Isolate-Specific Responses of the Nonhost Grass *Brachypodium distachyon* to the Fungal Pathogen *Zymoseptoria tritici* Compared with Wheat

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ABSTRACT

Septoria tritici blotch (STB) is an important foliar disease of wheat that is caused by the fungal pathogen *Zymoseptoria tritici*. The grass *Brachypodium distachyon* has been used previously as a model system for cereal–pathogen interactions. In this study, we examined the nonhost resistance (NHR) response of *B. distachyon* to two different *Z. tritici* isolates in comparison with wheat. These isolates vary in aggressiveness on wheat cultivar Remus, displaying significant differences in disease and pycnidia coverage. Using microscopy, we found that similar isolate-specific responses were observed for hydrogen peroxide accumulation and cell death in both wheat and *B. distachyon*. Despite this, induction of isolate-specific patterns of defense gene expression by *Z. tritici* did differ

between *B. distachyon* and wheat. Our results suggest that expression of the phenylalanine ammonia lyase *PAL* gene may be important for NHR in *B. distachyon*, while pathogenesis-related *PR* genes and expression of genes regulating reactive oxygen species may be important to limit disease in wheat. Future studies of the *B. distachyon*–*Z. tritici* interaction may allow identification of conserved plant immunity targets that are responsible for the isolate-specific responses observed in both plant species.

Keywords: *Brachypodium distachyon*, fungal, fungal pathogens, nonhost, pathogen recognition by plants, wheat, *Zymoseptoria tritici*

Septoria tritici blotch (STB) is caused by the ascomycete fungus *Zymoseptoria tritici*. This disease poses a major threat to wheat production worldwide. STB is a particular concern in countries with humid, temperate climates (Fones and Gurr 2015; Orton et al. 2017).

STB control relies on the use of resistant wheat varieties together with foliar fungicide applications. However, rapid evolution of *Z. tritici* to overcome host resistance and acquire fungicide tolerance is a challenge to control this disease (Cools and Fraaije 2013; Dooley et al. 2016).

The infection cycle of *Z. tritici* includes the entry of the fungus into the host leaf, colonization of plant tissue, and formation of the asexual and sexual fruiting bodies (Steinberg 2015). Following leaf contact, the asexual pycnidiospores (conidia produced from asexual pycnidia) or sexual ascospores (produced in pseudothecia) germinate to form hyphae (Dancer et al. 1999; Palmer and Skinner 2002). Studies have shown that *Z. tritici* may remain epiphytic for 4 to 10 days postinoculation (dpi) and prior to infection via the stomata (Fones et al. 2017; Haueisen et al. 2019). Following infection via the stomatal openings, hyphae colonize the apoplast of the mesophyll cell layer. The infecting hyphae show no evidence of forming either haustoria or other types of specialized feeding structures (Keon et al. 2007). The wheat host remains symptomless for 7 to 10 days and this is dependent

on the variety. Throughout this “latent” phase, there is little or no increase in fungal biomass (Kema et al. 1996; Rudd et al. 2015). It has been suggested that the fungus may utilize internal stores of nutrition such as lipids or fatty acids at this time (Rudd et al. 2015; Steinberg 2015).

The initial recognition of pathogen attack is mediated by the plant’s pattern recognition receptors that bind conserved pathogen-associated molecular patterns (PAMPs) such as fungal chitin (Miya et al. 2007; Robatzek et al. 2016). This is termed PAMP-triggered immunity (PTI). Pathogens can attempt to suppress PTI by secreting effector proteins, which in turn can be detected by plant resistance (R) gene products. However, effectors that evade recognition can manipulate plant cellular and metabolic processes to aid pathogen infection (Boller and He 2009). For example, the LysM domain of the *Z. tritici* effector Mg3LysM binds fungal chitin and acts to “cloak” the invading pathogen from the plant’s immune system (Marshall et al. 2011; Yang et al. 2013). Following the latent phase, *Z. tritici* switches to an aggressive necrotrophic growth stage (Eyal et al. 1987; Yang et al. 2015). This is characterized by an increase in fungal biomass, degradation of the host cell wall, a collapse of mesophyll tissue cells, and the accumulation of hydrogen peroxide (H₂O₂). The loss of cell integrity during the necrotrophic phase has been suggested to share similar characteristics to that of programmed cell death (PCD) (Yang et al. 2013). While PCD may reduce the spread of a biotrophic pathogen, it may also facilitate subsequent *Z. tritici* proliferation by providing a source of nutrition for the necrotrophic phase of growth (Keon et al. 2007). The mechanisms by which the necrotrophic phase is initiated are not entirely clear, although it has been suggested that the transition may be triggered by secreted effector proteins (Yang et al. 2015).

During the final stage of its lifecycle, *Z. tritici* produces asexual and sexual fruiting bodies inside the leaf substomatal cavity, termed pycnidia and pseudothecia, respectively. The pycnidia release asexual pycnidiospores that are dispersed to neighboring plants by rain splash (Steinberg 2015). Sexual reproduction occurs between two compatible *Z. tritici* strains (Mat1-1 and Mat1-2) and forms pseudothecia that appear 25 to 30 days after initial infection,

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generally, on senescent leaf tissue (Sánchez-Vallet et al. 2015; Slimane et al. 2012). These pseudothecia produce sexual ascospores dispersed by wind (Eyal et al. 1987).

Brachypodium distachyon is a grass species related to cereals in the Pooideae. *B. distachyon* has previously been explored as a model system for cereal–pathogen interactions, including for the study of STB (O’Driscoll et al. 2015; Omidvar et al. 2018). Here we compared the responses of *B. distachyon* (nonhost) and wheat (host) following challenge with two different *Z. tritici* isolates, IPO323 (Kema and van Silfhout 1997) and 560.11 (Lynch et al. 2016), using microscopy and gene expression studies. A better understanding of NHR in *B. distachyon* against STB may provide insights into *Z. tritici* isolate aggressiveness and the future identification of plant immunity targets.

MATERIALS AND METHODS

Plant and fungal material. Seeds of wheat (*Triticum aestivum* ‘Remus’) were kindly supplied by Prof. F. Doohan and seeds of *B. distachyon* (Bd21) were provided by Dr. C. Ng (School of Biology and Environmental Science, University College Dublin, Dublin, Ireland) (O’Driscoll et al. 2015; Stępień et al. 2004). Seeds of wild-type *B. distachyon* (Bd21) and the German spring wheat cultivar Remus were stratified at 4°C for 3 days and then incubated in the dark at room temperature for 4 days to allow germination. Germinated seeds were transferred to pots containing one-part vermiculite and three-parts peat soil. The pots were then placed in a growth chamber under a 16-h/8-h light/dark cycle at 24°C/12°C and watered every 2 days.

Z. tritici isolates IPO323 (Kema and van Silfhout 1997) and 560.11 (Lynch et al. 2016) were used for this study. Isolate 560.11 was isolated in Ireland in 2011 (Lynch et al. 2016) and isolate IPO323 was isolated in the Netherlands in 1981 (Kema and van Silfhout 1997). Prior to use, each isolate was cultured on potato dextrose agar (PDA) and grown at 20°C under white light supplemented with blue/black ultraviolet (UV-A) light under a 12-h/12-h light/dark cycle for approximately 7 days (O’Driscoll et al. 2015). *Z. tritici* cultures were grown under UV light to induce asexual sporulation in vitro (Cooke and Jones 1970; Holmes and Colhoun 1971; Kema and Annone 1991; Skinner 2001; Tiley et al. 2018; Zelikovitch and Eyal 1989). Fungal spores from PDA cultures were harvested using deionized water and the spore concentration was adjusted to 1×10^6 spores/ml in water containing 0.02% Tween-20 solution. Each plant was sprayed with 5 ml of spore suspension using hand-held mist spray bottles. Control plants were sprayed with 5 ml of 0.02% Tween-20 solution each. Inoculated plants were then covered with polythene bags for 48 h to ensure high humidity.

Microscopic analysis. For analysis of H₂O₂ accumulation, 3,3’-diaminobenzidine (DAB) staining was performed according to Thordal-Christensen et al. (1997). Leaf sections were placed in DAB (1 mg/ml), pH 3.8, for 4 h in the dark. Leaf samples from *Z. tritici*-challenged, 14-day-old wheat and *Brachypodium* seedlings were placed in 90% ethanol with heat for 10 min to remove chlorophyll. Dead cells and fungal structures were stained using trypan blue according to Koch and Slusarenko (1990) with a minor modification. Briefly, leaf sections were incubated in lactophenol with trypan blue (10 ml of lactic acid, 10 ml of glycerol, 10 ml of phenol, 10 ml of water, and 10 mg of trypan blue) for 10 to 20 min at 95°C. The leaves were rinsed with water three times and destained in chloral hydrate solution (2.5 g/ml of water) overnight. Leaf samples were mounted onto slides with 80% glycerol and examined under a light microscope (Leica DM5500B). For quantitative analysis, cells in the field of view (FoV) were examined at 40× magnification for 0, 2, 7, 9, and 21 dpi. An average of approximately 830 cells in Remus and 1,600 cells in *B. distachyon* were counted per FoV surrounding a spore for both DAB and trypan blue-stained epidermal, guard, and mesophyll cells. The FoV values for two

spores per each of the two leaves ($n = 4$) were counted. This was repeated independently twice ($n = 8$) and averaged. Images were collected over four independent experiments where two leaves (each from different plants) infected with each of the two isolates (560.11 and IPO323) were examined with a light microscope (Leica DM5500B). Images were recorded with a Leica DFC310FX digital camera.

Disease and pycnidia scores. Seedlings of the *Z. tritici*-susceptible wheat cultivar Remus and wild-type *B. distachyon* (Bd21) were inoculated with *Z. tritici* isolate IPO323 or 560.11. Inoculated plants were incubated under high humidity for 48 h and subsequently at normal humidity at 22°C under a 16-h/8-h light/dark cycle. Diseased leaves were harvested at 0, 10, 14, 17, and 21 dpi. The samples were boiled for 10 min in ethanol to remove chlorophyll and were then mounted on glass slides for scoring. High-resolution images of slides were obtained using a flatbed scanner (Epson XP-620). The coverage of necrosis or pigment browning and pycnidia was calculated using ImageJ software for the whole leaf area (Schneider et al. 2012). Two leaves, each from different plants, were used for each time point and this was repeated over three independent experiments.

For pycnidiospore counts, four 1-cm-long leaf sections each from three leaves from individual wheat and *B. distachyon* plants inoculated with *Z. tritici* or control plants were harvested at 31 dpi. Leaves were exposed to 100% humidity for 48 h to induce release of pycnidiospores. The leaf sections were immersed in sterile distilled water and vortexed for 15 s. The suspension was pipetted onto a hemocytometer for assessment of spores released from pycnidia of isolate 560.11 and IPO323 on *T. aestivum* ‘Remus’ and *B. distachyon*. This was repeated three times independently.

***B. distachyon* and wheat-extract agar.** *Z. tritici* isolates IPO323 and 560.11 were grown on Czapek Dox-V8 juice agar (46 g/liter of Czapek Dox agar, 200 ml/liter of V8 Original vegetable juice (Campbell’s), 3 g/liter of calcium carbonate, and 20 g/liter of agar) for 5 to 7 days under white light supplemented with blue/black UV-A light under 16-h/8-h light/dark cycles at 20°C (Kema and Annone 1991). The vegetative, yeast-like *Z. tritici* cells were used to inoculate wheat-extract agar (WEA) (37.5 g/liter of homogenized *T. aestivum* ‘Remus’ leaves of 21-day-old seedlings in 2% agar) and *B. distachyon*-extract agar (BEA) (37.5 g/liter of homogenized *B. distachyon* leaves of 21-day-old seedlings in 2% agar). The inoculated plates were wrapped in foil for 48 h and then incubated as above. Petri dishes were examined every 30 days for approximately 5 months using a Leica M205 C stereo microscope. Images were captured using Leica Application Suite software version 4.4.

RNA extraction and quantitative real-time PCR. For plant gene expression studies, wheat and *B. distachyon* leaves (14-day-old seedlings) were sampled at 0.5, 48, 96, and 144 h postinoculation (hpi) with either of the two *Z. tritici* isolates or following mock treatment (0.02% Tween-20). All samples were immediately frozen in liquid nitrogen and stored at –80°C until use. Total RNA was extracted from 100 mg of plant tissue using the RNeasy Plant Mini Kit (Qiagen, The Netherlands) and subjected to on-column DNase treatment (Sigma). Quantification of total RNA was carried out using a NanoDrop ND-1000 spectrophotometer. Reverse transcription of RNA (1 µg) was carried out using the Omniscript RT Kit (Qiagen) and oligo (dT) primer using the manufacturer’s instructions. Defense marker genes were analyzed by real-time quantitative reverse transcription PCR (qRT-PCR) with primers for the following: *pathogenesis-related* (PR) genes *PR1*, *PR2*, and *PR3*; *nonexpresser of PR gene 1* (*NPR1*); *superoxide dismutase* (*SOD*); *catalase* (*CAT*); *phenylalanine ammonia-lyase* (*PAL*); *12-oxophytodienoate reductase 3* (*OPR3*); and two reference genes, *actin* and *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*), in both wheat and *B. distachyon* (Table 1). qRT-PCR reactions were carried out in 12.5 µl including 1.25 µl of a 1:5 (vol/vol) dilution of cDNA, 0.2 µM of primers, and 1× SYBR Premix Ex Taq (Tli RNase H plus, RR420A; Takara). PCR

conditions were as follows: one cycle of 1 min at 95°C; 40 cycles of 5 s at 95°C and 20 s at 60°C; and a final cycle of 1 min at 95°C, 30 s at 55°C, and 30 s at 95°C for the dissociation curve. The normalized fold change was calculated for each time relative to mock using the $2^{-\Delta\Delta C_t}$ method described by Livak and Schmittgen (2001) and threshold cycle (C_t) reference = geometric mean (C_t GAPDH: C_t Tub). Two independent experiments were carried out. Each experiment included three leaves each from three plants per isolate per time point ($n = 6$). Average C_t and SEM values were calculated from six individual C_t values per isolate per time point.

Statistical analysis. For gene expression studies, statistical analysis was conducted using analysis of variance incorporating the Tukey significant difference test at $P \leq 0.05$ in GraphPad Prism (version 5.03 for Windows; GraphPad Software, San Diego, CA).

All other statistical analysis was performed using the R statistical package (R Core Team 2016). The proportion of disease symptoms and pycnidia per leaf area, the proportion of dead cells stained with trypan blue per FoV, and the proportion of cells showing the presence of H_2O_2 per FoV stained with DAB were analyzed using a generalized linear model with a quasi-binomial distribution (Crawley 2012). Multiple comparison tests were performed using the *emmeans* function of the *emmeans* package.

RESULTS

T. aestivum ‘Remus’ and *B. distachyon* were compared for their response to infection with an Irish isolate (560.11) and a Dutch isolate (IPO323) of *Z. tritici*.

Pathogenicity of the *Z. tritici* 560.11 and IPO323 isolates on wheat. Disease symptoms caused by the *Z. tritici* isolate 560.11 on wheat were more severe compared with those caused by IPO323 (Fig. 1A). At 21 dpi, disease coverage on Remus was significantly ($P < 0.05$) higher on plants inoculated with isolate 560.11 ($74 \pm 9\%$) than with IPO323 ($56 \pm 10\%$) (Fig. 1C). Pycnidia coverage on wheat infected with isolate 560.11 was also significantly ($P < 0.05$) higher at 14 dpi ($23 \pm 6\%$) and 21 dpi ($74 \pm 9\%$) compared with infection with IPO323 at 14 dpi ($2 \pm 1\%$) and 21 dpi ($45 \pm 9\%$), respectively (Fig. 1E).

***B. distachyon* nonhost response to *Z. tritici* isolates 560.11 and IPO323.** A macroscopic response was observed in

B. distachyon to infection with both of the *Z. tritici* isolates (Fig. 1B). *Z. tritici* isolates on *B. distachyon* caused the production of brown pigments (polyphenols) (Fig. 1B; Supplementary Fig. S1). Levels of pigment accumulation on *B. distachyon* plants inoculated with the 560.11 isolate were significantly ($P < 0.05$) higher at 14 dpi ($58 \pm 9\%$) and 21 dpi ($70 \pm 5\%$) compared with leaves inoculated with isolate IPO323 at 14 dpi ($32 \pm 6\%$) and 21 dpi ($44 \pm 8\%$) (Fig. 1D).

***Z. tritici* isolate interactions with the nonhost grass *B. distachyon*.** Infected leaves were examined microscopically to determine whether the symptoms observed on *B. distachyon* with *Z. tritici* isolate 560.11 were associated with any fungal colonization (Fig. 2). *Z. tritici* isolate 560.11 formed intracellular hyphae within the mesophyll cell layer of *B. distachyon* (Fig. 2F to J) comparable to that found in the wheat cultivar Remus (Fig. 2B to E). Typically, invading hyphae grow, branch, and fuse extensively in the substomatal cavities to form pycnidia, which are dark elliptical or round structures (Hilu and Bevee 1957; Shetty et al. 2003). Rarely, a few immature pycnidia-like structures were observed on *B. distachyon* inoculated with *Z. tritici* isolate 560.11 after 28 dpi (Fig. 2H to J), which were similar to those observed on Remus at 28 dpi (Fig. 2C to E). However, no pycnidiospores were released from the immature pycnidia in *B. distachyon*. Remus pycnidia released pycnidiospores via oozing cirrus, which were most abundant per square centimeter with isolate 560.11 (Supplementary Fig. S2).

***Z. tritici* pycnidia production on *B. distachyon* leaf extract.** The ability of *Z. tritici* to produce pycnidia in *B. distachyon* was investigated further by inducing asexual sporulation in vitro on WEA and BEA. This enabled the uncoupling of the *B. distachyon* nonhost response with the ability of this grass to support *Z. tritici* asexual sporulation.

Previous studies have shown that asexual sporulation can be established in *Z. tritici* by incubating the fungus on WEA under white light supplemented with fluorescent blue/black UV-A light, but sporulation cannot be established on water agar alone (Cooke and Jones 1970; Kema and Annone 1991; Skinner 2001; Tiley et al. 2018; Zelikovitch and Eyal 1989).

Both *Z. tritici* isolates IPO323 and 560.11 produced hyphal knots and pycnidia on WEA and BEA (Fig. 3). By 60 dpi, both *Z. tritici* isolates produced white lateral hyphae that radiated across the surface of the agar, away from the point of inoculation. These

TABLE 1. Primers and references used for the analysis of transcript profiles from *Zymoseptoria tritici*-infected leaves

Gene ^a	Forward primer	Reverse primer	Study
<i>TaPR1</i>	CAATAACCTCGGCTCTTCATCAC	TTATTTACTCGCTCGGTCCCTCTG	Casassola et al. (2015)
<i>TaGAPDH</i>	GGCCGGGATGTGCTCTGAACG	TGGTGCTGTGCATGTGACGG	Guo et al. (2011)
<i>Ta-ATub</i>	ATCTCCAACCTCCACAGTGTCG	TCATCGCCCTCATCACCGTC	Ding et al. (2016)
<i>TaCAT</i>	CCATGAGATCAAGGCCATCT	ATCTTACATGCTCGGCTTGG	Sheoran et al. (2015)
<i>TaMnSOD</i>	CAGAGGGTGCTGCTTTACAA	GGTCACAAGAGGGTCTCTGAT	Sheoran et al. (2015)
<i>TaPAL</i>	CAAGATGGTCGAGGCTTACC	CGAAGTCGATCATGAAGCAA	Casassola et al. (2015)
<i>TaOPR3</i>	TCGCCCTTCATGGACTACATG	TAGAGGATGCCGTGGTCTGTT	This study
<i>TaPR3</i>	CCTCCATTATCTCGCAGTCGCTC	CGCCGTAGTTGTAGAACCCTTG	Ding et al. (2016)
<i>TaPR2</i>	CGCCAACGTGTACCCTACTT	TCTCGGAAATCACACCTTCAC	Ding et al. (2016)
<i>BdTUA6</i>	ACCAACCTTGTGCCCTATCC	GTCACACAGTCAACAACTG	Hong et al. (2008)
<i>BdPAL</i>	ATTACGGCTATCCTTGCTGAGG	AGGAGCTTCCTTCCAAGATGTG	Gill et al. (2015)
<i>BdNPR1</i>	AGCTTCAACTCGACAGCAT	CGATCACCATCATTTGAGC	Kouzai et al. (2016)
<i>BdPR2</i>	CATCAACTCCATGCGGATCTAC	GGCGATGTACTTGATTTGACC	Gill et al. (2015)
<i>BdGADPH</i>	TTGCTCTCCAGCGATGAC	CTCCACGACATAATCGGCAC	Hong et al. (2008)
<i>BdPR1</i>	AGCTCTGGCATCATCAGCATCC	CGTTGTGTGGGTCCAGGAAATC	Mandadi and Scholthof (2012)
<i>BdPR3</i>	GCTCGGCTGATTGTCAACACG	TTGCCCGAACCACAAATATGCC	Mandadi and Scholthof (2012)
<i>BdOPR3</i>	ACCCATTCTTCTCGAATGATCCC	ACACGTGCAAGTACGGAAAGAAAG	Mandadi and Scholthof (2012)
<i>BdCAT</i>	GACTATTCTCCGTCAGGTTTC	CCTTCTCGATCAACATCTTCTC	Wang et al. (2017)
<i>BdMnSOD</i>	GCGCAATCAAGTTCAACGG	TCACCACCACCTCACTG	Glover-Cutter et al. (2014)
<i>ZtTUB</i>	ATCTACCGCGGAAAGGTGTCCA	TGGTCACCGACACGCTTAAAGAG	Rudd et al. (2015)

^a *Ta* = *Triticum aestivum*, *PR* = pathogenesis-related, *GAPDH* = glyceraldehyde 3-phosphate dehydrogenase, *ATub* = alpha tubulin, *CAT* = catalase, *MnSOD* = manganese-dependent superoxide dismutase, *PAL* = phenylalanine ammonia lyase, *OPR* = oxophytodieneoate reductase, *Bd* = *Brachypodium distachyon*, *TUA* = alpha tubulin, *NPR* = nonexpresser of *PR*, *Zt* = *Zymoseptoria tritici*, and *TUB* = beta tubulin.

hyphae formed white hyphal knots, which are the initial stages of pycnidia development. By 5 months postinoculation, the hyphal knots had developed into dark-brown, globose pycnidia that were approximately 50 to 100 μm in diameter (Fig. 3). These structures

resembled pycnidia observed in planta on wheat cultivar Remus isolates, and some oozed a cirrus-like substance. Only the *Z. tritici* 560.11 isolate consistently formed pycnidia on BEA over two independent experiments (Fig. 3).

Fig. 1. Progression of symptoms on **A**, *Triticum aestivum* ‘Remus’ and **B**, *Brachypodium distachyon* induced by *Zymoseptoria tritici* isolate IPO323 and 560.11. Infected leaves were cleared of chlorophyll by boiling in ethanol for 10 min and symptoms were recorded at 0, 10, 14, 17, and 21 days postinoculation (dpi). Two leaves each from a different plant were examined per time point. Images are representative of three independent experiments. Scale bars = 2 mm. **C**, Disease coverage on Remus and **D**, pigment coverage on *B. distachyon* at 0, 10, 14, 17, and 21 dpi. **E**, Pycnidia coverage on Remus. High-resolution images of diseased leaves (one per seedling of two seedlings) were obtained and coverage of symptoms and coverage of pycnidia were measured using ImageJ software. Each data point represents the mean $\pm$ SEM of three independent experiments. *P* values shown above bars are differences between isolates using the Tukey significant difference test at $P \leq 0.05$.

H₂O₂ accumulation and cell death during the host wheat–*Z. tritici* interaction. Wheat leaves inoculated with the two *Z. tritici* strains were stained with DAB (Thordal-Christensen et al. 1997) and trypan blue (Koch and Slusarenko 1990) to assess H₂O₂ accumulation and cell death, respectively, at 0, 2, 7, 9, and 21 dpi (Figs. 4 and 5; Supplementary Figs. 3B and 4B).

Following inoculation, the cells in the FoV surrounding the site of *Z. tritici* spores were assessed for H₂O₂ accumulation by DAB staining. The percentage of Remus cells with H₂O₂ accumulation following infection with isolate IPO323 significantly increased between 0 dpi ($1 \pm 0.2\%$) and 2 dpi ($12 \pm 2\%$), before peaking at 9 dpi ($25 \pm 6\%$). A similar pattern of H₂O₂ accumulation was observed following infection with isolate 560.11; cells stained for H₂O₂ production increased to $29 \pm 6\%$ at 2 dpi before peaking at 9 dpi ($44 \pm 7\%$) (Fig. 4A).

To determine responses in epidermal, guard, and mesophyll cells, these different cell types were assessed for the percentage of cells stained for H₂O₂ accumulation. No significant differences in the percentage of guard or epidermal cells accumulating H₂O₂ were found between the isolates at any of the time points. However, a significantly higher percentage of mesophyll cells was found to accumulate H₂O₂ in response to isolate 560.11 ($56 \pm 7\%$) compared with IPO323 ($16 \pm 3\%$) at 2 dpi. The highest percentage of H₂O₂ accumulation in wheat mesophyll cells was at 9 dpi in response to 560.11 ($73 \pm 4\%$), which was significantly higher than found in response to IPO323 ($32 \pm 4\%$) (Fig. 4B).

Following challenge with isolate IPO323, the percentage of dead cells stained by trypan blue in wheat significantly increased between 0 dpi ($0.3 \pm 0.2\%$) and 9 dpi ($17 \pm 5\%$) before leveling off. A significant increase in the percentage of dead cells was observed from 0 dpi ($0.2 \pm 0.2\%$) to 7 dpi ($5 \pm 2\%$) and between 9 dpi ($19 \pm 6\%$) and 21 dpi ($26 \pm 5\%$) following challenge with isolate 560.11 (Fig. 5A).

No significant differences in the percentage of cell death in guard or epidermal cells were found when comparing between the isolates. However, a significantly higher percentage of dead

mesophyll cells at 21 dpi was found in response to isolate 560.11 ($20 \pm 5\%$) compared with IPO323 ($7 \pm 1\%$) (Fig. 5B).

H₂O₂ accumulation and cell death during the *B. distachyon* nonhost–*Z. tritici* interaction. *B. distachyon* leaves were stained for H₂O₂ production with DAB (Thordal-Christensen et al. 1997) and for cell death using trypan blue (Koch and Slusarenko 1990) at 0, 2, 7, 9, and 21 dpi following challenge with isolate 560.11 and IPO323 (Figs. 4 and 5; Supplementary Figs. 3A and 4A).

Following inoculation, *B. distachyon* cells in the FoV surrounding the site of *Z. tritici* spores were assessed for H₂O₂ accumulation by DAB staining. After inoculation with isolate IPO323, the percentage of *B. distachyon* cells stained for H₂O₂ increased significantly between 0 dpi ($0.2 \pm 0.1\%$) and 7 dpi ($22 \pm 4\%$) before peaking at 9 dpi ($30 \pm 5\%$). A significant increase in the percentage of cells stained for H₂O₂ following 560.11 infection was observed earlier at 2 dpi ($28 \pm 4\%$) before peaking at 9 dpi ($48 \pm 5\%$) (Fig. 4A).

In *B. distachyon*, the percentage of epidermal cells stained for H₂O₂ accumulation in response to isolate 560.11 ($21 \pm 4\%$) was significantly higher than in response to isolate IPO323 ($2 \pm 1\%$) at 2 dpi (Fig. 4B). In contrast, such a significant difference was not observed in wheat (Fig. 4B). *B. distachyon* also had a significantly higher percentage of mesophyll cells stained for H₂O₂ accumulation in response to isolate 560.11 compared with IPO323 at 2, 7, 9 and 21 dpi. The highest percentage of DAB-stained mesophyll cells was observed in response to isolate 560.11 ($77 \pm 5\%$) at 9 dpi compared with $47 \pm 7\%$ in response to IPO323 (Fig. 4B).

Following challenge with isolate IPO323, the percentage of cells stained with trypan blue for cell death significantly increased between 0 dpi ($0.6 \pm 0.3\%$) and 7 dpi ($6 \pm 2\%$), with a further increase not observed until 21 dpi ($14 \pm 4\%$) (Fig. 5A). Following challenge with 560.11, a significant increase in the percentage of dead cells was observed between 0 dpi ($0.5 \pm 0.3\%$) and 7 dpi ($14 \pm 5\%$) before peaking at 21 dpi ($22 \pm 6\%$) (Fig. 5A).

No significant differences were observed in *B. distachyon* for epidermal or guard cell death with the exception of 7 dpi, in which a

Fig. 2. Images of *Zymoseptoria tritici* growth (isolate 560.11) in *Triticum aestivum* ‘Remus’ and *Brachypodium distachyon* leaves following staining with trypan blue. **A**, *Z. tritici* hypha interacting with a stomata in cultivar Remus at 2 days postinoculation (dpi). **B**, Hyphae growing in the apoplast along cultivar Remus mesophyll cells at 7 dpi. **C**, Immature pycnidia forming and mesophyll cell death at 21 dpi. **D and E**, Dark elliptical pycnidia in Remus stomata at 28 dpi. **F and G**, *Z. tritici* hyphae interacting with a stomata in *B. distachyon* at 9 dpi. **H**, Hyphae growing in the apoplast along mesophyll cells in *B. distachyon* at 9 dpi. **I**, Dark elliptical immature pycnidia formation in *B. distachyon* at 28 dpi. **J**, Same field of view as I but different field of plane, focus on hyphae growing in the apoplast along mesophyll cells in *B. distachyon*. Black arrowheads indicate hyphae interacting with a stomata, black arrows indicate hyphae, and white asterisks indicate pycnidia. Scale bars = 25 µm.

significantly higher percentage of dead guard cells was found in response to isolate 560.11 ($41 \pm 11\%$) compared with IPO323 ($13 \pm 4\%$) (Fig. 5B). In mesophyll cells, a significantly higher percentage of dead cells was found in response to isolate 560.11 at 9 dpi ($17 \pm 4\%$) and 21 dpi ($14 \pm 2\%$) compared with IPO323 at 9 dpi ($3 \pm 1\%$) and 21 dpi ($7 \pm 1\%$) (Fig. 5B).

Defense gene expression following challenge of wheat and *B. distachyon* with the two *Z. tritici* isolates. qRT-PCR was performed to assess the induction and relative defense gene expression in wheat and *B. distachyon* during the early stages after inoculation (0.5, 48, 96, and 144 hpi) with the two different *Z. tritici* isolates.

Salicylic acid signaling pathway marker gene expression. To explore a potential role for the salicylic acid (SA) signaling pathway, the expression of *PR1*, *PR2*, *NPRI*, and *PAL* was investigated.

TaPR1 transcripts were significantly induced in wheat inoculated with isolate IPO323 compared with mock controls at all time points analyzed. Wheat seedlings inoculated with isolate 560.11 had significantly induced *TaPR1* levels only at 144 hpi. Significant differences were observed between seedlings treated with the two different isolates at 96 hpi, where *TaPR1* levels increased 28-fold following isolate IPO323 infection compared with a fourfold induction following 560.11 infection. Conversely, at 144 hpi, *TaPR1* levels were significantly higher following isolate 560.11 infection (34-fold induction) compared with IPO323 (18-fold induction) (Fig. 6A).

Both *TaPR2* and *TaNPRI* transcripts were significantly higher in wheat plants infected with isolate IPO323 at 0.5 hpi compared with those inoculated with isolate 560.11 (Fig. 6C and E). *TaPR2* also had significantly higher levels following inoculation with isolate IPO323 than 560.11 at 144 hpi (Fig. 6C).

BdPR1 transcripts were only significantly induced at 144 hpi by inoculation with isolate IPO323 (Fig. 6B). No significant differences in relative transcript levels of *BdPR2* and *BdNPRI* were found in *B. distachyon* plants inoculated with either of the two *Z. tritici* isolates (Fig. 6D and G).

No significant induction of *TaPAL* in wheat was observed compared with the mock control with both isolates (Fig. 6F). However, a significant induction of *BdPAL* was observed in *B. distachyon* relative to the mock control at 48 and 96 hpi when challenged with isolate 560.11 and was significantly different compared with induction by isolate IPO323 at 48, 96, and 144 hpi (Fig. 6H).

Jasmonic acid signaling pathway marker gene expression. To explore the role of the jasmonic acid (JA) signaling pathway during *Z. tritici* infection, the expression of *PR3* and *OPR3* was investigated.

TaPR3 transcript accumulation was induced significantly at 0.5, 48, and 96 hpi in wheat plants infected with isolate IPO323 compared with mock inoculation and this was significantly different to induction by 560.11 at 0.5 hpi (Fig. 7A). In *B. distachyon* plants challenged with isolate IPO323, *BdPR3* transcripts were significantly induced relative to the mock control at 48 and 144 hpi. Following inoculation with the two different *Z. tritici* isolates, there was a significant difference in transcript accumulation of *BdPR3* at 48 and 96 hpi where isolate 560.11 induced a higher transcript level (Fig. 7B). Levels of *TaOPR3* and *BdOPR3* transcripts were not induced following infection with either *Z. tritici* isolate (Fig. 7C and D).

Marker gene expression for reactive oxygen species production and turnover. To further explore the production and turnover of reactive oxygen species (ROS; O_2^- and H_2O_2), the expression of *SOD* and *CAT* was investigated.

Significant induction of *TaSOD* was observed at all timepoints of wheat infected with isolate IPO323 and the transcript levels were significantly higher compared with infection with isolate 560.11

(Fig. 8A). *TaCAT* was significantly induced by isolate IPO323 at 48 hpi compared with the mock control and was significantly higher compared with inoculation of wheat with 560.11 (Fig. 8C). In *B. distachyon*, *BdSOD* was induced relative to the control at 48 and 144 hpi, but there was no significant difference between isolate inoculations (Fig. 8B). In contrast, *BdCAT* was induced and differentially expressed between *B. distachyon* plants inoculated with the two different isolates. *BdCAT* transcript levels were significantly higher at 0.5 hpi following challenge with isolate IPO323, while 560.11 induced higher transcript levels at 48 and 96 hpi (Fig. 8D).

DISCUSSION

In previous studies, symptoms caused by *Z. tritici* on *B. distachyon* were found to be similar to an incompatible interaction with the wheat host. Either no stomatal penetration was observed or infection hyphae were blocked in the substomatal cavities of *B. distachyon*. In both of these cases, no intracellular infection was detected with *Z. tritici* isolate IPO323 (Habig et al. 2020; O'Driscoll et al. 2015). However, in this study, we found isolate-specific responses when comparing the interaction between the wheat host and the nonhost grass *B. distachyon* using the *Z. tritici* isolates, 560.11 (Lynch et al. 2016) and IPO323 (Kema and van Silfhout 1997). During the early stages of infection, both isolates showed an increase in biomass on *B. distachyon*, suggested by an increase in *ZiTUB* transcripts between 0.5 and 48 hpi (Supplementary Fig. S5). Microscopic examination showed that although isolate IPO323 was unable to colonize *B. distachyon*, isolate 560.11 achieved stomatal penetration and intracellular growth. Furthermore, possible immature pycnidia-like formation occurred in *B. distachyon* following infection with isolate 560.11 but these were rare (Fig. 2). However, as the *B. distachyon* seedlings were not observed after 31 days, it is possible that these pycnidia do develop further and eventually mature. Further evidence that *B. distachyon* has the nutrients needed by *Z. tritici* to make pycnidia was supported by *Z. tritici* growth and pycnidia formation on BEA (Fig. 3). Although pycnidia-like structures were observed on *B. distachyon*, we were unable to obtain pycnidiospores from the immature pycnidia (Supplementary Fig. S2). A possibility for the

Fig. 3. *Zymoseptoria tritici* isolates IPO323 and 560.11 pycnidia formation on *Triticum aestivum* 'Remus' wheat extract agar (WEA) and *Brachypodium distachyon*-extract agar (BEA) at 5 months postinoculation. Isolates were incubated under white light supplemented with blue/black ultraviolet-A light on 16-h/8-h light/dark cycles at 20°C. Images are representative of two independent experiments and two technical repeats (plates). Black arrowheads indicate pycnidia and white arrowheads indicate cirrus.

lack of sporulation is that the smaller substomatal cavities in *B. distachyon* prevent mature pycnidia formation and/or pycnidiospore release, since the guard cells of *B. distachyon* are around half the size of those in wheat (Fig. 2). Immature nonsporulating pycnidia have been previously reported for *Z. tritici* infections of

chlorophyll-deficient wheat leaves silenced for wheat phytoene desaturase and the magnesium chelatase subunit H, demonstrating that pycnidia formation can be uncoupled from sporulation (Lee et al. 2015). The immature pycnidia we observed on *B. distachyon* were also similar to those formed by *ZtVf1* deletion mutants; *ZtVf1*

Fig. 4. A, Proportion of infected *Triticum aestivum* 'Remus' and *Brachypodium distachyon* cells with hydrogen peroxide (H_2O_2) accumulation. B, Proportion of Remus and *B. distachyon* epidermal, guard, and mesophyll cells with H_2O_2 accumulation. Leaves infected with IPO323 and 560.11 were stained with 3,3'-diaminobenzidine (DAB) at 0, 2, 7, 9, and 21 days postinoculation (dpi) and mounted onto microscope slides with 80% glycerol. For each experiment, fields of view for *Zymoseptoria tritici* spores were examined, two per each of two leaves (independent plants). Bars represent the mean of two independent experiments ($n = 8$) and error bars indicate $\pm$ SEM. Different letters denote significant differences between Remus/*B. distachyon* and IPO323/560.11 at each time point ($P \leq 0.05$) using a generalized linear model with a quasi-binomial distribution (Crawley 2012).

encodes for a transcription factor required for full virulence of *Z. tritici* (Mohammadi et al. 2017).

Since isolate 560.11 was able to penetrate and intracellularly colonize *B. distachyon*, this suggests that this isolate can partially overcome the preinvasive and initial PTI responses of *B. distachyon*.

Such PTI responses are hypothesized as the means by which NHR is conferred in distantly related plants, with nonhost pathogens lacking the capabilities to overcome PTI. This may be attributable to a lack of effector targets in the nonhost or an inability of the pathogen to manipulate nonhost plant targets (Stam et al. 2014).

Fig. 5. A, Proportion of dead cells following infection with *Triticum aestivum* 'Remus' and *Brachypodium distachyon*. B, Proportion of Remus and *B. distachyon* epidermal, guard, and mesophyll dead cells. Leaves infected with IPO323 and 560.11 were stained with 3,3'-diaminobenzidine (DAB) at 2, 7, 9, and 21 days postinoculation (dpi) and mounted onto microscope slides with 80% glycerol. For each experiment, fields of view for *Zymoseptoria tritici* spores were examined, two per each of two leaves (independent plants). Bars represent the mean of two independent experiments ($n = 8$) and error bars indicate $\pm$ SEM. Different letters denote significant differences between Remus/*B. distachyon* and IPO323/560.11 at each time point ($P \leq 0.05$) using a generalized linear model with a quasi-binomial distribution (Crawley 2012).

There is evidence that in some cases, such responses can be manipulated depending on the effector complement in the invading pathogen. For example, RXLR effectors from *Phytophthora infestans* were found to suppress PTI responses in both the tomato host and the nonhost *Arabidopsis* (Stam et al. 2014; Zheng et al. 2014). It has been proposed that effector-triggered immunity may also contribute to NHR, since isolate-specific responses were found in both *B. distachyon* and oat (*Avena sativa*) to isolates of the crown rust oat pathogen *Puccinia coronata* f. sp. *avenae* (Omidvar et al. 2018). It is therefore possible that the ability of isolate 560.11 to initiate infection in *B. distachyon* results from the ability of effectors secreted by this isolate to suppress plant immunity. Indeed, it is unlikely that isolate 560.11 is the only strain of *Z. tritici* with the capacity to do so, and future studies may be able to utilize the interactions of *B. distachyon* with aggressive isolates such as 560.11 to identify the effectors responsible.

ROS such as H_2O_2 are believed to play a role in both host and NHR responses to pathogen invasion (Hückelhoven and Kogel 2003; Nimchuk et al. 2003). For example, H_2O_2 accumulation at the site of *Z. tritici* penetration has been associated with resistance (Shetty et al. 2003, 2007). Despite this, significant epidermal H_2O_2 accumulation and cell death have also been reported at 13 dpi in a compatible *Z. tritici* reaction with the wheat cultivar Sevin (Shetty et al. 2003). Our results are in agreement with the accumulation of H_2O_2 promoting *Z. tritici* infection, since we observed a peak of H_2O_2 accumulation at 9 dpi in both the wheat and *B. distachyon* interaction with *Z. tritici*. In addition, the percentage of cells with H_2O_2 accumulation was higher in response to the aggressive isolate 560.11 (Fig. 4). Indeed, the H_2O_2 accumulation observed at 9 dpi correlates with the switch from the latent to necrotrophic phase (Eyal et al. 1987; Yang et al. 2015). SOD and CAT are known to break down superoxide radicals (O_2^-) to H_2O_2 and to break down H_2O_2 , respectively. The regulation of these enzymes plays a key

role in antioxidant defense of plants against pathogens (Mai et al. 2013; Zhang et al. 2008). Although wheat SOD (*TaSOD*) expression was significantly lower in leaves infected with *Z. tritici* isolate 560.11 than IPO323 over the majority of time points examined, *TaCAT* expression was significantly lower at 48 hpi. Similarly, significantly lower expression levels of *BdCAT* were observed at 0.5 hpi in *B. distachyon* inoculated with isolate 560.11 compared with IPO323. Therefore, a low H_2O_2 turnover by CAT early during infection by isolate 560.11 in wheat and *B. distachyon* may explain the H_2O_2 accumulation observed (Fig. 4).

Significant isolate-specific differences were found for H_2O_2 accumulation in mesophyll cells from 2 dpi and over the majority of timepoints assessed for both wheat and *B. distachyon*. In addition, significantly more epidermal cells stained for H_2O_2 were found in *B. distachyon* at 2 dpi following challenge with isolate 560.11. Together our data suggest that H_2O_2 accumulation in cells associated with *Z. tritici* occurs as early as 2 dpi in both a compatible host and nonhost response, and that this is promoted by aggressive isolates. Since the level of H_2O_2 accumulation in cells is related to the isolate, it may suggest recognition via PTI or the manipulation of PTI conserved between both wheat and *B. distachyon*.

Cell death caused by the accumulation of ROS may trigger the switch to the necrotrophic phase of the *Z. tritici* life cycle, providing nutrients and energy for the fungus to sporulate; conversely, early cell death may limit pycnidia formation (Lee et al. 2015; Shetty et al. 2003). Previous observations during an incompatible interaction in the resistant cultivar Stakado did not show cell death until 15 dpi (Shetty et al. 2003). In our study, we recorded significant increases in cell death from 2 dpi in the nonhost grass *B. distachyon* and from 7 dpi in the wheat host (predominantly in guard cells) (Fig. 5). It is conceivable that such early cell death in guard cells could arrest stomatal penetration and further studies

Fig. 6. Expression profile of pathogenesis-related (PR) genes **A and B**, *PR1*, **C and D**, *PR2*, **E and G**, *nonexpresser of PR gene 1 (NPR1)*, and **F and H**, *phenylalanine ammonia-lyase (PAL)* genes in *Triticum aestivum* in **A, C, E, and F** and *Brachypodium distachyon* in **B, D, G, and H** after infection by *Zymoseptoria tritici*. Real-time quantitative reverse transcription PCR was used to assess gene expression (fold change) in leaves infected with *Z. tritici* isolates IPO323 and 560.11 relative to mock inoculations. Normalization was carried out using *actin* and *glyceraldehyde 3-phosphate dehydrogenase* of *T. aestivum* and *B. distachyon*, respectively. Results represent the mean of three leaves (each from individual plants) per isolate per time point over two independent experiments ($n = 6$) (error bars indicate $\pm$ SEM). Means with the same letters are not significantly different, while means with asterisks are significantly different from the mock-treated control determined using the Tukey test at $P \leq 0.05$.

could elucidate the effects of guard cell death on attempted *Z. tritici* entry. Only at 21 dpi were isolate-specific differences in cell death observed on wheat, where cell death was higher following infection with isolate 560.11 (Figs. 1 and 5). The similar levels of cell death at earlier time points in wheat is unexpected, given that pycnidia formation was higher for isolate 560.11 at 14 dpi as well as 21 dpi (Fig. 1). Nonetheless, a previous study found that necrosis and pycnidia formation are largely independent (Karisto et al. 2018).

Polymerization of phenylpropanoid by peroxidase in the presence of H₂O₂ may form a chemical and/or physical barrier to *Z. tritici* (Barceló 1997; Ors et al. 2017). PAL catalyses the production of cinnamic acid from phenylalanine, which can be converted into phenolic compounds and lignin precursors. These compounds are in turn oxidized and polymerized into brown pigments (polyphenols) (Ngadze et al. 2012). Both *Z. tritici* isolates caused the production of such brown pigments (polyphenols) on *B. distachyon*. Despite the accumulation of brown pigmentation following infection with both *Z. tritici* isolates, browning was significantly higher for isolate 560.11 (Fig. 1B; Supplementary Fig. S1). This is consistent with the higher transcript levels of *BdPAL* (48 to 144 hpi) found in *B. distachyon* infected with isolate 560.11 (Fig. 6H). We found no induction of *TaPAL* by either *Z. tritici* isolate in

the susceptible wheat cultivar Remus (Fig. 6F). However, expression of wheat *TaPAL* was previously reported to be induced by *Z. tritici* and was higher in the STB semi-resistant wheat cultivar Premio compared with the susceptible cultivar Alixan (Ors et al. 2017).

Higher induction of *PR* genes against *Z. tritici* has been observed in resistant wheat cultivars, whereas little induction of these genes was observed in susceptible cultivars at initial stages of infection (Adhikari et al. 2007; Ray et al. 2003; Shetty et al. 2003). We observed that *TaPR1*, *TaPR2*, and *TaNPR1* were induced in wheat following *Z. tritici* infection and this was earlier and transcript levels were higher with the less aggressive isolate IPO323 (Fig. 6A, C, and E). This suggests that SA-mediated signaling is triggered in wheat possibly as a defense response against *Z. tritici*. In previous research, higher expression of *PR1* was observed in the resistant wheat cultivar Atigo compared with the susceptible cultivar Alixan (Ors et al. 2017).

The role of plant hormones in defense against pathogen infection has been well characterized (Bari and Jones 2009; Pieterse et al. 2009) but remains unclear with respect to STB. The JA signaling pathway mediates defense against necrotrophic and hemibiotrophic pathogens (Li and Yen 2008). We observed no differential expression of *TaPR3* and *TaOPR3* between the isolates on infected

Fig. 7. Expression profile of **A and B**, *pathogenesis-related 3 (PR3)* and **C and D**, *oxophytodienoate reductase 3 (OPR3)* genes in *Triticum aestivum* in A and C and *Brachypodium distachyon* in B and D after infection by *Zymoseptoria tritici*. Real-time quantitative reverse transcription PCR was used to assess gene expression (fold change) in leaves infected with *Z. tritici* isolates IPO323 and 560.11 relative to mock inoculations. Normalization was carried out using *actin* and *glyceraldehyde 3-phosphate dehydrogenase* of *T. aestivum* and *B. distachyon*, respectively. Results represent the mean of three leaves (each from individual plants) per isolate per time point over two independent experiments ($n = 6$) (error bars indicate $\pm$ SEM). Means with the same letters are not significantly different, while means with asterisks are significantly different from the mock-treated control determined using the Tukey test at $P \leq 0.05$.

Fig. 8. Expression of **A and B**, superoxide dismutase (SOD) and **C and D**, catalase (CAT) genes in *Triticum aestivum* in A and C and *Brachypodium distachyon* in B and D after infection by *Zymoseptoria tritici*. Real-time quantitative reverse transcription PCR was used to assess gene expression (fold change) in leaves infected with *Z. tritici* isolates IPO323 and 560.11 relative to mock inoculations. Normalization was carried out using *actin* and *glyceraldehyde 3-phosphate dehydrogenase* of *T. aestivum* and *B. distachyon*, respectively. Results represent the mean of three leaves (each from individual plants) per isolate per time point over two independent experiments ($n = 6$) (error bars indicate $\pm$ SEM). Means with the same letters are not significantly different, while means with asterisks are significantly different from the mock-treated control determined using the Tukey test at $P \leq 0.05$.

wheat, with the exception of *TaPR3* induction by isolate IPO323 at 0.5 hpi (Fig. 7A). *BdPR3* transcripts were induced in *B. distachyon* following *Z. tritici* inoculation and this was higher at 48 and 96 hpi with isolate 560.11 than IPO323 (Fig. 7B).

Taken together, the expression profiles of these defense marker genes suggest that induction of SA-mediated PR genes may inhibit STB disease progression in wheat but that aggressive isolates such as 560.11 may be able to interfere with this, conceivably, and this may be effector mediated. In contrast, the phenylpropanoid pathway appears to be more important for the *B. distachyon* NHR response against *Z. tritici*.

In conclusion, our findings show that there are similar isolate-specific responses of both *B. distachyon* and wheat to *Z. tritici*. The growth and formation of immature pycnidia of *Z. tritici* isolate 560.11 in both grasses may suggest that homologous susceptibility factors are conserved between *B. distachyon* and wheat. Such plant targets may be differentially manipulated by *Z. tritici* isolates, perhaps via the use of different effector repertoires. Future transcriptomic and genomic studies of the *B. distachyon* interaction with different *Z. tritici* isolates could reveal such effectors. These findings may also provide a basis to identify the genes responsible for *Z. tritici* recognition in this nonhost and for isolate-specific responses shared in both grass species.

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