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Benzothiadiazole-induced gene expression in wheat spikes does not provide resistance to *Fusarium* head blight

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Fusarium head blight (FHB) is a devastating disease of wheat throughout the world. FHB is primarily caused by the fungal pathogen *Fusarium graminearum*. In wheat, benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) induces the wheat chemically-induced (WCI) genes and provides resistance to several pathogens. The objectives of this study were two-fold: (1) to investigate the effects of BTH application and *F. graminearum* infection on selected defense response genes in wheat spikes; and (2) to study the potential of BTH for inducing FHB resistance in wheat. Wheat spikes were treated with BTH prior to anthesis and transcript accumulation and resistance to FHB were measured. BTH-treated wheat spikes were examined for expression of six defense response genes (PR-1, PR-2, PR-3, PR-4, PR-5 and peroxidase) and the five WCI genes that are induced by BTH in wheat leaves. All five WCI genes were induced by BTH in spikes; however none of the six defense response genes were induced. Conversely, the defense response genes were induced by *F. graminearum* infection, whereas the WCI genes were not. These data indicate that the pathway for induction of the defense response genes by *F. graminearum* infection is distinct from the BTH-induced pathway. In the disease evaluations of BTH-treated plants, we found that BTH did not provide significant Type I or Type II resistance to wheat spikes spray- or point-inoculated with *F. graminearum*, respectively. These data indicate that BTH application and the induction of WCI gene expression does not provide resistance to FHB.

Keywords: *Triticum aestivum*; *Fusarium graminearum*; benzo(1,2,3)thiodiazole-7-carbothioic acid S-methyl ester (BTH); *Fusarium* head blight.

INTRODUCTION

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.) Petch], is a major factor limiting wheat (*Triticum aestivum* L.) production worldwide [*6*]. In recent years, it has caused devastating losses in the upper midwest of the U.S.A. and around the world [*15*, *20*]. FHB develops when ascospores of *F. graminearum* infect wheat florets at anthesis. FHB dramatically reduces wheat grain yield and quality [*15*]. Starch and protein quality are diminished by mycotoxins such as deoxynivalenol and the estrogenic zearalenone produced by the fungus in infected kernels [*1*, *27*, *28*]. Currently, the only control methods are the combination of crop rotation with non-host crops, fungicide applications and use of partially-resistant cultivars [*15*, *16*].

There is a general lack of understanding of how resistance to FHB in partially-resistant genotypes is manifested. Five types of resistance to FHB have been proposed, although the focus has been principally on Type I, or resistance to initial infection, and Type II, resistance to spread in the spike [*17*]. In an attempt to understand the molecular basis of Type I and Type II resistance, defense response gene expression has been examined in wheat spikes during infection. In wheat spikes, infection by *F. graminearum* induces transcript accumulation of PR-1, PR-2 (β-1,3-glucanase), PR-3 (chitinase), PR-4, PR-5 (thaumatin-like protein) and peroxidase genes in both Type II-resistant and susceptible cultivars [*21*]. Interestingly, PR-4 and PR-5 transcripts accumulate earlier and in greater amounts in the Type II-resistant genotype. Moreover, β-1,3-glucanases and acidic chitinases were expressed earlier in Sumai 3 (Type II-resistant cultivar) than a susceptible Sumai 3 mutant [*14*]. In addition, defense response gene expression is systemic within wheat spikes of Type II-resistant and susceptible genotypes; colonized and uncolonized portions of the spike accumulate defense response gene transcripts [*22*]. To better understand the relationship between gene expression in wheat spikes and resistance to *F. graminearum* infection, we are studying the effect on FHB resistance of chemical applications that induce inherent defense responses.

Certain chemical treatments such as, salicylic acid (SA), 2,6-dicloroisonicotinic acid (INA) and benzo(1,2,3) thiodiazole-7-carbothioic acid S-methyl ester (BTH)

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induce systemic expression of plant defense response genes that may confer resistance to pathogen attack [7, 11, 24]. In wheat, BTH application induces a set of five genes referred to as the wheat chemically-induced (WCI) genes and provides resistance to powdery mildew (*Erysiphe graminis* f. sp. *hordei*; [8]). In maize, BTH application induces the expression of genes encoding PR-1 and PR-5 and provides resistance to downy mildew (*Peronosclerospora sorghi*; [19]). In barley, SA, BTH and INA treatments provide resistance to powdery mildew caused by *Blumeria graminis* f. sp. *hordei* [2, 12], and BTH and INA treatments induce a set of genes referred to as barley chemically-induced genes [2]. Moreover, INA treatment in barley induces the PR-1, chitinase and peroxidase genes [12]. These studies and other studies in dicot species indicate that BTH is useful for inducing defense response genes and controlling a range of diseases. However, the potential for BTH to induce WCI expression and the defense response genes in wheat spikes and provide FHB resistance is unexplored. In addition, the types of genes induced by *F. graminearum* infection vs. those induced by BTH have not been compared.

We have conducted studies investigate the molecular response in wheat spikes to BTH application and *F. graminearum* infection and to determine if BTH induces resistance to FHB. Our results show that the expression of BTH-induced genes is independent from *F. graminearum*-induced genes. In addition, we found that BTH application and induction of the WCI genes does not provide Type I or Type II resistance to FHB.

MATERIALS AND METHODS

Plant materials

The susceptible cv. Wheaton was used for all experiments. Seeds were surface-sterilized with 0·1 % NaOCl, and planted in 16 cm pots and grown in a growth chamber. The growth chamber was set at 60–80 % RH, a 16 h photoperiod of cool, white fluorescent and supplementary incandescent lamp illumination supplied at a total of 150 μE m^{-2} s^{-1}, and 20/16 (°C day/night) temperature. Plants were fertilized with mineral fertilizer at emergence (Zadoks stage 11) and the elongation stage (Zadoks stage 31) [31]. At the elongation stage, secondary tillers were trimmed.

BTH treatment

BTH and the blank formulation were gifts from Novartis Crop Protection, Inc. (Research Triangle Park, NC, U.S.A.). The blank formulation without BTH and water were used as controls. Plants including spikes were sprayed approximately 3 days before anthesis with 1 mM BTH, the blank formulation or water until runoff (about 40 ml per pot).

Pathogen preparation and inoculation

Monosporic isolate 3A-31 of *F. graminearum* was used in this study. The isolate was a gift from Ruth Dill-Macky, University of Minnesota and was collected from barley in Felton, MN, U.S.A. in July 1995. Cultures were initiated from a water suspension of macroconidia by inoculating mung bean agar plates [40 g l^{-1} mung beans (w/v) in distilled water, boiled for 15 min and filtered through four layers of cheese cloth; supplemented with 1·5 % agar (w/v)]. They were incubated for 1 week at 25 °C with a 12 h photoperiod of cool, white fluorescent light. Macroconidia were washed off from the colonized agar surface of each plate with 5 ml distilled water. The collected suspension was then filtered through four sterile cheesecloth layers. Spore counts were determined using a hemacytometer. The suspension was kept temporarily in a 4 °C refrigerator.

Two spray-inoculation experiments were conducted, each with 12 pots per treatment and six plants (one spike per plant) within each pot. For both spray-inoculation experiments, spikes were sprayed 3 days before anthesis with 1 mM BTH, the blank formulation, or water. At anthesis, wheat spikes were spray-inoculated with a water suspension containing 5×10^4 macroconidia per ml. The suspension was sprayed onto each spike with two passes using an air-brush sprayer (Model VL, Paasche Airbrush Co., Harwood Heights, IL, U.S.A.) operated at 15 psi (82·8 kPa). After inoculation, the pots were placed in a dew chamber (21 °C, approx. 100 % RH) for 2 days, then placed back in the growth chamber.

Two point-inoculation experiments also were conducted. For the first point-inoculation experiment, there were four pots for each treatment and five plants (one spike per plant) within each pot. For the second point-inoculation experiment, there were six pots for each treatment and five plants in each pot. For both experiments, spikes were sprayed 3 days before anthesis with 1 mM BTH, a blank formulation, or water. At anthesis, a 5 μl water suspension of *F. graminearum* conidia (1×10^5 conidia per ml) was injected into each of the two central spikelets [22]. After inoculation, the pots were placed in a dew chamber (21 °C, approx. 100 % RH, with continuous cool white fluorescent light) for 2 days, then placed back in the growth chamber.

Disease assessment and data analysis

For both spray-inoculation experiments, the percentage of infected spikelets was recorded daily from 6 to 12 days after *F. graminearum* inoculation. For both point-inoculation experiments, the percent of infected spikelets

(in entire spike) was recorded 11 days after *F. graminearum* inoculation. Infection was measured by counting the number of spikelets that exhibited visible head blight symptoms. The data from the four experiments were analysed separately using analysis of variance (ANOVA) from the SAS package [25].

RNA isolations and RNA gel blot analysis

For RNA gel blot analyses, RNA was isolated from BTH-treated plants. For BTH-treated plants, samples were combined from three spikes or three flag leaves at various times after treatment. RNA samples from *F. graminearum* spray-inoculated Wheaton spikes at 48 h after inoculation were the same as those used in Pritsch *et al*. [21]. The *F. graminearum*-inoculated plants were not treated with BTH. Frozen samples were ground into fine powder in liquid N_2, and total RNA was isolated using the LiCl precipitation procedure described by De Vries *et al*. [5]. Total RNA samples were fractionated in 1% agarose/formaldehyde gels and the gel was blotted onto nylon membranes (Zeta-Probe, BIO RAD, Hercules, CA, U.S.A.). Inserts of cDNA clones were isolated from preparative agarose gel electrophoresis of restriction digested plasmids. The probes were random prime labelled with [α-^{32}P]dCTP using prime-a-gene kit (Promega, Madison, WI, U.S.A.) according to the manufacturer's instruction. Prehybridization and hybridization were performed in 50% formamide at 42°C according to the membrane manufacturer's instructions. After hybridization, the membranes were washed at 42°C in 0·5 × SSC, 0·1% SDS.

Gene probes

Six defense response gene cDNAs were used as probes including two from wheat, peroxidase (plasmid pOX381; accession no. X56011; [23]), and PR-1-2 ([18]; accession no. EMBL AJ007349), and four from barley, [9] including PR-2 or β-1,3-glucanase (pBH72·11, accession number not available), acidic PR-3 or chitinase type II (HvCht2a, EMBL accession number X78671), PR-4 (pBH72-F6, EMBL accession number Y10814) and acidic PR-5 or thaumatin-like protein (pBH72-C6, accession number not available). All of these genes were shown to be induced in *F. graminearum*-infected wheat spikes [21]. The coding sequences for these genes were used as probes. Therefore, each probe provides an assessment of the expression of all highly-related genes that may be present within the family. Five wheat chemically-induced (WCI) cDNA probes were obtained from Novartis Crop Protection Inc. [8]. Based on a recent BLAST search, WCI-1 is similar to a jasmonate-induced gene from barley; WCI-2 is similar to lipoxygenases; WCI-3 does not exhibit similarity to any gene in the database; WCI-4 is similar to cysteine proteinases; and WCI-5 is similar to proteins with unknown function. All five genes are induced by BTH in wheat leaves [8]. The coding sequences of the WCI genes were used as probes.

RESULTS

Induced gene expression in wheat spikes

BTH has been shown to induce the expression of five wheat chemically-induced (WCI) genes in wheat leaves [8]. However, BTH induction of the WCI genes has not been shown in wheat spikes. In addition, the optimal concentration of BTH for inducing gene expression in spikes has not been shown. Therefore, the first objective was to determine if BTH induced the WCI genes in spikes. A series of BTH concentrations (0·1, 0·2, 0·5, 1, 2·5, 5 and 10 mM) was applied to spikes and sampled 4 days after application for RNA isolations. Spikes treated with 0·1 mM BTH had a very low level of WCI gene transcript accumulation at 4 days after treatment. However, spikes sprayed with 0·2–10 mM BTH exhibited a similar, high level of transcript accumulation for all five WCI genes at 4 days after treatment (data not shown). Taken together, we found that BTH induces the WCI genes in wheat spikes and that an appropriate concentration of BTH for future experiments was 1 mM as had been observed in other experiments (i.e. [8]).

To examine WCI gene expression in wheat spikes after BTH application and *F. graminearum* inoculation, RNA gel blot analysis was conducted on RNA samples isolated from spikes treated with BTH and infected with *F. graminearum*. Since leaves have been shown previously to express the WCI genes in response to BTH application (i.e. [8]), flag leaves were used in this experiment as a comparison. Plants including spikes were sprayed with BTH and a blank formulation 3 days before anthesis and WCI transcript accumulation was assessed in spikes and flag leaves at 0, 1, 2, 4, 6, 8 and 10 days after treatment. For the blank formulation, gene expression was examined only at 6 days after treatment. Also, an RNA sample from *F. graminearum* spray-inoculated spikes sampled 48 h after inoculation (h.a.i.) was included that had exhibited induction of defense response genes in our previous work [21]. No or very low transcript accumulation was observed in spikes and flag leaves from the blank formulation treatment (Fig. 1). However, transcripts accumulated for all five WCI genes in spikes and flag leaves 1 day after BTH treatment. By 2 days, transcript accumulation increased for all WCI genes and maintained a high level to 10 days. For the *F. graminearum*-inoculated spikes, transcripts were not detected for WCI-1, WCI-3, WCI-4 and WCI-5. A faint transcript was observed for WCI-2 but it was also observed in blank

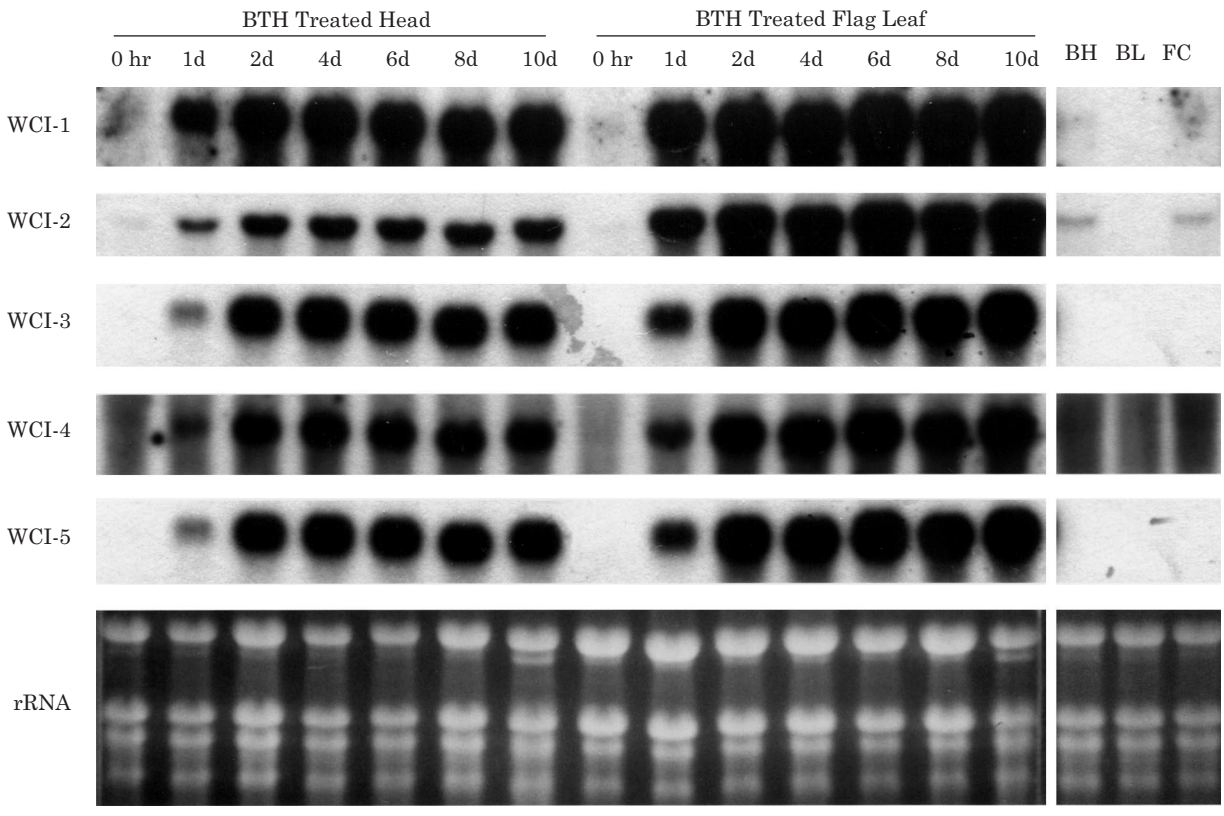

FIG. 1. BTH induces WCI gene expression in wheat spikes and flag leaves. Total RNA from cv. Wheaton spikes and flag leaves 0–10 days after application of 1 mM BTH. Total RNA from cv. Wheaton spikes (BH) and flag leaves (BL) 6 days after application of the blank formulation. Total RNA from cv. Wheaton spikes 48 h after *F. graminearum* spray-inoculation (FC, [*21*]). RNA gel blots were probed with five WCI cDNA clones. An ethidium bromide stain of the rRNA was used to verify similar RNA loading.

formulation control spikes. A second experiment was conducted, with RNA samples from spikes and flag leaves 0, 2, 4 and 6 days after BTH application, spikes 6 days after blank treatment, and spikes 48 h after *F. graminearum* inoculation (again from the authors' earlier work), providing similar results (data not shown). These data show that BTH induces accumulation of the WCI gene transcripts in wheat spikes and flag leaves within 1–2 days after treatment. In addition, these data show that WCI transcripts were not induced by *F. graminearum* within 48 h.a.i.

Previous work has shown that there are differences in the genes expressed in pathogen and BTH-treated plants (e.g. [*26*]). To determine if there is differential gene expression in BTH and *F. graminearum*-treated wheat spikes, RNA was isolated from BTH- and blank formulation-treated spikes at 0, 4 and 6 days after treatment and RNA gel blots were probed with PR-1, PR-2 (β-1,3-glucanase), PR-3 (chitinase), PR-4, PR-5 and peroxidase cDNAs. As controls, RNA samples from *F. graminearum*-inoculated spikes 48 h.a.i. and from water-sprayed spikes 4 days after treatment were included. As shown previously [*21*], transcripts for all six defense response genes were present in the *F. graminearum*-inoculated spikes (Fig. 2). The PR-1, PR-2, PR-4 and peroxidase hybridizations in the *F. graminearum*-inoculated lanes were intense because long exposure times were used to visualize the expression of the defense response genes in the BTH treatments. For the BTH and blank formulation treatments, transcripts did not appear to accumulate for PR-3, PR-5 and peroxidase. For PR-2 and PR-4, transcripts were abundant at 0 h and exhibited similar expression over time and were present in blank and water controls, indicating that the BTH treatment did not induce expression. PR-1 exhibited a low level of induction at 6 days after both the BTH and blank formulation treatment, indicating a possible developmental regulation or a response to other environmental factors. Taken together, our data indicate that the defense response genes PR-1, PR-2, PR-3, PR-4, PR-5 and peroxidase are not induced in spikes within 6 days after BTH treatment.

FHB disease assessment

BTH is a chemical inducer of disease resistance in many crop plants. Therefore, we were interested in examining whether BTH application would provide either Type I or Type II resistance to FHB. To determine if BTH

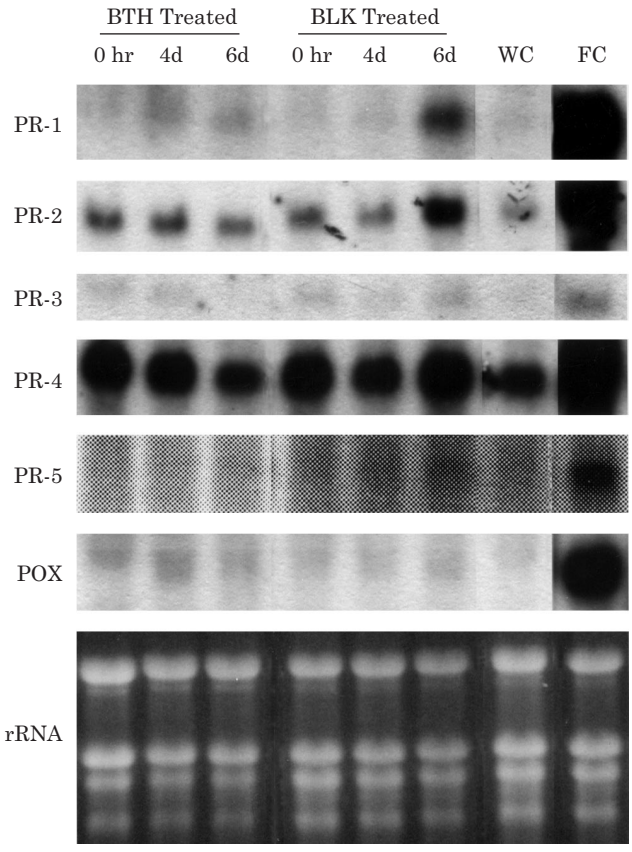

FIG. 2. BTH does not induce defense response gene expression in wheat spikes. Total RNA from cv. Wheaton spikes 0, 4 and 6 days after application of 1 mM BTH and the blank formulation. Total RNA from cv. Wheaton water-sprayed spikes 4 days after application (WC) and 48 h after *F. graminearum* spray-inoculation (FC, [*21*]). RNA gel blots were probed with PR-1, PR-2, PR-3, PR-4, PR-5 and peroxidase (POX). An ethidium bromide stain of the rRNA was used to verify similar RNA loading.

would provide Type I FHB resistance (resistance to initial infection) to the susceptible cv. Wheaton, 1 mM BTH, the blank formulation or water was applied to spikes 3 days before anthesis and then spikes, spray-inoculated with *F. graminearum* at anthesis. The percentage of diseased spikelets was recorded daily from 6 to 12 days after *F. gramineamm* inoculation. In two experiments, it was found that the BTH-treated spikes exhibited a slightly lower level of disease throughout the time period measured [Fig. 3(a) and (b)]. However, statistical analysis showed that there is no significant difference between the treatments and controls. In addition, the area under the disease progress curve was calculated for both experiments and statistical analysis showed that there was no difference between the treatments (data not shown). These data indicate that BTH application does not provide Type I resistance to spray-inoculated spikes.

To determine if the application of BTH would provide Type II FHB resistance (resistance to spread in the spike) to the susceptible cv. Wheaton, 1 mM BTH, a blank formulation or water was applied to wheat spikes approximately 3 days before anthesis and two central spikelets were point-inoculated at anthesis with *F. graminearum*. Eleven days after pathogen inoculation, the percentage of infected spikelets was recorded for each plant. In the two experiments conducted, it was found that over 40 % of the spikelets were infected following BTH treatment (Fig. 4). The percent infection was similar to the amount observed in the blank and water controls. Although the water controls exhibited somewhat less infection than the other treatments, there was no statistical difference between any of the treatments. These results indicate that BTH does not provide Type II resistance to point-inoculated spikes.

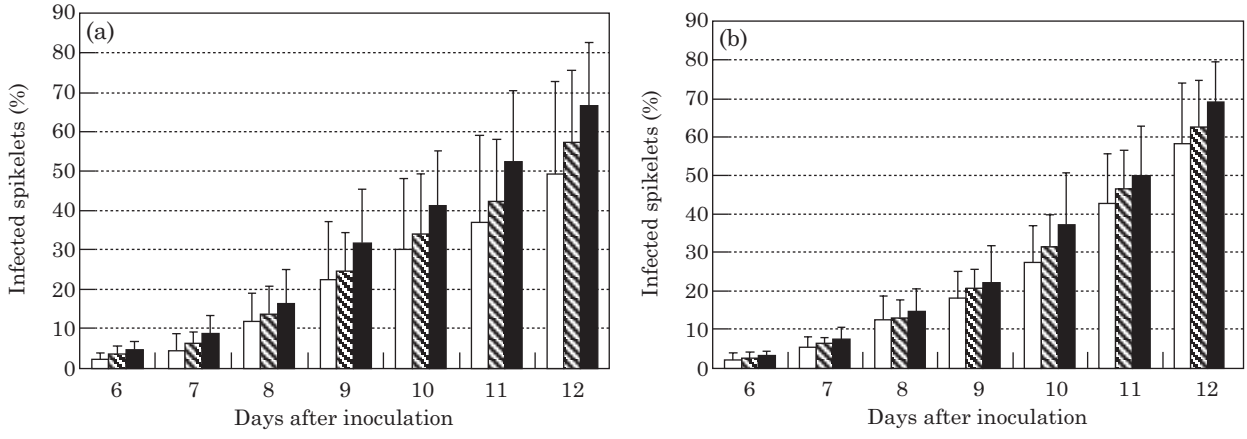

FIG. 3. BTH does not induce Type I resistance to FHB in spray-inoculated wheat spikes. Spikes from cv. Wheaton were treated with 1 mM BTH (□), a blank formulation (▨) and water (■) approximately 3 days before anthesis. At anthesis, wheat spikes were spray-inoculated with a *F. graminearum* conidia water suspension. From 6 to 12 days after inoculation, the percentage of infected spikelets was recorded. The data from two experiments were subjected to ANOVA using SAS. Bars represent the standard deviation of the mean. (a) Experiment 1; (b) experiment 2.

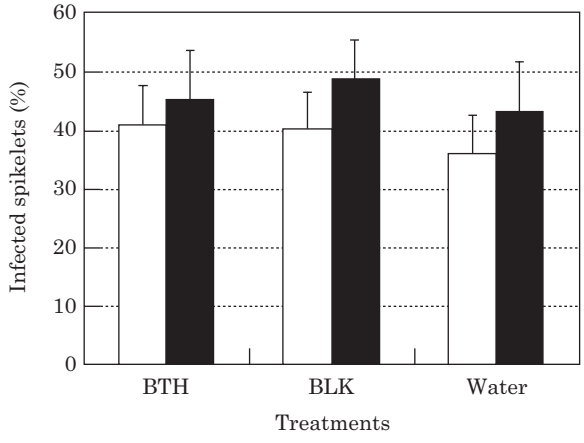

FIG. 4. BTH does not induce Type II resistance to FHB in point-inoculated wheat spikes. Spikes from cv. Wheaton were treated with 1 mM BTH, a blank formulation (BLK) and water approximately 3 days before anthesis. At anthesis, wheat spikes were point-inoculated into the central florets with a *F. graminearum* conidia water suspension. Eleven days after inoculation, the percentage of infected spikelets was recorded. The data from experiments 1 (□) and 2 (■) were subjected to ANOVA using SAS. Bars represent the standard deviation of the mean.

DISCUSSION

BTH and F. graminearum*-induced gene expression are different in wheat spikes*

Our results clearly show that BTH application on wheat spikes induced the expression of the five WCI genes characteristic of BTH treatment, but did not induce the expression of the six defense response genes (Figs 1 and 2). In addition, *F. graminearum* infection induced the expression of the defense response genes but did not induce the WCI genes at 2 days after inoculation (Figs 1 and 2). These results are similar to those obtained by Schaffrath *et al*. [*26*] in wheat leaves treated with BTH or powdery mildew; the WCI genes were induced by BTH application but were not induced by powdery mildew infection. These same authors showed that the wheat induced resistance (WIR) genes, WIR1 (putative cell wall protein), WIR2 (thaumatin-like protein) and WIR3 (peroxidase) were not induced in leaves by BTH. In a similar study, Molina *et al*. [*18*] showed that the defense response genes PR-1·1 and PR-1·2 were induced in *E. graminis*-infected wheat leaves but not in BTH-treated leaves. Moreover, in barley, BTH treatment induced a large set of chemically induced genes; however, application of *B. graminis* did not induce these genes [*2*]. In this study, the previous work has been extended by showing that this differential response exists in wheat spikes with BTH and the *F. graminearum* pathogen. In addition, we have also shown that three additional defense response genes, PR-2 (β-1,3-glucanase), PR-3 (chitinase) and PR-4 are not induced by BTH application. Taken together, our study strengthens the interpretation that BTH- and pathogen-induced genes are regulated through independent pathways in wheat.

Chemical activators of defense response genes induce different genes in wheat compared with other plants

Treatment of plants with chemical activators (e.g. SA, INA and BTH) of defense response genes induces a different set of genes in wheat than in other plants. BTH application in maize induces the expression of genes encoding PR-1 and PR-5 [*19*]. In barley, INA and BTH treatment induces a set of barley chemically-induced genes that are somewhat different from the WCI genes [*2*]. In addition, INA treatment of barley induces genes encoding PR-1, chitinase and peroxidase [*12*]. BTH treatment of tobacco has been shown to induce the expression of a large set of PR genes that are markers for systemic acquired resistance (SAR; [*7*, *24*]). In Arabidopsis, BTH has been shown to induce the expression of the SAR-associated genes, PR-1, PR-2 and PR-5 [*13*, *24*]. Therefore, it appears that in both Arabidopsis and tobacco, BTH induces the SAR signal transduction pathway. Taken together, our results indicate that the molecular response in wheat to chemical activators of defense response genes differs from barley, maize, Arabidopsis and tobacco.

Induced gene expression and FHB resistance

The two principle types of FHB resistance in wheat are Type I and Type II resistance. Type I and Type II mechanisms provide resistance to initial infection and spread of infection in the spike, respectively [*17*]. Type II resistance is the primary type of resistance that wheat breeders utilize to select for more resistant varieties. The susceptible cv. Wheaton used in this study does not possess a measurable level of Type I or Type II resistance. In these experiments, spikes were inoculated 3 days after BTH application. Our results show that the WCI genes are induced within 1 day after BTH treatment and this response does not provide a measurable level of Type I or Type II FHB resistance. Another experiment was also conducted in which BTH was applied 4, 6, 8 and 10 days prior to *F. graminearum* point-inoculation. No induction of Type II resistance was found in these plants (data not shown).

In other studies, the induction of defense response genes in wheat spikes after *F. graminearum* inoculation has been shown [*14*, *21*, *22*]. The induction of six defense response genes (PR-1, PR-2, PR-3, PR-4, PR-5 and peroxidase) after *F. graminearum* inoculation were documented in both the Type II-resistant cv. Sumai 3 and the susceptible cv.

Wheaton [*21*]. However, the expression of PR-4 and PR-5 were earlier and greater in the resistant cv. Sumai 3 than the susceptible cv. Wheaton. In addition, Li *et al*. [*14*] showed that the acidic chitinase and β-1,3-glucanase genes were expressed earlier in Sumai 3 than in a susceptible Sumai 3 mutant. In another study, it was found that accumulation of defense response gene transcripts is systemic in wheat spikes upon *F. graminearum* infection in both susceptible and resistant genotypes [*22*]. Uninoculated regions of point-inoculated spikes expressed the defense response genes but it does not provide protection to head blight fusaria in the susceptible cv. Wheaton. These data indicate that prior defense-response gene expression in uninoculated tissues does not induce FHB resistance. In addition, these data indicate that the systemic induction of defense response genes is not related to Type II resistance but more likely a general host response to infection. Based on the fact that BTH induces the WCI genes and *F. graminearum* infection separately induces the defense response genes, it is assumed that plants treated with BTH and subsequently inoculated with the fungus will express both the WCI genes and the defense response genes. However, it is unlikely that the combination will provide any resistance to FHB.

Development of FHB resistance in wheat may depend upon inducing or constitutively expressing defense response genes before *F. graminearum* infection. In fact, constitutive expression of rice PR-5 (thaumatin-like protein) in transgenic wheat delayed *F. graminearum* infection [*4*]. One of our original goals was to induce expression of the defense response genes before *F. graminearum* infection and therefore test the ability of the defense response genes to provide resistance. Unfortunately, BTH did not induce any of the defense response genes tested. To address this question directly in the future, we are developing transgenic wheat and barley plants with constitutively-expressed defense response genes (Muehlbauer, unpublished results). These plants will be tested for their ability to provide resistance to FHB.

Mechanisms of BTH-induced resistance

BTH-induced resistance is manifest through several potential mechanisms in various plants. In wheat, BTH application and the induction of the WCI genes is correlated with resistance to powdery mildew [*8*]. Resistance was thought to be associated with a lack of plant cell wall penetration and haustoria formation. In tomato, BTH application provides resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici*. The resistance in tomato was shown to be due to rapid and more efficient formation of protective layers at sites of potential fungal entry [*3*]. These include callose-enriched wall apposition and accumulation of phenolic-like compounds on both the host cell wall and the wall appositions. In addition, it has been shown that BTH activates parsley cells for augmented elicitation of defense responses. These defense responses are coumarin phytoalexin secretion and activation of genes encoding phenylalanine ammonia lyase (PAL; [*10*]). PAL has been proposed as a critical enzyme in the pathway leading to products that are related to plant defense, such as salicylic acid, phytoalexins and flavonoids [*29*]. However, in cucumber, BTH application did not provide protection against powdery mildew. Defense responses were weak and sporadic at infection sites on cell walls [*30*]. Since BTH application did not provide a measurable level of resistance to FHB in our work, we did not attempt to study the interaction between the pathogen and the plant in more detail. It is possible that the BTH-induced response is only one component of resistance to specific pathogens. Both a BTH-induced response and a specific pathogenic elicitation may be required to activate an effective defense response.

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