International Journal of Food Microbiology 439 (2025) 111250



Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro



Will climate change affect growth and ochratoxin A production of putative biocontrol knockout strains of *Aspergillus carbonarius*?

Belén Llobregat [a], Carla Cervini [b], Luis González-Candelas [a], Carol Verheecke-Vaessen [b], Ana-Rosa Ballester [a,*], Ángel Medina [b,*]

[a] Institute of Agrochemistry and Food Technology (IATA, CSIC), Paterna 46980, Valencia, Spain
[b] Magan Centre of Applied Mycology, Cranfield University, Cranfield, Beds MK43 0AL, UK

ARTICLE INFO

Keywords:
Aspergillus carbonarius
Biocontrol agent
Climate change
Kojic acid
Ochratoxin A
Pyranonigrin A

ABSTRACT

The research explored the effects of abiotic factors associated with climate change (CC) on the growth and metabolite production of wild-type *Aspergillus carbonarius* ITEM 5010 and three knockout mutants: one knockout in the first gene of the ochratoxin A (OTA) biosynthesis pathway (Δ*otaA*) and two in the *veA* and *laeA* genes (the latter knockout generated in this work) encoding VELVET complex proteins, which regulate metabolism. Variables examined were temperature (30 °C vs 37 °C), water activity (0.98 vs 0.90), and CO_2 levels (400 ppm vs 1000 ppm). Growth, OTA production, and other metabolites were evaluated on grape-based medium. The results showed that abiotic factors significantly influenced fungal growth and mycotoxin production, with a_w being the most critical parameter. At a_w 0.90, no growth was observed. A temperature of 37 °C combined with 1000 ppm CO_2 resulted in higher OTA production, indicating a greater health risk in predicted CC scenarios. Mutants of global regulatory factors showed altered metabolite production, with elevated OTA levels at 37 °C. The Δ*otaA* knockout mutant consistently showed no OTA production, suggesting its viability as a biocontrol agent under CC conditions. However, while OTA increased, other secondary metabolites, such as pyranonigrin A and kojic acid, decreased with rising temperatures in all strains. The research highlights the influence of abiotic factors related to CC on *A. carbonarius* growth and metabolite production, underlining the threat of increased mycotoxin production. This reinforces the need for resilient biocontrol strategies. The Δ*otaA* mutant has been identified as a potential biocontrol agent, demonstrating resistance to future environmental stresses associated with CC.

1. Introduction

Ochratoxin A (OTA) is a secondary metabolite produced by *Aspergillus* and *Penicillium* species and contaminates agricultural products like cereals, coffee, cocoa, and fruit-derived products. In grapes, OTA contamination is mainly attributed to *Aspergillus* section *Nigri*, especially *A. carbonarius*, while *A. niger* and *A. welwitschiae* are of minor importance (Battilani et al., 2003; Cabañes and Bragulat, 2018; García-Cela et al., 2016; Palumbo et al., 2011; Perrone et al., 2008). This mycotoxin has been described as neurotoxic, hepatotoxic, immunosuppressive, and possible carcinogen metabolite. In consequence, the International Agency for Research on Cancer (IARC) has classified OTA as possible carcinogen in humans (Group 2B) (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1993). Therefore, maximum limits have been set for OTA in different food. The European Commission has established the maximum levels for OTA in grape products of 2 μg/kg to ensure food safety (Commission Regulation (EC), 2023).

Understanding the molecular mechanisms underlying OTA biosynthesis is important for developing effective control strategies. The *otaA* gene, previously designated as AcOTA*pks*, encodes a polyketide synthase responsible for the first step of the OTA biosynthesis, playing a crucial role in OTA biosynthesis (Gallo et al., 2014; Maor et al., 2021; Wang et al., 2018). In addition to the gene cluster, several regulatory proteins, such as VeA and LaeA, were found to regulate genes involved in the secondary metabolite biosynthesis in *A. carbonarius* (Crespo-Sempere et al., 2013). These proteins are components of the heterotrimeric VelB/VeA/LaeA complex, also known as the VELVET complex, which has been previously characterized in several fungal species (Bayram et al., 2008; Bayram and Braus, 2012; Crespo-Sempere et al., 2013; Palmer et al., 2013). This complex is a key regulator of secondary metabolism and influences fungal development, including sexual and asexual growth, in response to light in *Aspergillus nidulans* (Bayram et al.,

* Corresponding authors.
E-mail addresses: ballesterar@iata.csic.es (A.-R. Ballester), a.medinavaya@cranfield.ac.uk (Á. Medina).

https://doi.org/10.1016/j.ijfoodmicro.2025.111250
Received 6 March 2025; Received in revised form 20 April 2025; Accepted 7 May 2025
Available online 8 May 2025
0168-1605/© 2025 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

2008). VeA functions as a global regulator, translocating from the cytoplasm to the nucleus in response to light signals. Once in the nucleus, VeA interacts with LaeA to regulate secondary metabolite production and with VelB to promote sexual development (Palmer et al., 2013).

Disruption of the *otaA* and *laeA* genes drastically reduced OTA levels in vitro and in vivo in *A. carbonarius* NRRL 368 (Maor et al., 2021). Similarly, no OTA production was observed in knockout mutants of the *otaA* and *veA* genes in *A. carbonarius* ITEM 5010, also in vitro and in vivo (Llobregat et al., 2022) and, furthermore, they were proposed as non-ochratoxigenic mutants that could be used as biocontrol agents (BCAs) for OTA mitigation. The EU's use of genetically modified organisms (GMOs) for biocontrol is still in its early stages, facing public concerns and opposition from countries like Germany, France, and Austria. While strict regulations exist, emerging frameworks for genomic technologies like CRISPR may enable future applications. The UK's exit from the EU has allowed for a more flexible approach, focusing on gene-editing techniques to promote sustainable farming. National regulations in the EU remain varied, with some countries refusing GMOs entirely (Directive (EU) 2015/412).

Production of mycotoxins may be affected by environmental conditions. OTA contamination in grapes varies significantly from season to season and is strongly related to geographical area, weather conditions, microclimate, and plant phenology (Visconti et al., 2008). The European Commission reported an increased risk of OTA contamination associated with global warming, highlighting higher incidence rates and concentrations of OTA in Southern European countries compared to Northern Europe (Commission Regulation (EC), 2002). García-Cela et al. (2015) conducted a study on the contamination of vineyards by *Aspergillus* section *Nigri*, comparing two different agro-climatic regions in Spain. The study revealed a significantly higher presence of the fungus in Andalucia, the Southern region, compared to Catalunya, the North-Eastern region.

Climate change (CC) and food security have received much attention in the last decade. This has led to a focus on how interacting abiotic factors such as elevated temperatures (+ 3–5 °C), fluctuations in wet and dry conditions (drought stress), and increased CO_2 levels (400 ppm vs. 800–1200 ppm) affect mycotoxin contamination in staple food crops (Magan et al., 2011; Medina et al., 2017). In the fungal species *A. carbonarius*, there is increasing evidence that new environmental conditions promoted by CC will have significant impacts. Oliveira et al. (2019) showed that CC is expected to increase the risk of OTA production in selected coffee-producing regions of Brazil, underlining the importance of implementing good agricultural practices. Similarly, Cervini et al. (2021) simulated a CC scenarios in high OTA risk areas of the Apulia region in Southern Italy, showing that elevated atmospheric CO_2 levels would increase the colonization rate of *A. carbonarius* ITEM 5010 and promote higher OTA production. Specifically, an increase of CO_2 up to 1000 ppm at current temperature conditions (15–28 °C) would lead to a 10.73 % rise in fungal growth. The authors also investigated the expression of two groups of genes: those directly involved in OTA biosynthesis, such as *otaA*, *nrps* or *bZIP,* and genes associated with the VELVET complex, including *laeA*, *veA* and *velB*. The expression of both gene groups increased, demonstrating that abiotic factors associated with CC lead to an increase in OTA production through gene regulation. This effect appears to involve both direct activation of genes within the biosynthesis cluster and up-regulation of global regulatory genes. Transcript levels of the AcOTA*nrps* gene increased almost 5-fold, while the gene encoding the bZIP transcription factor was overexpressed >2-fold. Similarly, the regulatory genes of the VELVET complex were significantly upregulated. These molecular findings were corroborated by the direct detection of increased OTA concentrations. This emphasises the fundamental role of the VELVET complex genes in the regulation of mycotoxin production, as already highlighted by Crespo-Sempere et al. (2013).

In addition to OTA, *A. carbonarius* produces a wide range of secondary metabolites, including pyranonigrin A and kojic acid. The production of these metabolites could also be influenced by different factors related to CC. Pyranonigrin A, known for its antioxidant properties and produced by several *Aspergillus* species (Miyake et al., 2008), plays a role in inhibiting the formation of reactive oxygen species (Miyake et al., 2010). On the other hand, the production of kojic acid, a metabolite recognized for its efficacy as a skin-lightening agent, has been extensively studied. Kojic acid, produced by *Aspergillus* and *Penicillium* species (Chib et al., 2023), is crucial in the cosmetic industry due to its ability to protect against UV radiation. In addition, kojic acid derivatives have demonstrated antibacterial, antifungal, antiproliferative and anti-inflammatory activities, making them valuable in medicine.

Despite their importance, studies on how future CC will affect the resilience of mycotoxigenic fungi and mycotoxin production are lacking. Understanding how these fungi will adapt to changing conditions and the implications for their growth and toxin production is crucial for developing strategies to mitigate the adverse effects of CC on food security. The aim of this research was to understand the impact of CC on the behaviour of different *A. carbonarius* knockout mutants that could be used as biocontrol agents. We have investigated the impact of temperature, a_w and CO_2 factors on specific gene knockout mutants, denoted as Δ*otaA,* Δ*veA* and Δ*laeA,* on growth, conidiation, OTA production and, to a lesser extent, the production of additional secondary metabolites.

2. Material and methods

2.1. Fungal strains, media preparation and inoculation

OTA-producer *A. carbonarius* ITEM 5010 strain was used as wild-type (wt), along with three knockout mutants: Δ*otaA* and Δ*veA*, previously described by Llobregat et al. (2022), and the Δ*laeA* mutant, generated in this work. All fungal strains were initially grown on potato dextrose agar (PDA) plates for 7 days at 25 °C. Conidia were detached using a sterile loop and placed in 1 mL of sterile water containing 0.05 % Tween 80 in a 2 mL tube. The concentration of conidia was determined with a hemacytometer, and the suspension was diluted with sterile water to reach a final concentration of 10^5 conidia/mL for each *A. carbonarius* strain. Each previously prepared conidial suspension was used to inoculate plates of grape juice basal medium (GJM). Five μL the 10^5 conidia/mL suspension was deposited in the centre of each plate. GJM with an a_w of 0.99 was prepared by diluting commercial pasteurised grape juice (composition per 100 mL: <0.5 g fat, <0.5 g protein, 15.3 g carbohydrate) with distilled water at a ratio of 50 % (*v*/v) and adding 1.2 % agar (Ioannidis et al., 2015). To obtain GJM with an a_w of 0.90, a ratio of 50: 24.6: 25.4 (v:v:v) of grape juice, water and glycerol, respectively, was used. The a_w of the medium was measured with an AquaLab 4TE water activity meter (Decagon Devices, Inc., Pullman, WA, USA). The pH of the medium, which was 3.2 and consistent with the natural pH of grape juice, did not require any additional pH adjustment. After 7 days post-inoculation (dpi), 6 mm plugs representing each colony section were extracted along the growth diameter and stored at −20 °C for mycotoxin analysis. Experiments were conducted with four replicates per treatment.

2.2. Construction and verification of the A. carbonarius laeA gene knockout strain

To construct the *laeA* gene replacement plasmid, approximately 1500 bp of the 5′ and 3′ flanking regions were amplified from *A. carbonarius* ITEM 5010 genomic DNA. Primer pairs (Table S1) were designed using Primer3 software (Untergasser et al., 2012), and amplification of 10 ng of genomic DNA was performed with High-Taq DNA polymerase following the manufacturer's instructions. PCR cycling conditions included an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 15 s, 58 °C for 20 s and 72 °C for 1 min and 30 s, with a final extension at 72 °C for 10 min. The pRF-HU2 vector,

compatible with the USER (Uracil-Specific Excision Reagent) cloning technique (Frandsen et al., 2008), was used to generate the plasmid pRF-HU2-*laeA*, as previously described (Gerin et al., 2018; Llobregat et al., 2022). The amplified upstream and downstream fragments were mixed with the digested pRF-HU2 vector in the presence of the USER enzyme mix and incubated according to the manufacturer's guidelines. This mixture was then used to transform chemically competent *Escherichia coli* DH5α cells. Kanamycin-resistant transformants were screened by PCR using specific primer pairs, and correct plasmid construction was confirmed by DNA sequencing.

Transformation of *Agrobacterium tumefaciens* AGL-1 cells was performed by introducing the plasmid into electrocompetent cells using a Gene Pulser apparatus (Bio-Rad, Richmond, CA, USA). Subsequently, *A. carbonarius* ITEM 5010 was transformed by co-culturing conidial suspensions with IMAS-induced *A. tumefaciens* cultures on agar plates containing hygromycin B and cefotaxime to select fungal transformants and inhibit bacterial growth. Hygromycin-resistant *A. carbonarius* colonies appeared after 3–4 days of incubation at 28 °C, from which monosporic cultures were obtained.

Extraction of genomic DNA from the transformants was followed by PCR analysis to confirm disruption of the *laeA* gene and insertion of the selection marker HygB. Quantitative PCR (qPCR) was performed to determine the number of T-DNA copies integrated into the genome, using specific primers designed for the upstream regions of the *laeA* gene, as previously described by Llobregat et al. (2022). The non-ribosomal peptide synthetase (*nrps*) gene served as a reference gene for qPCR (Crespo-Sempere et al., 2013), and the T-DNA copy number was calculated (Pfaffl, 2001). All primers used are described in Table S1. The Δ*laeA* knockout mutants were phenotypically characterized during in vitro growth as previously described (Llobregat et al., 2022). Growth area, conidiation and OTA production were analysed on PDA plates centrally inoculated with 5 μL of 10^5 conidia/mL. After 5 days of incubation at 28 °C, colony growth areas were scanned and analysed using ImageJ software, and three plugs were collected from the central, middle and peripheral regions of the colony for conidial quantification and OTA extraction.

2.3. Incubation conditions and CO_2 treatment

GJM agar plates inoculated with fungal spore suspensions were placed in sealed 12-L containers and maintained in environmental chambers. Three temperature simulations were carried out at: 25, at 30, and at 37 °C, the latter representing a more severe CC situation. Glycerol/water solutions were maintained inside the airtight compartments to regulate the relative humidity of the indoor atmosphere. In addition, these chambers included an outlet and an inlet with CO_2 flush valves. The chambers were flushed twice a day either with current (400 ppm) or increased (1000 ppm) CO_2 concentrations using specialty grade mixtures gas cylinders (British Oxygen Company, Guildford, Surrey, UK), with a flow rate of 3 L/min, to renew 3 times the air volume of the incubation chambers (Verheecke-Vaessen et al., 2019).

2.4. Growth measurement

Fungal growth was measured daily up to 7 days. Colony diameter was recorded as the mean (mm) of two centred orthogonal diameters. Colony diameters (mm) were plotted as a function of time (days). After 7 days of incubation, the maximum growth rate (μ_m, mm of diameter per day), maximum colony diameter (Dmax, mm), lag time (λ, days), and area under the curve (AUC) were determined in Rstudio using the model-free spline fit model implemented in the grofit package version 1.1.1–1 (Kahm et al., 2010). It should be noted that the term "lag phase" refers to the *visual lag phase*, that is, the point at which growth becomes visibly detectable to the naked eye, rather than the actual lag phase associated with the precise onset of conidial germination.

2.5. Ochratoxin A and other secondary metabolites analysis

To extract mycotoxin and other secondary metabolites, plugs were thawed (approximately 400 mg), and a solvent mixture consisting of acetonitrile, water and formic acid (79:20.9:0.1 % *v*/v/v) was added, maintaining a 1:4 ratio of sample to solvent. After solvent addition, the samples were shaken at 25 °C for 1.5 h and centrifuged at 16400 rpm for 5 min. A 500 μL aliquot of the supernatant was collected and stored at −20 °C until analysis. The LC-MS/MS system used in this study was a QTRAP 5500+ MS/MS system (SCIEX, Framingham, MA, USA) with a Turbo V electrospray ionisation (ESI) source, paired with an Exion LC AD system (SCIEX, Framingham, MA, USA). Both chromatographic separation and elution were carried out in the same way as in previous work conducted by the group (Isidro-Sánchez et al., 2020). The data acquisition process was conducted using Analyst® Data Acquisition version 1.6.3, while quantification was executed using MultiQuant™ version 3.0.3. Quantitative analysis was done by standard curve solution containing OTA and OTB. Other metabolites such as pyranonigrin A or kojic acid were measured qualitatively via peak area.

2.6. Data analysis

Statistical analyses were performed for each knockout mutant and the parental strain *A. carbonarius* ITEM 5010, using appropriate statistical tests based on the distribution and variance of the data. Normality and homoscedasticity were assessed using the Shapiro-Wilk and Levene tests, respectively. Data sets that met the assumptions of normality and homoscedasticity were analysed using parametric methods, specifically analysis of variance (ANOVA), followed by Tukey's post hoc test ($p < 0.05$). These analyses were performed in RStudio using the 'agricolae' package. For data sets that did not meet the assumptions, non-parametric tests were applied. The Kruskal-Wallis test was used, followed by a Wilcoxon post hoc test ($p < 0.05$).

3. Results

3.1. Characterization of laeA knockout mutants

To understand the behaviour of different potential non-mycotoxigenic BCAs under expected abiotic CC factors, the knockout mutant of the *laeA* gene in the OTA-producing *A. carbonarius* ITEM 5010 background was constructed. A pRFHU2-laeA gene replacement plasmid was generated, as described by Llobregat et al. (2022), using the USER-Friendly cloning strategy (Frandsen et al., 2008). Co-culture of *A. tumefaciens* cells carrying the gene replacement plasmid with *A. carbonarius* conidia resulted in hygromycin-resistant colonies approximately three days after transfer to PDA-selective plates. The correct integration of the T-DNA containing the hygromycin resistance marker replacing the target gene was confirmed by PCR amplification (Fig. S1). Phenotypic traits of the wt and the Δ*laeA*, including growth, conidiation and OTA production, were assessed on PDA plates at 5 dpi (Fig. 1). In general, the growth area of the Δ*laeA* (36.3 mm^2) was significantly higher than that of the wt (31.3 mm^2) (Fig. 1A). However, no statistically significant differences were observed in conidiation among them (Fig. 1B). Analysis of OTA production revealed that the three knockout mutants had markedly reduced OTA production under the conditions tested (Fig. 1C), with levels dropping dramatically from 961.6 for the wt to an average of 26.5 ppb for the knockout mutants. In view of the similar results obtained for the three Δ*laeA* knockout mutants analysed, we selected Δ*laeA*-3 for further analyses.

3.2. Impact of the interaction of abiotic factors related to climate change on growth of A. carbonarius strains

The growth of different *A. carbonarius* strains (wt, Δ*otaA*, Δ*veA* and Δ*laeA*) was monitored on a grape-based medium for a period of 7 days.

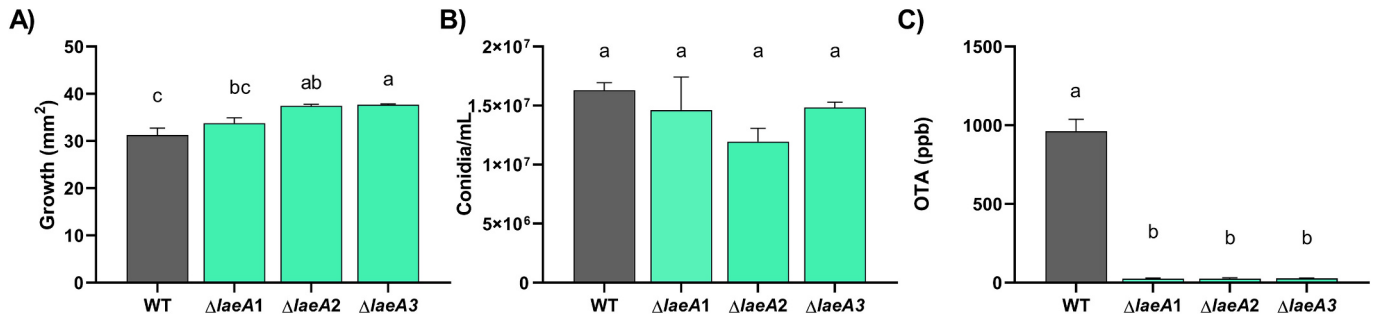


Fig. 1. Phenotypic characteristics of the wild-type strain of *A. carbonarius* ITEM 5010 (denoted 'wt', grey bars) and three *laeA* knockout mutants (denoted Δ*laeA*, green bars). Growth area (A), conidiation (B) and OTA production (C) were analysed on PDA plates centrally inoculated with 5 μL of 10^5 conidia/mL after 5 days of incubation at 28 °C. Colony growth areas were scanned and analysed using ImageJ software, and three plugs were collected from the central, middle and peripheral regions of the colony for conidial quantification and OTA extraction. Error bars indicate the standard error of the mean of at least three biological replicates. Bars marked with different letters within the same panel are statistically different according to one-way ANOVA followed by Tukey's test ($p < 0.05$).

Fig. 2 shows the effect of three-way interacting treatments (T × a_w × CO_2) on the lag phases (data are presented in Table S2). In general, the lag phases prior to growth of the different strains were lower than 1 day at a_w of 0.98, independently of the temperature and CO_2 concentration. However, a lower a_w of 0.90 significantly increased the lag phase to between 2 and 3 days. It is interesting to note that all strains were able to initiate growth even at 37 °C. The AUC is an integrated measure that combines the lag phase, the exponential growth phase and the stationary phase of a fungal growth curve. The AUC indicates that the growth of each strain under the different conditions tested were similar (Fig. 3, Table S3). This pattern was also observed when analysing the growth rate (μ, in mm/day) (Fig. S2, Table S4) and the maximum colony diameter (Dmax, Fig. S3). Considering temperature (25, 30 or 37 °C), the highest AUC values were observed at 30 °C. Although all strains were able to initiate the growth at 37 °C, as observed by the determination of the lag phase (Fig. 2), they were unable to achieve the expected growth of approximately 400 AUC observed at the other two temperatures (25 °C and 30 °C), which represent normal growth under optimal temperature and a_w conditions (Fig. 3). The increase of CO_2 concentration from 400 ppm to 1000 ppm did not affect significantly the growth of the strains in all the conditions tested. Statistical analysis revealed that a_w was essential for the normal development of all strains at 25 °C and 30 °C. Growth was significantly higher at a_w 0.98 compared to 0.90, with optimal growth observed at 30 °C and a_w 0.98, followed closely by 25 °C and a_w 0.98, irrespective of CO_2 levels.

3.3. Influence of different abiotic factors on Ochratoxin A and Ochratoxin B production from A. carbonarius strains on grape-based medium

OTA production in the different strains grown on grape-based medium after 7 days of incubation was analysed to observe the effect of the interaction of the different CC factors (Fig. 4, Table S5). The most remarkable observation was the significant difference in OTA production between the wt strain and Δ*veA* and Δ*laeA* knockout strains, with maximum values close to 1800 ppb for the wt and 40 ppb for the knockout mutants. As expected, no OTA production was detected in the Δ*otaA* due to the essentiality of this gene in the biosynthesis of the mycotoxin (data not shown).

Temperature had an important influence on the OTA production for the wt strain (Fig. 4A): both temperatures 25 °C and 37 °C increased mycotoxin levels (583.0 ppb for conditions 400 ppm at 25 °C; 0.98 a_w, and 1962.5 ppb for 1000 ppm at 37 °C; 0.98 a_w) compared to the normal growing temperature of 30 °C (maximum value of 94.4 ppb for CC conditions of 400 ppm at 30 °C; 0.98 a_w), with the most notable increase at 37 °C. The a_w in the medium was also a limiting factor in the mycotoxin production, with the lowest values (ranging from 47.4 ppb at 25 °C and 400 ppm to not detected at 37 °C and 400 and 1000 ppm) at 0.90 a_w. No OTA was detected at 37 °C with the lowest a_w. No effect on OTA levels was observed with different CO_2 flux at 25 °C and 30 °C and with the same a_w. However, higher levels of OTA were observed at 1000 ppm compared to that of 400 ppm at the highest temperature (37 °C).

Reduced levels of OTA were observed for both Δ*veA* and Δ*laeA* compared to the wt. under higher and lower temperatures related to CC, Δ*veA* strain was able to produce small amounts of OTA (a maximum value of 42.3 ppb for CC conditions of 400 ppm × 37 °C × 0.98 a_w) (Fig. 4B). It is worth mentioning that OTA values in the Δ*veA* mutant at 400 ppm × 25 °C × 0.90 a_w are close of those of the WT strain grown under the same conditions, 31.6 versus 47.4 ppb, respectively. Similar to what occurs for the wt and the Δ*veA* mutant, temperature was the climate factor that most affected the levels of OTA produced by the Δ*laeA* mutant (Fig. 4C). The highest OTA values (26.7 and 15.7 ppb for 400 ppm and 1000 ppm, respectively) were detected at 37 °C and 0.98 a_w. However, contrary to what has been observed for the wt strain, a CO_2 flux of 400 ppm at 0.98 a_w induced the highest mycotoxin production for Δ*laeA*. Notably, OTA produced by Δ*veA* and Δ*laeA* mutants was weakly detected at a low a_w of 0.90.

The concentrations of ochratoxin B (OTB) produced by the wt strain and the three mutants were also analysed when grown on grape-based medium (Fig. 5, Table S6). Similar to OTA production, no OTB levels were detected when Δ*otaA* was grown in any of the conditions assayed. The OTB levels were significantly higher, around 600 ppb for the wt strain (Fig. 5A) compared to those of the mutants (Figs. 5B-C), with maximum values of 13.2 and 10.6 ppb for Δ*veA* and Δ*laeA*, respectively. As indicated for OTA production, temperature was the abiotic factor that most strongly affected OTB production, with the highest values at 37 °C. Both the wt and the Δ*veA* strains were able to produce OTB with a low a_w of 0.90 and at 25 °C (Fig. 5A-B). At a CO_2 flux of 400 ppm the production of OTB was even higher in the Δ*veA* mutant than in the WT. Regarding the influence of CO_2 concentration, at the most stressful temperature of 37 °C, OTB values were higher when the wt strain was grown at 1000 ppm compared to 400 ppm, and the opposite effect was observed for the Δ*laeA*, where the higher values were detected at low CO_2 concentration.

3.4. Impact of the interaction of abiotic factors related to climate change on the production of other secondary metabolites by A. carbonarius

The wt of *A. carbonarius* and all knockout mutants were able to produce additional secondary metabolites on grape-based medium under different climate change conditions (Fig. 6). For pyranonigrin A (Fig. 6A, **Table S7**), the highest values were detected at 25 °C and an increase in temperature resulted in a strong decrease in the production of this metabolite. A 0.90 a_w reduced the amount of pyranonigrin A produced by all strains. In general, the levels of pyranonigrin A were independent of the CO_2 concentration, except for Δ*otaA,* in which an increase in the production of this compound was observed at 1000 ppm

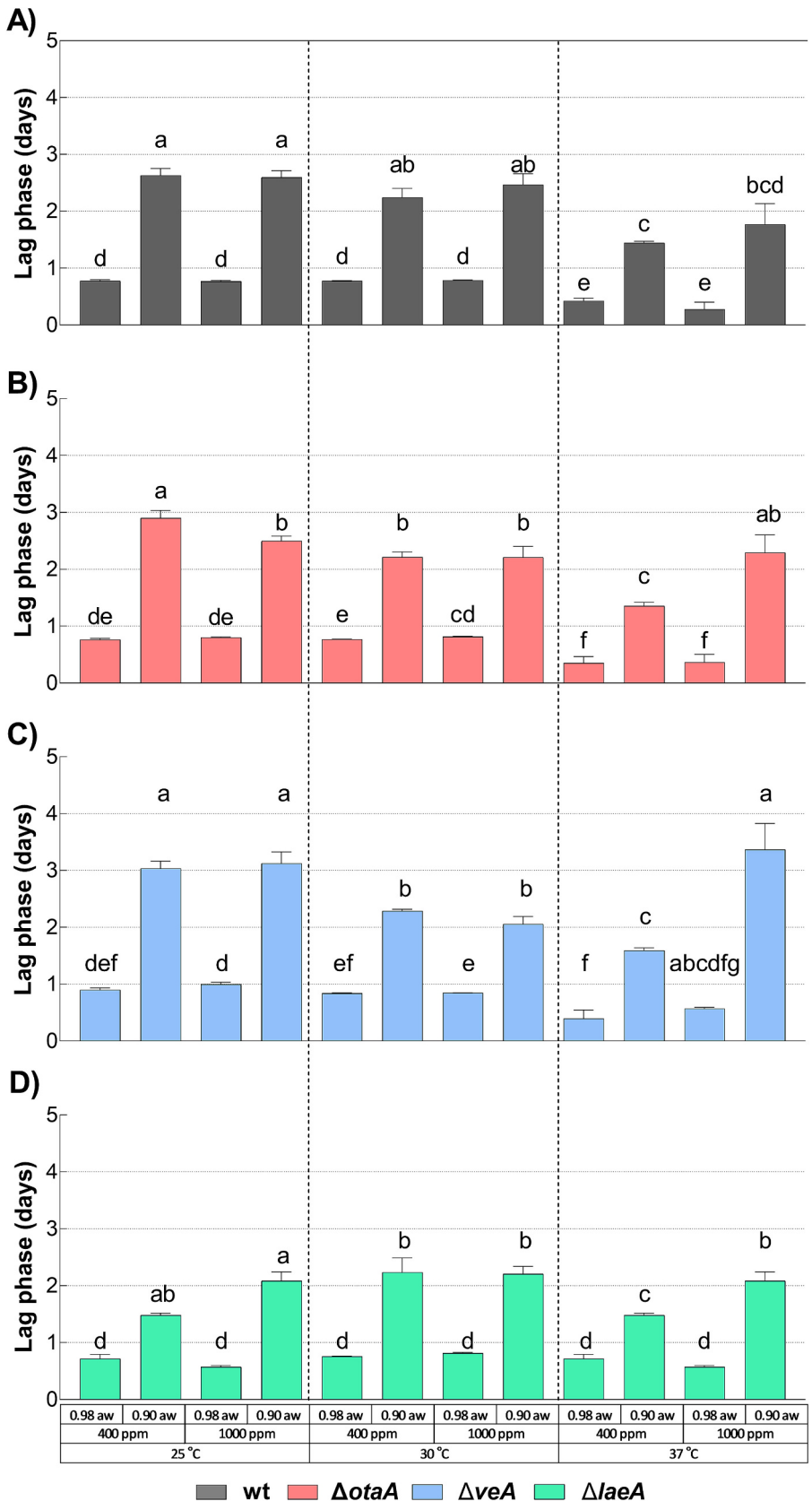
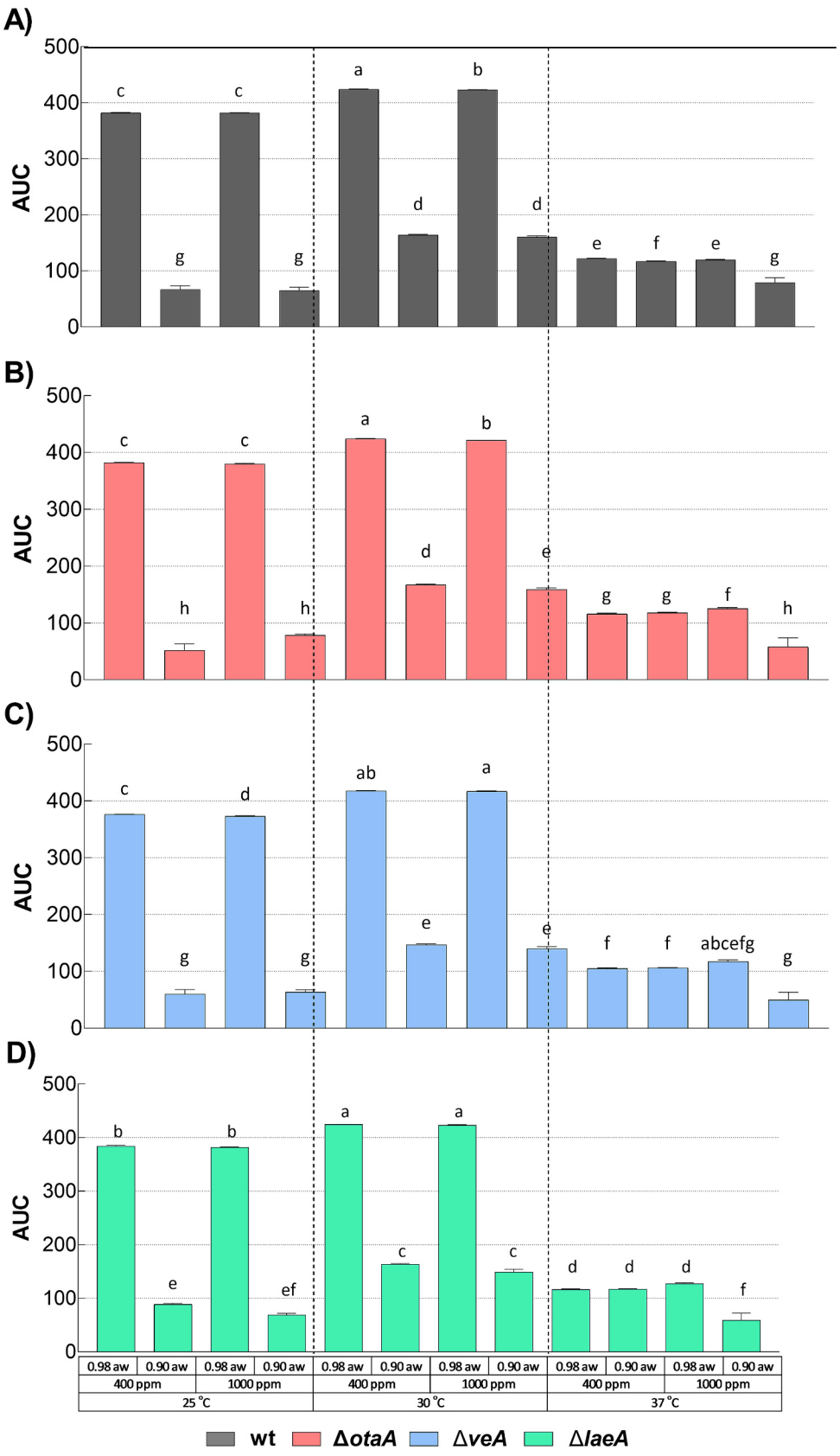


Fig. 2. Lag phase (days) of *A. carbonarius* ITEM 5010 (wt, grey bars) (A), Δ*otaA* (B, red bars), Δ*veA* (C, blue bars) and Δ*laeA* (D, green bars) knockout mutants at different temperature (25 - 30 and 37 °C), water activity (a_w of 0.90 and 0.98) and CO_2 levels (400 and 1,000 ppm). Bars marked with different letters within the same strain indicate statistically significant differences as determined by one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$) or Kruskal-Wallis test, followed by a Wilcoxon post hoc test ($p < 0.05$). Error bars indicate the standard error of the mean, calculated from at least three biological replicates. Data are presented in Table S2.



(caption on next page)

Fig. 3. Impact of abiotic factors of climate change on the growth of different strains, expressed as Area Under the Curve (AUC). Abiotic factors analysed: temperature (25 - 30 and 37 °C), water activity (a_w of 0.90 and 0.98), and CO_2 levels (400, 1,000 ppm). Strains: *A. carbonarius* ITEM 5010 (wt, grey bars) (A), and the Δ*otaA* (red bars, panel B), Δ*veA* (blue bars, panel C) and Δ*laeA* (green bars, panel D) knockout mutants. Bars marked with different letters within the same strain indicate statistically significant differences as determined by one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$) or Kruskal-Wallis test, followed by a Wilcoxon post-hoc test ($p < 0.05$). Error bars indicate the standard error of the mean, calculated from at least three biological replicates. Data are presented in Table S3.

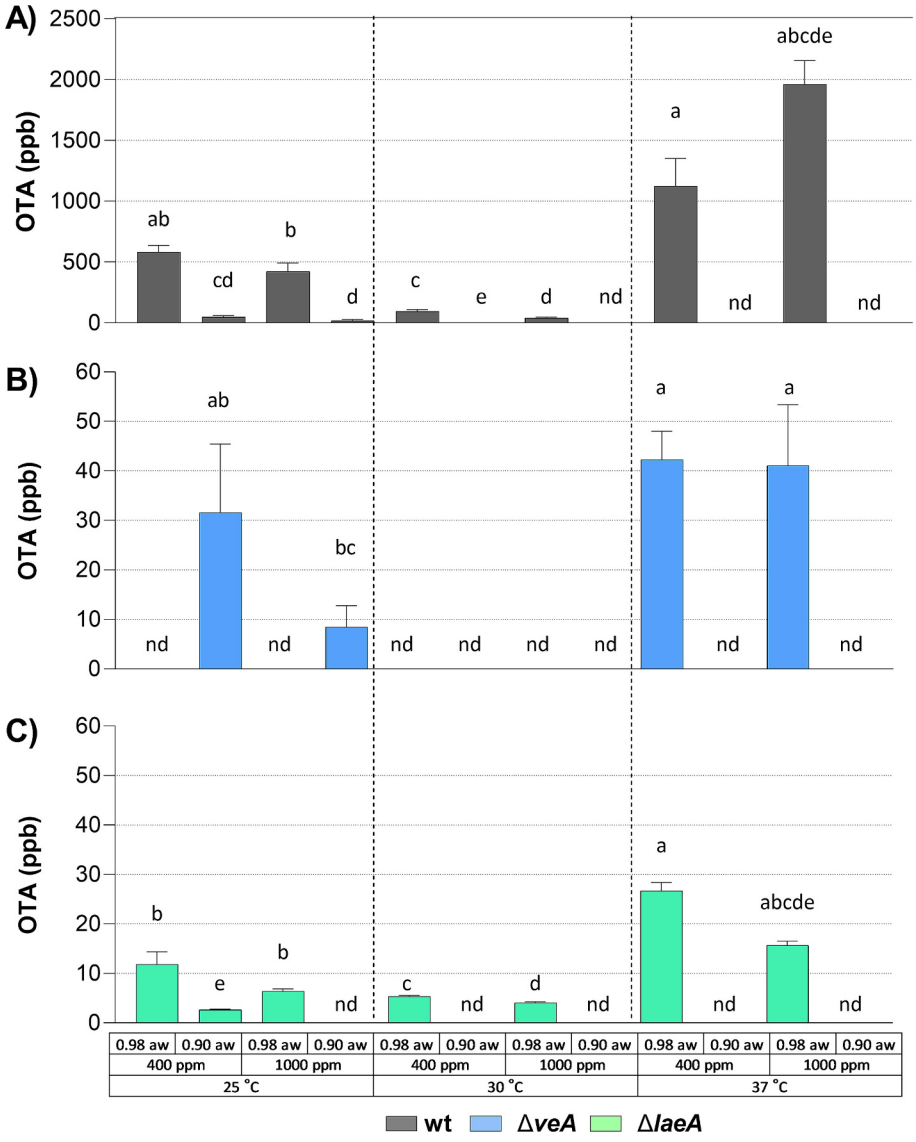


Fig. 4. Effect of three climate change-related abiotic factors (temperature, water activity, and CO_2 concentration) on ochratoxin A (OTA) (ppb) produced by *A. carbonarius* ITEM 5010 (wt) (A) and two knockout strains (Δ*veA* (B), Δ*laeA* (C)). Bars with different letters within a panel denote statistically significant differences based on one-way ANOVA with Tukey's post-hoc test ($p < 0.05$) or Kruskal-Wallis test, followed by a Wilcoxon post hoc test ($p < 0.05$). Error bars indicate the standard error of the mean from at least three biological replicates. Data are presented in Table S5.

compared to that at 400 ppm, independently of temperature. Regarding the different fungal strains analysed, the amounts of pyranonigrin A produced by the wt and the Δ*otaA* were similar in most of the conditions tested. However, Δ*veA* and Δ*laeA* produced a lower amount of this compound compared to the wt, with the greatest reduction observed in Δ*veA*.

The effect of CC-related abiotic factors on kojic acid produced by the different *A. carbonarius* strains was also analysed (Fig. 6B, Table S8). All strains produced the compound, independently of the condition tested. An increase in temperature lead to a reduction in metabolite production. In general, high concentration of CO_2 (1000 ppm) increased the amount of kojic acid produced at 25 °C and 37 °C. The water stress, with an 0.90 a_w resulted in the lowest metabolite production in all strains, independently of T and CO_2 concentration.

4. Discussion

Climate change is increasingly recognized as a significant driver of ecosystem disruption, with profound impacts on microbial behaviour, distribution and associated risks, such as mycotoxin contamination of food and feed. Variations in temperature, humidity and atmospheric composition can influence fungal physiology and secondary metabolism, amplifying mycotoxin risks under specific conditions (Medina et al., 2017; Paterson and Lima, 2010). While biological control is an

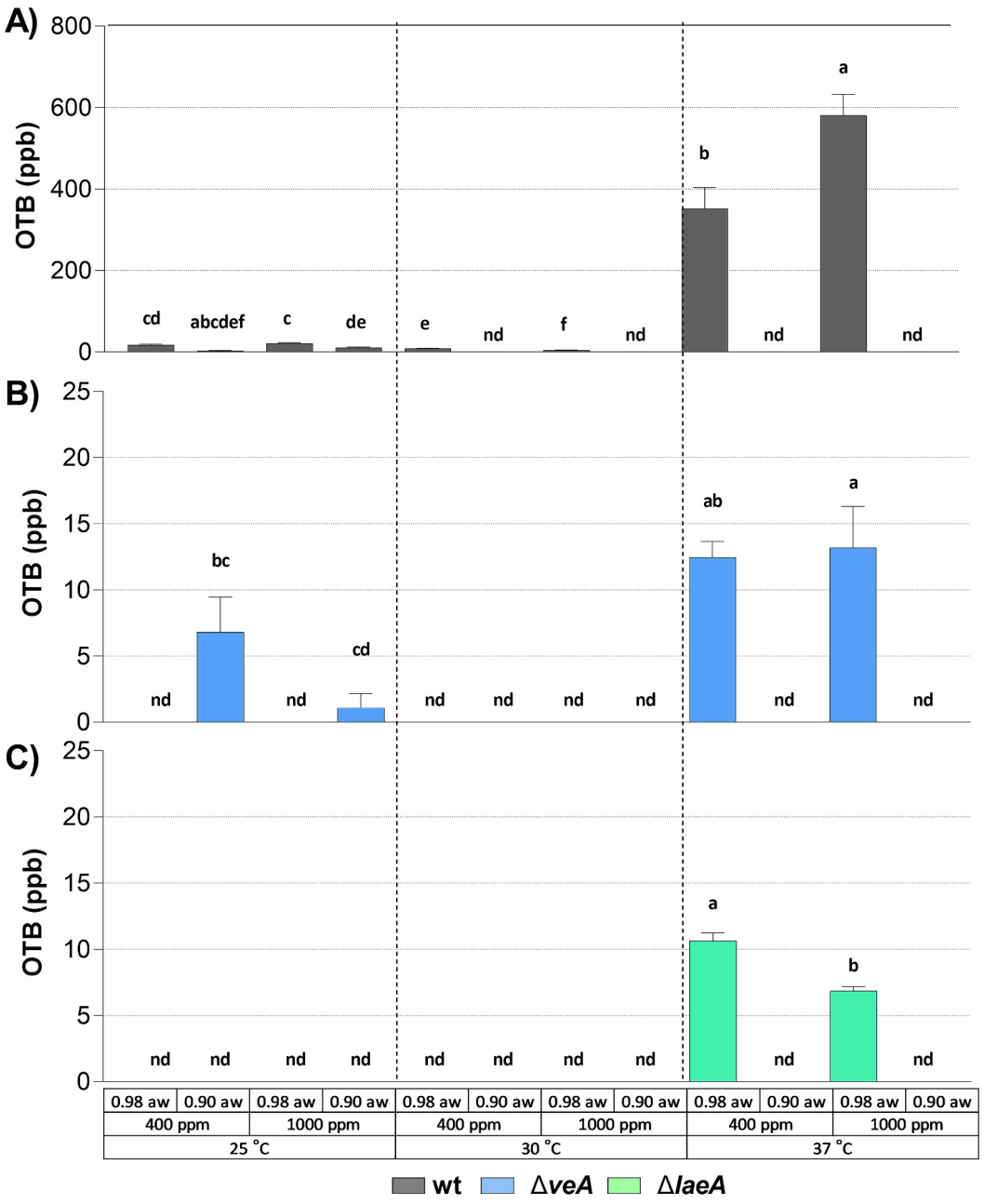


Fig. 5. Effect of temperatures (25 °C, 30 °C, 37 °C) x water activity (a_w of 0.90, 0.98) x CO_2 concentrations (400 ppm, 1000 ppm) on the production of ochratoxin B (OTB) (ppb) by *A. carbonarius* ITEM 5010 (wt) (A) and Δ*veA* (B), Δ*laeA* (C) knockout strains. Bars with different letters within a panel denote statistically significant differences based on one-way ANOVA with Tukey's post-hoc test ($p < 0.05$) or Kruskal-Wallis test, followed by a Wilcoxon post hoc test ($p < 0.05$). Error bars indicate the standard error of the mean from at least three biological replicates. Data are presented in Table S6.

established method to mitigate mycotoxin contamination, its reliability may be compromised by environmental changes induced by climate variability (Crosta et al., 2025; Zanon et al., 2023). This study represents the first analysis evaluating the performance of potential BCA knockout strains of *A. carbonarius* under CC scenarios. The Δ*otaA*, Δ*veA*, and Δ*laeA* strains, knockout mutants in genes directly or indirectly related to OTA biosynthesis (Llobregat et al., 2022), were analysed for their growth and secondary metabolite profiles under different temperatures, a_w, and CO_2 levels. Previous studies have shown that elevated CO_2 levels increase OTA production in *A. carbonarius* grown in a grape-based matrix (Cervini et al., 2021). Optimal OTA production occurred at temperatures ranging from 15 to 28 °C and a CO_2 concentration of 1000 ppm. To ensure that non-ochratoxigenic strains proposed as putative BCAs remain safe under varying environmental conditions, it is essential to evaluate their behaviour under different temperatures, a_w and CO_2 levels. Our experiments confirmed that OTA production by the wt strain increased significantly at 25 °C, and even more at 37 °C, especially when CO_2 levels increased from 400 ppm to 1000 ppm (Fig. 4).

While many studies have been conducted to understand the impact that temperature, CO_2 levels and a_w can have on fungal growth and mycotoxin production by the major toxigenic fungi such as *A. carbonarius*, *A. flavus*, *Fusarium asiaticum*, *Fusarium langsethiae*, and *Penicillium verrucosum* (Abdelmohsen et al., 2020; Cervini et al., 2024; Cervini et al., 2021; Garcia-Cela et al., 2021; Verheecke-Vaessen et al., 2019), very little is known about the impact of these interacting factors on putative BCAs. According to Gasperini et al. (2019), non-toxigenic *A. flavus* showed strong biocontrol potential against aflatoxin B_1 contamination, particularly in early maize development and post-

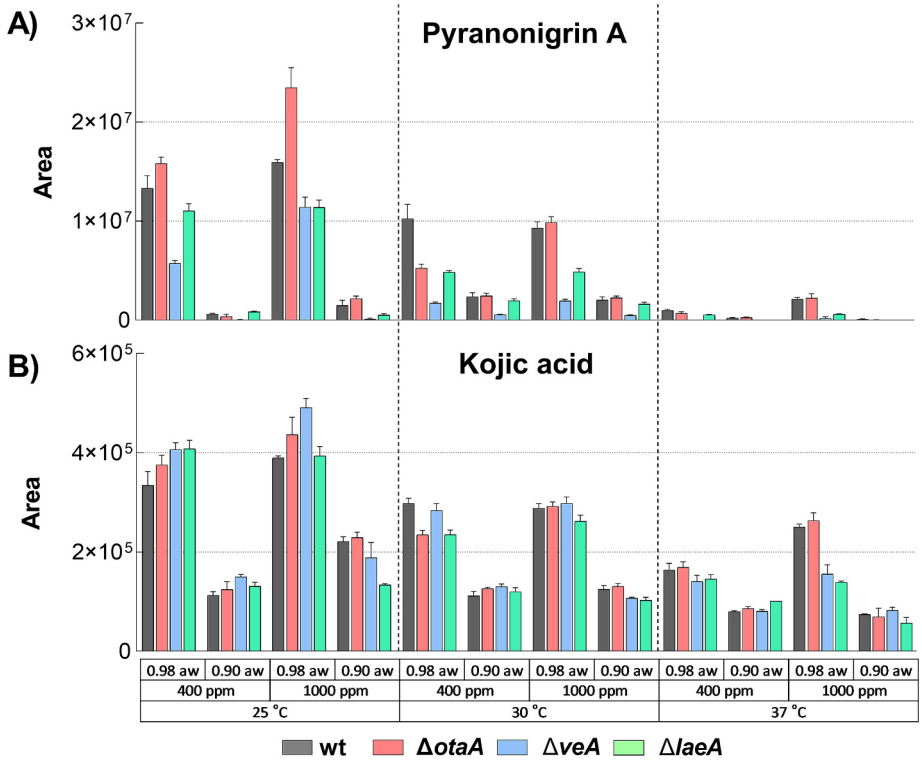


Fig. 6. Effect of three climate change-related abiotic factors (temperatures of 25 °C, 30 °C, 37 °C x water activity (a_w) of 0.90, 0.98, x CO_2 concentrations of 400 ppm, 1000 ppm) on pyranonigrin A (A) and kojic acid (B) levels by *A. carbonarius* ITEM 5010 (wt) and three knockout strains (Δ*otaA*, Δ*veA*, Δ*laeA*). Error bars indicate the standard error of the mean from at least three biological replicates. Values and statistical analysis are indicated in Tables S7 and S8.

harvest storage. While this strain effectively suppressed toxigenic *A. flavus* under optimal conditions, their efficacy decreased at later stages of maturation under climatic stress factors such as high temperatures (37 °C), high CO_2 (1000 ppm) and drought, posing limitations for field application. However, the biocontrol remains effective during storage at 30 °C, with improved performance in genetically modified maize cultivar, even under adverse climatic conditions. These findings underline the need to select resistant strains or adapt strategies to maintain aflatoxin management in changing climates (Gasperini et al., 2019). Pointing to the positive direction of these strategies, the study by Crosta et al. (2025) investigates the impact of simulated climate change conditions on the efficacy of a non-aflatoxigenic *A. flavus* biocontrol strain to reduce aflatoxin contamination in peanuts. The researchers tested different scenarios of temperature, a_w and CO_2 concentration, both in vitro (peanut-based medium) and in situ (peanut seeds). Results showed that the biocontrol strain remained effective under future climatic conditions, with aflatoxin B1 reductions of 55 % in vitro and 71 % in situ, and even greater suppression at higher temperatures (up to 76 % reduction in situ at 35 °C). These results suggest that climate change will not decrease, and may even increase, the effectiveness of biocontrol-based aflatoxin management in peanut.

The growth responses of various fungal species to abiotic factors show remarkable interspecific differences. For *A. flavus*, the combination of interacting CC factors had a minimal impact on its growth rate (Garcia-Cela et al., 2021). In contrast, *Penicillium verrucosum* showed reduced growth when the temperature was increased from 25 °C to 30 °C, indicating a reduced tolerance to elevated temperatures (Abdelmohsen et al., 2020). On the other hand, *F. asiaticum* showed stable growth under elevated CO_2 levels (1000 ppm) compared to standard conditions of 400 ppm, suggesting that CO_2 concentration has minimal influence on its growth rate (Cervini et al., 2024). Notably, our study corroborated these findings for *A. carbonarius*, showing that this fungus maintained comparable growth levels at a_w of 0.98 at both 25 °C and 30 °C, regardless of CO_2 concentration. These observations underline the species-specific nature of fungal growth responses to environmental factors and suggest that the projected CC scenario may have a limited impact on fungal growth per se, although other aspects such as mycotoxin production may be significantly affected.

The production of secondary metabolites, in particular mycotoxins, is significantly influenced by the interaction of CC factors such as temperature, a_w and CO_2 levels, with significant interspecific and intraspecific variability. In the case of *A. flavus*, aflatoxin B1 production increased significantly under conditions of low a_w (0.93), high CO_2 levels (1000 ppm) and high temperature (37 °C) (Garcia-Cela et al., 2021). On the other hand, although *P. verrucosum* showed inhibited growth at higher temperatures (30 °C vs. 25 °C), its OTA production responded strongly to abiotic factors. Elevated temperature and CO_2 levels (1000 ppm) markedly increased OTA synthesis, particularly at a_w levels of 0.98 and 0.90 (Abdelmohsen et al., 2020). *F. langsethiae* showed increased production of T-2/HT-2 toxins in oats under high temperature (30 °C) and a_w of 0.98, driving substantial increases in mycotoxin synthesis (Verheecke-Vaessen et al., 2019). Meanwhile, *F. asiaticum* showed significant intraspecific variability in mycotoxin production. Some strains showed higher toxin production under elevated CO_2 (1000 ppm) and a_w of 0.98 at 25 °C, while others showed preference for standard CO_2 (400 ppm) levels. Our study showed that *A. carbonarius* produced high levels of OTA under the most stressful combination of conditions, at the highest a_w of 0.98, elevated CO_2 (1000 ppm) and temperature (37 °C) (Fig. 4). These results emphasise the potential for increased mycotoxin contamination under different CC scenarios, with effects influenced by species, strain variability and specific environmental conditions.

The production of other secondary metabolites such as pyranonigrin A and kojic acid has been described by several *Aspergillus* and *Penicillium* species (Chib et al., 2023; Miyake et al., 2008). However, to the best of our knowledge, this study is the first to explore the involvement of CC in the production of these secondary metabolites by *A. carbonarius*. Under adverse CC conditions, pyranonigrin A production was significantly reduced (Fig. 6A). In addition, knockout mutants lacking the *veA* and

laeA genes showed reduced production of this compound. In contrast, based on the trend of the results, the environmental conditions predicted in the CC scenarios were expected to reduce kojic acid production overall (Fig. 6B). However, an increase in atmospheric CO_2 concentration up to 1000 ppm, combined with high a_w resulted in higher kojic acid production, independent of temperature variations.

Regarding the study of the behaviour of the different putative BCAs under CC conditions, the Δ*otaA* showed similar growth and production of secondary metabolites such as pyranonigrin A and kojic acid to the wt strain under all conditions tested. However, no production of OTA and OTB was detected in any of the analysed conditions, consistent with previous observations (Ballester et al., 2015). In contrast, the Δ*veA* and Δ*laeA* knockout mutants, which are components of the VELVET complex and serve as global regulators of metabolism, showed more pronounced differences. In our study, the *A. carbonarius* ITEM 5010 Δ*laeA* mutant did not show significant alterations in vegetative growth (Fig. 1A), in line with previous observations in the *A. carbonarius* UdL-TA 3.83 strain, where *laeA* deletion also did not compromise fungal growth; the growth rate of the mutant was comparable to that of the wt strain, or even slightly exceeded it (Crespo-Sempere et al., 2013). This pattern of maintenance or increase in hyphal extension after deletion of *laeA* is consistent with that observed in other filamentous fungi, such as *Aspergillus fumigatus* and *Fusarium oxysporum*, especially under light conditions (Bok and Keller, 2004). However, the impact on conidiation differed between strains. While the ITEM 5010 Δ*laeA* mutant showed no significant change in conidial production (Fig. 1B), the Δ*laeA* mutant of strain UdL-TA 3.83 demonstrated an approximately 40 % reduction in asexual spore production relative to wt (Crespo-Sempere et al., 2013). This strain-dependent variation in conidiation highlights the specific role of *laeA* in regulating fungal development. Furthermore, this variation in conidiation observed at the strain level is also evident across species. Specifically, while *A. nidulans* Δ*laeA* mutants exhibit minimal changes in spore production, *Penicillium chrysogenum* Δ*laeA* transformants show a pronounced reduction in conidiation, and *Fusarium fujikuroi* Δ*laeA* mutants display an approximate 30 % decrease in conidial output. These results underscore the species-specific regulatory role of LaeA in asexual development (Bok and Keller, 2004; Hoff et al., 2010; Wiemann et al., 2010). Finally, regarding mycotoxin production, in *A. carbonarius* UdL-TA 3.83, the Δ*laeA* mutant showed a profound reduction in OTA biosynthesis. Under dark conditions, OTA levels in the Δ*laeA* strain were reduced by 97 % relative to the wt strain (Crespo-Sempere et al., 2013). Our results agree with this observation, as OTA production in the Δ*laeA* strain was almost eliminated, leaving only a minimal basal level. Deletion of *laeA* has been shown to suppress secondary metabolite production in several fungal species. For example, in *A. fumigatus*, Δ*laeA* mutants show reduced gliotoxin production (Bok et al., 2005), while in *P. chrysogenum*, deletion of *laeA* causes an 80 % reduction in penicillin production (Hoff et al., 2010). In addition, different studies in *Aspergillus* species have shown that deletion of *laeA* decreases the synthesis of secondary metabolites such as aflatoxin, sterigmatocystin, lovastatin and protostadienol (Bayram et al., 2008; Bok et al., 2006; Bok and Keller, 2004; Lodeiro et al., 2009; Perrin et al., 2007).

In previous studies, it has been observed that the Δ*veA* strain did not produce OTA under the current growth conditions at 28 °C (Llobregat et al., 2022). The present study also showed that this mutant was not able to produce OTB. However, under stress conditions, low but detectable OTA and OTB levels were observed (Figs. 4B and 5B). In the case of the Δ*laeA* knockout, a basal level of OTA production was maintained in the different CC conditions analysed, increasing under the highest temperature of 37 °C (Fig. 4C). OTB was only detected at 37 °C and an a_w of 0.98, independent on the CO_2 concentration (Fig. 5C). The reduced production of secondary metabolites by the knockout mutants in the VELVET complex genes was also evident in the levels of pyranonigrin A when extreme CC conditions were analysed (Fig. 6A). In contrast, kojic acid production remains relatively constant in all strains tested, except under the 37 °C stress condition combined with 1000 ppm CO_2 and 0.98 a_w (Fig. 6B). Under these conditions, a clear disparity was observed between the wt and Δ*otaA* strains compared to Δ*veA* and Δ*laeA* knockouts. Overall results indicate that although mutations in regulatory VELVET genes may lead to a very high reduction in OTA and OTB levels under actual climate conditions, they are able to produce them under CC conditions. On the other hand, the disruption of the biosynthetic pathway represented by the *otaA* gene assures no OTA nor OTB production irrespective of the CC scenario. The production pattern of other secondary metabolites depends on each particular strain.

5. Conclusion

This study provides new evidence that CC significantly affects the growth of the wt strain *A. carbonarius* ITEM 5010, and the knockout strains, as well as the production of OTA and other secondary metabolites under combined CC conditions. In addition, we evaluated the responses of the proposed knockout mutants as BCAs under these conditions, analysing their behaviour in the context of predicted CC conditions. Our results indicate that the most severe effects on the production of OTA and secondary metabolites occurred under conditions of 37 °C, an a_w of 0.98, and a CO_2 concentration of 1000 ppm. Based on these findings and the responses of the various knockout strains proposed as BCA, the Δ*otaA* mutant emerges as the most suitable candidate. While the Δ*veA* and Δ*laeA* strains did not produce OTA under the current environmental conditions, they produced OTA under the more stressful conditions predicted by future climate scenarios, which undermined their efficacy as BCAs. In contrast, the Δ*otaA* mutant, which lacks the first gene of the OTA biosynthesis pathway, remained unable to produce OTA regardless of environmental changes. Moreover, the Δ*otaA* mutant demonstrated comparable biological efficacy to the wt strain (Llobregat et al., 2022), further supporting its potential as a BCA for both current and expected future environmental conditions.

However, to fully validate these findings, further in vivo analyses are essential. These studies should aim to confirm the stability and efficacy of the Δ*otaA* mutant as a BCA under real-world conditions, particularly those predicted in CC scenarios, and to test its efficacy also under competitive conditions. Moreover, our results with the VELVET mutants reinforce the need to check the targeted mycotoxin under CC scenarios in mutants affected in secondary metabolism, either natural or laboratory-made, which are to be deployed in the field as BCAs.

CRediT authorship contribution statement

Belén Llobregat: Writing – original draft, Visualization, Investigation, Formal analysis. **Carla Cervini:** Writing – review & editing, Formal analysis. **Luis González-Candelas:** Writing – review & editing, Supervision, Investigation, Funding acquisition. **Carol Verheecke-Vaessen:** Writing – review & editing, Formal analysis. **Ana-Rosa Ballester:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Formal analysis. **Ángel Medina:** Supervision, Funding acquisition.

Funding sources

This work was partially funded by projects PID2021-126005OBI00 (MICIU/AEI/10.13039/501100011033/FEDER, UE), AGROALNEXT/2022/028 (GVA, PRTR, MRR, NextGenerationEU), and PIE 202270I070 (CSIC). The Accreditation as Center of Excellence Severo Ochoa CEX2021–001189-S funded by MICIU/AEI/10.13039/501100011033 is also fully acknowledged. B.L. was a recipient of the predoctoral contract PRE2019–089326 (MICIU/AEI/FSE, UE).

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The technical assistance by Monika Jodkowska is greatly appreciated. The authors acknowledge the support of the publication fee by the CSIC Open Access Publication Support Initiative through its Unit of Information Resources for Research (URICI).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2025.111250.

Data availability

Data will be made available on request.

References

Abdelmohsen, S., Verheecke-Vaessen, C., Garcia-Cela, E., Medina, A., Magan, N., 2020. Solute and matric potential stress on *Penicillium verrucosum*: impact on growth, gene expression and ochratoxin A production. World Mycotoxin J. 13, 345–354. https://doi.org/10.3920/WMJ2019.2542.

Ballester, A.R., Marcet-Houben, M., Levin, E., Sela, N., Selma, C., Carmona, L., Wisniewski, M., Droby, S., González-Candelas, L., Gabaldón, T., 2015. Genome, transcriptome, and functional analyses of *Penicillium expansum* provide new insights into secondary metabolism and pathogenicity. Mol. Plant-Microbe Interact. 28, 232–248. https://doi.org/10.1094/MPMI-09-14-0261-FI.

Battilani, P., Pietri, A., Bertuzzi, T., Languasco, L., Giorni, P., Kozakiewicz, Z., 2003. Occurrence of ochratoxin A–producing fungi in grapes grown in Italy. J. Food Prot. 66, 633–636. https://doi.org/10.4315/0362-028X-66.4.633.

Bayram, Ö., Braus, G.H., 2012. Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. FEMS Microbiol. Rev. 36, 1–24. https://doi.org/10.1111/j.1574-6976.2011.00285.x.

Bayram, Ö., Krappmann, S., Ni, M., Bok, J.W., Helmstaedt, K., Valerius, O., Braus-Stromeyer, S., Kwon, N.-J., Keller, N.P., Yu, J.-H., Braus, G.H., 2008. VelB/Vea/LaeA complex coordinates light signal with fungal development and secondary metabolism. Science 320, 1504–1506. https://doi.org/10.1126/science.1155888.

Bok, J.W., Balajee, S.A., Marr, K.A., Andes, D., Nielsen, K.F., Frisvad, J.C., Keller, N.P., 2005. LaeA, a regulator of morphogenetic fungal virulence factors. Eukaryot. Cell 4, 1574–1582. https://doi.org/10.1128/EC.4.9.1574-1582.2005.

Bok, J.W., Keller, N.P., 2004. LaeA, a regulator of secondary metabolism in *Aspergillus* spp. Eukaryot. Cell 3, 527–535. https://doi.org/10.1128/EC.3.2.527-535.2004.

Bok, J.W., Noordermeer, D., Kale, S.P., Keller, N.P., 2006. Secondary metabolic gene cluster silencing in *Aspergillus nidulans*. Mol. Microbiol. 61, 1636–1645. https://doi.org/10.1111/j.1365-2958.2006.05330.x.

Cabañes, F.J., Bragulat, M.R., 2018. Black aspergilli and ochratoxin A-producing species in foods. Curr. Opin. Food Sci. 23, 1–10. https://doi.org/10.1016/j.cofs.2018.01.006.

Cervini, C., Naz, N., Verheecke-Vaessen, C., Medina, A., 2024. Impact of predicted climate change environmental conditions on the growth of *Fusarium asiaticum* strains and mycotoxins production on a wheat-based matrix. Int. J. Food Microbiol. 416, 110658. https://doi.org/10.1016/j.ijfoodmicro.2024.110658.

Cervini, C., Verheecke-Vaessen, C., Ferrara, M., García-Cela, E., Magistà, D., Medina, A., Gallo, A., Magan, N., Perrone, G., 2021. Interacting climate change factors (CO_2 and temperature cycles) effects on growth, secondary metabolite gene expression and phenotypic ochratoxin A production by *Aspergillus carbonarius* strains on a grape-based matrix. Fungal Biol. 125, 115–122. https://doi.org/10.1016/j.funbio.2019.11.001.

Chib, S., Jamwal, V.L., Kumar, V., Gandhi, S.G., Saran, S., 2023. Fungal production of kojic acid and its industrial applications. Appl. Microbiol. Biotechnol. 107, 2111–2130. https://doi.org/10.1007/s00253-023-12451-1.

Commission Regulation (EC), 2002. Commission Regulation (EC) No 472/2002 of 12 March 2002 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance).

Commission Regulation (EC), 2023. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA relevance).

Crespo-Sempere, A., Marín, S., Sanchis, V., Ramos, A.J., 2013. VeA and LaeA transcriptional factors regulate ochratoxin A biosynthesis in *Aspergillus carbonarius*. Int. J. Food Microbiol. 166, 479–486. https://doi.org/10.1016/j.ijfoodmicro.2013.07.027.

Crosta, M., Cervini, C., Medina, A., Battilani, P., 2025. Impact of simulated climate change conditions on *Aspergillus flavus* biocontrol effectiveness in peanut-based medium and peanut seeds. Int. J. Food Microbiol. 428, 110981. https://doi.org/10.1016/j.ijfoodmicro.2024.110981.

Frandsen, R.J.N., Andersson, J.A., Kristensen, M.B., Giese, H., 2008. Efficient four-fragment cloning for the construction of vectors for targeted gene replacement in filamentous fungi. BMC Mol. Biol. 9 (70), 70. https://doi.org/10.1186/1471-2199-9-70.

Gallo, A., Knox, B.P., Bruno, K.S., Solfrizzo, M., Baker, S.E., Perrone, G., 2014. Identification and characterization of the polyketide synthase involved in ochratoxin A biosynthesis in *Aspergillus carbonarius*. Int. J. Food Microbiol. 179, 10–17. https://doi.org/10.1016/j.ijfoodmicro.2014.03.013.

García-Cela, E., Crespo-Sempere, A., Gil-Serna, J., Porqueres, A., Marin, S., 2015. Fungal diversity, incidence and mycotoxin contamination in grapes from two agro-climatic Spanish regions with emphasis on *Aspergillus* species. J. Sci. Food Agric. 95, 1716–1729. https://doi.org/10.1002/jsfa.6876.

García-Cela, E., Marín, S., Reyes, M., Sanchis, V., Ramos, A.J., 2016. Conidia survival of *Aspergillus* section *Nigri*, *Flavi* and *Circumdati* under UV-A and UV-B radiation with cycling temperature/light regime. J. Sci. Food Agric. 96, 2249–2256. https://doi.org/10.1002/jsfa.7343.

Garcia-Cela, E., Verheecke-Vaessen, C., Gutierrez-Pozo, M., Kiaitsi, E., Gasperini, A.M., Magan, N., Medina, A., 2021. Unveiling the effect of interacting forecasted abiotic factors on growth and aflatoxin B1 production kinetics by *Aspergillus flavus*. Fungal Biol. 125, 89–94. https://doi.org/10.1016/j.funbio.2020.05.003.

Gasperini, A.M., Rodriguez-Sixtos, A., Verheecke-Vaessen, C., Garcia-Cela, E., Medina, A., Magan, N., 2019. Resilience of biocontrol for aflatoxin minimization strategies: climate change abiotic factors may affect control in non-GM and GM-maize cultivars. Front. Microbiol. 10, 2525. https://doi.org/10.3389/fmicb.2019.02525.

Gerin, D., González-Candelas, L., Ballester, A.-R., Pollastro, S., De Miccolis Angelini, R., Faretra, F., 2018. Functional characterization of the *alb1* orthologue gene in the ochratoxigenic fungus *Aspergillus carbonarius* (AC49 strain). Toxins 10, 120. https://doi.org/10.3390/toxins10030120.

Hoff, B., Kamerewerd, J., Sigl, C., Mitterbauer, R., Zadra, I., Kürnsteiner, H., Kück, U., 2010. Two components of a *VELVET* -like complex control hyphal morphogenesis, conidiophore development, and penicillin biosynthesis in *Penicillium chrysogenum*. Eukaryot. Cell 9, 1236–1250. https://doi.org/10.1128/EC.00077-10.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1993. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC monographs on the evaluation of carcinogenic risks to humans. World Health Organization, International Agency for Research on Cancer, Lyon.

Ioannidis, A.-G., Kogkaki, E.A., Natskoulis, P.I., Nychas, G.-J.E., Panagou, E.Z., 2015. Modelling the influence of temperature, water activity and sodium metabisulphite on the growth and OTA production of *Aspergillus carbonarius* isolated from Greek wine grapes. Food Microbiol. 49, 12–22. https://doi.org/10.1016/j.fm.2015.01.006.

Isidro-Sánchez, J., D'Arcy Cusack, K., Verheecke-Vaessen, C., Kahla, A., Bekele, W., Doohan, F., Magan, N., Medina, A., 2020. Genome-wide association mapping of *Fusarium langsethiae* infection and mycotoxin accumulation in oat (*Avena sativa* L.). Plant Genome 13, e20023. https://doi.org/10.1002/tpg2.20023.

Kahm, M., Hasenbrink, G., Lichtenberg-Fraté, H., Ludwig, J., Kschischo, M., 2010. **Grofit**: fitting biological growth curves with *R*. J. Stat. Softw. 33. https://doi.org/10.18637/jss.v033.i07.

Llobregat, B., González-Candelas, L., Ballester, A.-R., 2022. Ochratoxin A defective *Aspergillus carbonarius* mutants as potential biocontrol agents. Toxins 14, 745. https://doi.org/10.3390/toxins14110745.

Lodeiro, S., Xiong, Q., Wilson, W.K., Ivanova, Y., Smith, M.L., May, G.S., Matsuda, S.P.T., 2009. Protostadienol biosynthesis and metabolism in the pathogenic fungus *Aspergillus fumigatus*. Org. Lett. 11, 1241–1244. https://doi.org/10.1021/ol802696a.

Magan, N., Medina, A., Aldred, D., 2011. Possible climate-change effects on mycotoxin contamination of food crops pre- and postharvest. Plant Pathol. 60, 150–163. https://doi.org/10.1111/j.1365-3059.2010.02412.x.

Maor, U., Barda, O., Sadhasivam, S., Bi, Y., Levin, E., Zakin, V., Prusky, D.B., Sionov, E., 2021. Functional roles of LaeA, polyketide synthase, and glucose oxidase in the regulation of ochratoxin A biosynthesis and virulence in *Aspergillus carbonarius*. Mol. Plant Pathol. 22, 117–129. https://doi.org/10.1111/mpp.13013.

Medina, A., Akbar, A., Baazeem, A., Rodriguez, A., Magan, N., 2017. Climate change, food security and mycotoxins: do we know enough? Fungal Biol. Rev. 31, 143–154. https://doi.org/10.1016/j.fbr.2017.04.002.

Miyake, Y., Ito, C., Tokuda, H., Osawa, T., Itoigawa, M., 2010. Evaluation of flavoglaucin, its derivatives and pyranonigrins produced by molds used in fermented foods for inhibiting tumor promotion. Biosci. Biotechnol. Biochem. 74, 1120–1122. https://doi.org/10.1271/bbb.90955.

Miyake, Y., Mochizuki, M., Ito, C., Itoigawa, M., Osawa, T., 2008. Antioxidative pyranonigrins in rice mold starters and their suppressive effect on the expression of blood adhesion molecules. Biosci. Biotechnol. Biochem. 72, 1580–1585. https://doi.org/10.1271/bbb.80077.

Oliveira, G., Evangelista, S.R., Passamani, F.R.F., Santiago, W.D., Cardoso, M.D.G., Batista, L.R., 2019. Influence of temperature and water activity on ochratoxin A production by *Aspergillus* strain in coffee south of Minas Gerais/Brazil. LWT 102, 1–7. https://doi.org/10.1016/j.lwt.2018.12.032.

Palmer, J.M., Theisen, J.M., Duran, R.M., Grayburn, W.S., Calvo, A.M., Keller, N.P., 2013. Secondary metabolism and development is mediated by LlmF control of VeA subcellular localization in *Aspergillus nidulans*. PLoS Genet. 9, e1003193. https://doi.org/10.1371/journal.pgen.1003193.

Palumbo, J.D., O'Keeffe, T.L., Vasquez, S.J., Mahoney, N.E., 2011. Isolation and identification of ochratoxin A-producing *Aspergillus* section *Nigri* strains from California raisins. Lett. Appl. Microbiol. 52, 330–336. https://doi.org/10.1111/j.1472-765X.2011.03004.x.

Paterson, R.R.M., Lima, N., 2010. How will climate change affect mycotoxins in food? Food Res. Int. 43, 1902–1914. https://doi.org/10.1016/j.foodres.2009.07.010.

Perrin, R.M., Fedorova, N.D., Bok, J.W., Cramer, R.A., Wortman, J.R., Kim, H.S., Nierman, W.C., Keller, N.P., 2007. Transcriptional regulation of chemical diversity in *Aspergillus fumigatus* by LaeA. PLoS Pathog. 3, e50. https://doi.org/10.1371/journal.ppat.0030050.

Perrone, G., Varga, J., Susca, A., Frisvad, J.C., Stea, G., Kocsube, S., Toth, B., Kozakiewicz, Z., Samson, R.A., 2008. *Aspergillus uvarum sp. nov.*, an uniseriate black *Aspergillus* species isolated from grapes in Europe. Int. J. Syst. Evol. Microbiol. 58, 1032–1039. https://doi.org/10.1099/ijs.0.65463-0.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, 45e–445. https://doi.org/10.1093/nar/29.9.e45.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., Rozen, S. G., 2012. Primer3—new capabilities and interfaces. Nucleic Acids Res. 40, e115. https://doi.org/10.1093/nar/gks596.

Verheecke-Vaessen, C., Diez-Gutierrez, L., Renaud, J., Sumarah, M., Medina, A., Magan, N., 2019. Interacting climate change environmental factors effects on *Fusarium langsethiae* growth, expression of *Tri* genes and T-2/HT-2 mycotoxin production on oat-based media and in stored oats. Fungal Biol. 123, 618–624. https://doi.org/10.1016/j.funbio.2019.04.008.

Visconti, A., Perrone, G., Cozzi, G., Solfrizzo, M., 2008. Managing ochratoxin A risk in the grape-wine food chain. Food Addit. Contam. Part A 25, 193–202. https://doi.org/10.1080/02652030701744546.

Wang, Y., Wang, L., Wu, F., Liu, F., Wang, Q., Zhang, X., Selvaraj, J.N., Zhao, Y., Xing, F., Yin, W.-B., Liu, Y., 2018. A consensus ochratoxin A biosynthetic pathway: insights from the genome sequence of *Aspergillus ochraceus* and a comparative genomic analysis. Appl. Environ. Microbiol. 84. https://doi.org/10.1128/aem.01009-18.

Wiemann, P., Brown, D.W., Klei grewe, K., Bok, J.W., Keller, N.P., Humpf, H., Tudzynski, B., 2010. FfVel1 and FfLae1, components of a *velvet*-like complex in *Fusarium fujikuroi*, affect differentiation, secondary metabolism and virulence. Mol. Microbiol. 77, 972–994. https://doi.org/10.1111/j.1365-2958.2010.07263.x.

Zanon, M.S.A., Pena, G., Yerkovich, N., Bossa, M., Chiotta, M.L., Chulze, S.N., 2023. Aflatoxins and fumonisins in maize under a climate change scenario. Biocontrol strategies at the pre-harvest stage. Eur. J. Plant Pathol. 167, 551–567. https://doi.org/10.1007/s10658-023-02735-7.



https://dspace.org/

Will climate change affect growth and ochratoxin A production of putative biocontrol knockout strains of Aspergillus carbonarius?

Llobregat, Belén; Cervini, Carla; González-Candelas, Luis; Verheecke-Vaessen, Carol; Ballester, Ana-Rosa ...

2025-08-02
Elsevier
https://doi.org/10.1016/j.ijfoodmicro.2025.111250
Llobregat B, Cervini C, González-Candelas L, et al., (2025) Will climate change affect growth and ochratoxin A production of putative biocontrol knockout strains of Aspergillus carbonarius?. International Journal of Food Microbiology, Volume 439, August 2025, Article number 111250

Downloaded from a DSpace repository. https://dspace.org/