In vitro activity of isothiocyanates against Fusarium graminearum

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Received: 4 April 2021 | Accepted: 17 October 2021

DOI: 10.1111/ppa.13505

Plant Pathology WILEY

ORIGINAL ARTICLE

In vitro activity of isothiocyanates against *Fusarium graminearum*

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Funding information
European Union's Horizon 2020 - MyToolBox - 'Safe Food and Feed through an Integrated ToolBox for Mycotoxin Management, Grant/Award Number: 678012

Abstract

Isothiocyanates are biotoxic degradation products formed as a result of enzymatic hydrolysis of glucosinolates present in *Brassica* species. The application of biofumigant *Brassica* crops, as an alternative crop protection method for soilborne pathogens and pests is increasingly gaining interest. However, little is known of the potential of biofumigation to reduce the inoculum of *Fusarium* species affecting cereals. The aim of this study was to evaluate the antifungal activity of five isothiocyanates, namely allyl, benzyl, ethyl, 2-phenylethyl and methyl isothiocyanates, against germination and growth of *Fusarium graminearum* under in vitro conditions. Aromatic isothiocyanates were more inhibitory than the aliphatic isothiocyanates against mycelial growth, whereas the reverse was observed for conidial germination. Among the tested isothiocyanates, allyl and methyl isothiocyanates were more efficient overall, showing lower ED_{50} values (35–150 mg/L) for conidial germination and mycelial radial growth. The findings suggest that *Brassica* plants containing allyl and methyl glucosinolates could have a suppressive effect, reducing the inoculum of *F. graminearum* in soil prior to cereal production.

KEYWORDS
allyl isothiocyanate, biofumigation, *Brassica*, glucosinolates, head blight, wheat

1 | INTRODUCTION

The *Fusarium* genus contains several globally important pathogens and many species of *Fusarium* are recognized as wheat and maize pathogens. *Fusarium graminearum* is the most important causal agent of head blight in wheat, and stalk and ear rot in maize (McMullen et al., 2012). Fusarium head blight is mainly caused by species belonging to the *Fusarium graminearum* species complex and related species, such as *F. poae*, *F. culmorum* and *F. avenaceum*. These fungal pathogens can also cause seedling blight and foot rot in cereals (Parry et al., 1995). *F. graminearum* infection results in yield losses and reduced grain quality. Fusarium head blight can cause as high as 50% yield losses in cereals (Mielniczuk & Skwaryło-Bednarz, 2020). In the United States, Fusarium head blight resulted in yield losses worth $1.176 billion in 2015–2016 (Wilson et al., 2018). Reduction in wheat yield is due to shrivelled and a reduced number of kernels (McMullen et al., 2012). In addition, *F. graminearum* produces mycotoxins in these cereals, which is one of the most serious problems for consumer health and economic losses (Shephard, 2008; Wilson et al., 2018). The mycotoxins produced by *F. graminearum* that are of great concern are deoxynivalenol and zearalenone. Consumption of deoxynivalenol-contaminated food stimulates vomiting and it is

also associated with diarrhoea, nausea, headache, dizziness, gastroenteritis and ataxia in animals and humans (Sobrova et al., 2010). Zearalenone may cause severe reproductive disorders and it is linked to early onset of puberty in young children (Zhao et al., 2013). The European Commission set legislative limits for the *Fusarium* mycotoxins deoxynivalenol and zearalenone in unprocessed cereals and cereal products for human consumption in 2006. Since that time there have been several harvests across Europe when high numbers of crops of either wheat or maize have exceeded these legal limits, resulting in large costs to the cereal industry. For instance, in England in 2008, contamination exceeding limits of zearalenone and deoxynivalenol was reported in 29% and 13% of wheat samples, respectively (Edwards & Jennings, 2018). Concentrations of these mycotoxins, above the legal limits, were also detected in maize samples in Croatia in 2010; 28% of samples were contaminated with zearalenone and 50% samples with deoxynivalenol (Pleadin et al., 2013).

Historically, soil fumigation with synthetic pesticides has been used to manage soilborne pathogens. For example, methyl bromide was widely used as a soil fumigant until it was phased out under the Montreal Protocol and Clean Air Act in order to reduce damage to the ozone layer (Enebak, 2007). Metam sodium, which breaks down to methyl isothiocyanate (MITC), has been used for the control of Verticillium wilt of potato, caused by the soilborne fungus *Verticillium dahliae* (Tsror et al., 2005). However, fumigants are not used for treating *Fusarium* pathogens in cereal rotations, mainly due to cost constraints. The use of synthetic fungicides, particularly triazoles, has been the main source of control of *F. graminearum*. However, limited effectiveness of triazoles and concerns over use of pesticides have led to the pursuit of alternative crop protection strategies such as biofumigation. Biofumigation is a sustainable agricultural practice that generally uses volatile biotoxic compounds released from *Brassica* species (Kirkegaard et al., 1993). Plants belonging to the Brassicaceae including mustard, rapeseed, cabbage and rocket, rich in glucosinolates (GSL), are grown between cash crops, shredded thoroughly and then incorporated into the soil (Kirkegaard et al., 1993). Tissue maceration results in the release of GSL and the enzyme myrosinase, which are present separately in the intact tissues. This endogenous enzyme, in the presence of water, catalyses the breakdown of GSL into glucose and unstable aglycones, which are then converted into a variety of products such as organic thiocyanates, epithionitriles, nitriles and isothiocyanates (ITCs) (Wittstock & Halkier, 2002). Among these compounds, the focus of interest is the ITCs owing to their biopesticidal properties. These volatile compounds produce similar effects to synthetic chemical fumigants, such as metam sodium. ITCs have toxic effects against a broad range of noxious organisms including nematodes (Wood et al., 2017) and fungi (Smolinska et al., 2003). Each *Brassica* species has a specific GSL profile, which results in a corresponding ITC profile. Studies have linked GSL content with biotoxic effects on pathogens (Charron & Sams, 1999). Previous studies on individual ITCs have shown promising fungitoxic potential against pathogens such as *Rhizoctonia solani*, *Gaeumannomyces graminis* var. *tritici* and *Fusarium oxysporum* (Ramos-García et al., 2012; Sarwar et al., 1998; Smolinska et al., 2003). ITCs such as allyl isothiocyanate (AITC), benzyl isothiocyanate (BITC), propyl-isothiocyanate and phenyl isothiocyanate have been shown to inhibit conidial germination and mycelial growth of *F. oxysporum* (Ramos García et al., 2012). Similarly, Smolinska et al. (2003) reported complete inhibition of germination of *F. oxysporum* conidia with pure ITCs. Among the ITCs tested, ethyl ITC (EITC) and AITC significantly suppressed mycelial growth and chlamydospore germination. Whilst biofumigation has attracted significant interest, research on its potential application for reducing the inoculum of *Fusarium* species affecting cereals is scarce. *F. graminearum*, an ascomycete, produces ascospores (sexual spores) and conidia (asexual spores) and mainly overwinters as mycelium in infected crop debris (Yuen & Schoneweis, 2007). The present study was undertaken to investigate the inhibitory effect of pure ITCs against growth and germination of *F. graminearum* under in vitro conditions.

2 | MATERIALS AND METHODS

2.1 | Isothiocyanates

Pure AITC, BITC, EITC, MITC and 2-phenylethyl ITC (PEITC) were obtained from Sigma Aldrich. Sterile distilled water (SDW) was used to prepare the desired stock solutions of ITCs, which were diluted to the final concentration in media. Appropriate concentrations were determined, as described below, and varied from 0 to 5000 mg/L.

2.2 | *F. graminearum* isolates

Five strains of *F. graminearum* from UK wheat isolated in 2016 (FG2556, FG2498, FG2560, FG2502, FG2481) were provided by Fera Science Ltd. These isolates were confirmed as *F. graminearum* by species-specific PCR (Waalwijk et al., 2004).

2.3 | Assay on mycelia

The effect of each ITC on mycelial growth was evaluated using potato dextrose agar (PDA) (Merck) amended with ITC at 1.2, 4.9, 19.5, 78, 312.5, 1250, or 5000 mg/L. For uniform distribution of the ITC within the medium, ITC suspensions in SDW were first vortexed. This was then immediately transferred to molten PDA (at 50°C) and then shaken gently before pouring. Control plates contained PDA treated with SDW in place of ITC. Agar plugs of 7 mm in diameter were cut from the outer margin of the five *F. graminearum* isolates actively growing on PDA. These were transferred to the centre of 9-cm Petri dishes of all treatments with the mycelial surface facing the medium. Three replicates were used per treatment. The plates were incubated at 15°C in the dark and radial colony growth was measured after 9 days, just before untreated mycelia had reached the edge of the plates. Data were expressed as mycelial growth compared with the control. The experiment was conducted twice and the ED_{50} values

were calculated as described below. The experiment was repeated with treatment at the calculated ED_{50} values and hyphal morphology was observed using a light microscope (100× and 400× magnification).

2.4 | Assay on conidia

2.4.1 | Preparation of *F. graminearum* conidial suspension

Conidial suspensions were prepared following the method reported by Edwards and Seddon (2001) with some modifications. The five isolates of *F. graminearum* were subcultured onto PDA, using 10 plates for each isolate, and incubated at room temperature. After 14 days, conidia were harvested by the addition of 20–25 ml SDW to each plate and dislodging conidia using a sterile spreader. The suspension was filtered through Miracloth (EMD Millipore Corp.) to remove mycelia and the filtrate was centrifuged at 3000 × *g* for 10 min. Supernatant was removed and conidia resuspended in 15 ml SDW and centrifuged at 3000 × *g* for 10 min. Conidia were washed twice and resuspended in 15 ml SDW. Conidia of all five isolates were mixed in the final conidial suspension. Conidia were counted using an Improved Neubauer counting chamber (Weber 99, Scientific International) and the concentration adjusted to 10^5 spores/ml.

2.4.2 | Assessment of conidial germination

The five ITCs under investigation were tested for their efficacy at inhibiting the germination of *F. graminearum* conidia. Fourfold dilutions of each ITC were prepared by mixing 500 µl of stock solution in 1.5 ml SDW in sterile 2-ml Eppendorf tubes. The suspensions were vortexed and immediately pipetted for the serial dilution. Subsequently, 1 ml of each ITC dilution was mixed with 900 µl conidial suspension and 100 µl potato dextrose broth (Oxoid) to give final ITC concentrations of 5000, 1250, 312.5, 78, 19.5, 4.9 and 1.2 mg/L. The suspensions were vortexed each time and immediately transferred to the tubes. For controls, SDW was used instead of ITC. For each suspension, 20 µl were pipetted onto a flame-sterilized microscope glass slide, which was placed in a 9-cm Petri dish containing two 85-mm diameter filter paper disks (Fisherbrand) moistened with 2 ml SDW. Four replicates were used for each treatment and the plates were incubated for 16–17 h at 12°C in the dark. The slides were observed under 100× magnification and a minimum of 100 conidia per replicate were counted to calculate percentage germination. Conidia were considered germinated if the length of the germ tube was equal to or greater than the conidial width (Manners, 1966). The experiment was conducted twice.

2.5 | Data analysis

Genstat (18th edition) was used to determine the ED_{50} of each ITC from the percentage of inhibition of mycelial growth and conidial germination. Each treatment in the mycelium assay was represented by a mean of the three replicates taken for each of the five isolates. The concentrations were converted to $\log_{10}$ scale to fit nonlinear curves. Data were best fitted with logistic curves with separate lines for each ITC, while the ED_{50} and 95% confidence interval values were generated from the fitted curve. All data were fitted using ITC as a group.

3 | RESULTS

3.1 | In vitro effects of isothiocyanates on mycelial growth

Different responses of *F. graminearum* were observed according to type and concentration of ITC. All tested ITCs except EITC showed 100% inhibition of radial growth at the highest two concentrations, 5000 and 1250 mg/L. PEITC and BITC showed complete inhibition at 312.5 mg/L. AITC (Figure 1) and MITC at 78 mg/L, on average, inhibited the radial growth by 20%–60% and 45%–65%, respectively. EITC showed the weakest inhibition (ED_{50}, c.1964 mg/L) and at concentrations ranging from 1.2 to 312.5 mg/L showed 10%–20% stimulation compared to the control. AITC and PEITC also showed slight stimulation at the lowest concentrations. The calculated mean ED_{50} values for the tested ITCs from the two experiments showed some variability but were, however, within a fourfold order of magnitude (Table 1). As evident from the ED_{50} values, BITC (c.5 mg/L) and PEITC (c.26 mg/L) showed the strongest inhibition. The fitted curves accounted for 91.6% and 85.8% of the observed variance ($p < 0.001$) within the data from experiments 1 (Figure 2) and 2 (Figure S1), respectively. The five isolates showed little variation in their responses to ITCs, apart from one isolate (FG2481), which showed greater variation in response to EITC (Figure 3).

Microscopic examination (100× and 400×) of the mycelial growth of *F. graminearum*, when exposed to the five ITCs at their respective calculated ED_{50} values, showed no observable difference in hyphal morphology compared to the control (Figure S2).

3.2 | In vitro effects of isothiocyanates on conidial germination

The fitted curves accounted for 99.8% and 99.9% of the deviance within the data from experiments 1 (Figure 4) and 2 (Figure S3), respectively. All tested ITCs (except PEITC) showed 100% inhibition at the highest concentration, 5000 mg/L, when compared to the control treatment (100% germination). At 1250 and 312.5 mg/L, AITC (Figure S4) and MITC showed complete inhibition of conidial germination, whereas 1250 mg/L of EITC was found to be comparatively less effective, with around 50% inhibition. However, at 19.5 mg/L or lower concentrations, none of the tested ITCs appeared to have any effect on conidial germination. PEITC showed no inhibitory response on conidial germination at any concentration, therefore no

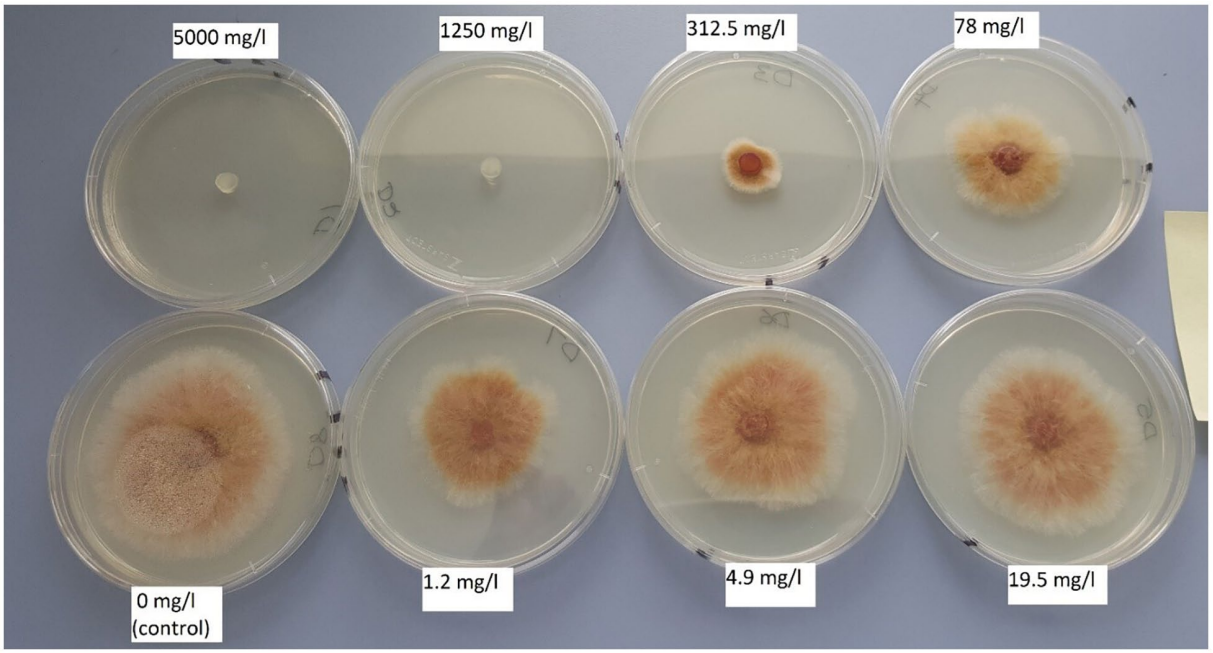

FIGURE 1 Cultures of *Fusarium graminearum* after 9 days of exposure to different concentrations of allyl isothiocyanate at 15°C (Experiment 1)

TABLE 1 Effective dose values at 50% (ED_{50}) of isothiocyanates for mycelial growth and conidial germination of *Fusarium graminearum*

	$ED_{50\ (mg/L)}$[a]			
	Mycelial growth		Conidial germination	
Isothiocyanate	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Allyl	69 [19–269]	129 [56–240]	135 [129–141]	62 [58–66]
2-Phenylethyl	11 [7–20]	41 [21–110]	>5000	>5000
Methyl	35 [<1–110]	91 [20–224]	151 [148–155]	45 [43–47]
Benzyl	7 [3–12]	2 [<1–13]	3020 [2951–3090]	2951 [2884–3020]
Ethyl	2951 [1905–4169]	977 [138–3631]	1288 [1202–1413]	1549 [1413–1660]

[a]For mycelial growth and conidial germination, inhibitory activity was evaluated at 15°C and 12°C respectively; [95% confidence interval].

ED_{50} values were determined for this ITC. For the other ITCs, as with the mycelial experiments, the calculated mean ED_{50} values from the two experiments showed some variability but were, however, within a fourfold order of magnitude (Table 1). Among the tested ITCs, AITC and MITC were the most efficient, showing lower ED_{50} values, c.99 mg/L and c.98 mg/L, respectively (Table 1). As evident from the ED_{50} values, BITC (c.2986 mg/L) and EITC (c.1419 mg/L) were the least effective for inhibition of conidial germination.

4 | DISCUSSION

The present study has demonstrated that the sensitivity of *F. graminearum* varied according to ITC type and concentration tested. Aliphatic ITCs (AITC, MITC, EITC) were more toxic than the aromatic ITCs (PEITC, BITC) in the conidial assay, with AITC and MITC causing complete inhibition at 312.5 mg/L. MITC is generally considered to be a synthetic ITC although it has been detected in tissues of *Brassica napus*, *B. juncea* and *B. campestris* (Ojaghian et al., 2012). In contrast to our results, a previous study found that ITC at lower concentrations (0.1–2 mg/L) were completely inhibitory to *F. oxysporum* conidia in a shorter duration of 7 h (Ramos García et al., 2012). This suggests that the response of *F. graminearum* to ITC differs from other *Fusarium* species. Mari et al. (2008) also reported low ED_{50} values of AITC (0.17 mg/L) and BITC (0.12 mg/L) for conidial germination of *Monilinia laxa*.

In the mycelial assay of the present study, results were the reverse of the conidial assay, with aromatic ITCs showing lower ED_{50} values than aliphatic ITCs. Sarwar et al. (1998) reported that aromatic ITCs, when incorporated in to media, showed stronger

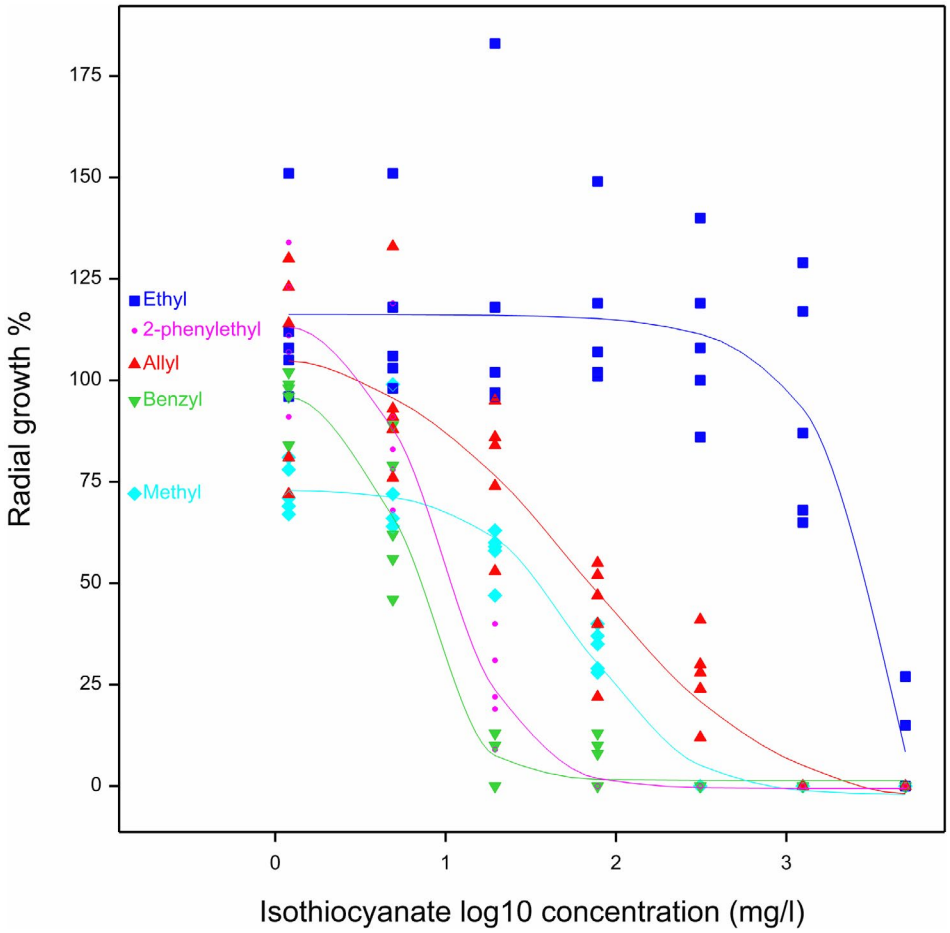

FIGURE 2 Dose–response curves for ethyl, 2-phenylethyl, allyl, benzyl and methyl isothiocyanates at concentrations 5000, 1250, 312.5, 78, 19.5, 4.9 and 1.2 mg/L (presented on $\log_{10}$ scale) on mycelial growth of *Fusarium graminearum* isolates as a percentage of growth in the untreated control. Data from Experiment 1 are presented here

toxicity against fungal pathogens but were less toxic in volatile form due to their lower volatility. The slightly higher ED_{50} value for AITC (c.99 mg/L) recorded in our mycelial assay was unexpected. Possibly, as AITC is highly volatile, some of the volatiles might have been lost when the media plates were prepared, resulting in a higher ED_{50} value than the those obtained for aromatic ITCs and MITC (c.63 mg/L). Previously, Ojaghian et al. (2012) found MITC and AITC to be the most effective in inhibiting mycelial growth of *Sclerotinia sclerotiorum* and the two ITCs were completely inhibitory at c.500 μg/L. Neubauer et al. (2014) observed that microsclerotia of *V. dahliae* were more sensitive to aromatic ITCs than aliphatic ITCs, consistent with the present mycelial assay; they recorded lower LD_{50} values for BITC (0.36 μg/L) and PEITC (0.46 μg/L) than MITC (0.72 μg/L) and AITC (1.45 μg/L). Smolinska et al. (2003) reported that the inhibitory effect of various ITCs on mycelial growth of *F. oxysporum* was fungistatic rather than fungicidal. However, in the present study, mycelial plugs from the treatments that showed complete inhibition when transferred to fresh PDA did not grow (data not shown), indicating a fungicidal effect. The toxicity of an ITC depends on its molecular structure but is not strictly dependent on the aliphatic or aromatic structure (Smolinska et al., 2003). Moreover, the varying responses of different ITCs observed in different studies could be due to their mode of action, different side chains and penetrability, and sensitivity of the target fungal species.

We found that the extent of mycelial growth inhibition by ITCs varied slightly among isolates. However, the greatest variation was observed among isolates when exposed to EITC. Such variation in mycelial growth inhibition of different isolates of the same species in the presence of ITC had been observed previously for *F. oxysporum* (Smolinska et al., 2003). AITC and PEITC at very low concentrations appeared to stimulate growth of *F. graminearum* in our mycelial assay, as the developing colonies were slightly larger than the control plates. Such stimulation has been observed in other studies with fungicides. For example, fosetyl-aluminium at the recommended rate stimulated radial growth of *Lecanicillium longisporum* (Shah et al., 2009). Although our assays showed inhibition of mycelial growth and conidial germination of the pathogen after exposure to ITCs, the susceptibility of conidia was less consistent than the mycelia. This in contrast to other studies, where conidia were found to be more sensitive than mycelia to ITCs (Mari et al., 2008; Ramos-García et al., 2012). However, in an in vitro assay with fungicides (Masiello

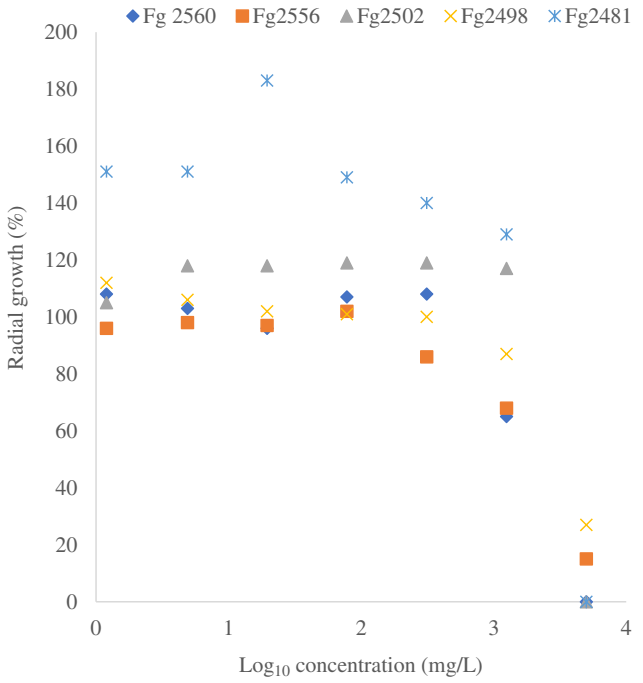

FIGURE 3 Mycelial growth of *Fusarium graminearum* isolates (FG2560, FG2556, FG2502, FG2498, FG2481) exposed to ethyl isothiocyanates at concentrations 5000, 1250, 312.5, 78, 19.5, 4.9 and 1.2 mg/L (presented on $\log_{10}$ scale) as a percentage of growth in the untreated control (Experiment 1)

et al., 2019), the ranking of sensitivity of conidia and mycelia of *F. graminearum* differed according to fungicide. A study on *Alternaria brassicicola* suggested that ITCs alter mitochondrial function, trigger production of reactive oxygen species and possibly disrupt metabolic pathways in fungal cells (Calmes et al., 2015). The variation in sensitivity at the different development stages could be due to differences in structure and physiology and mode of action of the antifungal compounds.

The results from the conidial and mycelial assays suggest that AITC and MITC are the most inhibitory ITCs against *F. graminearum* under laboratory conditions. The precursor GSL of AITC is sinigrin. This GSL comprises about 98%–99% of the total GSL content of some *Brassica* species such as *B. juncea* and *B. nigra* (Matteo, 2017; Ngala et al., 2015). Charron and Sams (1999) detected volatile compounds emitted from macerated leaves of *B. juncea* placed in a jar and determined that AITC was the predominating compound (>90%) in the volatiles measured by gas chromatography. As sinigrin (AITC precursor) is the dominant GSL in *B. juncea*, this species would be a strong candidate for biofumigation against *F. graminearum* under field conditions. In a field experiment, incorporation of *B. juncea* reduced *Spongospona subterranea* (powdery scab) inoculum by 27% and also reduced disease incidence and severity of powdery scab and common scab (caused by *Streptomyces scabies*) by 40% and >20%, respectively (Larkin & Griffin, 2007). In the same study, an in vitro assay showed that macerated leaf tissues of *B. juncea* resulted in 73% inhibition of *F. oxysporum* and 80% inhibition of *Fusarium sambucinum*. In the field, researchers have detected GSL at concentrations high enough to produce ITC concentrations comparable to the ED_{50} values determined in the present study (Gimsing & Kirkegaard, 2006). Sinigrin, at concentration of 90 μmol/g and 147 μmol/g, has been detected in *B. juncea* leaves (Ngala et al., 2015) and *B. nigra* defatted seed meal (Matteo, 2017), respectively. In contrast, lower concentrations of sinigrin ranging from 15 to 27 μmol per g tissue (freeze-dried) have also been observed previously (Charron & Sams, 1999; Neubauer et al., 2014). However, it has been indicated that a GSL concentration of around 13 μmol/g (dry weight) would be able to produce at least 50 mg/L of AITC (Wood et al., 2017), suggesting that the ED_{50} value for AITC determined in the present study (c.99 mg/L) might be achievable in the field. Gimsing and Kirkegaard (2006) also reported high ITC release efficiency in field following incorporation of *Brassica* species; an ITC release efficiency as high as 56% was recorded for *B. juncea* and 26% for *B. napus*. However, it should be kept in mind that such high ITC concentrations were only achievable by following practices such as thorough pulverization and heavy watering to maximize ITC release under field conditions.

In the present study, ITCs were mixed with water and not dissolved in a solvent such as ethanol because *F. graminearum* cultures when exposed to ethanol alone resulted in abnormal colony growth of the fungus. Unlike the assay on mycelium where treatments were incubated at 15°C, the conidial assay was performed at 12°C. This temperature was selected for logistical reasons because, at 15°C, germination tubes grew longer and it was not possible to distinguish individual conidia after overnight incubation. As the response of all five isolates was broadly similar in the assay on mycelia, conidia of all five isolates were mixed in the final conidial suspension in the conidial assay. Moreover, during the preliminary runs for the conidial experiment, the five isolates responded similarly when treated separately with ITC. Data from mycelial and conidial assays were broadly similar in the repeated experiments with the aromatic ITCs. However, the ED_{50} values determined for the aliphatic ITCs were less consistent among the two experiments for each assay. Due to these ITCs being highly volatile, slight differences in temperature may have affected their volatility. Vapour pressure of AITC was reported to increase rapidly with temperature, for example, 136 to 2267 Pa from 5 to 50°C (Lim, 1999), indicating its high volatile properties. Similarly, the vapour pressure of EITC is estimated to increase from 1150 Pa at 20°C to around 1500 Pa at 25°C (Yaws & Satyro, 2015), suggesting this ITC may also be sensitive to slight differences in temperature. Moreover, the vapour pressure of EITC can reach as high as 5000 Pa at 50°C (Yaws & Satyro, 2015), a volatilization rate more than double that of AITC at the same temperature (Lim, 1999), indicating the highly volatile nature of EITC. This may explain the discrepancies observed for EITC in the mycelial assay where the ITCs were added to molten medium at 50°C. Delaquis and Sholberg (1997) have argued that due to high volatility and weak water solubility of ITCs, the test concentrations may not remain constant throughout the experiment. Variation in volatilization may explain the discrepancies observed for ITCs between our two experiments.

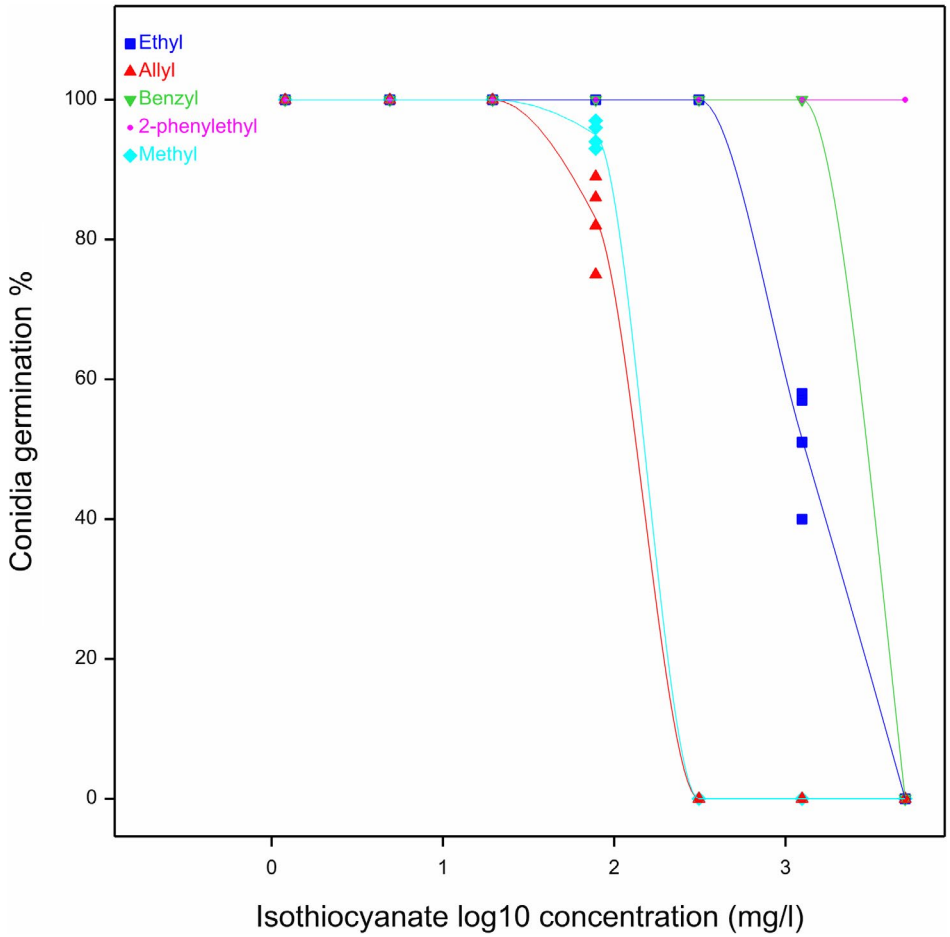

FIGURE 4 Dose–response curves for ethyl, allyl, benzyl, 2-phenylethyl and methyl isothiocyanates at concentrations 5000, 1250, 312.5, 78, 19.5, 4.9 and 1.2 mg/L (presented on $\log_{10}$ scale) on germination of *Fusarium graminearum* conidia as a percentage of germination in the untreated control. Data from experiment 1 are presented here

The present findings have demonstrated the antifungal potential of ITCs against *F. graminearum*, particularly MITC and AITC. There are ongoing issues with the use of synthetic fumigants, where some fumigants are already banned and others are likely to be banned in the near future; thus, biofumigation could provide a "green" alternative. The relatively high ED_{50} values of ITCs recorded in the present study might be achievable in the field depending on factors such as agronomic practices and *Brassica* species used. Nevertheless, the outcome suggests that *Brassica* species rich in sinigrin and glucocapparin, such as *B. juncea* and *B. napus*, respectively, could have a suppressive effect, reducing the inoculum of *F. graminearum* in soil prior to cereal production.

ACKNOWLEDGEMENTS

This study has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 678012 (MyToolBox – "Safe Food and Feed through an Integrated ToolBox for Mycotoxin Management"). Thanks are due to Dr Phil Jennings of Fera Science Limited, UK for providing *F. graminearum* isolates. The authors are grateful to Dr Fabio Veronesi and Dr Edwin Harris for advice on statistical analysis, and Danielle Henderson-Holding for technical support. The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Ashiq, S., Edwards, S.G., Fatukasi, O., Watson, A. & Back, M.A. (2021) In vitro activity of isothiocyanates against *Fusarium graminearum*. *Plant Pathology*, 00, 1–8. https://doi.org/10.1111/ppa.13505