Chemical Activation of the Ethylene Signaling Pathway Promotes *Fusarium graminearum* Resistance in Detached Wheat Heads

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ABSTRACT

Plant signaling hormones such as ethylene have been shown to affect the host response to various pathogens. Often, the resistance responses to necrotrophic fungi are mediated through synergistic interactions of ethylene (ET) with the jasmonate signaling pathway. On the other hand, ET is also an inducer of senescence and cell death, which could be beneficial for some invading necrotrophic pathogens. *Fusarium graminearum*, a causative agent in Fusarium head blight of wheat, is a hemibiotrophic pathogen, meaning it has both biotrophic and necrotrophic phases during the course of infection. However, the role of ET signaling in the host response to *Fusarium* spp. is unclear; some studies indicate that ET mediates resistance, while others have shown that it is associated with susceptibility. These discrepancies could be related to various aspects of different experimental designs, and suggest that the

role of ET signaling in the host response to FHB is potentially dependent on interactions with some undetermined factors. To investigate whether wheat genotype can influence the ET-mediated response to FHB, the effect of chemical treatments affecting the ET pathway was studied in six wheat genotypes in detached-head assays. ET-inhibitor treatments broke down resistance to both initial infection and disease spread in three resistant wheat genotypes, whereas ET-enhancer treatments resulted in reduced susceptibility in three susceptible genotypes. The results presented here show that the ET signaling can mediate FHB resistance to *F. graminearum* in different wheat backgrounds.

Keywords: disease resistance, ethylene, Fusarium head blight, plant defense, plant hormone, wheat.

Fusarium graminearum and related species are fungal pathogens responsible for Fusarium head blight (FHB) disease of important cereal crops, including wheat. The disease has caused severe crop damage and yield loss worldwide (Del Ponte et al. 2012; McMullen et al. 2012; Vančo et al. 2007). The fungus produces a series of toxins (Chelkowski 2014), including trichothecenes, which are potent inhibitors of protein synthesis in eukaryotic cells (McLaughlin et al. 1977). Trichothecenes accumulate in the kernels of FHB-infected wheat (Foroud and Eudes 2009). Because consumption of trichothecenes is harmful to humans and animals (Pestka 2010; Rocha et al. 2005), it is necessary to devise strategies to eliminate their contamination of food. Cultivation of FHB-resistant cereals is integral to the most effective disease management strategies for reducing toxin contamination of wheat (Foroud et al. 2014). Different forms of resistance have been reported (Boutigny et al. 2008; Mesterházy 1995), including resistance to initial infection (type 1) and resistance to disease spread within the wheat spike (type 2), first described by Schroeder and Christensen (1963).

FHB resistance can be mediated, in part, through different hormone signaling pathways. Ethylene (ET), jasmonate (JA), and salicylic acid (SA) are the classic hormones involved in disease resistance although, more recently, abscisic acid, auxins, and others have been shown to contribute to the defense response (Denancé et al. 2013). For example, abscisic acid can promote susceptibility to FHB in wheat, while the opposite has been observed for

gibberellic acid (Buhrow et al. 2016; Gordon et al. 2016; Qi et al. 2016). There is discord in the literature on the role of the classical defense hormones in the FHB response. The reported discrepancies may be related, in part, to the pathogen's lifestyle, which involves a short biotrophic phase followed by a necrotrophic one. In the classical examples reported mainly for dicotyledonous plants, SA is generally involved in mediating resistance to biotrophic pathogens, while JA and ET often work together to facilitate resistance to necrotrophs (Glazebrook 2005). That being said, deviations in the role of plant defense hormones have been reported between dicots and monocots (De Vleeschauwer et al. 2013; Hükelhoven et al. 1999; Molina et al. 1999).

Various studies have shown that SA is involved in FHB resistance (Diethelm et al. 2014; Makandar et al. 2006, 2012), although there are also reports where exogenous application of SA or benzothiadiazole (SA analog) did not influence host disease response (Li and Yen 2008; Yu and Muehlbauer 2001). It has been suggested that these discrepancies may be related to the method of application (Makandar et al. 2012). In that context, it is interesting that SA directly inhibits *F. graminearum* growth and germination on synthetic media (Qi et al. 2012).

JA has been implicated in both FHB resistance (Gottwald et al. 2012; Li and Yen 2008; Qi et al. 2016; Sun et al. 2016; Wang et al. 2018) and susceptibility (Makandar et al. 2010). Importantly, the timing of JA accumulation can differentiate between the two responses and appears to involve antagonistic interactions with the SA pathway (Ameye et al. 2015; Makandar et al. 2010, 2012). It has been proposed that the dichotomous role of JA signaling in the FHB response is related to the hemibiotrophic nature of the pathogen: JA is thought to contribute to susceptibility during the biotrophic phase but can mediate resistance once the pathogen has entered the necrotrophic part of its lifecycle (Ameye et al. 2015; Ding et al. 2011).

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It is not known whether ET participates in a JA-ET-mediated resistance to the necrotrophic phase of FHB. The mixed reports in the literature do not identify a clear role for ET in disease response. Gene expression studies have implicated ET signaling in *Fusarium* resistance in wheat spikes (Gottwald et al. 2012; Li and Yen 2008) and leaves (Zhang et al. 2007). However, studies involving genetic or chemical manipulation of the ET-signaling pathway have suggested that ET signaling can enhance (Chen et al. 2009), reduce (Gillespie et al. 2012; Li and Yen 2008; Michniewicz et al. 1990), or have no effect (Sun et al. 2016) on *Fusarium* infection of wheat tissues. These studies differed vastly in experimental design and tissues tested, which may explain some of the differences in reported outcomes. It may also be relevant that ethephon (ETp), a chemical precursor of ET (also described as ethrel) used in some of these reports, has been found to inhibit vegetative growth, sporulation, and spore germination of *F. culmorum* (Michniewicz et al. 1983).

As is the case for SA signaling, variations in experimental design may explain the differences reported on the role of ET in FHB—although one experiment showed that silencing the ET-signaling pathway either had no effect or led to resistance or susceptibility in wheat, depending on the hosts' genetic background (Foroud 2011). Based on these results, it was hypothesized that the reported differences concerning the role of hormones in FHB disease might be related to which pathways are naturally activated in the *Fusarium*–host interaction, and that cross-talk among these pathways may explain the genotype-dependent response observed by Foroud (2011). In order to test the effect of genotype, we screened six wheat lines for their response to *F. graminearum* when treated with different chemicals that positively or negatively affect ET signaling in FHB. In this study, the results support a role for ET signaling in wheat resistance to FHB.

MATERIALS AND METHODS

Plant material. Four Canadian hexaploid wheat cultivars (Awesome, Roblin, Superb, and Tenacious) and two doubled-haploid wheat lines (GS-1-EM0040 and GS-1-EM0168) were used in the current study. These six genotypes carry different levels of FHB resistance and susceptibility, which are summarized in Table 1. Plants were grown in 3.8-liter pots (2 seeds/pot) with Cornell mix (Boodley and Shelldrake 1977) in a greenhouse at 22 and 18°C (day and night, respectively) with a 16-h photoperiod. Pots were watered daily as needed and fertilized biweekly. In order to prevent powdery mildew or aphid infestation, plants were treated at the five- to seven-leaf stage with Tilt (Syngenta Crop Protection Canada) and Intercept (Bayer Crop Science Canada), as previously described (Foroud et al. 2012).

***F. graminearum* strain, mycelial growth, and spore production.** The *F. graminearum* strain GZ3639 (provided by Dr. Robert Proctor from Agriculture Research Services, United

States Department of Agriculture) was used in this work. For spore production, the fungus was first grown on potato dextrose agar, and a 10-mm mycelial plug was used to inoculate 100 ml of CMC broth (1.5% carboxymethylcellulose [Sigma C1011], 0.1% NH_4NO_3 , 0.1% KH_2PO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.1% yeast extract) (Cappellini and Peterson 1965) in 250-ml Erlenmeyer flasks. The culture was incubated at 27°C for 7 days under gentle agitation (180 rpm). Macroconidia were collected by filtering through cheesecloth and the filtrate spun at $3,220 \times g$ in a swinging bucket rotor at 4°C for 10 min. The supernatant was poured off and conidia were washed with sterile double-distilled (dd) H_2O three times by centrifugation ($3,220 \times g$, 10 min, 4°C). The conidia were then resuspended in sterile dd H_2O and counted using a hemocytometer. The suspension was diluted to 100,000 macroconidia ml^{-1} prior to inoculation. A new batch of inoculum was prepared for each experimental repetition and was used within 7 days of preparation.

Preparation of chemicals affecting ET signaling. Four chemicals affecting the ET-signaling pathway were used: (i) 2-chlorophosphonic acid (ETp) (Sigma) is a chemical releaser of ET together with chloride and phosphonate (Yang 1969), (ii) 1-aminocyclopropane-1-carboxylic acid (ACC) (Sigma) is the biological precursor to ET (Adams and Yang 1979), (iii) cyclopropane-1,1-dicarboxylic acid (CDA) (Sigma) an inhibitor of ACC oxidase activity (Dourtoglou et al. 2000), and (4) 1-methylcyclopropene (MCP) (generously provided by AgroFresh Inc.) binds to ET receptors, thereby inhibiting the activation of the ET response. All chemical solutions were dissolved in water and prepared within 20 min of application. Chemical solutions were diluted to a final concentration of 100 μM in the culture medium for detached-head assays described below.

Detached-head assay for FHB resistance assessment. The detached-head assay was adapted from Chen et al. (2009) and employed for the six wheat genotypes described above. Tillers were cut at midanthesis 12 cm from the base of spikes, and the stems were sprayed and wiped down with 75% ethanol, rinsed with water, then placed in a 15-ml falcon tube with culture medium. The medium was prepared according to the recipe for an in vitro spike assay (5% sucrose, 0.04% L-glutamine, and 0.05% morpholinoethanesulfonic acid, pH 6.2) described by (Ganeshan et al. 2010) and filter sterilized with 0.20- μm polyethersulfone membranes. To assess the effect of chemical treatments the medium was supplemented with 100 μM ETp, ACC, CDA, or MCP. Control (CTL) reactions without chemical treatment were also included. The tubes were wrapped with parafilm to prevent the media from evaporating and then placed in a tube rack inside a Mondi 1020 Propagation Tray (no holes) covered with a 20 cm Mondi Propagation Dome (clear plastic). Each container housed a total of five spikes corresponding to one plant and treatment (representing five replicates), and the experiment was repeated three times. Prior to inoculation, the detached spikes were incubated inside the container for 2 h. The heads were then point or dip inoculated. To assess resistance to

TABLE 1. Description of wheat lines

Wheat line	Class ^a	Parentage	Type 1 ^b	Type 2 ^c	Reference
Awesome	SP	93FHB37/2*Andrew//SWS366	I	S	Harpinder Randhawa, Agriculture & Agri-Food Canada
Roblin	HRS	RL4302/RL4356//RL4359/RL4553	S	S	Campbell and Czarnecki (1987)
Superb	CWRS	Grandin*2/AC Domain	I	S	Townley-Smith et al. (2010)
Tenacious	CPS	HY665/BW346	R	MR	Brown et al. (2015)
GS-1-EM0040	CWRS	CIMMYT11/Superb*2	R ^d	MR	Foroud et al. (2012)
GS-1-EM0168	CWRS	CM82036/Superb*2	MR	R	Foroud et al. (2012)

^a Wheat class: SP = special purpose, HRS = hard red spring, CWRS = Canadian Western red spring, and CPS = Canadian Prairie Spring.

^b Type 1 resistance based on Fusarium head blight (FHB)-index from field trials. Disease rating index: R = resistant, MR = moderately resistant, I = intermediate, MS = moderately susceptible, and S = susceptible.

^c Type 2 resistance determined by point inoculation experiments in the greenhouse; ratings for Awesome and Tenacious based on results presented here; ratings for Superb, GS-1-EM0040, and GS-1-EM0168 based on results presented in (Foroud et al. 2012).

^d R value based on field data for severity rating in field trials (Foroud et al. 2012) but greenhouse experiments shows MS (Foroud 2011).

disease spread, the spikes were point inoculated by pipetting 10 μ l of an *F. graminearum* spore suspension (100,000 macroconidia ml^{-1}) into a single floret of each head. The dip inoculation method was designed to provide a uniform exposure of the spike to the inoculum and to differentiate initial infection from disease spread. For dip inoculation, wheat spikes were immersed in a spore suspension (50,000 macroconidia ml^{-1}) for 1 s. The containers were placed in a greenhouse at 22 and 18°C (day and night, respectively) with a 16-h photoperiod.

Disease was assessed as the number of diseased spikelets below the inoculation point (point inoculation) or the percentage of diseased spikelets per head (dip inoculation) at 3, 6, and 9 days after inoculation (DAI). Area under the disease progress curve (AUDPC) was calculated as described by Madden et al. (2017).

Quantitative analysis of ACC accumulation in detached wheat heads. Detached heads of Superb and GS-1-EM0168 were prepared as described above, except without inoculation. The detached heads were placed in tubes with culture medium (CTL treatment) or culture medium supplemented with 100 μM ETp, ACC, CDA, or MCP. Spikes were harvested 1 day after cutting or exposure to the different treatments, frozen in liquid nitrogen, and stored at -80°C until further analysis. Five heads from each treatment and plant line were pooled together for ACC extraction for each of three replicates, and the experiment was repeated twice.

ACC extraction and quantification was carried out through the Hormone Profiling service facility at the National Research Council of Canada (Saskatoon, Canada) (https://www.nrc-cnrc.gc.ca/eng/solutions/advisory/plant_hormone.html). The extraction and purification protocol was performed according to Lulsdorf et al. (2013). ACC was derivatized with phenyl isothiocyanate according to Chauvaux et al. (1997), with modifications. Samples were chromatographed by ultraperformance liquid chromatography (ACQUITY UPLC system; Waters Inc.) and delivered by electrospray ionization to a Micromass Quattro Premier XE quadrupole tandem mass spectrometer coupled with a Z-spray interface (Waters Inc.). The data were analyzed by MassLynx v4.1 (Waters Inc.) control software using the Multiple Reaction Monitoring function. Chromatographic traces were quantified offline using QuanLynx v4.1 software (Waters Inc.). Each trace was integrated and the resulting ratio of signals (nondeuterated analytes versus deuterated internal standards [d_4 -ACC and PTH- d_4 -ACC]) compared with a calibration curve generated from standard solutions to determine the amount of analyte present in each sample.

Statistical analysis. All statistical analyses were carried out in SAS 2.0.4. For disease assays, least-square means (LSMeans) were calculated for three datasets to compare the treatment effects within each of the six wheat genotypes: (i) AUDPC of point-inoculated spikes, (ii) AUDPC of dip-inoculated spikes, and (iii) percent diseased spikelets of dip-inoculated spikes rated at 3 DAI. Comparisons of the resistant versus susceptible response to treatments were also carried out on datasets i and iii, where data were pooled across all resistant or susceptible lines. For ACC quantification, LSMeans was calculated for each treatment, within each of the two wheat genotypes assessed. A general linear model analysis of variance of the LSMeans was employed for each of the comparisons made. Means were separated using Fisher's least significant difference and the type 1 error rate (α) was set at 0.05 or less. Fold change was calculated by dividing the treatment value by the CTL value.

RESULTS

Effect of ET-related treatments on initial infection and disease progression in dip-inoculated wheat spikes. To determine whether ET signaling affects the FHB response of six wheat genotypes, detached-head assay experiments were carried out in the presence or absence of ET enhancers (ACC and ETp) or inhibitors (CDA and MCP). A novel dip-inoculation method was

employed to provide uniform exposure of the head to the spores, enabling an assessment of any treatment-related changes in disease establishment. Disease evaluation at 3 DAI can provide an approximation of resistance to initial infection (Fig. 1). In the absence of chemical treatments, initial infection was observed at a mean of 1.00 ± 0.102 diseased spikelets per head among the three resistant lines (Tenacious, GS-1-EM0040, and GS-1-EM0168), which translates to $6.58 \pm 0.78\%$ (Table 2). There was no effect of ET enhancer treatments, whereas ET inhibitors resulted in a significant increase in initial infection: 1.36-fold ($P = 0.0348$) and 1.73-fold ($P < 0.0001$) increase for CDA and MCP, respectively, compared with the CTL. When looking at the resistant lines individually, initial infection was unaffected by any of the treatments in Tenacious, and GS-1-EM0168 was only affected by MCP, which showed a significant increase in diseased spikelets (Fig. 1). Results for GS-1-EM0040 showed a breakdown of resistance in response to both ET inhibitor treatments.

In the susceptible cultivars (Awesome, Roblin, and Superb), the mean initial infection was 3.00 ± 0.102 diseased spikelets per head ($17.22 \pm 0.798\%$) (Table 2). In this case, ET enhancer treatments resulted in a lower level of initial infection by 0.52- and 0.44-fold ($P < 0.0001$) in the ACC and ETp treatments, respectively. When analysis was carried out by cultivar (Fig. 1), Superb deviated from the effect observed in the susceptible group. The trend was consistent—ACC and ETp treatments resulted in the lowest level of initial infection at 3 DAI; however, MCP also showed a significant reduction (0.66-fold; $P < 0.0001$) when compared with the CTL and the CDA treatment.

A similar trend was observed in disease progression of dip-inoculated spikes over the course of 3 to 9 DAI (Fig. 1) (AUDPC). ET enhancer treatments had no effect compared with the CTL for resistant genotypes but resulted in significant reduction of disease progression in the susceptible genotypes compared with the CTL ($P < 0.0001$). The effect of ACC and ETp were comparable, except in Superb, where the ETp treatment had a larger AUDPC compared with ACC treatment (1.69-fold difference; $P < 0.0001$), suggesting that ETp had a stronger effect in this genotype.

ET inhibitors resulted in a significant ($P < 0.0001$) increase in disease progression compared with the CTL, except in the case of the CDA treatment in Tenacious, which showed a 1.93-fold increase in disease progression at $P = 0.0021$ (Fig. 1) (AUDPC). ET inhibitors had no effect on disease progression in Awesome or Superb but both MCP and CDA did further increase susceptibility in Roblin (1.35- and 1.36-fold difference for CDA and MCP, respectively, compared with the CTL; $P < 0.0001$).

Effect of ET-related treatments on disease spread in point-inoculated wheat spikes. Point inoculation was used to assess the effect of ET-inhibitor or -enhancer treatments on disease spread in six wheat genotypes. The trends were similar to what was observed for both initial infection and disease progression in dip-inoculated wheat. With some genotype-specific variation, the ET-enhancer treatments had little to no effect in the resistant genotypes, whereas inhibitor treatments, without exception, significantly increased disease spread compared with the CTL ($P < 0.0001$) (Fig. 2; Table 2). In each of the three susceptible genotypes, a significant reduction in disease spread was observed in the ET-enhancer treatments compared with the CTL (Fig. 2), where an average 0.51- and 0.60-fold decrease was observed in the ACC and ETp treatments, respectively ($P < 0.0001$) (Table 2). The ET inhibitors had no effect on disease spread in any of the susceptible cultivars (Fig. 2), although the mean value for the effect of MCP on the susceptible genotypes (Awesome, Roblin, and Superb) showed a 1.11-fold increase in disease spread ($P = 0.0020$).

ACC accumulation in spikes of chemically treated detached wheat heads. Detached heads of Superb and GS-1-EM0168 were transferred to culture media with or without 100 μM ACC, CDA, ETp, or MCP, as carried out in the detached-head assay experiment, but without *F. graminearum* inoculations. Spikes were

collected for ACC quantification 24 h later in order to determine whether any of the treatments affected the ACC concentration within the spike. ACC treatments led to an increase in ACC content within the spikes of both wheat genotypes compared with the CTL

(Table 3). For Superb, a 2.29-fold increase was observed ($P = 0.0343$), and a 2.05-fold increase was observed in GS-1-EM0168 ($P = 0.0403$). The higher concentration of ACC within the spikes suggests that the ACC was taken up by the plant and translocated to

Treatment	'Roblin'	'Awesome'	'Superb'	GS-1-EM0040	'Tenacious'	GS-1-EM0168
CTL	a	a	a	b	a	b
ACC	b	b	c	b	a	b
ETp	b	b	c	b	a	b
CDA	a	a	a	a	a	b
MCP	a	a	b	a	a	a

Fig. 1. Effect of chemical treatments on dip-inoculated wheat heads. Detached wheat heads were transferred to culture media with or without (control [CTL]) 100 μ M 1-aminocyclopropane-1-carboxylic acid (ACC), cyclopropane-1,1-dicarboxylic acid (CDA), ethephon (ETp), or 1-methylcyclopropene (MCP) 2 h prior to dip inoculation with *Fusarium graminearum* spores. Disease progression was rated as the percentage of diseased spikes within a spike at multiple days after inoculation (DAI), and area under the disease progress curve was calculated for general linear model (GLM) analysis (least-square means [LSMeans] standard error 8.89; $P < 0.0001$). Disease ratings as the percentage of diseased spikelets at 3 DAI were also compared in a separate GLM analysis, and are presented in the table (LSMeans standard error 0.160; $P < 0.0001$). Different letters represented within each chart or column within the table indicate statistically different results for each plant line ($P < 0.001$).

the inflorescence. There was no significant effect on ACC content in other treatments compared with the CTL, except in the case of the MCP treatment in GS-1-EM0168 (2.36-fold change; $P=0.0072$). In this case, an increase in ACC was observed, exceeding the levels found in the ACC treatment. No significant difference was observed in ACC content between the CTL treatments for Superb versus GS-1-EM0168 ($P=0.6232$).

DISCUSSION

The role of ET signaling in the FHB response was assessed in six wheat genotypes with different levels and types of resistance (Table 1). The disease assays were carried out on detached wheat heads with their stems immersed in media supplemented with or without 100 μ M ACC, CDA, ETp or MCP. ACC and ETp are enhancers of the ET pathway, where the latter is a chemical releaser of ET and the former a biosynthetic precursor. CDA and MCP are chemical inhibitors of the ET pathway, where the latter blocks the ET receptors and the former is a biosynthesis inhibitor. To determine whether chemical treatments could be taken up by the stem and translated into a response in the wheat heads, uninoculated spikes of Superb and GS-1-EM0168 were harvested for ACC quantification 24 h after the heads were placed in the media with or without the chemical treatments. This enabled assessment of how each of the four chemical treatments affected ACC content within the spikes. As expected, an increase in ACC abundance was observed in the heads treated with ACC compared with the CTL (Table 3). Both positive and negative feedback loops have been reported for ET (Wang et al. 2002); interestingly, although ETp treatments in both Superb and GS-1-EM0168 resulted in an apparent reduction in ACC content (0.50- and 0.35-fold difference, respectively), these differences were not significant. CDA had no effect on ACC accumulation; however, MCP resulted in an increase in ACC content in GS-1-EM0168 (2.36-fold difference; $P=0.0072$). This may be a result of a feedback mechanism, where blocking ET perception with MCP (as opposed to inhibiting its biosynthesis with CDA) may result in an upregulation of ACC production in this genotype.

The detached-head assays, both point and dip inoculations, showed a trend consistent with a role for ET signaling in FHB resistance. An effect was observed for initial infection, as assessed by disease evaluation 3 days after dip inoculation (Fig. 1; Table 2), where ET enhancers improved resistance in the susceptible cultivars

but did not offer further protection in the resistant genotypes. However, ET inhibitors did reduce resistance to initial infection in some instances, although none of the treatments affecting the ET pathways were able to break down the strong type 1 resistance in dip-inoculated Tenacious. In the CTL treatment, Tenacious did not differ in type 1 resistance compared with the other two resistant lines; however, unlike in Tenacious, inhibition of ET signaling resulted in some loss of resistance at 3 DAI in these two lines (Fig. 1).

The conserved resistance to initial infection in Tenacious, regardless of treatment, might be related to undetermined structural or physical properties mediating type 1 resistance in this genotype. Inhibition of the ET pathway did increase disease spread in this cultivar (as seen in the point inoculation data) (Fig. 2), which may explain the higher percentage of diseased spikelets observed in dip-inoculated Tenacious by 6 DAI, despite the lack of difference at 3 DAI (Fig. 1). Inhibition of the ET pathway also increased disease spread in the other two resistant genotypes, whereas ET-enhancer treatments had no effect (Fig. 2). Meanwhile, ET enhancers improved type 2 resistance in the susceptible genotypes, whereas inhibition had no effect in these cultivars. These trends suggest that the ET-signaling pathway may be naturally activated in resistant wheat and not in susceptible wheat, and that ET contributes to both type 1 and type 2 resistances. Previous work in gene expression analysis, where upregulation of *ACC OXIDASE* was observed in the resistant cultivars Sumai 3 and Dream, also suggests that the ET pathway is upregulated by *F. graminearum* in some resistant genotypes (Gottwald et al. 2012; Li and Yen 2008).

The results presented in this study and elsewhere (Gillespie et al. 2012; Gottwald et al. 2012; Li and Yen 2008) suggest that ET plays a role in wheat resistance to FHB. However, there is also evidence that contradicts this (Chen et al. 2009; Sun et al. 2016). In this context, it may be relevant that similar discrepancies have been reported about the role of other plant hormones in the host response to FHB. For example, SA signaling has been identified in resistance in some reports (Diethelm et al. 2014; Makandar et al. 2006, 2012) and, in other cases, as having no effect (Li and Yen 2008; Qi et al. 2012; Yu and Muehlbauer 2001). Meanwhile, JA signaling has been implicated in FHB resistance (Gottwald et al. 2012; Li and Yen 2008; Qi et al. 2016; Sun et al. 2016) and susceptibility (Makandar et al. 2010). These inconsistencies are thought to be related to different experimental designs such as treatment method or timing. Similar explanations may be offered with regards to the role of ET signaling in the host response to *F. graminearum*.

In both the current study and that presented by Chen et al. (2009), detached-head assays with chemical treatments affecting ET signaling were employed but the experiments yielded different conclusions regarding the role of ET in the FHB response. Despite the similarities in experimental design, many variables were different. Among these differences, the assays presented by Chen et al. (2009) were carried out at lower temperatures (15 and 12°C day and night, respectively) compared with those presented herein (22 and 18°C day and night, respectively). In addition, the setup differed in a manner that may affect the relative humidity, which is an important consideration in FHB assays. Each of these differences could alter the rate of disease initiation and progression, which could interact with a potential effect of timing of ET accumulation and disease response. Furthermore, different chemical treatments were employed in the two studies. Two ET pathway enhancers and two inhibitors were compared herein, including MCP, an inhibitor of ET perception. Chen et al. (2009) also used an inhibitor of ET perception but, in that case, silver thiosulfate was used. Silver ions interact with ET receptors by blocking the copper cofactor binding site. At the time, it was not known that silver ions can also increase auxin efflux (Strader et al. 2009). Because many enzymes have metallic cofactor binding sites, it is plausible that silver ions may also interfere with other biochemical pathways. Interestingly, auxin is produced by *F. graminearum* during the course of infection but

TABLE 2. Effect of ethylene enhancer and inhibitor treatments on Fusarium head blight initial infection and disease spread^a

Treatment, lines ^b	Initial infection ^c	Disease spread ^d
Resistant		
CTL	6.58	8.83
ACC	6.49	7.43*
ETp	6.69	7.67
CDA	8.97*	14.10***
MCP	11.4***	16.40***
Susceptible		
CTL	17.22	18.07
ACC	8.94***	9.20***
ETp	7.53***	10.9***
CDA	19.22	18.8
MCP	16.66	20.07**

^a Asterisks indicate significant differences (*, **, and *** = $P < 0.05$, 0.01, and 0.0001, respectively) between control (CTL) and treatments calculated across three resistant or three susceptible lines.

^b ACC = 1-aminocyclopropane-1-carboxylic acid, ETp = ethephon, CDA = cyclopropane-1,1-dicarboxylic acid, and MCP = 1-methylcyclopropene.

^c Initial infection measured as the percentage of diseased spikelets per head following dip inoculation measured at 3 days after inoculation (least-square means [LSMeans] standard error = 0.769, $P < 0.0001$).

^d Disease spread measured as area under the disease progress curve for point-inoculated heads (LSMeans standard error = 0.455, $P < 0.0001$).

exogenous application of the auxin indole acetic acid can delay spore germination, inhibit mycelial growth, and alter hyphal branching patterns (Luo et al. 2016). Exogenous application of indole acetic acid was shown to improve FHB resistance in barley (Petti et al. 2012); meanwhile, the same effect was not observed in wheat (Qi et al. 2016). The potential implications of endogenous auxin export from host cells on the *Fusarium*–host interaction are unknown. Regardless of whether this influenced the disease response observed by Chen et al. (2009) in their silver thiosulfate treatments, the authors did provide substantial experimental evidence, including investigations of genetic knockdown and knockout lines of wheat and *Arabidopsis*, that support a role for ET signaling as a susceptibility factor. Additionally, the same group recently reported the ability of a number of *F. graminearum* and

TABLE 3. 1-Aminocyclopropane-1-carboxylic acid (ACC) accumulation in chemically treated wheat spikes

Treatment ^b	ACC (ng/g of spike) ^a	
	Superb	GS-1-EM0168
CTL	1,305 ± 546	1,702 ± 599
ACC	2,988 ± 546*	3,492 ± 599*
ETp	658 ± 546	591 ± 546
CDA	1,337 ± 546	2,306 ± 546
MCP	1,568 ± 546	4,040 ± 546**

^a Asterisks indicate significant differences (* and ** = $P < 0.05$ and 0.01 , respectively) between control and treatments within each wheat line.
^b CTL = control, ACC = 1-aminocyclopropane-1-carboxylic acid, ETp = ethephon, CDA = cyclopropane-1,1-dicarboxylic acid, and MCP = 1-methylcyclopropane.

Fig. 2. Effect of chemical treatments on point-inoculated wheat heads. Detached wheat heads were transferred to culture media with or without (control [CTL]) 100 μ M 1-aminocyclopropane-1-carboxylic acid (ACC), cyclopropane-1,1-dicarboxylic acid (CDA), ethephon (ETp), or 1-methylcyclopropane (MCP) 2 h prior to point inoculation with *Fusarium graminearum* spores. Disease spread was assessed at multiple days after inoculation and area under the disease progress curve was calculated for general linear model analysis (least-square means standard error 0.671; $P < 0.0001$). Different letters represented within each chart indicate statistically different results for each plant line ($P < 0.001$).

F. culmorum isolates to produce ET in axenic cultures (P. Nicholson, personal communications), although it is not yet known whether this occurs in planta or affects disease progression.

By contrast, the results presented here demonstrate that the ET pathway can mediate wheat resistance to FHB, and this is supported by some reports (Gillespie et al. 2012; Gottwald et al. 2012; Li and Yen 2008). Meanwhile, another study reported no effect of ET signaling on FHB resistance (Sun et al. 2016). Foroud (2011) observed an apparent genotype-specific influence on the role of ET signaling in the FHB response. In the latter, it was proposed that the dissimilarities in the literature may be a result of genotype-specific differences, likely governed by an interaction with other biochemical or hormone-signaling pathways that are naturally activated in an individual genotype's response to FHB. In order to explore the possibility of a genotype-dependent role of ET signaling in the current work, six wheat genotypes with different levels of resistance and susceptibility were assessed in the detached-head assays; however, the anticipated genotype-specific effect was not observed here. Although some genotype-specific variation was observed in response to specific treatments, the trend was consistent across genotypes in supporting a role for ET in the resistance response.

Given the discrepancies in the literature, the story is perhaps more complex and warrants additional attention. For instance, it is not known how the ET pathway interacts with other signaling pathways. ET pathway inhibition did not alter the high level of resistance in Tenacious, indicating that other factors are also at play—which is consistent with the fact that FHB resistance is quantitative. It is known that other hormone pathways are involved in FHB resistance or susceptibility but the interaction of these pathways with ET is unknown in this context. JA and ET often work together in mediating resistance to necrotrophs (Glazebrook 2005). It was earlier proposed that JA signaling may be involved in resistance to disease spread but not initial infection (Foroud et al. 2012). This has not been tested but it is understood that JA-signaling, although involved in FHB resistance, is a susceptibility factor in the early stages of infection (Ameye et al. 2015; Makandar et al. 2006, 2012). Presumably, the dichotomous role of JA is concomitant with the switch from the biotrophic to necrotrophic phase of the pathogen's lifestyle. The results presented here support a role for ET signaling in resistances to both initial infection and disease spread. Additional research is needed to assess the effect of timing on the activation of ET signaling, as well as the interaction of ET with JA or other signaling pathways in the context of both type 1 and type 2 FHB resistances. This information could help to determine whether the influence of ET signaling on the host response is dependent on the pathogen lifestyle as it pertains to the infection process.

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