agricultural settings (Ma and Michailides 2005; Sanglard
328 2016).

329 Transporters also can be important for the export of fungal secondary metabolites. These
330 metabolites are typically produced by gene clusters encoding enzymes for the biosynthesis of the
331 metabolites themselves as well as transporters presumed to allow for metabolite secretion. When these
332 metabolites are mycotoxins, export mediated by gene cluster-encoded transporters has been suggested
333 to be important to avoid self-inhibition. However, deletion mutants for the transporters in mycotoxin
334 gene clusters sometimes can secrete nearly wild-type levels of the toxin and show little or no adverse
335 impact on growth during toxin-inducing conditions (Chang et al. 2004; Proctor et al. 2003). This has led
336 to the idea that additional secretion mechanisms may be operative within the cell that also function to
337 export toxins (O'Mara et al. 2020). For the trichothecene biosynthetic gene cluster in *F. graminearum*,
338 deletion of the cluster-associated MFS transporter, Tri12, indeed has been shown to have minimal effect
339 on secretion of DON and related metabolites (Menke et al. 2012). Deletion analysis of an ABC
340 transporter Abc1 and components of vesicular transport pathways each suggested that alternate
341 pathways for DON secretion were possible (O'Mara et al. 2020). Here we sought to test the impact of
342 mutation on multiple transporters in *F. graminearum,* alone and in combination, on pathogenicity to
343 wheat and DON accumulation *in planta*.

344 None of the transporter deletion mutants used in this study presented altered growth or
345 macroscopic changes in phenotype when tested on laboratory media (Table S2 and Figure S3) indicating
346 that the individual and combined effect of these transporters on the normal vegetative growth of *F.*
347 *graminearum* is minimal. This suggests that these transporters may be associated with other functions
348 such as the transport of secondary metabolites, which by definition are only produced during limited
349 parts of the life cycle and are not necessary for primary metabolic function of an organism (Keller et al.

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350 2005). While Tri12 is encoded within the trichothecene biosynthetic gene cluster, genes for the *Mfs1*
351 and *Abc6* lie within the biosynthetic genes clusters for other known natural products; the mycotoxin
352 Fusarin C and the siderophore Malonichrome, respectively (Sieber et al. 2014; Oide et al. 2014). *Abc1* is
353 located near (~25 kb) a predicted polyketide synthase, *Pks29*, and an O-methyltransferase (Sieber et al.
354 2014), and has been suggested to be involved in the transport of numerous *F. graminearum* secondary
355 metabolites including trichothecenes and zearalenone (Abou Ammar et al. 2013; Lee et al. 2011). Taken
356 together, there is correlative evidence that these transporters may be involved to varying degrees in
357 secondary metabolite transport but not in primary vegetative growth.

358 By expressing these transporters in yeast, we sought to directly test their ability to transport
359 DON and related compounds by measuring the ability of strains to overcome DON toxicity. Yeast strains
360 expressing Fusarium transporters were also tested for sensitivity to a panel of chemicals including the
361 wheat phytoalexin BOA, which may impact pathogenicity toward wheat (Kettle et al. 2015). The yeast
362 strain YZGA515 is deficient in all major multidrug resistance transporters (Poppenberger et al. 2003) and
363 is completely inhibited by 10 ppm 15-ADON and 60 ppm DON (Figure 2), the major trichothecenes
364 produced by the wild type strain used in this study. As predicted, the *F. graminearum* Abc1 transporter
365 performed nearly as well as its yeast ortholog, the Pdr5 multidrug resistance transporter, for increasing
366 resistance of YZGA515 to DON, 15-ADON, and 3-ADON (Figure 2). The other *F. graminearum*
367 transporters tested were not able to increase resistance to DON or 15-ADON although Abc6 may confer
368 resistance at higher levels of 3-ADON. Surprisingly, the MFS transporters Mfs1 and especially Tri12,
369 seemed to allow for greater sensitivity at DON and 15-ADON at lower concentrations.

370 Additionally, yeast strains expressing *FgTri12*, *FgAbc1*, *FgAbc6*, or *ScPdr5* provided similar levels
371 of increased resistance to BOA (Figure 4). This observation is consistent with the idea that multiple *F.*
372 *graminearum* MFS and ABC transporters are capable of transporting BOA and thereby conferring
373 redundant modes of resistance to the phytoalexin. Previously, a *Δabc1* mutant of *F. graminearum* failed

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374 to show increased sensitivity to BOA *in vitro* (Gardiner et al. 2013). However, the concentration of BOA
375 used in that study was equal to the highest concentration used in the present study (500 ppm), at which
376 all strains tested here were sensitive. Additionally, several single ABC transporter mutants of *F.*
377 *graminearum* did not grow at significantly different rates compared to wildtype on multiple
378 concentrations of BOA (Abou Ammar et al. 2013). Based on our results in yeast, previously published
379 results may have not detected an effect of individual transporters *in situ* due to functional redundancies
380 of other MFS and ABC transporters.

381 Except for Abc1, there is a poor correspondence between the ability of the transporters to
382 confer DON resistance in yeast and the ability contribute to DON accumulation by *Fusarium*. This may
383 reflect a fundamental difference in the manner in which these transporters act in *Fusarium*. The other
384 membrane-bound transporters studied here (Tri12, Mfs1, and Abc6) may be indirectly associated with
385 DON accumulation, rather than more directly involved in DON export as with Abc1. While the ABC
386 multidrug resistance transporters are expected to be targeted to the plasma membrane in yeast and
387 *Fusarium* (Egner et al. 1995; Lee et al. 2011) we have previously noted that Tri12 in *Fusarium* localizes to
388 motile vesicles that may fuse with the vacuole or the plasma membrane (Menke et al. 2012). Because
389 Tri12 and Mfs1 expressed in yeast actually increases sensitivity to DON, our hypothesis is that Tri12 may
390 facilitate DON uptake by these vesicles that may then be transported to the vacuole for sequestration or
391 to the plasma membrane for export. Previously we noted that the plasma membrane localized SNARE
392 protein Sso2, essential for subapical vesicular exocytosis, was required for wildtype DON accumulation
393 (O'Mara et al. 2020). Moreover, combining mutations for *Abc1* and *Sso2* reduced DON accumulation in
394 an additive manner indicating that both vesicular transport and direct export via the multidrug
395 transporter Abc1 contributed to DON export in a non-redundant manner.

396 In conclusion the ABC transporter Abc1 plays a significant role in the export of the trichothecene
397 DON both *in vitro* and *in planta* and may act as the primary membrane-bound transporter involved in

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398 DON export. Two other membrane-bound transporters studied here, Tri12 and Abc6, show significant
399 involvement in DON accumulation either *in vitro* or *in planta* but may act indirectly in conjunction with
400 vesicular transport mechanisms or by allowing for greater virulence necessary for maximum DON
401 accumulation in wheat by transport of BOA or other small molecules. Disruption of *Abc1* through novel
402 management techniques such as host-induced or spray-induced gene silencing (HIGS and SIGS
403 respectively) (Koch et al. 2016; Qi et al. 2019), combined with other management techniques in an
404 integrated pest management system, may provide better control of FHB.

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417 **Conflicts of Interest**

418 The authors declare that there are no conflicts of interest.

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419 **Literature Cited**

420 Abou Ammar, G., Tryono, R., Döll, K., Karlovsky, P., Deising, H. B., and Wirsel, S. G. R. 2013. Identification
421 of ABC transporter genes of *Fusarium graminearum* with roles in azole tolerance and/or virulence. PLoS
422 One. 8:e79042 Available at:
423 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3823976&tool=pmcentrez&rendertype=ab
424 stract.

425 Alexander, N. J., McCormick, S. P., and Hohn, T. M. 1999. TRI12, a trichothecene efflux pump from
426 *Fusarium sporotrichioides*: Gene isolation and expression in yeast. Mol. Gen. Genet. 261:977–984.

427 Bahadoor, A., Brauer, E. K., Bosnich, W., Schneiderman, D., Johnston, A., Aubin, Y., et al. 2018. Gramillin
428 A and B: Cyclic lipopeptides identified as the nonribosomal biosynthetic products of *Fusarium*
429 *graminearum*. J. Am. Chem. Soc. 140:16783–16791 Available at:
430 http://pubs.acs.org/doi/10.1021/jacs.8b10017 [Accessed August 1, 2019].

431 Cappellini, R. A., and Peterson, J. L. 1965. Macroconidium formation in submerged cultures by a non-
432 sporulating strain of *Gibberella zeae*. Mycologia. 57:962 Available at:
433 http://www.jstor.org/stable/3756895?origin=crossref [Accessed March 26, 2018].

434 Cavalheiro, M., Pais, P., Galocha, M., and Teixeira, M. C. 2018. Host-pathogen interactions mediated by
435 MDR transporters in fungi: As pleiotropic as it gets! Genes (Basel). 9 Available at:
436 https://pubmed.ncbi.nlm.nih.gov/30004464/ [Accessed September 23, 2020].

437 Chang, P. K., Yu, J., and Yu, J. H. 2004. aflT, a MFS transporter-encoding gene located in the aflatoxin
438 gene cluster, does not have a significant role in aflatoxin secretion. Fungal Genet. Biol. 41:911–920.

439 Coleman, J. J., and Mylonakis, E. 2009. Efflux in fungi: La piece de resistance. PLoS Pathog. 5.

440 Coleman, J. J., White, G. J., Rodriguez-Carres, M., and Vanetten, H. D. 2011. An ABC transporter and a
441 cytochrome P450 of Nectria haematococca MPVI are virulence factors on pea and are the major
442 tolerance mechanisms to the phytoalexin pisatin. Mol. Plant. Microbe. Interact. 24:368–376.

443 Desjardins, A. E., Proctor, R. H., Bai, G., McCormick, S. P., Shaner, G., Buechley, G., et al. 1996. Reduced
444 virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. Mol. Plant-
445 Microbe Interact. 9:775–781 Available at:
446 http://www.apsnet.org/publications/mpmi/backissues/Documents/1996Abstracts/Microbe09-775.htm.

447 Dunham, M. J., Gartenberg, M. R., and Brown, G. W. 2015. *Methods in yeast genetics and genomics : a*
448 *Cold Spring Harbor Laboratory course manual*. 2015 Editi. Cold Spring Harbor Laboratory Press.

449 Egner, R., Mahé, Y., Pandjaitan, R., and Kuchler, K. 1995. Endocytosis and vacuolar degradation of the
450 plasma membrane-localized Pdr5 ATP-binding cassette multidrug transporter in Saccharomyces
451 cerevisiae. Mol. Cell. Biol. 15:5879–5887.

452 Fleissner, A., Sopalla, C., and Weltring, K.-M. 2002. An ATP-binding cassette multidrug-resistance
453 transporter is necessary for tolerance of Gibberella pulicaris to phytoalexins and virulence on potato
454 tubers. Mol. Plant. Microbe. Interact. 15:102–108.

455 Fried, H. M., and Warner, J. R. 1981. Cloning of yeast gene for trichodermin resistance and ribosomal
456 protein L3. Proc. Natl. Acad. Sci. U. S. A. 78:238–42 Available at:
457 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=319027&tool=pmcentrez&rendertype=abst

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458 ract.

459 Fuchs, U., Czymmek, K. J., and Sweigard, J. A. 2004. Five hydrophobin genes in *Fusarium verticillioides*
460 include two required for microconidial chain formation. Fungal Genet. Biol. 41:852–864 Available at:
461 https://www.sciencedirect.com/science/article/pii/S1087184504000672 [Accessed March 26, 2019].

462 Gale, L. R., Harrison, S. A., Ward, T. J., O'Donnell, K., Milus, E. A., Gale, S. W., et al. 2011. Nivalenol-type
463 populations of *Fusarium graminearum* and *F. asiaticum* are prevalent on wheat in southern Louisiana.
464 Phytopathology. 101:124–134 Available at: http://apsjournals.apsnet.org/doi/10.1094/PHYTO-03-10-
465 0067 [Accessed March 26, 2018].

466 Gardiner, D. M., Osborne, S., Kazan, K., and Manners, J. M. 2009. Low pH regulates the production of
467 deoxynivalenol by *Fusarium graminearum*. Microbiology. 155:3149–3156.

468 Gardiner, D. M., Stephens, A. E., Munn, A. L., and Manners, J. M. 2013. An ABC pleiotropic drug
469 resistance transporter of *Fusarium graminearum* with a role in crown and root diseases of wheat. FEMS
470 Microbiol. Lett. 348:36–45.

471 Garreau de Loubresse, N., Prokhorova, I., Holtkamp, W., Rodnina, M. V., Yusupova, G., and Yusupov, M.
472 2014. Structural basis for the inhibition of the eukaryotic ribosome. Nature. 513:517–522 Available at:
473 http://dx.doi.org/10.1038/nature13737 [Accessed January 18, 2018].

474 Goswami, R. S. 2012. Targeted Gene Replacement in Fungi Using a Split-Marker Approach. Methods
475 Mol. Biol. 835:255–269 Available at: http://link.springer.com/10.1007/978-1-61779-501-5 [Accessed
476 May 8, 2019].

477 Goswami, R. S., and Kistler, H. C. 2005. Pathogenicity and *in planta* mycotoxin accumulation among
478 members of the *Fusarium graminearum* species complex on wheat and rice. Phytopathology. 95:1397–
479 1404.

480 Gulshan, K., and Moye-Rowley, W. S. 2007. Multidrug resistance in fungi. Eukaryot. Cell. 6:1933–1942.

481 Harris, L. J., and Gleddie, S. C. 2001. A modified Rpl3 gene from rice confers tolerance of the *Fusarium*
482 *graminearum* mycotoxin deoxynivalenol to transgenic tobacco. Physiol. Mol. Plant Pathol. 58:173–181.

483 Hothorn, T., Bretz, F., and Westfall, P. 2008. Simultaneous inference in general parametric models.
484 Biometrical J. 50:346–363.

485 Imazaki, I., and Kadota, I. 2015. Molecular phylogeny and diversity of Fusarium endophytes isolated
486 from tomato stems. FEMS Microbiol. Ecol. 91:fiv098 Available at:
487 http://www.ncbi.nlm.nih.gov/pubmed/26298015 [Accessed March 26, 2019].

488 Keller, N. P., Turner, G., and Bennett, J. W. 2005. Fungal secondary metabolism — from biochemistry to
489 genomics. Nat. Rev. Microbiol. 3:937–947 Available at:
490 http://www.nature.com/doifinder/10.1038/nrmicro1286.

491 Kettle, A. J., Batley, J., Benfield, A. H., Manners, J. M., Kazan, K., and Gardiner, D. M. 2015. Degradation
492 of the benzoxazolinone class of phytoalexins is important for virulence of Fusarium pseudograminearum
493 towards wheat. Mol. Plant Pathol. 16:946–962 Available at: http://doi.wiley.com/10.1111/mpp.12250
494 [Accessed June 26, 2018].

495 Klittich, C., and Leslie, J. F. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella*
496 *fujikuroi*). Genetics. 118:417–23 Available at:

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497 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1203296/pdf/ge1183417.pdf [Accessed October 22,
498 2019].

499 Koch, A., Biedenkopf, D., Furch, A., Weber, L., Rossbach, O., Abdellatef, E., et al. 2016. An RNAi-based
500 control of *Fusarium graminearum* infections through spraying of long dsRNAs involves a plant passage
501 and is controlled by the fungal silencing machinery ed. Savithramma P. Dinesh-Kumar. PLoS Pathog.
502 12:e1005901 Available at: http://dx.plos.org/10.1371/journal.ppat.1005901 [Accessed May 15, 2018].

503 Kovalchuk, A., and Driessen, A. J. 2010. Phylogenetic analysis of fungal ABC transporters. BMC Genomics.
504 11:177 Available at: http://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-11-177
505 [Accessed November 20, 2018].

506 Lee, S., Son, H., Lee, J., Lee, Y. R., and Lee, Y. W. 2011. A putative ABC transporter gene, *ZRA1*, is required
507 for zearalenone production in Gibberella zeae. Curr. Genet. 57:343–351.

508 Lofgren, L. A., LeBlanc, N. R., Certano, A. K., Nachtigall, J., LaBine, K. M., Riddle, J., et al. 2018. *Fusarium*
509 *graminearum*: pathogen or endophyte of North American grasses? New Phytol. 217:1203–1212
510 Available at: http://doi.wiley.com/10.1111/nph.14894 [Accessed April 18, 2018].

511 Ma, Z., and Michailides, T. J. 2005. Advances in understanding molecular mechanisms of fungicide
512 resistance and molecular detection of resistant genotypes in phytopathogenic fungi. Crop Prot. 24:853–
513 863.

514 Menke, J., Dong, Y., and Kistler, H. C. 2012. Tri12p Influences virulence to wheat and trichothecene
515 accumulation. Mol. Plant-Microbe Interact. 25:1408–1418.

516 Menke, J., Weber, J., Broz, K., and Kistler, H. C. 2013. Cellular development associated with induced
517 mycotoxin synthesis in the filamentous fungus *Fusarium graminearum*. PLoS One. 8:e63077.

518 Nakajima, Y., Koseki, N., Sugiura, R., Tominaga, N., Maeda, K., Tokai, T., et al. 2015. Effect of disrupting
519 the trichothecene efflux pump encoded by FgTri12 in the nivalenol chemotype of *Fusarium*
520 *graminearum*. J. Gen. Appl. Microbiol. 61:93–96 Available at:
521 https://www.jstage.jst.go.jp/article/jgam/61/3/61_93/_article.

522 O'Mara, S. P., Broz, K., Boenisch, M., Zhong, Z., Dong, Y., and Corby Kistler, H. 2020. The *Fusarium*
523 *graminearum* t-SNARE Sso2 is involved in growth, defense, and DON accumulation and virulence. Mol.
524 Plant-Microbe Interact. 33:888–901.

525 Oide, S., Berthiller, F., Wiesenberger, G., Adam, G., and Turgeon, B. G. 2014. Individual and combined
526 roles of malonichrome, ferricrocin, and TAFC siderophores in *Fusarium graminearum* pathogenic and
527 sexual development. Front. Microbiol. 5:759 Available at:
528 http://journal.frontiersin.org/article/10.3389/fmicb.2014.00759/abstract [Accessed May 20, 2020].

529 Pasquali, M., and Kistler, C. 2006. *Gibberella zeae* ascospore production and collection for microarray
530 experiments. J. Vis. Exp. 1:115 Available at: http://www.ncbi.nlm.nih.gov/pubmed/18704186 [Accessed
531 March 26, 2018].

532 Perlin, M. H., Andrews, J., and Toh, S. S. 2014. *Essential letters in the fungal alphabet: ABC and MFS*
533 *transporters and their roles in survival and pathogenicity*. Elsevier. Available at:
534 http://dx.doi.org/10.1016/B978-0-12-800271-1.00004-4.

535 Pestka, J. J. 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. Anim. Feed Sci.
536 Technol. 137:283–298 Available at: http://linkinghub.elsevier.com/retrieve/pii/S0377840107002209.

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537 Poppenberger, B., Berthiller, F., Lucyshyn, D., Sieberer, T., Schuhmacher, R., Krska, R., et al. 2003.
538 Detoxification of the Fusarium mycotoxin deoxynivalenol by a UDP-glucosyltransferase from *Arabidopsis*
539 *thaliana*. J. Biol. Chem. 278:47905–47914 Available at:
540 http://www.jbc.org/lookup/doi/10.1074/jbc.M307552200.

541 Proctor, R. H. 1995. Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin
542 biosynthetic gene. Mol. Plant-Microbe Interact. 8:593 Available at:
543 http://www.ncbi.nlm.nih.gov/pubmed/8589414 [Accessed October 2, 2019].

544 Proctor, R. H., Brown, D. W., Plattner, R. D., and Desjardins, A. E. 2003. Co-expression of 15 contiguous
545 genes delineates a fumonisin biosynthetic gene cluster in Gibberella moniliformis. Fungal Genet. Biol.
546 38:237–249.

547 Proctor, R. H., McCormick, S. P., Alexander, N. J., and Desjardins, A. E. 2009. Evidence that a secondary
548 metabolic biosynthetic gene cluster has grown by gene relocation during evolution of the filamentous
549 fungus Fusarium. Mol. Microbiol. 74:1128–1142.

550 Puhalla, J. E., and Spieth, P. T. 1983. Heterokaryosis in *Fusarium moniliforme*. Exp. Mycol. 7:328–335
551 Available at: https://www.sciencedirect.com/science/article/pii/0147597583900178 [Accessed October
552 22, 2019].

553 Qi, P. F., Zhang, Y. Z., Liu, C. H., Zhu, J., Chen, Q., Guo, Z. R., et al. 2018. Fusarium graminearum ATP-
554 binding cassette transporter gene FgABCC9 is required for its transportation of salicylic acid, fungicide
555 resistance, mycelial growth and pathogenicity towards wheat. Int. J. Mol. Sci. 19.

556 Qi, T., Guo, Jia, Peng, H., Liu, P., Kang, Z., and Guo, Jun. 2019. Host-induced gene silencing: A powerful
557 strategy to control diseases of wheat and barley. Int. J. Mol. Sci. 20:206 Available at:
558 http://www.mdpi.com/1422-0067/20/1/206 [Accessed April 17, 2019].

559 R Core Team. 2018. R: A language and environment for statistical computing. Available at:
560 https://www.r-project.org/.

561 Rocha, O., Ansari, K., and Doohan, F. M. 2005. Effects of trichothecene mycotoxins on eukaryotic cells: A
562 review. Food Addit. Contam. 22:369–378 Available at:
563 http://www.tandfonline.com/doi/abs/10.1080/02652030500058403.

564 Rodriguez Estrada, A. E., Hegeman, A., Corby Kistler, H., and May, G. 2011. In vitro interactions between
565 *Fusarium verticillioides* and *Ustilago maydis* through real-time PCR and metabolic profiling. Fungal
566 Genet. Biol.

567 Sanglard, D. 2016. Emerging threats in antifungal-resistant fungal pathogens. Front. Med. 3:1 Available
568 at: http://www.ebi.ac.uk/interpro/entry/IPR011701/ [Accessed September 23, 2020].

569 Seong, K.-Y. Y., Pasquali, M., Zhou, X., Song, J., Hilburn, K., McCormick, S., et al. 2009. Global gene
570 regulation by Fusarium transcription factors Tri6 and Tri10 reveals adaptations for toxin biosynthesis.
571 Mol. Microbiol. 72:354–367 Available at: http://onlinelibrary.wiley.com/store/10.1111/j.1365-
572 2958.2009.06649.x/asset/j.1365-
573 2958.2009.06649.x.pdf?v=1&t=hv4dt99w&s=f7b520d8ee2c82b3abbb3cb2768a01898c6ba636.

574 Sieber, C. M. K., Lee, W., Wong, P., Münsterkötter, M., Mewes, H. W., Schmeitzl, C., et al. 2014. The
575 *Fusarium graminearum* genome reveals more secondary metabolite gene clusters and hints of
576 horizontal gene transfer. PLoS One. 9:e110311 Available at:
577 http://www.ncbi.nlm.nih.gov/pubmed/25333987 [Accessed November 8, 2019].

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578 Wachowska, U., Packa, D., and Wiwart, M. 2017. Microbial inhibition of Fusarium pathogens and
579 biological modification of trichothecenes in cereal grains. Toxins (Basel). 9:408 Available at:
580 http://www.ncbi.nlm.nih.gov/pubmed/29261142 [Accessed March 26, 2019].

581 Wang, Q., Chen, D., Wu, M., Zhu, J., Jiang, C., Xu, J.-R., et al. 2018. MFS Transporters and GABA
582 Metabolism Are Involved in the Self-Defense Against DON in Fusarium graminearum. Front. Plant Sci.
583 9:438 Available at: http://journal.frontiersin.org/article/10.3389/fpls.2018.00438/full [Accessed April
584 13, 2018].

585 Waweru, B., Turoop, L., Kahangi, E., Coyne, D., and Dubois, T. 2014. Non-pathogenic *Fusarium*
586 *oxysporum* endophytes provide field control of nematodes, improving yield of banana (Musa sp.). Biol.
587 Control. 74:82–88 Available at: https://www.sciencedirect.com/science/article/pii/S1049964414000784
588 [Accessed March 26, 2019].

589 Wilson, W., Dahl, B., and Nganje, W. 2018. Economic costs of Fusarium Head Blight, scab and
590 deoxynivalenol. World Mycotoxin J. 11:291–302 Available at:
591 https://www.wageningenacademic.com/doi/10.3920/WMJ2017.2204 [Accessed January 14, 2019].

592 Wipfle, R., McCormick, S. P., Proctor, R. H., Teresi, J. M., Hao, G., Ward, T. J., et al. 2019. Synergistic
593 phytotoxic effects of culmorin and trichothecene mycotoxins. Toxins (Basel). 11:555 Available at:
594 https://www.mdpi.com/2072-6651/11/10/555 [Accessed May 12, 2020].

595 Yin, Y., Wang, Z., Cheng, D., Chen, X., Chen, Y., and Ma, Z. 2018. The ATP-binding protein FgArb1 is
596 essential for penetration, infectious and normal growth of Fusarium graminearum. New Phytol.
597 219:1447–1466.

598 Zhang, X.-W., Jia, L.-J., Zhang, Y., Jiang, G., Li, X., Zhang, D., et al. 2012. In planta stage-specific fungal
599 gene profiling elucidates the molecular strategies of *Fusarium graminearum* growing inside wheat
600 coleoptiles. Plant Cell. 24:5159–76 Available at:
601 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3556981&tool=pmcentrez&rendertype=ab
602 stract.

603

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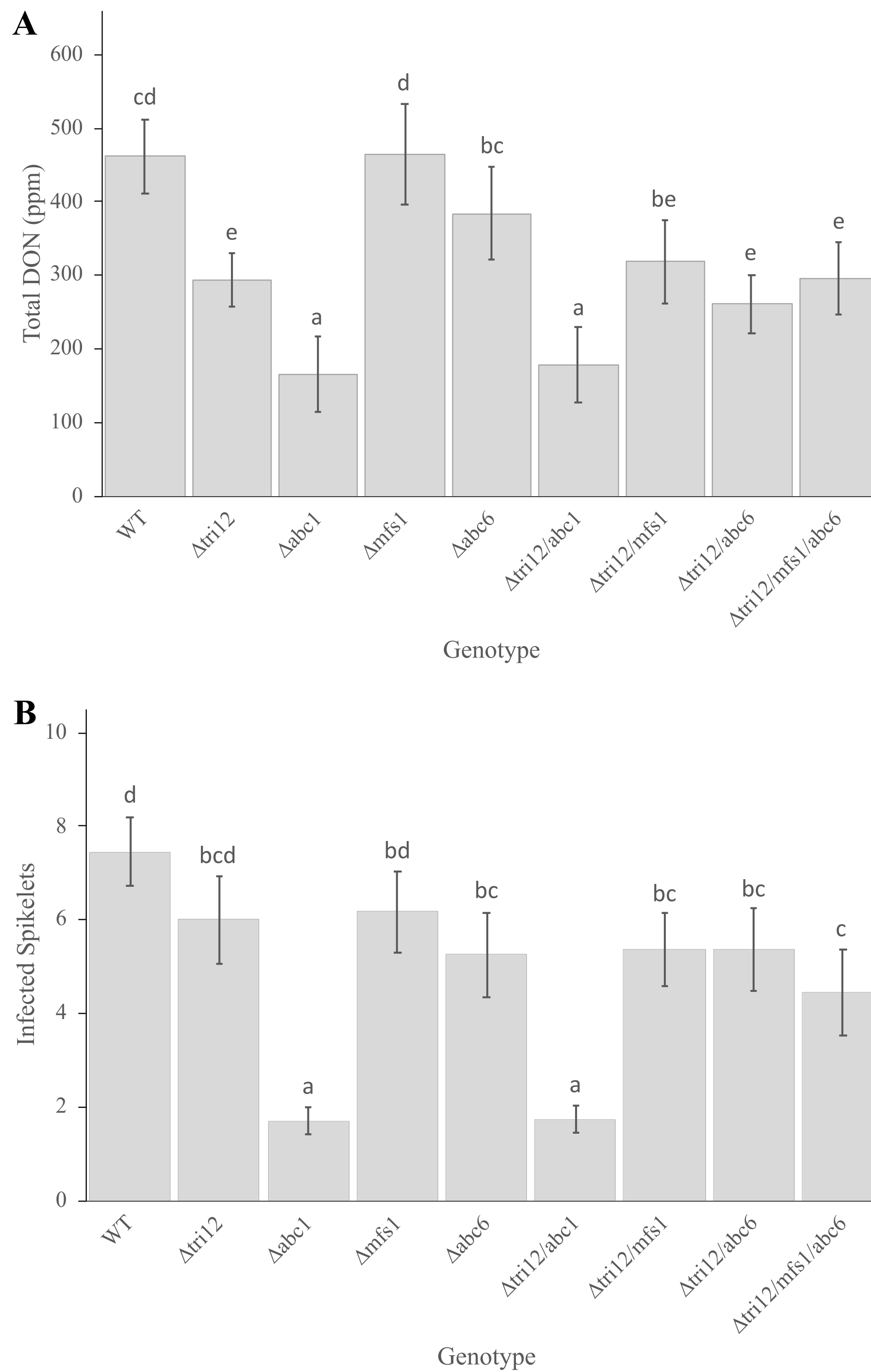
604 **Figures**



605 **Figure 1: Total DON accumulation *in planta* and pathogenicity of *F. graminearum* transporter mutants.** Total
606 DON accumulation (A) and disease progression (B) 14 days post inoculation (n=40). Mean + 95% CI. Data analyzed
607 by one-way ANOVA. Genotypes with the same letter are not significantly different as determined by a Tukey's post
608 hoc analysis.

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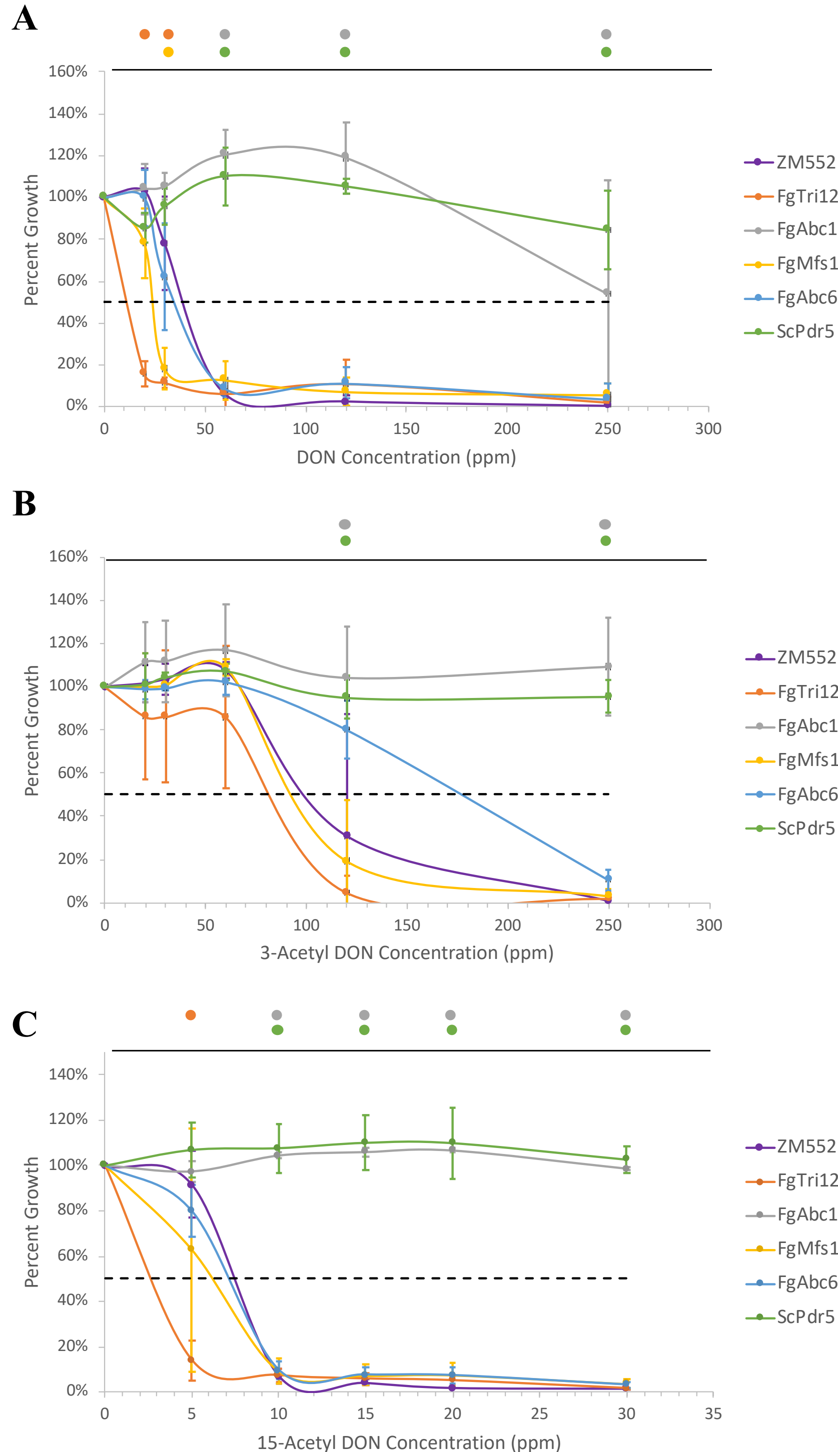

609 **Figure 2: Growth of yeast YZGA515 transformants exposed to exogenous DON and ADON.** Growth of *S. cerevisiae*
610 strain YZGA515 expressing *F. graminearum* transporters after 5 days exposure to exogenous (A) DON, (B) 3-ADON,
611 and (C) 15-ADON. Measurements are the average (n=3) percent growth compared to the 0 ppm concentration ± 95%
612 confidence interval. Dashed black line indicates EC50. Colored dots above graph indicate genotypes which are
613 significantly different ($p<0.05$) from the ZM552 control at each concentration. One-way ANOVA conducted on raw
614 optical density (600 nm) measurements at each concentration.

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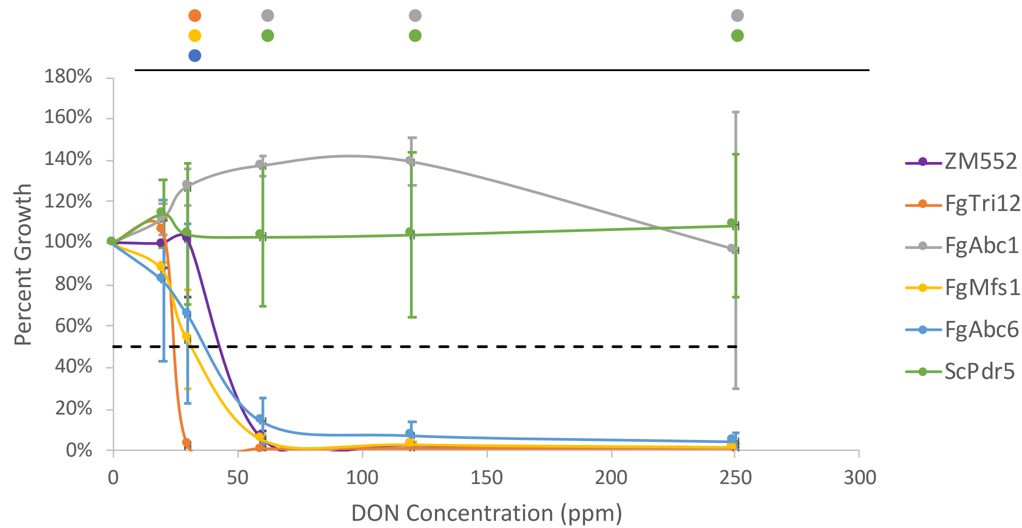
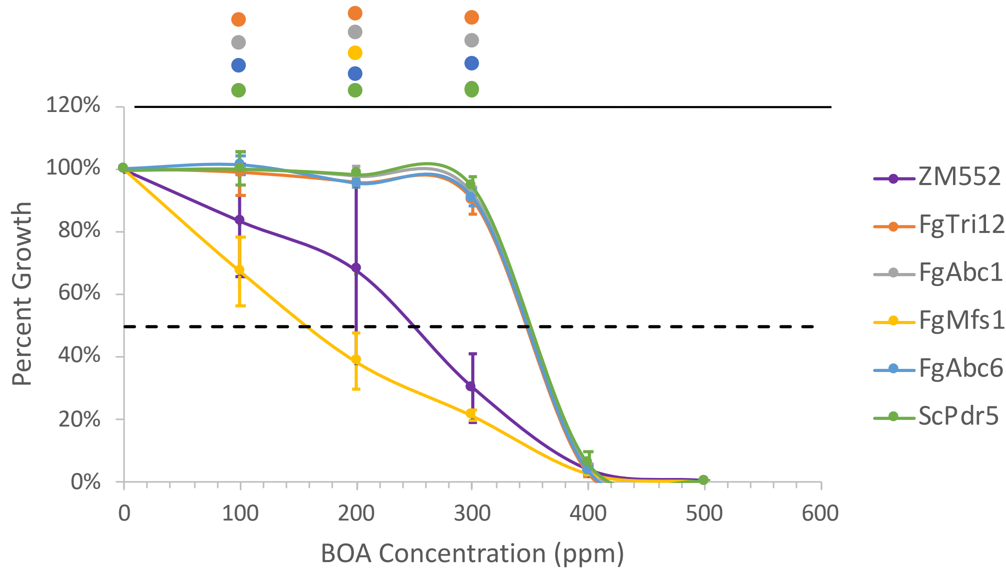


615 **Figure 3: Growth of yeast YZGA515 transformants exposed to exogenous DON at pH 2.5.** Growth of *S. cerevisiae*
616 strain YZGA515 expressing *F. graminearum* transporters after five days exposure to exogenous DON/ADON at pH
617 2.5. Measurements are the average (n=3) percent growth compared to the 0 ppm concentration ± 95% confidence
618 interval. Dashed black line indicates EC50. Colored dots above graph indicate genotypes which are significantly
619 different ($p<0.05$) from the ZM552 control at each concentration. One-way ANOVA conducted on raw optical density
620 (600 nm) measurements at each concentration.



621 **Figure 4: Growth of yeast YZGA515 transformants exposed to exogenous BOA.** Growth of *S. cerevisiae* strain
622 YZGA515 expressing *F. graminearum* transporters after three days exposure to the plant phytoalexin 2-
623 benzoxazolinone (BOA). Measurements are the average (n=3) percent growth compared to the 0 ppm concentration
624 ± 95% confidence interval. All *F. graminearum* transporter transformants showed increased resistance to BOA,
625 except for the FgMfs1 lines which was more sensitive. Dotted black line indicates EC50. Colored dots above graph
626 indicate genotypes which are significantly different ($p<0.05$) from the ZM552 control at each concentration. One-
627 way ANOVA conducted on raw optical density (600 nm) measurements at each concentration.

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Supplemental Information

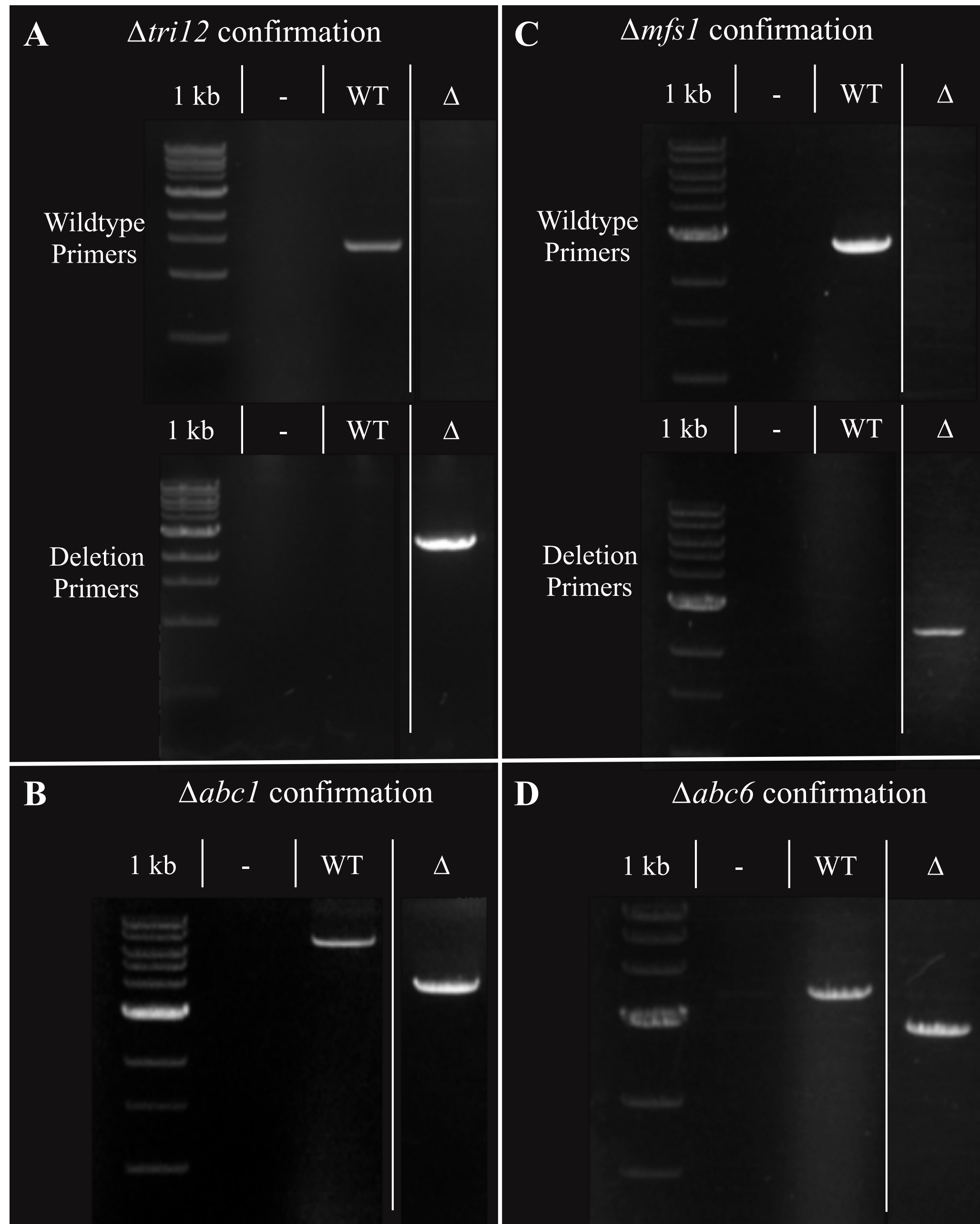

Figure S1: Confirmation PCR of *F. graminearum* deletion mutants. Size discrepancy or presence/absence determination of proper genetic deletions for (A) *Δtri12* knockout, (B) *Δabc1* knockout, (C) *Δmfs1* knockout, and (D) *Δabc6* knockout. Size discrepancy primers flank 5' and 3' of manipulated locus. Presence/absence wild type amplification primers bind upstream to manipulated locus and end of native gene; deletion amplification primers bind upstream to the target locus and end of antibiotic resistance gene. Vertical lines between lanes indicate removed lanes. 1 kb = 1 kb ladder, - = No DNA, WT = *F. graminearum* PH1 DNA, Δ = deletion mutant DNA.

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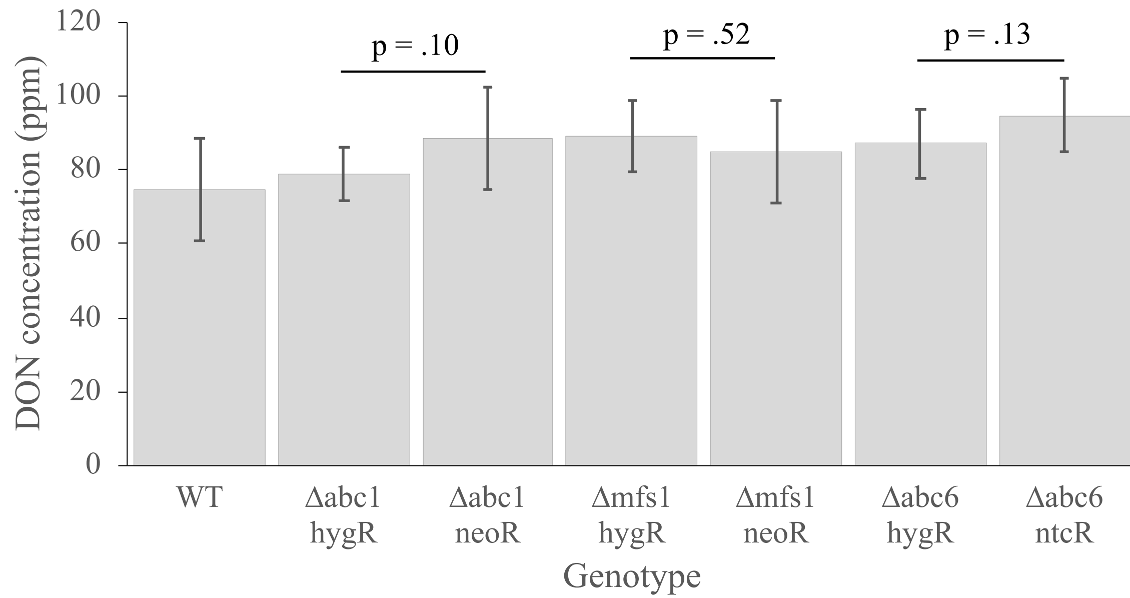

Figure S2: *In vitro* DON accumulation comparison of independently deleted *F. graminearum* transporters. Transporter deletions were conducted twice, using different antibiotic selectable markers, to confirm mutant phenotypes. Data analyzed by Student's T-test. All comparisons between independent transformation mutants showed insignificant differences in DON accumulation.

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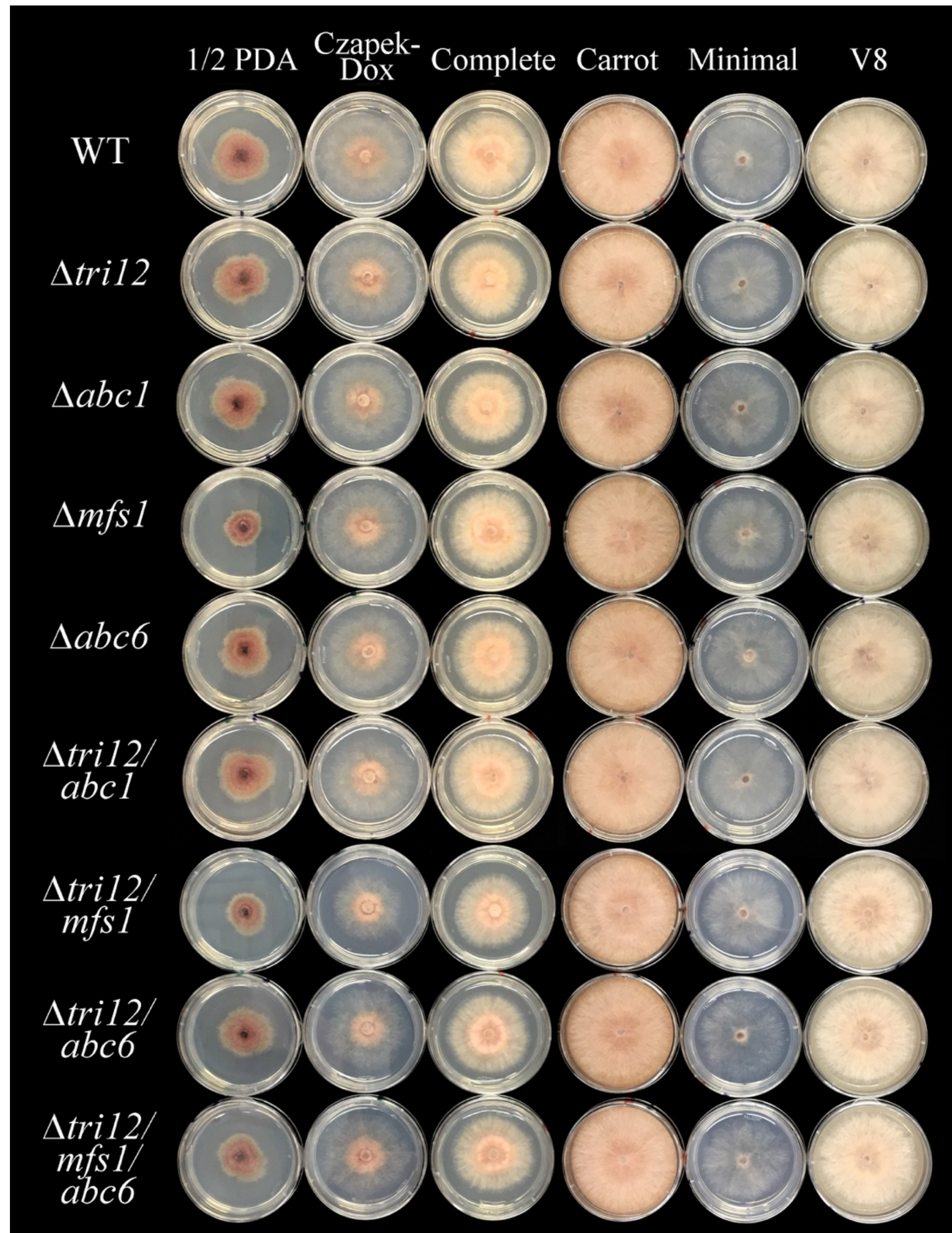

Figure S3: Phenotype of *F. graminearum* genotypes on laboratory media. Gene deletion did not result in any overt phenotypic changes in *F. graminearum* mutants, including multi-knockout mutants.

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Figure S4: Growth of *F. graminearum* transporter expressing yeast on xenobiotics. Relative growth of transformed yeast YZGA515 expressing *F. graminearum* transporters compared to an empty vector control. Green cells indicated more growth of transformant compared to control; red cells indicate less growth. 1=Amino acid analog, 2=Antibacterial, 3=Antibiotic, 4= Anticancer, 5=Antifungal, 6=Antipsychotic, 7=Antiseptic, 8=Chelating Agent, 9=Metal salt, 10=Nucleotide analog, 11= Polyketide, 12=Viologen.

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Table S1: Oligonucleotide primers used for amplification, deletion, and confirmation of F. graminearum mutants.

Purpose and oligonucleotide	Sequence (5'-3')nt	Remarks
PCR based amplification of *F. graminearum* DNA flanks		
FGSG_04580 LF1F	AATTCCCTCTCCTTTATGCACAAG	Used for all Abc1 deletion setups
FGSG_04580 LF2R NEO	GGGAACCAATTTGAGTACCCAATTCTATGTTTGCGCTGCAAGACAG	Used for Abc1 deletion with neoR construct
FGSG_04580 RF3F NEO	GCCTAGTTTCTCGGTACTATGCATATGCTCTTTCTGCCTCACCTTC	Used for Abc1 deletion with neoR construct
FGSG_04580 RF4R	ACATGTTTGCTATCGTCGGC	Used for all Abc1 deletion setups
FGSG_07802 LF1F	AGGAGATGTTGTCAGTATCCACG	Used for all Mfs1 deletion setups
FGSG_07802 LF2R NEO	GGGAACCAATTTGAGTACCCAATTCGGGACGCGAAAGAATTTTACAGT	Used for Mfs1 deletion with neoR construct
FGSG_07802 RF3F NEO	GCCTAGTTTCTCGGTACTATGCATATAATTTTGTTGAAACGCCCAGGA	Used for Mfs1 deletion with neoR construct
FGSG_07802 RF4R	AGCATCAACGGAACTGTCAAGA	Used for all Mfs1 deletion setups
FGSG_11028 LF1F	TGGTGCCATTTTGATCGGTCTA	Used for all Abc6 deletion setups
FGSG_11028 LF2R NAT	TGACTTTTACCATTTCACCGCAGCCACGGAGACAGCAAACTC	Used for Abc6 deletion with ntcR construct
FGSG_11028 LF2R NAT	TATAGTGAGTCGTATTACGCGCGCTTACTGTGTGGCTTGATCCTTCA	Used for Abc6 deletion with ntcR construct
FGSG_11028 RF4R	GACAGAACCAACAGACAATGCC	Used for all Abc6 deletion setups
PCR based amplification of antibiotic resistance cassette		
CK_216 NEO/F	GAATTGGGTACTCAAATTGGTTCCC	Amplification of first 2/3 of NPT construct
CK_217 NE/R	ATGTTCTTCGTCCAGATCATCCTGA	Amplification of first 2/3 of NPT construct
CK_218 EO/F	CCTGCTCATCACCTTTTCTCACATA	Amplification of last 2/3 of NPT construct
CK_219 NEO/R	ATATGCATAGTACCGAGAAACTAGGC	Amplification of last 2/3 of NPT construct
AL12-5_NAT/F	TGCGGTGAAATGGTAAAAGTCA	Amplification of first 2/3 of NAT construct
AL12-6 NA/R	GTTGACGTTGGTGACCTCC	Amplification of first 2/3 of NAT construct
AL12-7_AT/F	TCCTTCACCACCGACACC	Amplification of last 2/3 of NAT construct
AL12-8_NAT/R	AGGTAGTTCTGGTCCATTGGT	Amplification of last 2/3 of NAT construct
PCR based confirmation of genetic knockouts		
FGSG_04580/F	CCAGCCTTGACAATGACGTT	Confirmation of *Δabc1* knockout
FGSG_04580/R	GAAACACCATTCCGCCCG	Confirmation of *Δabc1* knockout

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FGSG_07802/F	CGTTCCTACTACCACTGCGA	Confirmation of *Δmfs1* knockout
FGSG_07802/R	TCTTGGGGAAGTCAGCGATT	Confirmation of *Δmfs1* knockout
FGSG_07802 2R IG	TCAATCTCTGACGACACCTT	Confirmation of *Δmfs1* knockout
FGSG_11028/R	CCAATCCCGTGCTTCTGATG	Confirmation of *Δabc6* knockout
FGSG_11028/F	TCCTTGTGTTCATCGTCGGA	Confirmation of *Δabc6* knockout
FGSG_03541/F	CTCACTCCAGCACTTGTCCA	Confirmation of *Δtri12* knockout
FGSG_03541/R	GCAACCGCCTATAAGATCCA	Confirmation of *Δtri12* knockout
Tri12 GFPScreen5p	TATATGGCTCACGGCTTTCC	Confirmation of *Δtri12* knockout
Heterologous expression of *F. graminearum* transporters		
FgTri12 attB1 F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACCGCCACCGTCCAT	Amplification of *Tri12* from pUC-IDT plasmid
FgTri12 attB2 R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTATGACTGAGTTTTGTCGTC	Amplification of *Tri12* from pUC-IDT plasmid
FgMfs1 attB1 F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGGCTATGGATTCA	Amplification of *Mfs1* from pUC-IDT plasmid
FgMfs1 attB2 R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAATCCCTTACAACACCCCT	Amplification of *Mfs1* from pUC-IDT plasmid
FgAbc6 attB1 F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCAACCAATGACGGT	Amplification of *Abc6* from pUC-IDT plasmid
FgAbc6 attB2 R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCACAGAGAAATTTTGGCAGC	Amplification of *Abc6* from pUC-IDT plasmid
M13 Forward	TGTAAAACGACGGCCAGT	Amplification of plasmid inserts
M13 Reverse	CAGGAAACAGCTATGACCATG	Amplification of plasmid inserts

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Table S2: Colony area (mm^2) of *F. graminearum* genotypes grown on laboratory media. Mean ± standard deviation (n=3) of *F. graminearum* colonies grown for 3 days on laboratory media. No significant differences in colony area were found between genotypes for each medium (p>0.05).

	Medium					
Strain	**1/2 PDA**	**Czapek-Dox**	**Complete**	**Carrot**	**Minimal**	**V8**
WT	213 ± 52	886 ± 56	709 ± 104	2013 ± 100	830 ± 20	609 ± 49
Δtri12	445 ± 204	1033 ± 94	843 ± 36	2031 ± 87	909 ± 75	585 ± 16
Δabc1	358 ± 22	912 ± 83	753 ± 126	1929 ± 67	822 ± 76	530 ± 15
Δmfs1	333 ± 26	1013 ± 118	812 ± 40	2027 ± 56	923 ± 51	555 ± 7
Δabc6	327 ± 11	928 ± 128	794 ± 18	2062 ± 123	845 ± 57	551 ± 30
Δtri12/abc1	200 ± 16	883 ± 81	710 ± 14	1923 ± 17	747 ± 30	579 ± 23
Δtri12/mfs1	187 ± 44	823 ± 248	678 ± 228	1969 ± 29	798 ± 87	588 ± 71
Δtri12/abc6	245 ± 105	944 ± 316	489 ± 187	1880 ± 58	807 ± 92	450 ± 133
Δtri12/mfs1/abc6	266 ± 147	898 ± 282	714 ± 251	1942 ± 70	753 ± 54	561 ± 48