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Impact of predicted climate change environmental conditions on the growth of *Fusarium asiaticum* strains and mycotoxins production on a wheat-based matrix

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ABSTRACT

Fusarium asiaticum is a predominant fungal pathogen causing Fusarium Head Blight (FHB) in wheat and barley in China and is associated with approximately £201 million in annual losses due to grains contaminated with mycotoxins. *F. asiaticum* produces deoxynivalenol and zearalenone whose maximum limits in cereals and cereals-derived products have been established in different countries including the EU. Few studies are available on the ecophysiological behaviour of this fungal pathogen, but nothing is known about the impact of projected climate change scenarios on its growth and mycotoxin production. Therefore, this study aimed to examine the interacting effect of i) current and increased temperature (25 vs 30 °C), ii) drought stress variation (0.98 vs 0.95 water activity; a_w) and iii) existing and predicted CO_2 concentrations (400 vs 1000 ppm) on fungal growth and mycotoxin production (type B trichothecenes and zearalenone) by three *F. asiaticum* strains (CH024b, 82, 0982) on a wheat-based matrix after 10 days of incubation. The results showed that, when exposed to increased CO_2 concentration (1000 ppm) there was a significant reduction of fungal growth compared to current concentration (400 ppm) both at 25 and 30 °C, especially at 0.95 a_w. The multi-mycotoxin analysis performed by LC-MS/MS qTRAP showed a significant increase of deoxynivalenol and 15-acetyldeoxynivalenol production when the CH024b strain was exposed to elevated CO_2 compared to current CO_2 levels. Zearalenone production by the strain 0982 was significantly stimulated by mild water stress (0.95 a_w) and increased CO_2 concentration (1000 ppm) regardless of the temperature. Such results highlight that intraspecies variability exist among *F. asiaticum* strains with some mycotoxins likely to exceed current EU legislative limits under prospected climate change conditions.

1. Introduction

Within global food chains, wheat (*Triticum* spp.) is one of the world's most crucial crop not only for its nutritional properties but also for its economic value linked with import/export market around the world. In 2021/2022, approximately one-third of the world's total area for cereal agriculture was dedicated to wheat cultivation, with a global production volume of >771 M tonnes (FAOSTAT, 2023). However, wheat production is challenged by a range of diseases, caused by different phytopathogens and pests, especially fungi, causing on average of 10–28 % yield losses globally (Savary et al., 2019).

Among the fungal pathogens, *Fusarium graminearum* species complex (FGSC) are the etiological agent of Fusarium Head Blight (FHB) of wheat. The FGSC consists of at least 16 phylogenetically different species with *F. graminearum* being the best-known causative agent of FHB (O'Donnell et al., 2008; Sarver et al., 2011). *F. asiaticum* is morphologically indistinguishable from *F. graminearum* (O'Donnell et al., 2004) however, it differs from it in some aspects, such as the geographic distribution and chemotypes. Indeed, while *F. graminearum* is cosmopolitan, growing mainly in cooler areas (<15 °C, average temperature/year) and has been isolated from maize, *F. asiaticum* is confined to eastern Asian countries, grows in warmer areas (>15 °C, average temperature/year) and it has been found in rice and wheat (Jang et al., 2019; Xu et al., 2021). The pathogens of FHB not only cause a serious plant disease but also threaten public health due to mycotoxins contamination of the infected crops. FHB mycotoxins include zearalenone (ZEN) and type B-trichothecenes (nivalenol (NIV), deoxynivalenol (DON), and its acetyl derivatives, 3-acetyldeoxynivalenol (3-

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ADON) and 15-acetyldeoxynivalenol (15-ADON)) (Jennings, 2007). Exposure to trichothecenes can cause immunological problems, vomiting, skin dermatitis and hemorrhagic lesions while ZEN exposure causes uterine changes (hyperplasia and hypertrophy) and infertility (Rai et al., 2020; Ganesan et al., 2022). Due to such demonstrated detrimental effect on human and animal health, the levels of DON and ZEN in cereal crops are regulated in the European Union (EU, 2023/915), while it is not the case for NIV and the acetyl derivatives for which no regulation exist so far.

Fungi within the FGSC produce different mycotoxins depending on the geographic distribution and environmental conditions. For instance, in China all *F. graminearum* isolates produce 15-ADON, whereas *F. asiaticum* may produce 3-ADON, NIV, and 15-ADON (Xu et al., 2021). Garcia-Cela et al. (2022) found that optimum growth happened at 25 °C/0.98 a_w while marginal growth was observed at 35 °C/0.90 a_w for three *F. asiaticum* strains grown on a wheat-based matrix. Mycotoxins production differed between the strains and toxins with ZEN optimum production found at 30 °C/0.93–0.95a_w DON, 3-ADON and NIV at 0.98a_w/20–30 °C.

In the sixth Assessment Report (AR6) of the Intergovernmental Panel on Climate Change (IPCC) it has been reported that emissions of greenhouse gases (GHG) have caused an increase of the global surface of +1.1 °C since 1850–1900 and that atmospheric CO_2 concentrations reached 410 ppm (ppm) in 2019. The AR6 also published five Shared Socioeconomic Pathways (SSPs) which are climate change (CC) scenarios based on socioeconomic global changes up to 2100. The SSP2–4.5 (*middle of the road*) is considered the most likely to happen and is characterized by intermediate GHG emissions, CO_2 concentration in the air reaching ~600 ppm and temperature increase within 2.1–3.5 °C by 2100. The SSP5–8.5 scenario (*taking the highway*), the most catastrophic one, is characterized by very high GHG emissions, CO_2 concentration reaching at least 1000 ppm and temperature increase within 3.3–5.7 °C by 2100. Under such predicted CC scenario cereal:pathosystems will be impacted resulting in loss of productivity and quality of crops hindering food security (IPCC, 2023). Moreover, the changes of climatic conditions may shift the geographic distribution and the dominance of pathogenic species, including FGSC, and associated mycotoxins (Vaughan et al., 2016).

Many studies have been conducted to simulate the impact of combined CC abiotic factors (T x a_w x CO_2) on the growth and mycotoxins production by different fungal species in various crops (Abdelmohsen et al., 2021; Baazeem et al., 2021; Cervini et al., 2021; Verheecke-Vaessen et al., 2022) but little is known with respect to the FGSC, including *F. asiaticum*.

Thus, the objective of this study was to examine for the first time the effect of three-way interacting climatic-related abiotic factors of temperature (25 vs 30 °C), drought stress (0.98 vs 0.95 a_w) and elevated CO_2 (400 vs 1000 ppm) on fungal growth and type B trichothecenes (DON, 3-ACDON, 15-ACDON, NIV) and ZEN production by three *F. asiaticum* strains on a wheat-based matrix. Such conditions were chosen as representative, on average, of optimum current climate occurring during the flowering (anthesis) and grain filling stages of wheat (25 °C x 0.98 a_w) and projected CC scenario characterized by increased temperature (30 °C), mild drought stress (0.95 a_w) and higher atmospheric CO_2 concentrations (1000 ppm) compared to now (400 ppm).

2. Materials and methods

2.1. Fungal strains

Three strains of *Fusarium asiaticum* (CH024b, 082, 0982) were used in this study. They were isolated in China from 2002 to 2005 in three different regions and from different hosts, as reported by Garcia-Cela et al. (2022). The strains were kept at −20 °C in a glycerol:water (30:70, *v*/v) solution in the Mycoteque of the Applied Mycology Group at Cranfield University.

2.2. Media preparation and inoculation

A basal Wheat Based Medium (WBM; 0.99 water activity, (a_w)) was prepared by adding 3 % milled wheat in distilled water and supplemented with 2 % agar. The a_w of WBM, measured by an AquaLab Series 4TE (Decagon Devices, Inc., Washington, USA) water activity meter at 25 °C, was adjusted to 0.98 and 0.95 by adding the required grams of glycerol. The media were autoclaved (121 °C, 15 min) and subsequently 20 ml were poured into 9 cm diameter sterile Petri plates.

Fungal strains were sub-cultured on WBM (0.99 a_w) and grown at 25 °C in darkness for 10 days. After this period, agar discs (3 mm diameter) were taken from the colonies with a sterile cork-borer and centrally inoculated in the WBM treatment plates (Kahla et al., 2023). Four replicates per strain per each a_w were incubated at 25 and 30 °C in separated climatic chambers to be flushed with CO_2 at 400 and 1000 ppm.

2.3. Incubation conditions and CO_2 treatment

The in vitro agar cultures were placed in 12-l airtight containers and incubated at 25 °C and 30 °C for 10 days. The containers included a beaker (500 ml) of water/glycerol solution to minimize moisture loss. The boxes were flushed twice a day with current (400 ppm) and increased (1000 ppm) CO_2 concentrations by using specialty grade mixture cylinders (British Oxygen Company Guildford, Surrey, UK), with a flow rate of 3 $l \cdot min^{-1}$ to renew 3× the air volume of the incubation chambers (Cervini et al., 2021).

2.4. Growth measurements

Fungal growth was assessed daily for 10 days and expressed as the average measurement (mm) of two orthogonal diameters of the centrally inoculated colonies. The colonies' diameters were plotted against time (days) and the linear regression model was used to calculate the maximum growth rate (μ_{max}, $mm \cdot day^{-1}$). The square of the linear correlation coefficient was ≥0.98.

2.5. Multi-mycotoxins analysis by LC-MS/MS

The ability of the isolates to produce type B trichothecenes (deoxynivalenol (DON), nivalenol (NIV), 3-acetil-deoxynivalenol (3-ADON), 15-acetil-deoxynivalenol (15-ADON)) and zearalenone (ZEN) was tested with LC-MS/MS qTRAP. After 10 days of incubation, approximately 350 mg plugs were taken along the two orthogonal diameters of the fungal colonies and placed in pre-weighed 2 ml tubes and kept at −20 °C until extraction. Four-times the weight of the plugs of acetonitrile:water:formic acid (79:20:0.1 *v*/v/v) solution was added to the tubes. Samples were incubated and agitated at 25 °C for 90 min at 300 rpm on a rotary shaker (miniShaker VMR, Leighton Buzzard, UK) followed by centrifugation for 10 min at 22600*g* (Centrifuge 5417S Eppendorf, Stevenage, UK). The supernatants (500 μl) were transferred to previously labelled HPLC silanized vials and 1 μl from each sample was injected into an Exion LC series HPLC linked to a 6500 + qTRAP-MS system in Electrospray Ionisation (ESI) mode (Sciex Technologies, Warrington, UK). Details about the method used were reported by Isidro-Sánchez et al. (2020). Data acquisition was conducted with Analyst® version 1.6.3, and quantification through MultiQuant™version3.0.3. DON, 3-ADON, 15-ADON and ZEN, were quantified using internal standards and their amount expressed as ng/g. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on Malachová et al. (2014).

2.6. Statistical analysis

Statistical analysis was performed using JMP Pro (version 17, SAS Institute Inc., 2019, Cary, NC, USA). Data were firstly checked for normal distribution, using the Shapiro-Wilk test, and homoscedasticity,

using Levene test. All datasets failed the requirements for parametric tests; therefore, the Kruskal-Wallis non-parametric test was performed to evaluate the significant effect (p-value: <0.05) of the variables tested (T, a_w, CO_2) on both fungal growth and mycotoxins data by the three *F. asiaticum* strains. A non-parametric comparison for each pair using the Wilcoxon method was performed to detect whether significant differences occurred (p-value: ≤0.05). Statistical analysis was only conducted on conditions where growth/mycotoxin production happened during the 10 days of incubation.

3. Results

3.1. Effect of simulated climate change conditions on growth measurements

Table 1 shows the effect of interacting abiotic factors of temperature, water activity and CO_2 on the maximum fungal growth rates (μ_{max}, $mm{\cdot}day^{-1}$) of the three *F. asiaticum* strains. Overall, growth rates were similar among the strains examined. At 25 °C fungal growth was faster compared to 30 °C at both 0.95 and 0.98 a_w. High water availability conditions (0.98 a_w) favored fungal growth compared to dry-simulated ones (0.95 a_w). The exposure to increased CO_2 concentrations (1000 ppm) resulted in no significant differences of growth rates at both temperatures (25 and 30 °C) at 0.98 a_w while it caused a significant reduction at 0.95 a_w, regardless of the different temperatures tested.

Statistical analysis showed that such interacting CC-related factors (T x a_w x CO_2) had a significant effect on the growth rates of all the three *F. asiaticum* strains (<0.0001*** ≤ p ≤ 0.0002***) (Table S1).

3.2. Effect of simulated climate change conditions on the production of type B trichothecenes

A different intraspecies mycotoxin-producing ability was observed, with the 0982 strain not producing any type B trichothecenes. NIV hasn't been detected in any of the conditions by none of the strains.

Table 1
Maximum growth rate (μ_{max}, $mm{\bullet}day^{-1}$) of *F. asiaticum* CH024b, 082, 982 on wheat-based media under the effect of combined T x a_w x CO_2 conditions after 10 days of incubation.

Strains	Temperature	a_w	CO_2 ppm	μ_{max} ($mm{\bullet}day^{-1}$) ± SD
Ch024b	25 °C	0.95	400	13.45 ± 0.71 [b]
			1000	6.86 ± 0.18 [d]
		0.98	400	16.49 ± 0.77 [a]
			1000	16.75 ± 0.62 [a]
	30 °C	0.95	400	9.97 ± 0.60 [c]
			1000	5.02 ± 0.13 [d]
		0.98	400	14.07 ± 1.79 [b]
			1000	12.68 ± 0.17 [b]
082	25 °C	0.95	400	17.56 ± 0.83 [b]
			1000	11.07 ± 0.60 [c]
		0.98	400	21.60 ± 0.79 [a]
			1000	21.55 ± 0.24 [a]
	30 °C	0.95	400	11.53 ± 0.69 [c]
			1000	5.59 ± 0.48 [d]
		0.98	400	16.85 ± 0.27 [b]
			1000	16.59 ± 0.21 [b]
0982	25 °C	0.95	400	20.25 ± 0.60 [a]
			1000	8.96 ± 0.20 [d]
		0.98	400	20.51 ± 0.34 [a]
			1000	18.73 ± 0.22 [b]
	30 °C	0.95	400	9.85 ± 0.20 [d]
			1000	5.61 ± 0.17 [e]
		0.98	400	16.24 ± 0.68 [c]
			1000	15.48 ± 1.03 [c]

a_w: water activity; SD: standard deviation. Different letters indicate significant differences (p < 0.05) between a_w x CO_2 concentrations within every temperature.

Fig. 1 shows that 3-ADON was produced by both CH024b and 82 strains that behaved differently when exposed to such combined CC factors. The former produced 3-ADON only at 0.98 a_w. When incubated at 25 °C, CH024b produced this metabolite at both 400 and 1000 ppm CO_2 levels in similar concentration (no significant differences), while at 30 °C, 3-ADON was produced only when this strain was exposed to increased CO_2 level (1000 ppm) with no significant differences compared to 25 °C. The 82 strain produced 3-ADON only at 400 ppm. At 25 °C, its production was enhanced by higher water availability (0.98 a_w) compared to 0.95 a_w, resulting in the highest significant amount produced (13.20 ng/g).

As shown in Fig. 2, 15-ADON was only produced by the CH024b strain. A significantly higher amount of this mycotoxin was observed at 0.98 a_w when the strain was exposed to 1000 ppm compared to current 400 ppm, with no significant differences observed between the different temperatures tested. In moderate drought stress simulated conditions (0.95 a_w) this strain produced 15-ADON only at 400 ppm, with no production detected at 1000 ppm independently of the temperature.

Fig. 3 shows that DON was produced by both CH024b and 82 strains, with the latter producing lower concentrations compared to the former. The exposure of CH024b strain to the combined effect of elevated CO_2 (1000 ppm) and high-water availability (0.98 a_w) resulted in significantly higher levels of DON compared to those observed at 400 ppm, with an overall enhanced production at 25 °C vs 30 °C. No significant differences were found in the amount of DON produced by the 82 strain when incubated under these combined abiotic factors.

Statistical analysis revealed that the exposure to combined T x a_w x CO_2 conditions significantly influenced the type B trichothecenes production by the *F. asiaticum* strains (<0.0001*** ≤ p ≤ 0.043*) (Table S2).

3.3. Effect of simulated climate change conditions on the production of zearalenone

Fig. 4 shows the different ZEN producing behaviour by three *F. asiaticum* strains under the effect of interacting climate change factors. Interestingly, ZEN was produced only at 0.95 a_w. Regardless of the temperature tested, the 982 strain produced a significantly higher amount of ZEN when exposed to 1000 ppm vs 400 ppm. The exposure to elevated CO_2 concentration (1000 ppm) stimulated ZEN production at 30 °C/0.95 a_w by the 82 strain that didn't produce this mycotoxin in the same conditions at 400 ppm. No significant differences were found in the amount of ZEN produced by the other strains. Table S2 shows that ZEN production by CH024b, 82, 0982 *F. asiaticum* strains was significantly influenced by the effect of combined T x a_w x CO_2 abiotic factors (p = 0.0042**).

4. Discussion

This is the first study investigating the impact of interacting climate change (CC) abiotic factors (T x a_w x CO_2) on the ecophysiology of three *F. asiaticum* strains grown on wheat-based matrix. Overall, the three strains shared the same growth conditions when exposed to the effect of such combined CC factors. The exposure to increased CO_2 concentration (1000 ppm) resulted in no significant differences or significant reduction of the growth rates compared to current CO_2 level (400 ppm) at 0.98 and 0.95 a_w, respectively regardless of the different temperature tested. Such data constitute new evidence about the ecophysiology of *F. asiaticum* and are partially in agreement with previous CC studies conducted on other fungal species. Verheecke-Vaessen et al. (2022) reported that the growth rate of three *F. langsethiae* strains grown on an oat-based medium for 10 days was significantly reduced at all the temperatures tested (25, 30 and 34 °C) regardless of the a_w treatment when exposed to 1000 ppm. While no studies are currently available on the effect of three-ways interacting CC factors on the growth of *F. asiaticum*, lot of attention has been given to unveil optimum growth conditions of FHB related

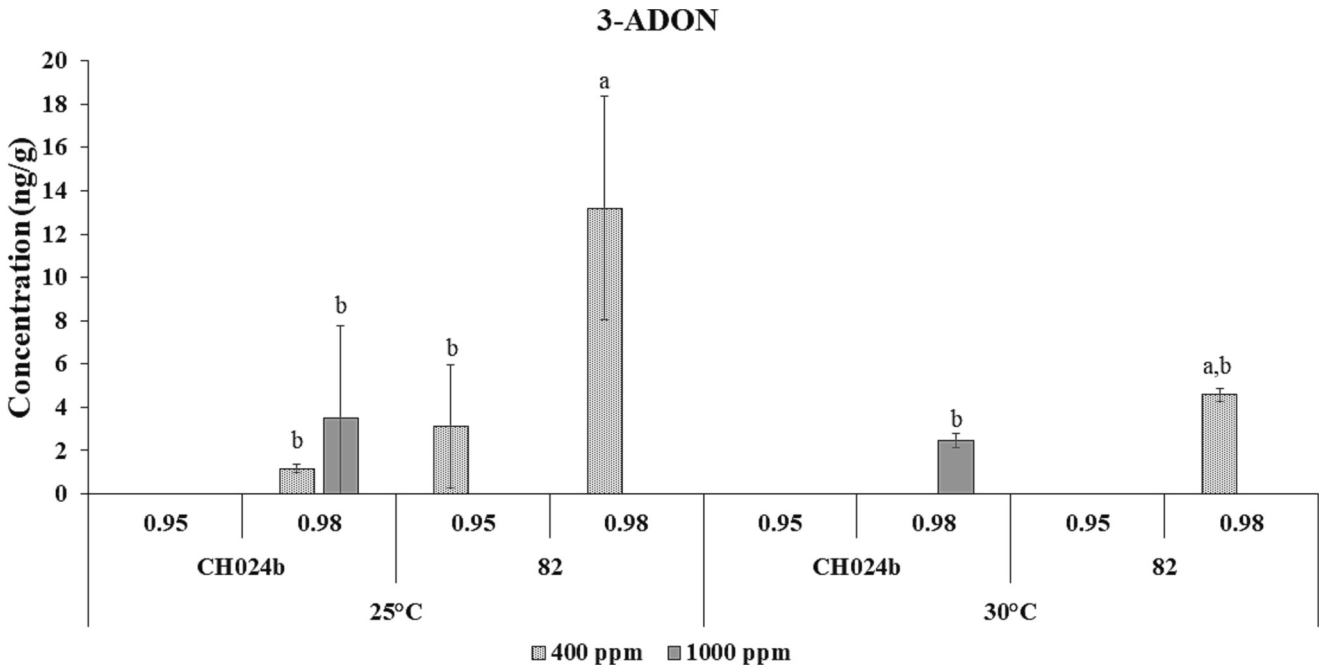

Fig. 1. 3-acetyl deoxynivalenol (3-ADON) production by *F. asiaticum* CH024b and 82 under the effect of interacting conditions of temperature (25 vs 30 °C) x CO_2 (400 vs 100 ppm) x water activity (0.95 vs 0.98 a_w) after 10 days of incubation on wheat-based matrix. Different letters indicate significant differences ($p < 0.05$).

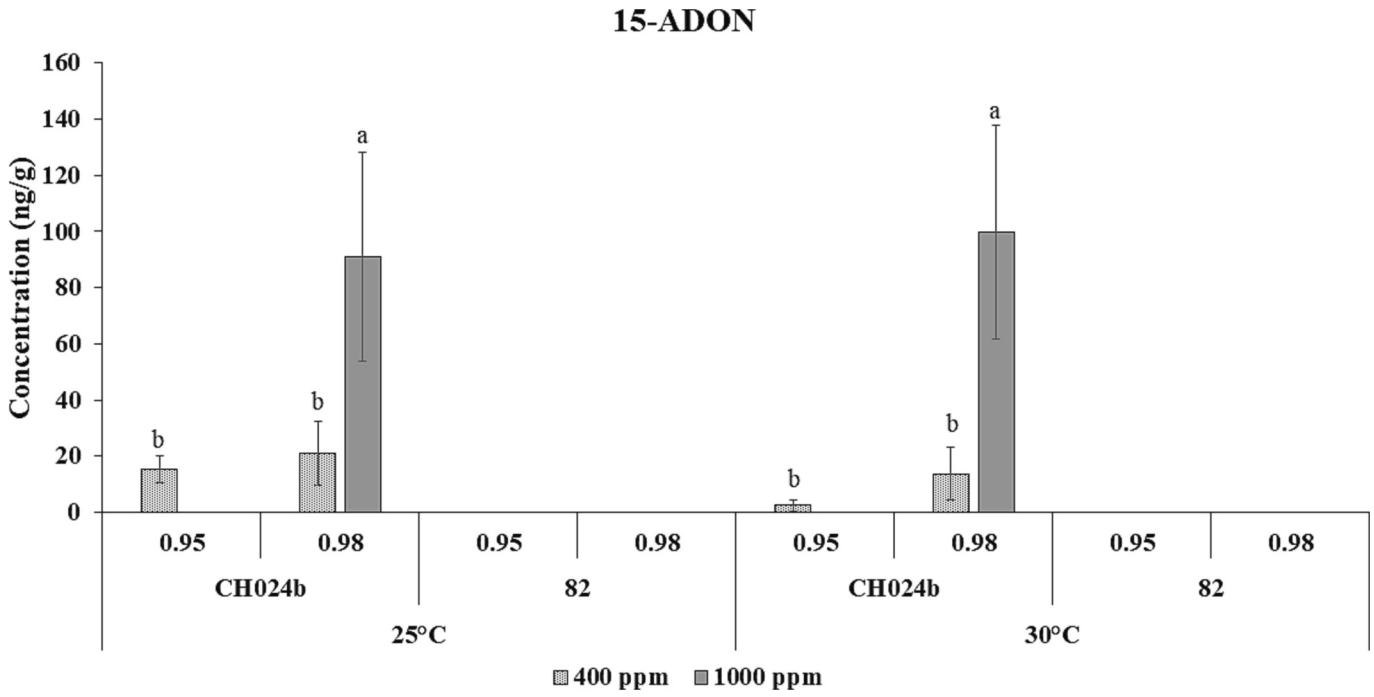

Fig. 2. 15-acetyl deoxynivalenol (15-ADON) production by *F. asiaticum* CH024b and 82 under the effect of interacting conditions of temperature (25 vs 30 °C) x CO_2 (400 vs 100 ppm) x water activity (0.95 vs 0.98 a_w) after 10 days of incubation on wheat-based matrix. Different letters indicate significant differences ($p < 0.05$).

species, including *F. asiaticum*. Garcia-Cela et al. (2022) studied the combined effect of temperature x a_w on the growth rate of the same three *F. asiaticum* strains. Similarly, the authors reported that maximum growth rate was found at 25 °C/0.98 a_w. Ramirez et al. (2006) when studying the effect of interacting temperature (5–30 °C) and a_w (0.90–0.995) on the ecophysiology of two *F. graminearum* strains isolated from wheat ears in Argentina, reported that maximum growth rates were obtained at the 0.995 a_w/25 °C, with no intraspecies differences observed. Brennan et al. (2003) found that the growth of *F. graminearum*, *F. culmorum* and *F. poae*, isolated from infected wheat seed, was stimulated when temperature increased from 10 to 25 °C and reached its maximum at 25 °C. However, this study was conducted on Potato Dextrose Agar (PDA) and didn't included changes in water activities.

Nevertheless, fungal growth isn't synonymous with production of mycotoxins, and it may happen that environmental conditions suitable for the growth could result in reduced or absence of mycotoxins production, or vice versa. Therefore, in this study the impact of interacting

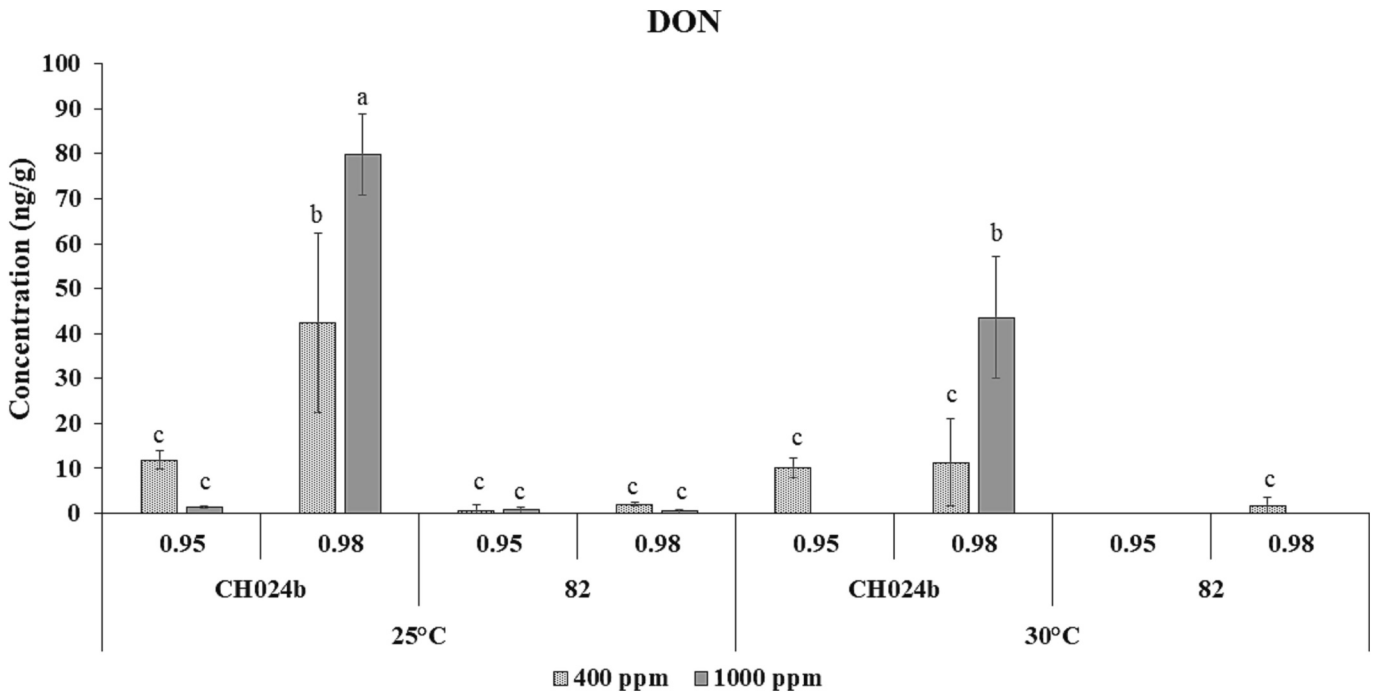

Fig. 3. Deoxynivalenol (DON) production by *F. asiaticum* CH024b and 82 under the effect of interacting conditions of temperature (25 vs 30 °C) x CO_2 (400 vs 100 ppm) x water activity (0.95 vs 0.98 a_w) after 10 days of incubation on wheat-based matrix. Different letters indicate significant differences ($p < 0.05$).

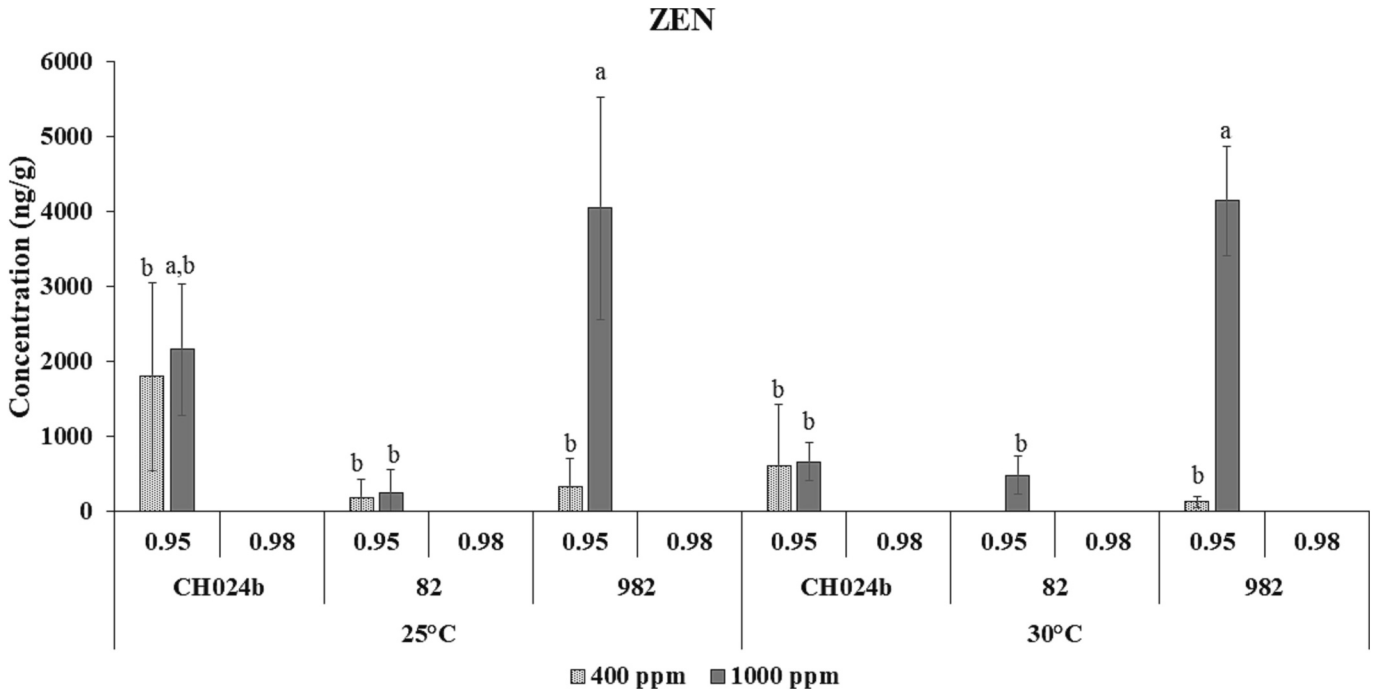

Fig. 4. Zearalenone (ZEN) production by *F. asiaticum* CH024b, 82, 982 under the effect of interacting conditions of temperature (25 vs 30 °C) x CO_2 (400 vs 100 ppm) x water activity (0.95 vs 0.98 a_w) after 10 days of incubation on wheat-based matrix. Different letters indicate significant differences ($p < 0.05$).

CC factors on ZEN, and type B trichothecenes (DON, 3-ACDON, 15-ACDON, NIV) by *F. asiaticum* strains was also investigated.

Among *Fusarium* mycotoxins, DON has been reported as the most frequently occurring one usually highest in wheat and maize, although concentration may vary depending on weather conditions during cereal growing seasons (World Mycotoxin Survey, 2023; Luo et al., 2021). Usually, for both wheat and maize, seasons with wet weather, followed by increased rainfall, influence higher contamination frequency and high concentration of DON. Furthermore, when DON is highly produced, 3-ADON and 15-ADON were also found (Pleadin et al., 2017; Kos et al., 2020). A high intraspecies variability was observed among the three strains in terms of both mycotoxins profile and amount produced, with 0982 strain producing only ZEN.

In our study, CH024b strain was the only one whose DON and 15-ADON concentrations were enhanced by high water availability conditions (0.98 a_w) and increased CO_2 concentration (1000 ppm), with a

significant major production of DON at 25 vs 30 °C. In a recent in situ study, it has been found that exposure to elevated CO_2 determined an increase of DON accumulation in plants infected by *F. graminearum* (Hay et al., 2021). Optimum conditions of DON production have been reported to occur within 25–30 °C and at high water activity (0.98–0.99 a_w) by *F. asiaticum* strains (Garcia-Cela et al., 2022), and other species within FGSC (Hope et al., 2005; Ramirez et al., 2006). 3-ADON was highly produced by the 82 strain at 0.98 a_w/25 °C under current CO_2 concentration (400 ppm), which was significantly different compared to 0.98 a_w/30 °C. These findings are partially in agreement with a previous study by Garcia-Cela et al. (2022) where they also found that the 82 strain was the major 3-ADON among the other strains, but its highest production occurred at 0.98 a_w/30 °C instead of 0.98 a_w/25 °C.

In the present study, ZEN was produced by all three strains only at moderate water stress (0.95 a_w) with no significant differences between the different temperatures (25 vs 30C). For the strain 982, the exposure to combined CC including elevated CO_2 levels resulted in significantly higher ZEN production compared to current CO_2. The role of moderate water stress on stimulation of ZEN production by different FGSC has already been shown. Garcia-Cela et al., 2022 also found a reduction of ZEN produced by the same three *F. asiaticum* strains grown on WBM when water activity increased from 0.93 to 0.98 with no significant differences observed between 25 and 30 °C. In another study, it has been also reported that ZEN production by *F. incarnatum* on sorghum seeds was enhanced by low a_w values (0.91, 0.94 a_w) in relation to temperature changes (Lahouar et al., 2017). In the present study, NIV was not detected in none of the conditions tested by none of the *F. asiaticum* strains. Contrasting findings exist about optimum conditions for NIV production by different FGSC (Garcia-Cela et al., 2022; Shen et al., 2012; Nazari et al., 2018; Schöneberg et al., 2019). While previous studies confirmed the dominance of *F. asiaticum* with NIV genotype in rice agrosystems (Gomes et al., 2015; Yang et al., 2018) it is not clear yet the distribution of *F. asiaticum* chemotypes in wheat.

In summary, this study has provided new evidence on the possible ecophysiological behaviour of three *F. asiaticum* strains when exposed to projected CC scenario. While fungal growth was similar among the three strains and resulted to be significantly reduced by the exposure at high CO_2 concentration at 0.95 a_w, mycotoxin production was subjected to higher intraspecies variability. Indeed, the exposure to elevated atmospheric CO_2 levels may trigger some F. *asiaticum* strains to produce higher concentrations of DON and ZEN compared to current climatic conditions. The latter would result in 40-fold increased concentration compared to the EU legislative limit fixed for unprocessed cereals (EU, 2023/915), especially under moderate water stress conditions (0.95 a_w). These types of data, implemented by a higher number of fungal strains and by future in situ studies, will be beneficial to develop accurate predictive models on the potential impact and possible increase in risks of type B trichothecenes and ZEN in wheat growing areas where *F. asiaticum* has already been reported or to outline new possible areas of occurrence. Either way such information can be considered by farmers, food industries, and policy makers to implement mycotoxins controlling strategies to make sure they are resilient enough against expected climate changes.

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CRediT authorship contribution statement

Carla Cervini: Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Naoreen Naz:** Investigation. **Carol Verheecke-Vaessen:** Writing – review & editing. **Angel Medina:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2024.110658.

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