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Exogenous abscisic acid and gibberellic acid elicit opposing effects on *Fusarium graminearum* infection in wheat

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ABSTRACT

While the roles of salicylate (SA) and jasmonate (JA) have been well-characterized in Fusarium head blight (FHB) infected cereals, the role of other phytohormones remains more ambiguous. Here, the association between an array of phytohormones and FHB pathogenesis in wheat is investigated. Comprehensive profiling of endogenous hormones demonstrated altered cytokinin, gibberellin (GA), and JA metabolism in a FHB-resistance cultivar; while challenge by *Fusarium graminearum* increased abscisic acid (ABA), JA, and SA in both FHB-susceptible and –resistant cultivars. Subsequent investigation of ABA or GA co-application with fungal-challenge increased and decreased FHB spread, respectively. These phytohormone induced effects may be attributed to alteration of the *F. graminearum* transcriptome as ABA promoted expression of early-infection genes including hydrolases and cytoskeletal reorganization genes, while GA suppressed nitrogen metabolic gene expression. Neither ABA nor GA elicited significant effects on *F. graminearum* fungal growth or sporulation in axenic conditions nor do these phytohormones affect trichothecene gene expression, deoxynivalenol mycotoxin accumulation, or SA / JA biosynthesis in *F. graminearum*-challenged wheat spikes. Finally, the combined application of GA and paclobutrazol, a *Fusarium* fungicide, provided additive effects on reducing FHB severity, highlighting the potential for combining fungicidal agents with select phytohormone-related treatments for management of FHB infection in wheat.

Key words: Fusarium head blight, jasmonic acid, salicylic acid, deoxynivalenol, grain yield, paclobutrazol, 3'-hexysulfanyl-ABA, mycotoxin

INTRODUCTION

Fusarium head blight (FHB) is a devastating disease of wheat and other small grain cereals caused by species in the *Fusarium* genus with the most prevalent species being *F. graminearum* and *F. culmorum* (Parry et al., 1995; Edwards, 2004). *F. graminearum*'s hemibiotrophic lifestyle involves establishment on and penetration into its host where optimal fitness and virulence is dependent on nitrogen metabolism (Divon et al., 2006; Namiki et al., 2001; Seong et al., 2005) and biosynthesis of virulence factors like the trichothecene mycotoxin deoxynivalenol (DON) (McCormick et al., 2003; Gale et al., 2003; Kang et al., 1999). DON is not only phytotoxic, reducing grain quality and yield, but also poses a threat to human and animal health when consumed (Antonissen et al., 2014; Mostrom et al., 2007). FHB is manifested as bleaching or necrotic browning of spike tissue ultimately producing shriveled and/or discolored grain (McMullen et al., 1997). In spite of extensive wheat breeding efforts, only a limited number of FHB-resistant cultivars have been developed, where 'Sumai 3' and its derivatives exhibit the greatest resistance (reviewed in Bai and Shaner, 2004). The use of chemical fungicides has also been investigated; however, these reports highlight the inconsistency and reduced efficacy of available fungicides in FHB-susceptible wheat varieties (Mesterhazy et al., 2011).

Phytohormones commonly associated with plant defense against pathogens, including salicylic acid (SA) and jasmonic acid (JA) (reviewed in Vleesschauwer et al., 2014), have recently been investigated to elucidate their roles in FHB resistance. Both SA and JA have been reported to possess antifungal activity against *F. graminearum* (Qi et al., 2012; Qi et al., 2016; Sun et al., 2016) and also mediate induced defense responses *in planta* through their signaling activities (Qi

et al., 2016; Sun et al., 2016; Makandar et al., 2006). In fact, independent studies have demonstrated that overexpression of an SA-signaling gene in the otherwise susceptible wheat cultivar, 'Bobwhite', is sufficient to promote FHB resistance (Makandar et al., 2006), and related SA-signaling genes may serve as markers for resistance in a diverse set of winter wheat cultivars (Diethelm et al. 2014). Although SA and JA signaling have been reported to contribute to FHB resistance, the role of JA has also been reported to contribute to FHB susceptibility, specifically during the early stages of infection (Makandar et al., 2010; Ding et al., 2011; Makandar et al., 2012; Ameye et al 2015). Together, these studies suggest an infection model where SA promotes resistance during early *F. graminearum* biotrophic growth, while JA promotes resistance only in the later necrotrophic infection (Makandar et al., 2010; Ding et al., 2011; Makandar et al., 2012; Ameye et al 2015).

While the roles of SA and JA signaling in the FHB disease response have been well-characterized, the role of other phytohormones remains more ambiguous. By combining differential gene expression and exogenous hormone application, ET has been reported to be associated with FHB-resistance (Li & Yen, 2008), FHB-susceptibility (Chen et al., 2009), or have no effect on pathogenesis (Sun et al., 20160). As well, isolated studies of exogenous auxin (IAA) or epibrassinolied application immediately prior to *Fusarium* infection have been reported to reduce FHB disease severity and yield loss (Petti et al., 2012; Ali et al., 2013). Finally, gibberellic acids (GAs) have been reported to induce pathogen-resistance associated gene expression (Casacuberta et al., 1992) and promote resistance to ascomycota fungal infections (Eshel et al., 2002; Tanaka et al., 2006), though the effect of GA has not previously been investigated in relation to FHB. The reports in this field to date have also suggested a complex

interaction among phytohormones in the FHB-response potentially involving crosstalk between SA, JA, IAA, and abscisic acid (ABA) signaling pathways (Qi et al., 2016; reviewed in Yang et al., 2015).

The work described herein is focused on elucidating the roles of ABA, GA, and other less-well characterized hormones in the FHB-wheat interaction. Unique associations between phytohormones and FHB resistance were identified by evaluating endogenous and *Fusarium*-induced phytohormone profiles in FHB-resistant ('Sumai 3') and susceptible ('Fielder') *Triticum aestivum* wheat cultivars. Exogenous co-application of diverse phytohormones was subsequently investigated and included independent application of ABA, IAA, GA, and the cytokinin zeatin (Z). Effects of these phytohormones on axenic *F. graminearum* growth and sporulation as well as FHB spread, DON biosynthesis and accumulation, and hormonal crosstalk in *F. graminearum*-challenged wheat spikes are evaluated. Finally, the effects of applying phytohormone analogs and combining phytohormones and fungicide treatments are also discussed. This work highlights the potential value of combining phytohormone-related treatments with existing fungicidal applications as part of an agronomic strategy for mitigating FHB pathogenesis in wheat.

RESULTS

Hormone profiling of susceptible and resistant wheat cultivars in F. graminearum-challenged and -unchallenged spikes

Phytohormone signaling and metabolism have been well characterized in a variety of wheat lines shortly after *Fusarium*-challenge with these studies supporting an infection model where SA promotes resistance during early *F. graminearum* growth, while JA promotes resistance only in the necrotrophic infection (Qi et al., 2012; Qi et al., 2016; Makandar et al., 2006; Makandar et al., 2010; Makandar et al., 2012; Ding et al., 2011; Ameye et al., 2015; Sun et al., 2016). Toward detecting possible involvement of these classical hormones in responses at later stages in FHB disease progression, as well as any roles for the non-classical hormones (Supplemental Figure 1), constitutive and *Fusarium*-induced hormonal changes were compared between the susceptible and resistant *T. aestivum* wheat cultivars 'Fielder' and 'Sumai 3', respectively, at 14 days post infection. This time point was selected to allow FHB to spread from the site of *F. graminearum* challenge to the adjacent tissue along the spike . Interestingly, in unchallenged cultivars, the resistant 'Sumai 3' demonstrated at least two-fold higher levels of ABA and its metabolites 7'-OH ABA and ABA glucose ester, as well as SA, JA, and JA-isoleucine (JA-Ile) compared to the FHB-susceptible 'Fielder' (Table 1). 'Sumai 3' also exhibited higher cytokinin metabolism based on the 20-fold lower levels of the cytokinin biosynthetic precursor, Z-riboside, and higher content of the cytokinin metabolite Z-*O*-glucoside. Finally, 'Sumai 3' displayed higher accumulation of the bioactive GA1 biosynthetic precursor GA19 and no detectable bioactive GA4 biosynthetic precursor GA24 compared with 'Fielder', suggesting different endogenous GA metabolism pathways may be functioning in these cultivars (for a review of the GA biosynthetic pathways see Yamaguchi et al., 2008). Levels of other phytohormones and their metabolites, including IAA, did not differ between the unchallenged cultivars.

Toward investigating the effect of FHB on these profiles, FHB-resistant 'Sumai 3' and -susceptible 'Fielder' spikes were challenged with *F. graminearum* spores by point inoculation and phytohormone responses were quantified. Fourteen days after *F. graminearum*-challenge, ABA and JA as well as their respective metabolites were increased by more than 50 % and 200 %, respectively, while conjugated SA levels were depleted by approximately 50 % compared to unchallenged 'Fielder' spikes (Table 1). Similar responses in the SA and ABA metabolic pathways were observed in 'Sumai 3', whereas no changes in JA or related metabolites were observed in this line. The absence of a *Fusarium*-induced response in JA and related metabolites may be due to the JA biosynthetic pathway being activated in 'Sumai 3' prior to *Fusarium* challenge and / or constitutively greater JA pools accumulating in this FHB-resistant cultivar. Ultimately, the intrinsic differences in phytohormone profiles between 'Fielder' and 'Sumai 3' cultivars may be due to physical or developmental differences inherent to each line. However, these differences may also provide insight into how these cultivars respond to biotic stresses like FHB. Consensus between phytohormone profiles, signaling, and effects of exogenous applications provide complementary approaches to describe the fundamental roles of phytohormones in response to pathogens.

Co-application of exogenous ABA or GA promotes and reduces FHB symptoms respectively, in the FHB-susceptible cultivar, 'Fielder'

Based on the findings that FHB-susceptible and -resistant cultivars have unique phytohormone profiles and that these phytohormone profiles are responsive to *Fusarium*-challenge, the direct effect of phytohormones on FHB disease symptoms and mycotoxin accumulation were evaluated

in susceptible 'Fielder' spikes. Phytohormones ABA, GA, IAA, and Z were co-inoculated with purified *F. graminearum* spores, while SA and JA were not included in this study as the effects of their application to wheat heads have been previously reported elsewhere (Qi et al., 2015; Qi et al., 2016; Makandar et al., 2012). FHB spread exhibited a degree of variability between independent trials and, as such, was represented as the average of three experiments. Co-application of 1.0 mM ABA increased the rate of FHB spread especially from symptomatic tissue to an adjacent asymptomatic spikelets (Figure 1), in a statistically significant manner throughout at least one third of the challenged 'Fielder' spike (Supplemental Figure 2). Conversely, co-application of 1.0 mM GA reduced the spread of FHB most significantly from seven to ten days after *Fusarium*-challenge (Figure 1). This single application of GA was not sufficient to prevent disease spread; however, the delay in spread manifested itself in beneficial grain attributes including reduced DON contamination and increased yield (Figure 1B-C). ABA did not have a significant effect on either grain quality or quantity. Neither IAA nor Z elicited any significant changes in FHB response or DON accumulation in either spikes or grain (Supplemental Figure 3).

The reduction of FHB disease symptoms in 'Fielder' spikes treated with GA was consistent with reduced detection of *F. graminearum* gene expression in challenged spikelets. Two florets from a central spikelet were co-inoculated with *F. graminearum* spores supplemented with either 1.0 mM ABA or GA, and the abundance of *F. graminearum* was monitored based on the expression of its constitutively expressed actin gene relative to the wheat *RNPQ* reference gene (Qi et al., 2012) in the challenged spikelet and the adjacent basipetal node. Although actin has been widely reported as a housekeeping gene, the possibility of non-specific semi-quantitative RT-PCR

amplification as well as actin expression changes during the course of infection cannot be ignored. To this end, three additional genes encoded by *F. graminearum*, but not wheat, were also quantified: trichothecene biosynthetic cluster genes *tri4*, *tri5*, and *tri11*. Five days after *Fusarium*-challenge, just prior to the phenotypic observation of FHB disease spread, the application of GA was found to consistently reduce the levels of detectable *F. graminearum* actin and *tri* gene expression in 'Fielder' spike tissue (Figure 2A), while ABA application had no significant effect. This reduced gene expression, in addition to the corresponding reduced FHB disease symptoms (Figure 1), is consistent with reduced *Fusarium* fungal spread upon GA co-application.

Co-application of ABA or GA does not affect trichothecene biosynthetic gene expression or deoxynivalenol accumulation in Fusarium-challenged 'Fielder' spikes

The virulence of *F. graminearum* is dependent on its ability to biosynthesize trichothecene mycotoxins, including deoxynivalenol (DON). Co-application studies demonstrated that in addition to modulating FHB phenotypic symptoms, GA also affects the accumulation of DON in isolated grains (Figure 1). This DON accumulation may either be a correlative symptom of limiting FHB spread or a direct effect on DON biosynthesis or accumulation. Five days after *Fusarium*-challenge, when *tri4* and *tri*5 are reported to be maximally expressed (Brown et al., 2011), neither GA nor ABA affected *tri4*, *tri5*, or *tri11* gene expression relative to the actin reference gene expression (Figure 2A). To expand on this, the effects of phytohormone treatments on expression of all of the 22 trichothecene biosynthetic genes in the pathogen, were monitored in 'Fielder' spikes by transcriptome sequencing 24 hours after *Fusarium*-challenge.

205 Upon ABA or GA co-application, no significant change in expression was observed ($p < 0.01$
206 with fold change in expression > 2; Supplemental Table 1). Subsequently, the effects of 1.0 mM
207 ABA or GA co-application on DON accumulation were monitored through direct toxin
208 quantification. 'Fielder' spikes were challenged with *F. graminearum* spores in the presence and
209 absence of 1.0 mM ABA or GA at each spikelet along the length of the 'Fielder' spike, and DON
210 was monitored five days post challenge by LC-MS. Co-application of ABA or GA did not affect
211 the accumulation of DON in 'Fielder' spikes (Figure 2B).
212
213 ***ABA promotes the expression of hydrolases and cytoskeletal reorganization genes previously***
214 ***reported in early F. graminearum infection while GA represses genes involved in F.***
215 ***graminearum nitrogen metabolism***
216
217 Although ABA and GA do not affect *F. graminearum tri* gene expression, these phytohormones
218 may modulate FHB infection by altering other *Fusarium* virulence- or infection-related gene
219 expression. To assess this possibility, the transcriptome of *F. graminearum* was evaluated in
220 challenged 'Fielder' spikelets in the presence and absence of 1.0 mM ABA or GA. As many as
221 140 unique *F. graminearum* genes were expressed in 'Fielder' spikelet tissue 24 hours after
222 *Fusarium*-challenge. Of these, 24 were significantly differentially expressed upon co-application
223 of ABA and six were differentially expressed upon co-application of GA relative to that of tissue
224 challenged with *F. graminearum* alone (Supplemental Table 2). Upon ABA co-application, two
225 glucosidases (FGSF_01621; FGSF_13861), two hydrolases (FGSF_11366; FGSF_02875), and
226 cytoskeletal reorganization (FGSF_03563) gene expression were induced, potentially associated
227 with promotion of plant cell wall degradation and hyphae insertion into the host wheat cells.

228 Interestingly, when comparing genes reported to be highly expressed in diverse *F. graminearum*
229 strains during early wheat spike infection (Stephens et al., 2008; Menke 2011; Lysoe et al.,
230 2011), nine of these genes were observed in this work to not only be expressed but induced two-
231 to five-fold upon ABA co-application (Table 2). Alternatively upon GA co-application, a
232 calcium-dependent aldoxime dehydratase (FGSF_06482), calcium transporting ATPase
233 (FGSG_08985), ATP-dependent oxoprolinase (FGSF_04902), and nitroalkane oxidase
234 (FGSF_02378) gene expression was repressed by approximately 65 – 75 % while a 5'-
235 nucleotidase (FGSF_00259) was induced over two fold (Supplemental Table 2). As these
236 enzymes are involved in nitrogen metabolism, either regulating inorganic nitrogenous
237 compounds or amino acids, it is plausible that GA application hinders *F. graminearum* nitrogen-
238 dependent bioenergetics and / or cellular redox (reviewed in Audenaert et al., 2013). In fact,
239 gibberellin biosynthesis and nitrogen metabolism have been reported as opposing metabolic
240 pathways in *Fusarium moniliforme* (Mihlan et al., 2003). The remaining *F. graminearum* genes
241 differentially expressed upon application of these hormones are poorly characterized, but are
242 putatively linked to amino acid, carbohydrate, and lipid metabolism based on DNA sequence
243 similarity with characterized genes.
244
245 Based on the observation that ABA and GA affect *F. graminearum* early-infection gene
246 expression, mycelium growth and sporulation were monitored to determine whether the ABA-
247 and GA-mediated modulation of FHB spread (Figure 1) is the direct effect of these compounds
248 on *F. graminearum* viability, as opposed to phytohormone-induced host-resistance.
249 Paclobutrazol (PBZ), a triazole fungicide that inhibits ergosterol biosynthesis in *Fusarium* fungal
250 species (Vanden Bossche et al., 1989), was used as a positive control for fungicidal activity. The

presence of 1.0 mM ABA, IAA, GA, or Z did not affect spore germination or mycelium production on rich media from isolated spores or fungal plugs (Figure 3A-B). Furthermore, these phytohormones also did not affect *F. graminearum* growth in rich liquid media (Figure 3C) or production of spores in the presence of cellulose (Figure 3D). However, ABA, GA, and Z did alter the fungal morphology of *F. graminearum* in liquid media where the formation of condensed fungal masses, rather than diffuse mycelia, was observed (Figure 3E).

The opposing effects of ABA and GA on FHB spread are not related to modification of the classical SA or JA defense phytohormone pathways in 'Fielder' spikes

Due to extensive hormonal cross-talk among phytohormone signaling pathways during fungal disease responses (Vleesschauwer et al., 2014), the effects of hormone application on FHB pathogenesis may elicit synergistic or antagonistic interactions with additional classical defense responsive phytohormones. To test the effects on JA and SA phytohormone content, 'Fielder' spikes were challenged with *F. graminearum* spores in the presence and absence of 1.0 mM ABA or GA by point inoculation at two central spikelets. The phytohormone content from the treated individual 'Fielder' spikes was determined seven days after Fusarium-challenge, when the differential effects of ABA and GA on disease response are most obvious (Figure 1; Supplemental Figure 2). The phytohormone profile of *Fusarium*-challenged spikes revealed an increase in JA accumulation as compared to unchallenged spikes, consistent with a previous report (Buhrow et al., 2016), while the co-application of ABA or GA with *F. graminearum* did not alter JA or SA phytohormone content, compared to pathogen-challenge alone (Table 3).

Phytohormone applications may serve as a stand-alone or integrative approach to mitigating FHB in susceptible wheat cultivars

Based on the observation that ABA and GA differentially modulate FHB spread in 'Fielder', one strategy to address the agronomic and economic costs of *F. graminearum* wheat infection is the development of potent, stable phytohormone analogs. However, the utility of phytohormone and phytohormone analog applications is dependent on the effect of these molecules on grain yield and quality. To investigate the effects of hormone treatments on grain production and viability independently of FHB infection, 1.0 mM IAA, ABA, GA, Z, or solvent control were applied to 'Fielder' spikes at each spikelet during anthesis. There were no visible phenotypic differences in mature grain harvested from phytohormone treated spikes compared to those from control spikes (Figure 4A). Although ABA, IAA, and GA led to a slight, but significant reduction, while Z led to a slight, but significant increase in yield (Figure 4B). Seed germination rates were increased by both GA and Z spike treatments and decreased by ABA spike treatment as compared to solvent-treated controls (Figure 4C). These results suggest that single applications of ABA, GA, or related chemical analogs during anthesis have only minor effects on development of wheat grains. Thus, their application may be a viable approach for the modulation of FHB severity which has a much more significant impact on grain yield and quality.

An ABA antagonist, 3'-hexasulfanyl-(+)-ABA (AS6), has recently been synthesized and characterized (Takeuchi et al., 2014). AS6 competitively binds to *Arabidopsis thaliana* ABA receptors and, through its hexysulfanyl group, sterically hinders the formation of ABA receptor: protein phosphatase 2C complexes and subsequent signaling (Takeuchi et al., 2014). To test

297 whether co-application of AS6 is capable of reducing FHB phenotypic spread, 'Fielder' spikes
298 were challenged with *F. graminearum* supplemented with 1.0 mM AS6. AS6 did not
299 significantly alter FHB spread, DON contamination, or yield (Supplemental Figure 4). The lack
300 of AS6 efficacy on FHB spread may be a result of reduced chemical stability, bioavailability,
301 metabolic half-life, or wheat receptor affinity compared to ABA.
302
303 An alternative strategy to address the costs of *F. graminearum* infection is the combined
304 application of novel phytohormone modulators and existing *Fusarium* fungicides such that
305 multiple metabolic pathways are targeted. GA may inhibit FHB spread by disrupting *Fusarium*
306 nitrogen acquisition and metabolism; while PBZ has been shown to target ergosterol-rich fungal
307 cell membranes (Vanden Bossche et al., 1989). To investigate whether GA and PBZ can be
308 combined to more effectively reduce *Fusarium* pathogenesis, 'Fielder' spikes were *F.*
309 *graminearum*-challenged at a central spikelet where the inoculum was supplemented with 1.0
310 mM GA, 10 µM PBZ, or the combination of GA and PBZ. To enable this experiment, it was
311 necessary to dissolve PBZ, and thus GA as well, in 10 % (v/v) methanol rather than the initial
312 selected deionized water solvent. The application of alternative solvent systems may affect the
313 rate of FHB spread in the absence of fungicide or phytohormone co-application; however any
314 apparent differences in spread using water (Figure 1; Supplemental Figure 2) or 10% (v/v)
315 methanol solvents (Figure 5) were not statistically significant in this work. Co-application of 1.0
316 mM GA or 10 µM PBZ alone moderately reduced FHB spread in spikelets beginning seven days
317 post inoculation; while the co-application of 1.0 mM GA and 10 µM PBZ further reduced FHB
318 phenotypic symptoms in *F. graminearum*-challenged 'Fielder' spikes (Figure 5). The additive

effects of GA and PBZ are recapitulated by reduced DON contamination in 'Fielder' spikes and grain and increased yield (Figure 5B-C).

DISCUSSION

FHB is a costly disease affecting wheat and other small grain cereal development and commercial viability. Consistent with previous observations, phytohormone profiling in this work describes the induction of SA and JA metabolism, but additionally implicates ABA biosynthesis and metabolism upon *F. graminearum*-challenge in both FHB-susceptible and –resistant cultivars. Unique to the FHB-resistant 'Sumai 3', cytokinin and gibberellin metabolism is promoted in unchallenged spike tissue suggesting phytohormones not traditionally reported to be involved in biotic stress may provide underlying physiological characteristics related to inherent resistance. This possibility may be further explored by comparing hormone profiles of multiple wheat varieties with varying degrees of FHB susceptibility.

In addition to the classical defense response phytohormones, the application of ABA and GA were also shown to modulate pathogenesis in wheat with GA promoting resistance and ABA promoting susceptibility. During the preparation of this work, Qi et al. (2015) reported that multiple applications of millimolar concentrations of racemic ABA solutions, rather than the single application of (+)-ABA in this work, with and after *F. graminearum*-challenge, are capable of increasing FHB symptoms in the susceptible cultivar 'Roblin'. In both reports, these effects were observed strongest in the early stages of FHB spread from challenged tissue to an adjacent asymptomatic spikelet. This specific response suggests that the ABA-elicited effects are

342 limited to early infection spread or have limited duration or localization. Together these findings
343 strengthen the idea that ABA plays a role in promoting FHB and further suggest that an ABA
344 antagonist, similar to the recently reported AS6, may be exogenously applied to reduce FHB
345 spread in diverse susceptible wheat lines.
346
347 The mechanism of ABA and GA modulation of FHB disease progression was evaluated in terms
348 of a direct effect on the *F. graminearum* fungus and also with respect to previously reported *F.*
349 *graminearum*-elicited wheat phytohormone responses. The co-application of ABA promotes *F.*
350 *graminearum* early-stage gene expression, potentially promoting wheat pathogenesis by cell wall
351 degradation and fungal insertion into challenged spikes. Additionally, the co-application of GA
352 represses early-stage *F. graminearum* nitrogen metabolic gene expression, potentially reducing
353 bioenergetic resources and redox regulation required for the newly infecting fungal cells.
354 Although ABA and GA elicit *Fusarium* gene expression changes that would be expected to
355 promote and repress FHB pathogenesis, respectively, these phytohormones may also elicit later
356 stage regulation of fungal expression events and / or act on the wheat host. The application of
357 ABA and GA did not have a significant effect on *F. graminearum* growth or sporulation in
358 axenic conditions. Reports of ABA on *F. graminearum* axenic growth are mixed, where in rich
359 media no growth defects are observed (this work; Petti et al., 2012), while high micromolar
360 concentrations of ABA inhibited mycelia growth but not spore germination in minimal media
361 (Qi et al., 2015). Finally, ABA and GA application did not alter trichothecene gene cluster
362 expression, DON accumulation, or SA or JA biosynthesis in *F. graminearum* challenged spikes.
363

364 Although the application of (+)-ABA and GA (predominantly GA3) affect FHB spread in
365 'Fielder' spikes, the identities of the bioactive molecules responsible for eliciting these effects
366 remain unknown. ABA is a sesquiterpene phytohormone, synthesized in plants by the
367 carotenoid-dependent, plastidal non-mevalonate biosynthetic pathway (Nambara & Marion-Poll
368 2005). Additionally, a number of plant symbiotic and pathogenic fungal species have also been
369 shown to biosynthesize ABA (Siewers et al., 2006; Crocoll et al., 1991; Dorffling et al., 1984)
370 with an *F. graminearum* strain reported to biosynthesize low nanogram concentrations of ABA
371 in axenic culture (Qi et al., 2015). Alternatively, GA is a diterpenoid phytohormone that is
372 biosynthesized through the methylerythritol phosphate pathway where more than 20 gibberellins
373 have been identified (Yamaguchi, S., 2008). A number of fungal and bacterial species are also
374 capable of synthesizing gibberellins (MacMillan, J., 2002) including mating populations of
375 *Fusarium fujikuroi* (Yabuta, Y., 1935). Furthermore, GA has been reported to be moderately
376 unstable in aqueous solution (Pryce 1973a; Pryce 1973b); therefore, the use of alternative solvent
377 systems, such as the 10 % methanol applied in this work to investigate the combined contribution
378 of GA and PBZ, may alter the chemical stability and metabolism of exogenously applied GA.
379 Together these reports highlight the possibility that the bioactive molecules responsible for
380 eliciting changes on FHB spread may be wheat- or *F. graminearum*-derived phytohormone
381 metabolites or their chemical degradative products. Toward elucidating the metabolic fate and
382 duration of exogenous and endogenous phytohormones, the hormone profiles arising from co-
383 application of labelled phytohormones to *F. graminearum*-challenged 'Fielder' spikes are being
384 evaluated.
385

In summary, the phytohormone profiling documented in this work supports the biosynthesis of SA, JA, and ABA in response to *F. graminearum*-challenge, and that this effect is independent of the degree of FHB-susceptibility of the wheat cultivar. ABA and GA were shown to modulate FHB spread in an opposing manner when co-applied to susceptible 'Fielder' spikes where ABA was shown to promote *F. graminearum* gene expression linked to early wheat infection and GA was shown to repress fungal nitrogen metabolism; however a full understanding of the associated mechanisms remains to be determined. Finally, based on the combined effect of GA and PBZ on limiting FHB spread, this work supports an agronomic strategy of combining phytohormone-related treatments with existing fungicides and biocontrol agents for the management of FHB in wheat. Interestingly, PBZ itself may reduce FHB disease progress by not only acting as a fungicide but by modulating wheat phytohormone biosynthesis and signaling as this molecule has been reported to inhibit GA (Brock et al., 2011) and ABA (Norman et al., 1986) biosynthesis in fungal plant pathogens and SA biosynthesis *in planta* (Leon et al., 1995). Overall, this study implicates the role of diverse phytohormones in regulating FHB pathogenesis in wheat potentially by eliciting wheat responses and acting on the *F. graminearum* transcriptome.

MATERIALS AND METHODS

Chemicals and Phytohormones

Indole 3-acetic acid, gibberellin A3, racemic zeatin, and paclobutrazol were purchased from Sigma-Aldrich (St. Louis, MO). The National Research Council Hormone Profiling Facility provided (+)-ABA while 3'-hexasulfanyl-(+)-ABA was synthesized as described (Takeuchi et al., 2014) and provided by Kenneth Nelson and Suzanne Abrams at the University of

409 Saskatchewan. ABA and its chemical analogs were assessed by NMR prior to acquisition.
410 Phytohormone stocks were solubilized in deionized water as sodium salts by 1.0 N NaOH
411 titration and stored at -20 °C in amber vials. Working solutions were made in deionized water
412 and pH was adjusted, as needed, to 7.0 with 0.1 N HCl. PBZ, and GA for direct comparison,
413 stocks were solubilized in 50% (v/v) methanol and working solutions were made by dilution in
414 deionized water with the final solution having no more than 10% (v/v) methanol. Chemdraw Pro
415 v11 (CambridgeSoft, Waltham, MA) was used to depict chemical structures.
416
417 **_F. graminearum_ inoculum preparation**
418 *F. graminearum* GZ3639 (Proctor et al., 1995) was propagated on potato dextrose agar (PDA;
419 Sigma-Aldrich) at 25 ° C for five days. To obtain spores, carboxymethylcellulose liquid media
420 (CMC; Sigma-Aldrich) was inoculated with a marginal 5 mm square PDA plug and grown for
421 five days at 27 ° C, shaking at 180 rpm. Spores were isolated by filtering through one layer of
422 cheese cloth and 25 µm Miracloth filter (EMD Millipore; Billerica, MA), washed three times
423 with sterile water, and quantified using a haemocytometer and light microscopy.
424
425 **_F. graminearum_ growth and sporulation quantification**
426 The effects of ABA, IAA, GA, Z, PBZ, or the respective solvent (deionized water or 10 % (v/v)
427 methanol) were evaluated for their ability to alter *Fusarium* growth and sporulation. PDA plates
428 were supplemented with 1.0 mM ABA, IAA, GA, or Z or 100 µM PBZ or solvent controls and
429 inoculated with marginal 5 mm square PDA plugs or 500 isolated *F. graminearum* spores. Radial
430 growth was monitored in triplicate as the average of three diameter measurements, at
431 approximately 45 ° angles, over the course of seven consecutive days. Potato dextrose broth

(PDB; Sigma-Aldrich) 100 mL cultures supplemented with 1.0 mM ABA, IAA, GA, or Z or 100 µM PBZ were inoculated with 500 isolated spores and shaken at 180 rpm and 27 ° C for seven days. Fungal mass was measured in triplicate after vacuum filtration through 11 µm filter paper and lyophilization. CMC 50 mL cultures supplemented with 1.0 mM ABA, IAA, GA, or Z or 100 µM PBZ were inoculated with marginal 5 mm square PDA plugs and shaken at 180 rpm and 27 ° C for five days. *Fusarium* sporulation was quantified in triplicate as the number of spores isolated after successive cheesecloth and 25 µm Miracloth filtering per mL culture.

Propagation of plants and grain preparation

T. aestivum 'Fielder' and 'Sumai 3' were grown in Sunshine^{R} Mix 8 (Sungrow Horniculture, Agawam, MA) and maintained in climate controlled chambers with a 16 photoperiod, at 25 ° C followed by 8 h of dark at 16 ° C every day. Plants were watered as needed and fertilized biweekly with 20-20-20 (N-P-K). At the two-leaf stage plants were treated Intercept™ (Bayer Crop Science, Calgary, AB) as a powdery mildew and aphid preventative. For preparation of grain, wheat was allowed to mature naturally under normal growing conditions and grain was manually isolated.

Phytohormone-only application to 'Fielder' spikes for phenotypic assessment

'Fielder' spikes were treated at each spikelet with 10 µL of 1.0 mM ABA, IAA, GA, Z, or deionized water (mock) during anthesis. Wheat was incubated in climate controlled conditions as described above until spikes naturally matured. Grain from each treatment was manually isolated where spikes were dried and grain harvested from each spikelet using a forceps, including the

small *Fusarium* damaged kernels. The resulting samples were characterized for yield and germination as described below.

Fusarium-challenge of 'Fielder' and 'Sumai 3' spikes

During anthesis, two florets from a central spikelet were point inoculated with 10 µL of 5.0 x 10^4 *F. graminearum* GZ3639 spore suspension or deionized water (mock). To promote infection, wheat plants were transferred to climate controlled chambers with 90 % humidity for 72 hours, with the same light and temperature conditions as described above, and then returned to ambient humidity. Challenged spikes were monitored for phenotypic evidence of FHB in each spikelet of all inoculated spikes for 14 days. Spike and grain tissue were harvested from *F. graminearum*-challenged and unchallenged treatment groups, where individual spikes were independently processed while grain samples were prepared by allowing spikes to dry and then pooling harvested grain within a treatment group into 1.0 g biological replicate samples. Tissues were analyzed for phytohormone content, DON contamination, and yield.

Hormone profiling of _Fusarium_-challenged 'Fielder' and 'Sumai 3' spikes

'Fielder' and 'Sumai 3' spikes were *F. graminearum*-challenged as described above. Fourteen days post challenge, five biological replicates comprised of one individual spike each of mock-innoculated 'Fielder,' *Fusarium*-inoculated 'Fielder,' mock-inoculated 'Sumai 3,' and *Fusarium*-inoculated 'Sumai 3' were flash frozen and ground in liquid nitrogen. Phytohormones were extracted from individual replicate spikes and quantified by UPLC/ESI-MS/MS at the NRC Plant Biotechnology Institute as described (Abrams et al., 2003; Lulsdorf et al., 2013; Galka et al., 2005; Ross et al., 2004; Zaharia et al., 2005).

477

478 **DON quantification in *Fusarium*-infected 'Fielder' spikes and grain**

479 'Fielder' spikes were *F. graminearum*-challenged as described under '*Fusarium*-challenge of
480 'Fielder' and 'Sumai 3' spikes', and spikes and grain were sampled. In particular ten spikes, with
481 each spike representing one biological replicate, were analyzed individually at the indicated
482 times post-challenge. For grain sampling, remaining plants were allowed to mature and all grain
483 from each treatment group was isolated using forceps and pooled. This pooled grain was mixed
484 and three 1.0 g replicates were made from the pooled, mixed total sample. In both instances,
485 each individual sample was ground under liquid nitrogen and solvent-extracted in five volumes
486 of 84 % (v/v) acetonitrile by shaking at 220 rpm, 25° C for two hours. Extracted DON was
487 quantified relative to a commercial standard (Sigma-Aldrich; St. Louis, MO) by LC-MS
488 modified from Plattner et al. (2003). Briefly, DON was separated from co-extracted molecules
489 with a Waters 2695 LC coupled with a Waters Symmetry C18 column (100 x 2.1 mm ID, 3.5
490 µm) at 25° C. The mobile phase was composed of (A) 0.3% (v/v) acetic acid and (B) 95% (v/v)
491 methanol: 0.3% (v/v) acetic acid under gradient elusions at 0-7 min: 99% A, 7-25 min: 67% A
492 33% B, 25-30 min 99% A. Mass spectrometry analysis was performed using a Waters 3100
493 Mass Detector fitted with ESI in negative ion mode and an optimized 40 V cone voltage. DON
494 was detected at *m/z* of 355.3 Da and analyzed using Empower Pro Software (Waters, Milford,
495 MA).

496

497 **Grain yield and germination determination**

498 Fielder' spikes were phytohormone-treated or *F. graminearum*-challenged as described above.
499 Thousand Grain Weight (TGW) was selected as the yield metric and calculated, in triplicate, as

five times the mass of 200 grains randomly selected from the pooled grain per treatment group as described previously (Pask et al., 2012). For germination studies, triplicate groups of twenty seeds from each treatment group were washed with a 5% (v/v) hypochlorite solution, imbibed in deionized water, and incubated in the dark at 25° C for ten days. Germination was evaluated by the visual presence of a coleorhiza.

Co-application of phytohormones with *Fusarium*-challenge to 'Fielder' spikes

'Fielder' spikes were *Fusarium*–challenged as described above in the section entitle '*Fusarium*-challenge of 'Fielder' and 'Sumai 3' spikes', but the inoculum was supplemented with hormones or PBZ. For co-inoculation with hormones, compounds were solubilized in deionized water and a total of six treatments were assessed: 1.0 mM ABA, IAA, GA, Z, AS6 or deionized water (mock). Each co-inoculum was applied to a minimum of 30 spikes during anthesis, where two florets from a central spikelet were inoculated, and repeated in triplicate. For co-inoculation with PBZ, compounds were solubilized in 10 % (v/v) methanol and a total of four treatments were assessed: 1.0 mM GA, 10 µM PBZ, 10 µM PBZ / 1.0 mM GA or 10% (v/v) methanol (mock). For PBZ co-inoculations, each treatment was applied to a minimum of fifteen spikes and repeated in triplicate. In both co-application experiments, inoculated spikes were rated for the number of diseased spikelets over a fourteen-day period. Seven days post inoculation, five spikes (biological replicates) from the ABA, GA, and mock treatment groups were analyzed for phytohormone content as described above. Fourteen days after inoculation, ten spikes (biological replicates) from each treatment were analyzed for DON contamination with the exception of the PBZ co-application experiments where only five spikes were analyzed for DON content. The

522 remaining wheat was allowed to mature and grain from each treatment characterized for yield
523 and DON contamination as described above.
524
525 **RNA sequencing and *F. graminearum* expression analysis**
526 Total RNA was extracted from five 'Fielder' spikelets (biological replicates) each challenged
527 with *F. graminearum* in the presence and absence of 1.0 mM ABA or GA, at 24 hours and five
528 days post challenge. RNA was purified using the RNeasy Plant Mini Kit (Qiagen, Mississauga,
529 CA) and treated with DNaseI (Qiagen, Mississauga, CA) according to the manufacture's
530 instruction. RNA quantity and quality were evaluated using NanoDrop ND-8000 (NanoDrop,
531 Wilmington, DE) and agarose gel electrophoresis.
532
533 Total RNA isolated from 'Fielder' spikelets 24 hours after being challenged with *F.*
534 *graminearum* in the presence and absence of 1.0 mM ABA or GA were sequenced. RNA library
535 construction and HiSeq RNA sequencing were performed at the National Research Council
536 Canada DNA Sequencing Facility (Saskatoon, SK). RNA libraries were prepared using 1.0 μg
537 total RNA and the TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, CA) according to the
538 manufacturer's instructions. Library quality was assessed on the 2100 Bioanalyzer (Agilent
539 Technologies Inc., Santa Clara, CA) equipped with a DNA 1000 chip. Library concentrations
540 were determined by qPCR using the KAPA SYBR FAST ABI Prism qPCR Kit (Kapa
541 Biosystems, Wilmington, MA) and the StepOnePlus Real-Time PCR System (Applied
542 Biosystems, Foster City, CA). RNA samples were multiplexed at a sequencing depth of five
543 libraries per lane. Equimolar concentrations of the libraries were pooled and a final concentration
544 of 12 pM was used for clustering in cBOT (Illumina) flowcell lanes. The samples were then

545 sequenced (2 x 101 cycles, paired-end reads) on the HiSeq2500 (Illumina) using the TruSeq SBS
546 Kit v3-HS 200 cycles Kit (Illumina). Raw RNA sequence reads were processed with
547 Trimmomatic v0.32 (Bolger et al. 2014) to remove Truseq3 adaptor sequences, low quality bases
548 selected using five base averaging and a quality threshold of 20, and reads with fewer than 50
549 bases. The trimmed reads were mapped against the *F. graminearum* PH-1 (FG3) genome
550 (Fusarium Comparative Sequencing Project, Broad Institute of Harvard and MIT
551 (http://www.broadinstitute.org/)) using STAR v2.4.0j (Dobin et al., 2012). Per sample GTF files
552 were merged using cuffmerge from the Cufflinks v2.1.1 suite (Trapnell et al., 2010). Read counts
553 were calculated using HTSeq 0.6.0 (Anders et al 2014) htseq-count in unstranded mode.
554 Comparisons between individual treatments and control were evaluated using the DESeq2 v1.6.3
555 (Love et al 2014) Wald statistic. *F. graminearum* genes were annotated using information
556 extracted from two public sources: (1) the Protein Extraction, Description and Analysis Tool
557 version 3 (PEDANT 3) from the Munich Information Center for Protein Sequences (MIPS) and
558 (2) the MycoCosm tool from the Joint Genome Institute (Cuomo et al., 2007), which hosts an
559 annotated copy of the genome of *F. graminearum* strain PH-1 (NRRL 31084) sequenced by the
560 BROAD Institute. The RNASeq data generated by this work is available at the NCBI SRA under
561 BioSample accession numbers: SAMN04386757, SAMN04386758, SAMN04386759 for *F.*
562 *graminearum*, *F. graminearum* + GA and *F. graminearum* + ABA treatments, respectively (to be
563 released to the public May 1, 2016).
564
565 **Relative *F. graminearum* gene expression determined by semi-quantitative RT-PCR**
566 Total RNA isolated from 'Fielder' spikelets five days after being challenged with *F.*
567 *graminearum* in the presence and absence of 1.0 mM ABA and GA were selected for relative

gene expression analysis. cDNA was synthesized from 0.5 µg RNA using the Superscript III reverse transcriptase kit (Invitrogen, Carlsbad, CA). *F. graminearum actin* (GenBank XM_011328784.1) and trichothecene biosynthetic cluster genes (Kulik et al., 2012) *tri4* (GenBank: AAK33083.1), *tri5* (GenBank: AAK33084.1), and *tri11* (GenBank: AAK33080.1) were compared to the wheat heterogeneous nuclear ribonucleoprotein Q (*hn-PNP-Q*, Ta.10105; Qi et al., 2012) reference gene using comparative C_T ($\Delta\Delta C_T$ method; Livak and Schmittgen, 2001). StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA) and StepOne Software v2.3 (Thermo Fisher Scientific Inc., Carlsbad, CA) were used to quantify and analyze relative expression. All primers in this work were designed using Primer3 v0.4.0 (Untergasser et al., 2012) and are listed in Supplemental Table 3.

Statistical analysis

Phytohormone content differences in 'Fielder' and 'Sumai 3' with and without *Fusarium*–challenging were analyzed with two-way ANOVA with Sidak post-hoc comparisons. Unless otherwise noted, all other data (*Fusarium* growth and sporulation, grain germination, spikelet infection, DON contamination, yield, and relative gene expression) was analyzed with one-way ANOVA with Dunnett post-hoc comparisons. Comparisons were performed with GraphPad Prism 6 (GraphPad Software, Inc. La Jolla, CA).

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800 **TABLE LEGENDS**

801 **Table 1: Phytohormone profiles differ between FHB susceptible 'Fielder' and resistant**
802 **'Sumai 3' with and without *F. graminearum*–challenging.** Values represent the average
803 phytohormone content of five biological wheat spike replicates with standard deviation at 14
804 days post *Fusarium*-challenging or mock inoculation. Phytohormones that were not detected in
805 three or more samples are noted N.D. Phytohormone differences between wheat cultivars († $p \leq$
806 0.05, p †† $\leq$ 0.01, ††† $p \leq$ 0.001, †††† $p \leq$ 0.0001) and changes induced upon *Fusarium*-
807 challenging inoculation (* $p \leq$ 0.05, ** $p \leq$ 0.01, *** $p \leq$ 0.001, **** $p \leq$ 0.0001) were
808 evaluated by two-way ANOVA with Sidak post-hoc comparisons.

	Mock		***Fusarium*-challenged**	
Phytohormones (ng/g)	**'Fielder'**	**'Sumai 3'**	**'Fielder'**	**'Sumai 3'**
ABA	150 ± 31	280 ± 97 †	260 ± 77	420 ± 73 *††
7'-OH ABA	8.4 ± 2.9	75 ± 37 ††††	14 ± 6.6	85 ± 5.5 ††
ABA glucose ester	18 ± 15	98 ± 71 ††	26 ± 23	67 ± 19
Dihydrophaseic acid	28 ± 10.	20. ± 3.5	71 ± 65	38 ± 12
Phaseic acids	130 ± 34	51 ± 23	210 ± 98 *	82 ± 19 ††
Total ABA Catabolites	180 ± 62	240 ± 130	460 ± 190 **	270 ± 56
cis-zeatin-riboside	150 ± 36	3.8 ± 1.1 ††††	112 ± 12 *	5.2 ± 0.80 ††††
cis-zeatin	2.4 ± 1.2	N.D.	3.8 ± 3.5	1.5 ± 0.30
trans-zeatin	N.D.	2.2 ± 0.73 ††††	N.D.	2.4 ± 0.76 ††††
cis-zeatin-*O*-glucoside	94 ± 18	180 ± 32 ††††	79 ± 17	130 ± 10. **††
trans- zeatin-*O*-glucoside	41 ± 19	110 ± 46 †††	46 ± 8.7	81 ± 11
IAA	4320 ± 1090	5160 ± 1670	4090 ± 1270	6280 ± 3190
IAA-Aspartate	129 ± 244	40 ± 18	2400 ± 3170	2860 ± 3210
GA19	27 ± 7	49 ± 16 †	30 ± 8	38 ± 19
GA24	14 ± 7	N.D. ††††	8 ± 2	N.D. †
GA44	12 ± 2	9 ± 3	12 ± 4	5 ± 2 ††
SA	122 ± 32	245 ± 61 ††	137 ± 56	195 ± 27
Conjugated SA	1120 ± 77	1200 ± 219	682 ± 48 ***	880 ± 102 **
JA	23 ± 3	67 ± 17 ††††	64 ± 12 ****	71 ± 10
JA-Isoleucine	11 ± 5	21 ± 7 ††	20 ± 4 *	18 ± 3

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Table 2: Genes involved in early *F. graminearum* infection are induced by ABA co-application. Three previous studies characterized *F. graminearum* genes expressed in early infection. The co-application of ABA results in a significant expression increase in at least nine of these genes. [1]Stephens et al., 2008; [2]Menke 2011; [3]Lysoe et al., 2011

F. graminearum Gene ID: MIPS annotation	Crown Rot and FHB[1]	*In vivo* infection[2]	Global FHB[3]	ABA induced
FGSG_03632: related to cellulose binding CEL1	√	√	√	√
FGSG_07642: related to monooxigenase		√	√	√
FGSG_10989: conserved hypothetical protein			√	√
FGSG_10991: related to benzoate 4-monooxygenase cytochrome P450		√	√	√
FGSG_10992: related to polysaccharide deacetylase		√	√	√
FGSG_10993: related to selenocysteine lyase		√		√
FGSG_11366: conserved hypothetical protein		√		√
FGSG_11397: related to desaturase			√	√
FGSG_11399: related to oxidoreductase		√	√	√

Table 3: ABA and GA co-application do not affect SA or JA hormone profiles in *Fusarium*-challenged 'Fielder' spikes. Values represent the average phytohormone content of five biological 'Fielder' spike replicates with standard deviation, at seven days post *Fusarium*-challenge or after mock inoculation. Phytohormone differences between ABA or GA co-application and the mock control were evaluated by one-way ANOVA with Dunnett post-hoc comparisons. A significant increase in JA content (* $p < 0.05$) was observed upon *F. graminearum* challenging compared to mock (deionized water) treatment.

		***Fusarium*-challenged**		
Phytohormones (ng/g)	**Mock**	**Mock**	**ABA treated**	**GA treated**
SA	56 ± 11	71 ± 25	66 ± 11	53 ± 11
Conjugated SA	667 ± 87	809 ± 333	528 ± 127	478 ± 102
JA	21 ± 3	39 ± 12 *	65 ± 34	55 ± 10
JA-Ile	21 ± 5	23 ± 7	21 ± 8	21 ± 2

FIGURE LEGENDS

Figure 1: Co-application of ABA and GA elicit opposing effects on FHB spread and infection in 'Fielder'. Two florets on a central spikelet were *F. graminearum*-challenged where the inoculum was supplemented with 1.0 mM ABA, IAA, GA, or Z and compared to non-supplemented (Mock) (A) Spikelet infection. Following inoculation each of the three sequentially adjacent spikelets from the site of inoculation were evaluated for FHB symptoms on a daily basis where the *Fusarium*–challenged spikelet is denoted spikelet position 1. The infection rate at each spikelet position was tabulated individually, with values representing the average of three independent experiments, each composed of a minimum of 30 spikes, with standard error (left column). These rates were also normalized to that of the non-supplemented mock (right column). (B) DON accumulation. Ten biological replicate spikes harvested at 14 dpi or three 1.0 g biological replicate grain samples were analyzed by LC-MS for the presence of DON. Values represent the average DON detected in each biological replicate with standard deviation. Only the co-application of GA decreased DON contamination compared to the mock-treated control. (C) Yield analysis. Thousand Grain Weight for 'Fielder' spikes challenged with *F. graminearum* and each phytohormone was calculated using three biological replicate grain samples with standard deviation. Only the co-application of GA increased yield compared to mock-treated samples. Changes upon phytohormone application were evaluated by one-way ANOVA with Dunnett post-hoc comparison (* $p \leq 0.05$).

Figure 2: *F. graminearum* detection, but not DON contamination, is reduced in 'Fielder' upon GA co-application. Five days after inoculation, (A) *F. graminearum* gene expression

relative to the endogenous wheat *hn-RNP-Q* was detected in spikelets and (B) DON contamination was detected in wheat spikes inoculated in all spikelets. Values represent the average of three independent experiments, each composed of three samples, with standard error. Changes between hormone-treated and the mock control were evaluated by one-way ANOVA with Dunnett post-hoc comparisons (* $p \leq 0.05$).

Figure 3: *F. graminearum* axenic growth and sporulation are unaffected by phytohormones. Phytohormone supplementation did not affect *F. graminearum* (A) spore germination and mycelia production on rich media, (B) mycelia growth on rich media, (C) mycelia growth in rich liquid culture, nor (D) sporulation. PBZ was applied as a positive fungicidal control throughout. Values represent the average of three independent experiments, each composed of three samples, with standard error. Changes between hormone-treated and the solvent control were evaluated by one-way ANOVA with Dunnett post-hoc comparisons (* $p \leq 0.05$ **** $p \leq 0.0001$). (E) *F. graminearum* morphology in rich liquid culture upon no hormone, ABA, IAA, GA, or Z (left to right) supplementation.

Figure 4: Phytohormone application to 'Fielder' florets affects yield and germination. (A) Grain phenotypes resulting after mock, ABA, IAA, GA, or Z (left to right) applications to florets during anthesis. (B-C) Values represent the average of three biological replicate grain samples, sufficient for Thousand Grain Weight calculation or 20 germinated grains, from a representative experiment with standard deviation. Changes between hormone-treated and the mock control samples were evaluated by one-way ANOVA with Dunnett post-hoc comparisons (*$p \leq 0.05$ ** $p \leq 0.001$).

892

893 **Figure 5: Co-application of PBZ and GA reduce *F. graminearum* infection severity in**
894 **'Fielder'.** Two florets on a central spikelet were *F. graminearum*-challenged where the
895 inoculum was supplemented with and without 1.0 mM GA, 10 μM PBZ, or the combination of
896 GA and PBZ. (A) Spikelet infection. Following inoculation each of the six sequentially adjacent
897 spikelets from the site of inoculation were evaluated for FHB symptoms on a daily basis where
898 the *Fusarium*–challenged spikelet is denoted spikelet position 1. The infection rate at each
899 spikelet position was tabulated individually, with values representing the average of three
900 independent experiments, each composed of a minimum of 15 spikes, with standard error. (B)
901 DON contamination. Five biological replicate spikes harvested at 14 dpi or three 1.0 g biological
902 replicate grain samples were analyzed by LC-MS for the presence of DON. Values represent the
903 average DON detected in each biological replicate with standard deviation. (C) Yield analysis.
904 Thousand Grain Weight for each inoculation condition was calculated using three biological
905 replicate grain samples with standard deviation. Changes between hormone-treated and the mock
906 control were evaluated by one-way ANOVA with Dunnett post-hoc comparisons (* $p \leq 0.05$).
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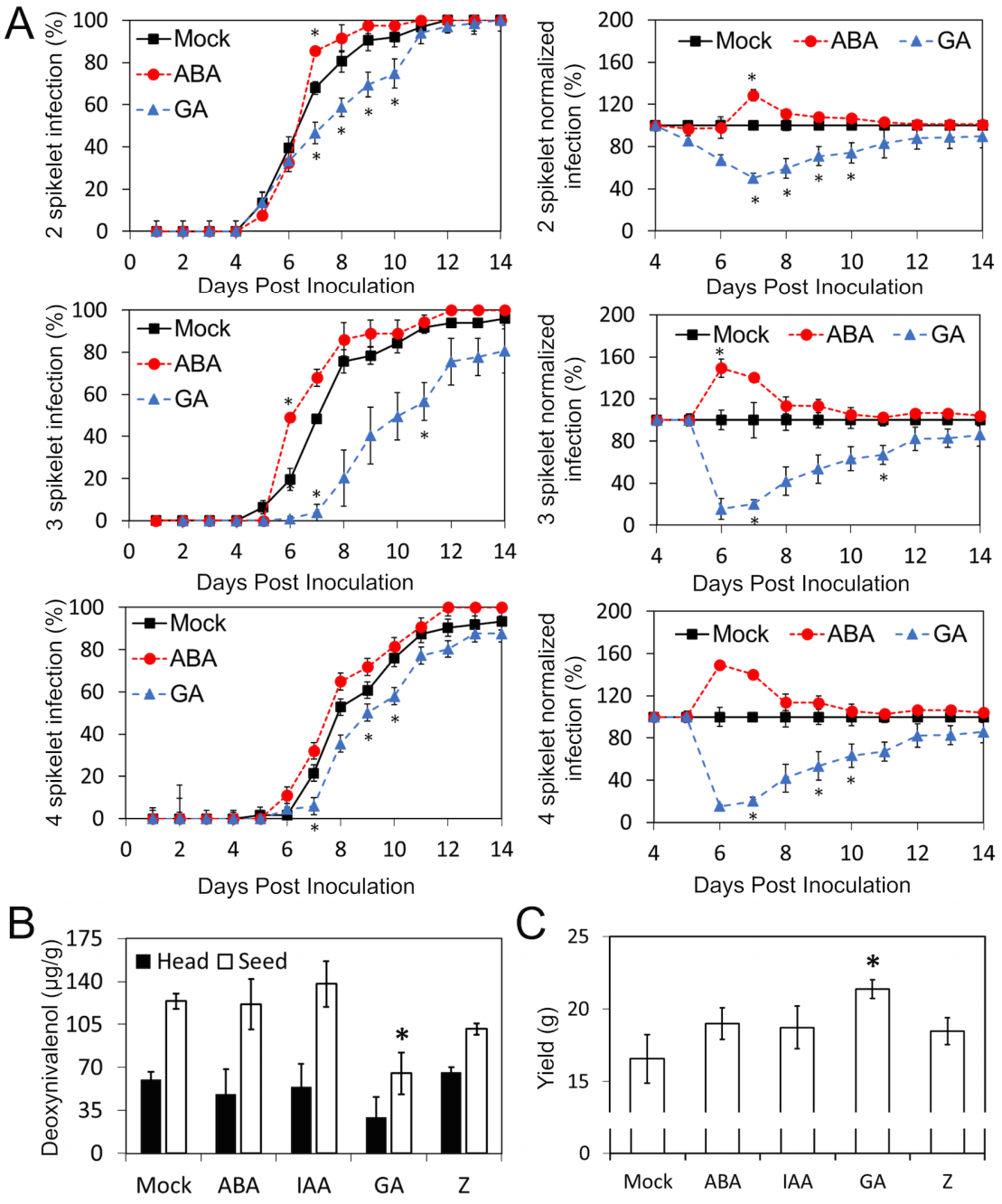
908 Figure 1



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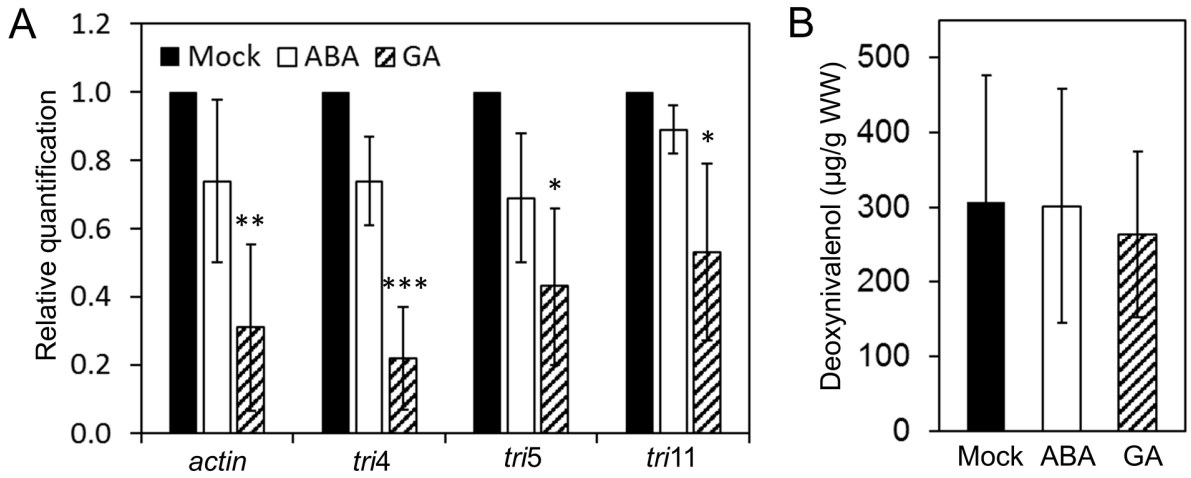
913 Figure 2



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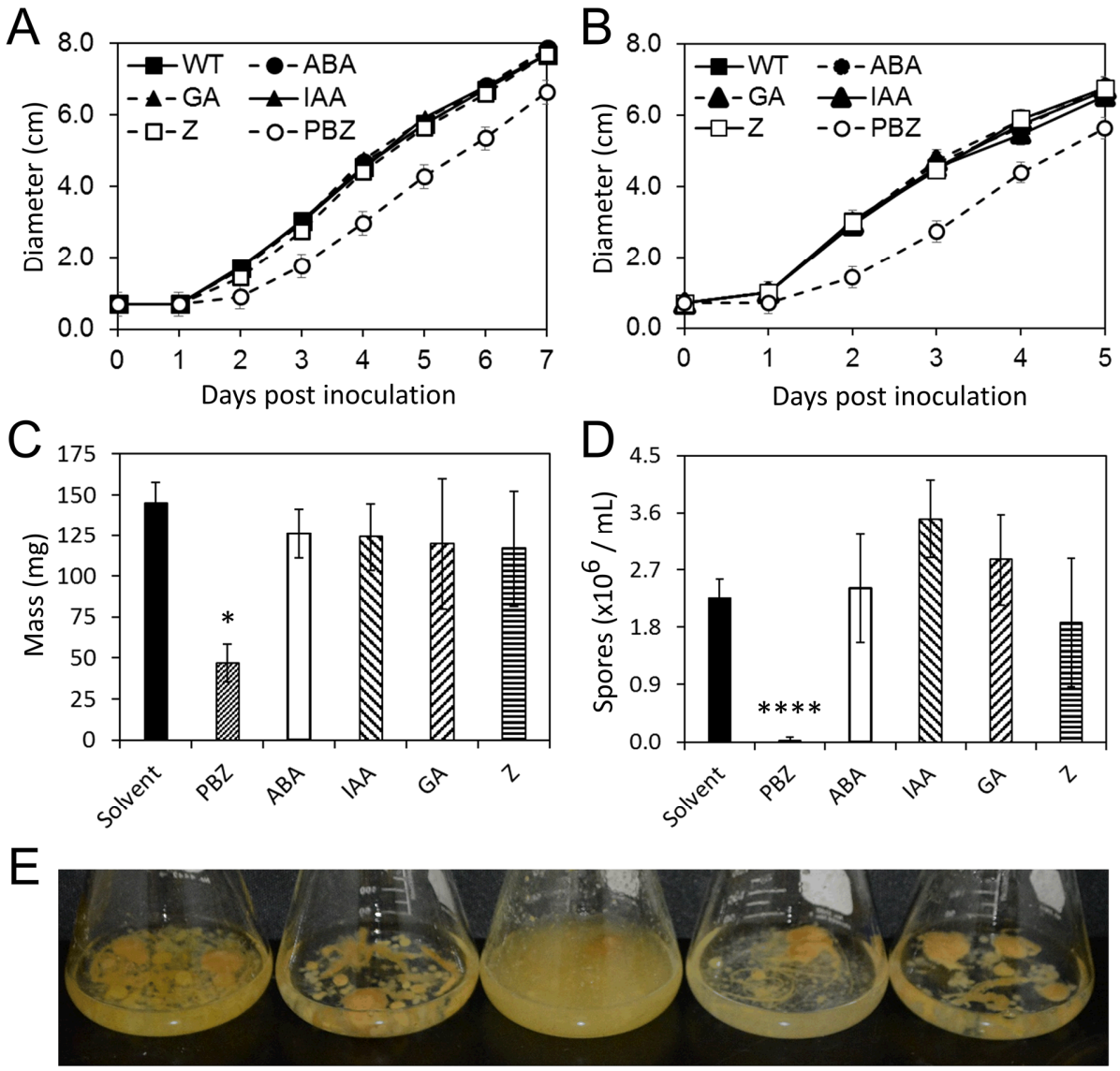

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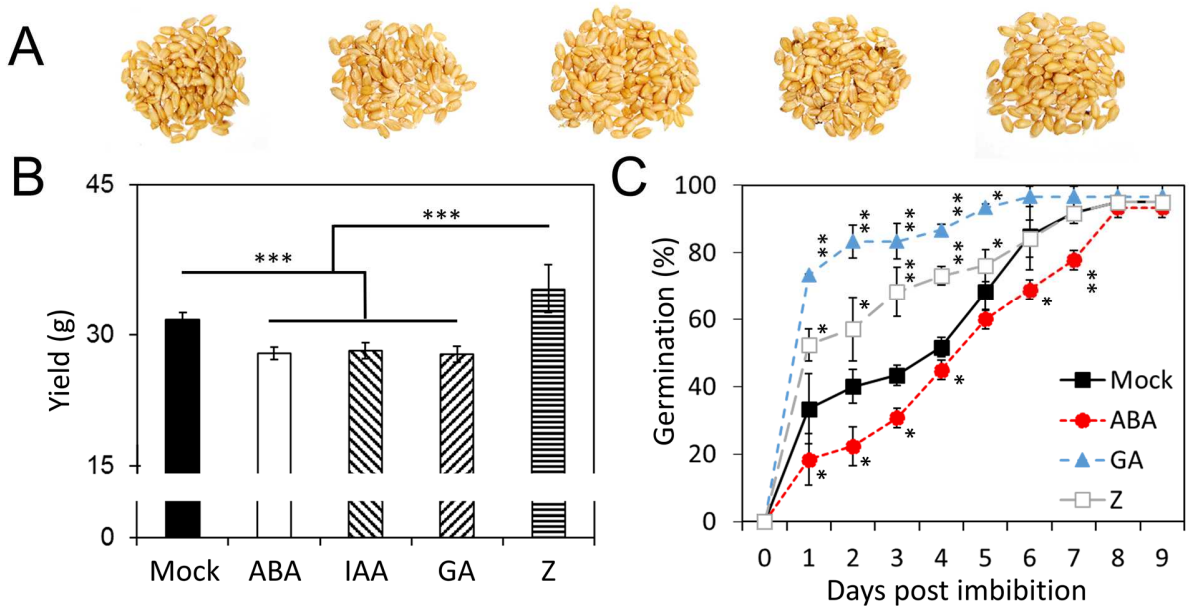

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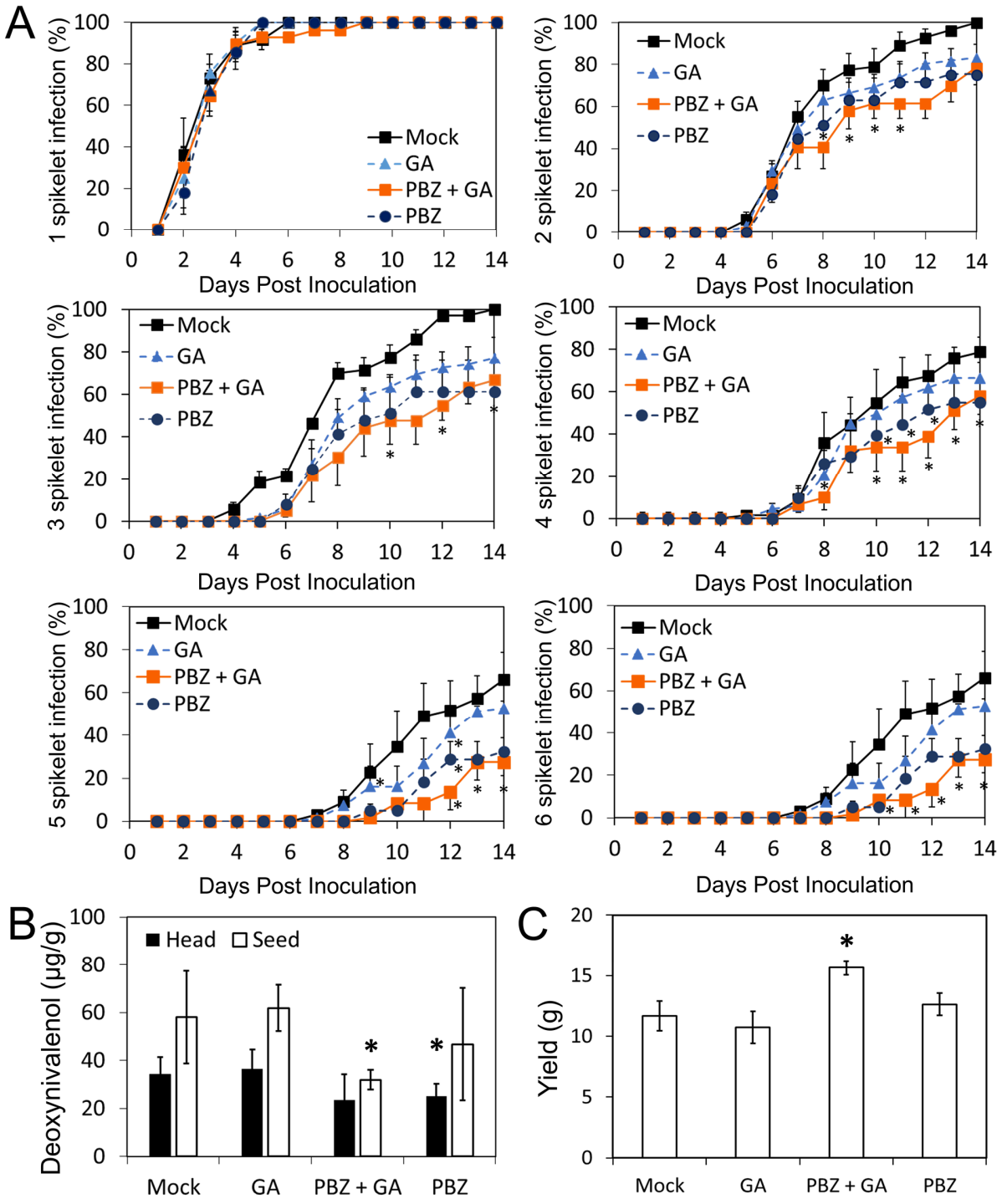
922 Figure 5



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