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Fusarium graminearum and zearalenone in wheat: A water activity–temperature model

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ABSTRACT

Zearalenone (ZEN) is a nonsteroidal estrogenic mycotoxin produced primarily by *Fusarium graminearum*, posing significant threats to agricultural grain production. When ZEN levels exceed regulatory limits, grains face rejection, and its harmful effects on the female reproductive system raise health concerns. Despite its importance, there is a lack of information on the ecophysiological conditions that promote *F. graminearum* colonisation and ZEN production in wheat grains. This study aimed to develop and validate predictive models for the growth of *F. graminearum* and ZEN accumulation in wheat. For this purpose, two strains isolated from wheat were inoculated in agar wheat-based medium supplemented with glycerol to adjust the water activity (a_w) to five different values of 0.88, 0.91, 0.94, 0.97 and 0.99. The cultures were incubated at 4, 6, 8.5, 15, 20, 25, 30 and 35 °C, the colony growth was measured daily, and ZEN accumulation assessed at day 10, 20 and 30. To analyse the growth kinetics of *F. graminearum*, the fungal growth rate (μ) and lag time (λ) were calculated, applying the Cardinal/Rosso, Davey, and Gibson models. These techniques, commonly used in secondary modelling, were enhanced through variable transformation, with the square root transformation yielding optimal results in the Cardinal models. The outcome showed probabilistic model accuracy for growth ranging 65–79 % and ZEN production ranging 45–77 % on internal and external data set. Optimum temperature for ZEN production was 25–30 °C in media and wheat. In wheat, a higher a_W was required for both growing (0.92 a_w) and ZEN production compared to media (0.90 a_w). Probabilities of growth over 80 % were predicted in the range of 0.90–0.95 a_w at 16–34 °C after 30 days. In conclusion, to avoid mycotoxin contamination in wheat an $a_w < 0.89$ should be maintained, and temperatures in the range 18–31 °C should be avoided ($P < 0.5$). The integration of predictive models into decision support systems could assist farmers in identifying pre-harvest contamination risks and in optimising harvesting and drying practices to minimise post-harvest contamination. This study highlights the importance of understanding the ecophysiological profiles of mycotoxigenic species like *F. graminearum* to mitigate contamination risks and optimise storage conditions in wheat.

1. Introduction

Grasslands account for approximately 30 % of the Earth's landmass and represent one of the most biodiverse and geographically widespread plant families, primarily comprising grasses. Among these grasses, wheat (*Triticum* spp.) originally domesticated in the Mediterranean and West Asia regions, is widely cultivated for its seed (Jones et al., 2016). In 2023, global wheat production reached 787 million tonnes (FAOSTAT,

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2024). Cereal crops in the field are often susceptible to fungal infections; in addition, harvested cereal grains can deteriorate markedly during the post-harvest management stages. Economic and trade implications arise from fungal contaminations as approximately 18 % of wheat production is lost due to fungal invasion (Al-Hazmi and Gomaa, 2012). *Fusarium* head blight (FHB), a devastating plant disease that affects wheat caused mainly by a few members of the *F. graminearum* species complex (FGSC), is a major threat to agricultural grain production, food safety, and animal health (Karlsson et al., 2021; Shah et al., 2018; Vaughan et al., 2016). Although *F. graminearum* infection is mainly considered a pre-harvest disease, spores can persist on the surface of the kernels and germinate in silos under favourable moisture conditions. In fact, *Fusarium* is commonly isolated in storage cereals (Del Palacio et al., 2016; Felšöciová et al., 2021, Meng et al., 2023).

Aside from the significant economic losses, *F. graminearum* contamination poses a serious health risk due to the potential contamination with zearalenone (ZEN) and type B trichothecenes (for e.g. Deoxynivalenol (DON)) damaging human and animals. ZEN long-term high-dose exposure may cause severe toxic effects in mammals, disturb the reproductive system, and induce endocrine disorders, while long-term low-dose exposure causes endocrine disorders, which may lead to metabolic disorders, and increase the risk of metabolic syndrome-related diseases (Han et al., 2022). Human exposure to DON is associated with vomiting, loss of appetite, diarrhoea, impairments to the immune system, disruptions in endocrine function, genetic damage, potential for cancer development, and in severe cases, fatality (Murtaza et al., 2023). To protect the consumer, the European Union has set maximum limits for these toxins in food for human consumption of final wheat products and unprocessed wheat at ranges of 20–100 μg/kg and 200–1750 μg/kg, respectively, as well as in animal feed between 0.5 and 2 mg/kg and 0.9–8 mg/kg for both toxins (European Commission, 2006; 2023).

Wheat is typically harvested during the warmer months when the grain moisture content is naturally below the safety level of 14–15 % (Magan and Aldred, 2007) eliminating the need for drying in most cases. Once the wheat is harvested, grains are transported and stored in silos or warehouses during variable periods of time until final use. Magan et al. (2010) reviewed different strategies for limiting mycotoxins in stored wheat, including the use of modified atmosphere storage, chemical preservation systems and biocontrol agents. Some of these approaches had been effective in reducing the fungal contamination and mycotoxin production at lab scale, but there is still a lack of transfer of these strategies into applied knowledge useful for the industry but also socially accepted from the public perception. Water activity (a_w) and temperature (T) are the two most important abiotic factors determining fungal colonisation and mycotoxin production by fungi. Knowledge of the optimum and marginal conditions of $a_w \times T$ for different toxigenic fungi is critical for understanding the relative risks of spoilage and toxin contamination. Currently, temperature and sometimes relative humidity (RH) sensors are employed in silos and during transport to monitor these two key parameters in stored cereals. Species-specific models of ecophysiological conditions for optimum and marginal conditions for growth/mycotoxin production could be effectively utilised in conjunction with real-time sensor systems for the improvement of post-harvest management decisions while reducing food spoilage.

There are some data on fungal growth and ZEN production by *F. graminearum*. Previously tested environmental conditions for *F. graminearum* growth are summarised in Fig. 1, but only few studies were carried out in wheat (Hope et al., 2005; Ramirez et al., 2006; Marin et al., 2024). Therefore, there is no detailed knowledge of the full range of $a_w \times T$ for *F. graminearum* colonisation of wheat grain and production of ZEN. In the last decade, kinetic and probabilistic models had been used to predict fungal growth and mycotoxin production. However, most of these models have focused on *Aspergillus* genus (Astoreca et al., 2012; Marín et al., 2012; 2024). Regarding environmental conditions, predictive models have been developed for *Fusarium verticilloides, Fusarium proliferatum*, *Fusarium asiaticum* and *F. graminearum* (Cambaza et al., 2019; Garcia-Cela et al., 2022; Samapundo et al., 2005) while a probabilistic model has focused on *Fusarium langsethiae* (Verheecke-Vaessen et al., 2021).

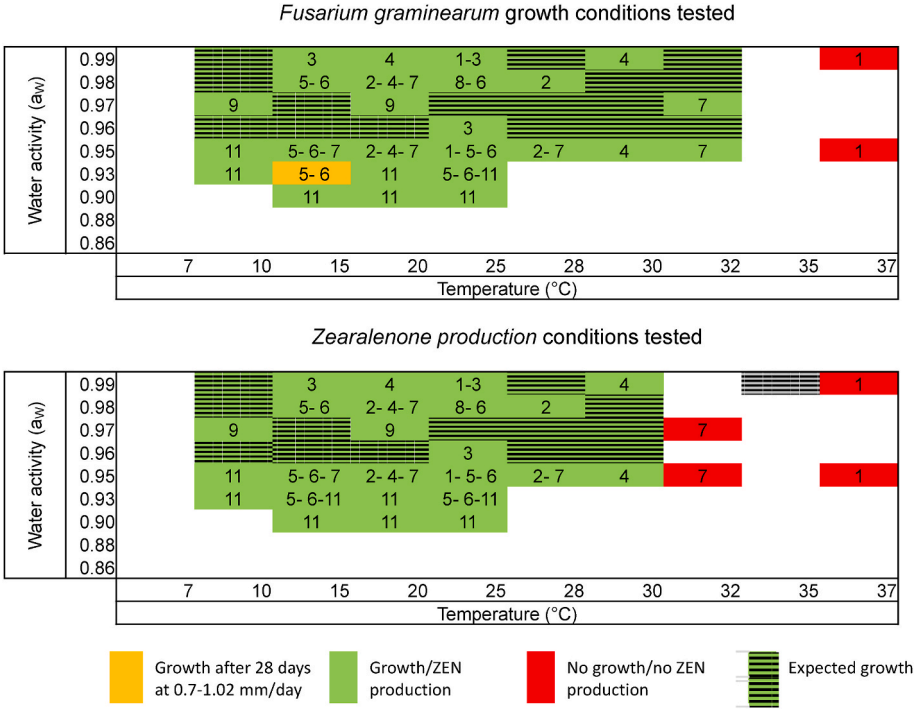

Fig. 1. Summary of publications reporting *Fusarium graminearum* growth assessment. Numbers in the graph indicate the publication 1) Armando et al., 2013, 2) Habschied et al., 2011, 3) Kokkonen et al., 2010, 4) Velluti et al., 2004, 5) Velluti et al., 2000, 6) Velluti et al., 2001, 7) Llorens et al., 2004, 8) Jiménez et al., 1996, 9) Etcheverry, 1998, 10) Ramirez et al., 2006 and 11) Garcia-Cela et al., 2018.

The aim of this study was to determine the effects of the interactions of a_w, temperature and incubation time on the growth of two *F. graminearum* strains and ZEN production in wheat-based media. In addition, mathematical models for fungal growth were validated in irradiated wheat kernels.

2. Material and methods

2.1. Experimental design

A full factorial design was used in which three factors were assayed: isolate, water activity (a_w) and temperature (T). Two growth parameters, fungal growth (μ) and lag time (λ) were recorded at each condition as response variables. The a_w levels assayed were 0.88, 0.91, 0.94, 0.97 and 0.99 a_w and the incubator Temperatures were 4, 6, 8.5, 15, 20, 25, 30 and 35 °C. Three Petri plates of each were measured daily for 30 days.

2.2. Fungal isolates and preparation of the inoculum

F. graminearum strains Fg 08/091 and Fg 08/111 isolated from UK wheat were kindly supplied by Prof. S. Edwards, Harper Adams University, Shropshire, UK. Cultures of this strain were maintained in glycerol:water (67:33) at -20 °C at Cranfield University and sub-cultured when required for experimental use.

2.3. Media

The basic medium used in this study was wheat-based media (3 % milled wheat + 1.5 % agar w/w) with five different a_w. The a_w of the medium was modified to 0.88, 0.91, 0.94, 0.97 and 0.99 by adding 623 g/L, 465 g/L, 308 g/L, 150 g/L and 0 g/L of glycerol, respectively and verified with an AquaLab Series 4 TE (Decagon Devices, Inc., WA, USA) with an accuracy ±0.003. The medium was autoclaved and poured into 9 cm sterile Petri dishes. The aw of each medium was verified with an AquaLab Series 4 TE (Decagon Devices, Inc., WA, USA) with an accuracy ±0.003.

2.4. Inoculation and incubation conditions

Ten Petri plates of *F. graminearum* strains were grown on V8 agar (V8®, 175 mL; $CaCO_3$, 3 g; $ZnSO_4{\cdot}7H_2O$, 0.01 g; $CuSO_4{\cdot}5H_2O$, 0.005 g; agar, 20 g/L) and incubated at 25 °C for 10 days to obtain heavily sporulated cultures. Following the incubation, 3 ml of sterile Tween®80 (0.05 %) was poured onto the Petri plate colonies and then gently scraped with a sterile Drigalsky's spatula. The liquid was then transferred from one Petri plate to another until all 10 Petri plates were processed. Finally, the liquid was recovered and collected in a sterile Falcon tube. After homogenising, the number of spores per ml was then determined using a Thoma counting chamber, and the final concentration was adjusted to $1x10^5$ spores/ml in Tween®80 (0.005 %). Five μL were centrally inoculated on each Petri plate.

2.5. Zearalenone quantification

2.5.1. Wheat-based media

Three agar plugs (diameter 4 mm) of each colony were removed from the colonies after 10, 20 and 30 days and placed in an Eppendorf. Samples were then extracted with 1 mL of acetonitrile and the vials were shaken for 5 s and allowed to rest. After 60 min, the Eppendorf were shaken again and extract filtered (Millex-HV 0.45 μm 25 mm, Millipore Corporation. Bedford, U.S.A.) into another vial and stored at 4 °C until analysis by HPLC (Agilent Technologies, Palo Alto, CA, USA). Mobile phase of acetonitrile–water (60:40 v/v), adjusted at pH 3.2 with acetic acid. The mobile phase flow rate was 1 mL/min. Excitation and emission wavelengths were set at 274 and 455 nm, respectively. Detection limit of the analysis was 10 ng/g.

2.5.2. Wheat

ZEN was quantified following the method described by Portell et al. (2020). Briefly, samples were dried at 60 °C for 24 h and then milled in a laboratory blender (Waring Commercial, Christian, UK). Samples were extracted by adding 500 μL of acetonitrile:water:formic acid (79:20.9:0.1, v:v:v) to 100 (±10) mg of milled wheat and agitated for 90 min at 300 rpm at 25 °C on a rotary shaker (miniShaker VWR, Leighton Buzzard, UK). Then, samples were centrifuged for 10 min at 22,600 g (Centrifuge 5417S Eppendorf, Stevenage, UK), and 1 μL of the supernatant was injected into an Exion LC series HPLC linked to a 6500+ qTRAP-MS/MS system in Electrospray Ionisation (ESI) mode (Sciex Technologies, Warrington, UK). Detection limit of the analysis was 0.26 ng/g.

2.6. Mathematical and statistical methods

The mathematical methods employed to analyse fungal growth dynamics under different environmental conditions largely follow the methods described by Marin et al. (2012). Analysis of variance (ANOVA) was employed to assess the effects of temperature, water activity, strain, and their interactions on fungal growth (mm/day) and lag phase (days), with significant differences subsequently determined using Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$) using Statgraphics Centurion 18 (Manugistics, Inc, Maryland, USA).

2.6.1. Kinetic model

A typical two-step modelling approach, including primary and secondary modelling, was employed to quantify the effect of T and a_w on kinetic parameters of two strains of *F. graminearum*. Initially, estimates of the growth rates of the fungi were obtained by plotting colony diameter changes against time. For each treatment, a non-linear regression was applied to estimate the maximum growth rate (μ_{max}, mm/day), lag phase before growth (λ, day), and maximum colony diameter, if applicable, by fitting the experimental data to the primary model of Baranyi and Roberts (1994) using Statgraphics ® Centurion (Manugistics, Inc, Mayland, USA).

$$D = \mu_{max}A - ln\left\{1 + \frac{[exp(\mu_{max}A)] - 1}{exp(D_{max})}\right\}$$

$$A = t + \left(\frac{1}{\mu_{max}}\right) + ln[exp(-\mu_{max}t) + exp(-\mu_{max}\lambda) - exp(-\mu_{max}t - \mu_{max}\lambda)] \quad (1)$$

Analysis of variance (ANOVA) was applied to μ_{max} and λ repeated data to establish the significance of the assayed factors (a_w, temperature, strain).

Next, the radial growth rate (μ_{max}) was modelled as a function of temperature and a_w level using a variety of models:

The Arrhenius–Davey model, originally introduced by Davey (1989), serves as the simplest linear model used in this study, focusing on the individual effects of temperature on fungal growth kinetics. This model was subsequently extended by Panagou et al. (2003), introducing a more comprehensive approach, while maintaining simplicity, begins to account for the separate influences of a_w and T on fungal growth, without explicitly modelling their interactive effects without directly modelling potential interactions.

$$\mu_R = a_0 + a_1a_w + a_2a_w^2 + \frac{a_3}{T} + \frac{a_4}{T^2} \quad (2)$$

where T is absolute temperature (K).

The polynomial model includes an interaction term between temperature and a_w on fungal growth dynamics, facilitating an exploration of interacting relationships between these parameters,

$$\mu_R = a_0 + a_1a_w + a_2a_w^2 + a_3T + a_4T^2 + a_5Ta_w \quad (3)$$

Following the work of Gibson et al. (1994), as well as using a_w data directly in these models and the transformation of a_w with: $\sqrt{1-a_w}$ can ensure desirable estimation properties in the parameter estimation process including linearisation and normalisation of the data while improving homoscedasticity.

Finally, a multifactorial cardinal model based on previous cardinal models (Rosso et al., 1993; Rosso and Robinson, 2001) was applied.

$$\mu_{max}(T, a_w) = \mu_{opt} \cdot \tau(T) \cdot \rho(a_w) \quad (4)$$

where

$$\tau(T) = \left(\frac{(T - T_{min})^2 \cdot (T - T_{max})}{(T_{opt} - T_{min}) \cdot \left[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T) \right]} \right)$$

$$\rho(a_w) = \left(\frac{(a_w - a_{wmin})^2 \cdot (a_w - 1)}{(a_{wopt} - a_{wmin}) \cdot \left[(a_{wopt} - a_{wmin})(a_w - a_{wopt}) - (a_{wopt} - 1)(a_{wopt} + a_{wmin} - 2a_w) \right]} \right)$$

Where.

T_{min} is the temperature below which growth is no longer observed

T_{max} is the temperature above which no growth occurs

T_{opt} is the temperature at which maximum growth rate equals its optimal value μ_{opt}

a_{wmin} is the a_w below which growth is no longer observed

a_{wmax} is the a_w above which no growth occurs

a_{wopt} is the a_w at which maximum growth rate equals its optimal value μ_{opt}

To model the fungal growth kinetics, critical thermal and moisture parameters were determined. These parameters include T_{min} and T_{max}, representing the lower and upper temperature limits beyond which growth is not observed, and a_{wmin}, defining the lower limit of a_w required for growth. Similarly, T_{opt} and a_{wopt} were determined as the conditions at which the maximum growth rate (μ_{opt}) occurs, aligned with the optimal values for temperature and water activity, respectively. For simplification and following the work of Sautour et al. (2001), the parameter a_{wmax} was set to 1, which did not significantly affect the estimation accuracy of other model parameters. The estimation of these cardinal values, along with the optimal radial growth rates, was facilitated through bespoke modelling software utilizing Python's SciPy library (Oliphant, 2007). This software applied multivariable nonlinear regression techniques based on the Marquardt algorithm, ensuring efficient and accurate fitting of models to the experimental data.

Our approach followed the methodology described by Zwietering et al. (1996), applying the gamma concept to assume factor independency and applying a progressive modelling strategy. This required selecting the temperature level that yielded the highest growth rate (μ_{max}) from the dataset and then estimating a_{wmin} and a_{wopt} using a model akin to the Rosso model for a_w. Subsequently, T_{min}, T_{max}, and T_{opt} were determined for the optimal level of a_w using a temperature-based Rosso model. Finally, a single joint optimisation step was carried out to further improve parameter estimates. This stepwise process culminated in the recalibration of μ_{opt} at the newly estimated cardinal points for both temperature and a_w, allowing for a better understanding of fungal growth kinetics under specified environmental conditions.

To improve stability in the estimation process and mitigate issues related to homogeneity, the application of a square-root transformation to the dependent variable, or growth rate, is also considered in modelling these dynamics. This approach is valuable in ensuring a uniform variance across the range of predictor variables, a principle known as homoscedasticity, which is crucial for the reliability of statistical inferences. The necessity for transforming the growth rate variable can be empirically assessed by examining the correlation between the mean growth rate and its variance across various levels of temperature and a_w. A significant correlation between the variance and the untransformed growth rate data would indicate heteroscedasticity, thereby justifying the application of a square-root transformation to achieve homogeneity and improve the model's estimation accuracy and interpretability. This methodological step enhances the robustness of the models, facilitating more accurate predictions of microbial growth under diverse environmental conditions.

2.6.2. Modelling of the growth/no growth and zearalenone production/no production interface

For each treatment, growth data were converted into probabilities of growth by assigning the value of 1 in the case where visible fungal growth/toxin production was recorded, and 0 in the case of absence of growth during the overall period of the experiment. The resulting data were fitted to a logistic regression model (Ratkowsky and Ross, 1995) to determine the growth/no growth boundaries of the fungi under the different a_w/T levels assayed. The model employed was a full second-order logistic regression model (Battey et al., 2002) that also includes the linear term for time:

$$LogitP = ln\left(\frac{P}{1-P}\right) = b_0 + b_1 a_w + b_2 T + b_{11} a_w^2 + b_{22} T^2 + b_{12} a_w T + b_3 time$$

Where b_i are the coefficients to be estimated. The equation was fitted by using Statgraphics Centurion 18 (Manugistics, Inc, Maryland, USA) logistic regression procedure. The automatic variable selection option with a backward stepwise factor selection method was chosen to identify the significant effects ($P < 0.05$). The predicted growth/no growth interfaces for $P = 0.1$, 0.5 and 0.9 were calculated after 30 days and plotted using Microsoft Excel 2013 Solver.

2.7. Validation of growth models

Validation was carried out directly in wheat with modified a_w. The combinations were chosen near the growth/no growth boundaries to validate the models under those conditions in which prediction may be a key point in food safety (0.88, 0.90, 0.92, 0.95 a_w vs 5, 10, 15, 20, 25, 30, 35 °C) for 30 days. Wheat grain which was gamma irradiated with 12–15 kGy was used in these studies. The irradiated grain retained germinate capacity while being free of contaminating microorganisms. Sterile wheat grains were adjusted to the required a_w levels by aseptically adding amounts of sterile distilled water to the grain in sterile bottles (6, 8, 10 and 12 mL of sterile distilled water per 100 g of irradiated wheat kernels). The bottles were cooled down to 4 °C for 48 h with periodic hand shaking during this time. Final a_w values of each substrate were confirmed with an AquaLab Series 4 TE (Decagon Devices, Inc., WA, USA) with an accuracy ± 0.003. Then wheat kernels were poured in Petri plates forming a single layer. Then 5 μL of a spore suspension ($1x10^5$ spores/mL) was centrally inoculated. Plates with the same a_w were enclosed in sealed containers along with beakers containing water-glycerol solution of the same a_w as the plates which were renewed periodically to maintain constant a_w. For each condition, three Petri dishes were inoculated. Growth assessment was carried out as for

the wheat-based medium experiment. In addition, a set of data previously published in wheat was used.

For the kinetics model given the two strains, a total of 6 experiments were carried out for each temperature and a_w condition. To compare, contrast and validate the different models, for each temperature/a_w pair, a test sample was randomly selected to form part of the test dataset, the remaining 5 samples were used to estimate the parameters of the model. Given the estimated model parameters, summary statistics were calculated to characterise the error between the predicted data and the observed test dataset. In addition, the Bias and Accuracy Factor measures were used to further quantify the performance of the predictive models. The Bias Factor indicates whether a model tends to overestimate or underestimate observed values whereas the Accuracy Factor quantifies the closeness of the predictions to the observed values, considering both the systematic and random errors.

Furthermore, to assess the accuracy of the probabilistic models, an external dataset from previous publications was used. This dataset included growth data from Ramirez et al. (2006), and ZEN production data from Armando et al. (2013), Garcia-Cela et al. (2018); Habschied et al. (2011); Kokkonen et al. (2010).

3. Results

3.1. Kinetic primary model in wheat-based media

Although both strains grew under identical conditions, there was occasionally a one-day growth delay observed between them, as highlighted in Table 1 and Supplementary Fig. 1. However, the differences between μ_{max} and λ among the two strains tested were not significant ($p > 0.05$). Longer λ were observed under conditions of low a_w, while the shortest λ was associated with rapid fungal growth. No growth was observed at 0.88 a_w and 4 or 35 °C. Overall, μ_{max} increased with both a_w and temperature, peaking at 25 °C. Maximum radial growth rate (μ_{max}) and λ were estimated through Baranyi's primary model for the two *F. graminearum* strains in wheat-based media, R^2 was always higher than 0.85. In general, a lag-linear curve was observed, with some exception occurring at 6 °C/0.97a_w and 15–20 °C/0.91 a_w.

3.2. Secondary modelling for the effects of a_w and temperature on the growth rate and time to visible growth

Cardinal/Rosso, Davey and Gibson models were fitted with the maximum growth rate obtained from kinetic model from both fungal strains combined to describe the effect of a_w and temperature on fungal growth. Fig. 2 shows the experimental growth rate data, and the response surface plots for the three different models or approaches evaluated to describe the response of the fungus to the environmental variables examined. Transforming variables, whether dependent or independent, can significantly improve the fit of a linear model by aligning the data more closely with the assumptions underlying linear regression analysis. Most notably, linear models assume that the relationship between the independent and dependent variables is linear, the residuals (differences between observed and predicted values) are normally distributed, and the variance of these residuals is constant across all levels of the independent variables (homoscedasticity). Many real-world datasets, however, exhibit non-linear relationships, skewed

Table 1
Estimated maximum growth rates (μmax) and time to visible growth (λ) for *Fusarium graminearum* isolates on wheat agar medium at different temperature (T) and water activity (aw) levels.

T(°C)	a_w	Fg08/091		Fg08/111	
		μmax (mm/day) ± SD	λ (day) ± SD	μmax (mm/day) ± SD	λ (day) ± SD
4	0.97	–	–	–	–
4	0.99	–	–	–	–
6	0.91	–	–	–	–
6	0.94	–	–	–	–
6	0.97	1.89 ± 0.14^{a}	5.45 ± 0.33^{a}	2.17 ± 0.03^{a}	4.08 ± 0.14^{a}
6	0.99	3.96 ± 0.02^{b}	1.92 ± 0.22^{b}	3.63 ± 0.07^{b}	1.13 ± 0.04^{b}
8.5	0.91	–	–	–	–
8.5	0.94	1.04 ± 0.05^{a}	10.13 ± 0.25^{a}	1.20 ± 0.05^{a}	10.18 ± 0.21^{a}
8.5	0.97	2.34 ± 0.07^{b}	1.07 ± 0.20^{b}	3.58 ± 0.01^{b}	1.94 ± 0.19^{b}
8.5	0.99	6.02 ± 0.25^{c}	1.48 ± 0.29^{b}	6.03 ± 0.20^{c}	1.57 ± 0.96^{b}
15	0.88	–	–	–	–
15	0.91	0.90 ± 0.04^{a}	7.23 ± 0.31^{a}	0.91 ± 0.11^{a}	5.92 ± 0.69^{a}
15	0.94	2.54 ± 0.08^{b}	1.31 ± 0.22^{b}	2.57 ± 0.11^{b}	0.93 ± 0.28^{b}
15	0.97	4.03 ± 0.08^{c}	0.32 ± 0.33^{c}	6.77 ± 0.12^{c}	0.33 ± 0.13^{b}
15	0.99	11.85 ± 0.12^{d}	0.71 ± 0.49^{b}	11.20 ± 0.35^{d}	0.48 ± 0.05^{b}
20	0.88	–	–	–	–
20	0.91	1.05 ± 0.04^{a}	1.10 ± 0.12^{a}	1.16 ± 0.21^{a}	1.14 ± 0.89^{a}
20	0.94	3.87 ± 0.05^{b}	0.57 ± 0.07^{b}	4.80 ± 0.00^{b}	1.82 ± 0.06^{ab}
20	0.97	8.60 ± 0.14^{c}	0.43 ± 0.22^{b}	11.64 ± 0.43^{c}	0.72 ± 0.19^{b}
20	0.99	14.57 ± 0.03^{d}	0.61 ± 0.01^{b}	14.41 ± 0.04^{d}	0.10 ± 0.05^{b}
25	0.88	–	–	–	–
25	0.91	1.11 ± 0.05^{a}	0.81 ± 0.11^{a}	*	*
25	0.94	4.54 ± 0.10^{b}	0.64 ± 0.19^{a}	5.64 ± 0.19^{a}	1.47 ± 0.11^{b}
25	0.97	11.87 ± 0.44^{c}	0.56 ± 0.12^{bc}	16.67 ± 0.33^{b}	1.02 ± 0.05^{c}
25	0.99	15.88 ± 0.37^{d}	0.27 ± 0.11^{c}	16.46 ± 0.26^{c}	0.20 ± 0.03^{d}
30	0.88	–	–	–	–
30	0.91	0.53 ± 0.01^{a}	0.82 ± 0.15^{a}	0.46 ± 0.01^{a}	1.42 ± 0.47^{a}
30	0.94	2.42 ± 0.97^{b}	0.71 ± 0.81^{a}	3.72 ± 0.04^{b}	0.85 ± 0.48^{ab}
30	0.97	10.26 ± 0.10^{c}	0.85 ± 0.07^{a}	8.43 ± 0.08^{c}	0.33 ± 0.03^{b}
30	0.99	13.49 ± 0.08^{d}	0.73 ± 0.03^{a}	15.36 ± 0.13^{d}	0.32 ± 0.06^{b}
35	0.88	–	–	–	–
35	0.91	–	–	–	–
35	0.94	–	–	–	–
35	0.97	–	–	–	–
35	0.99	–	–	–	–

- No growth was observed for 30 days. Average of three plates. SD: standard deviation * Missing data. For each strain and temperature level, different letters mean significant differences ($p < 0.05$) among growth at the different aw levels according to Tukey HSD test.

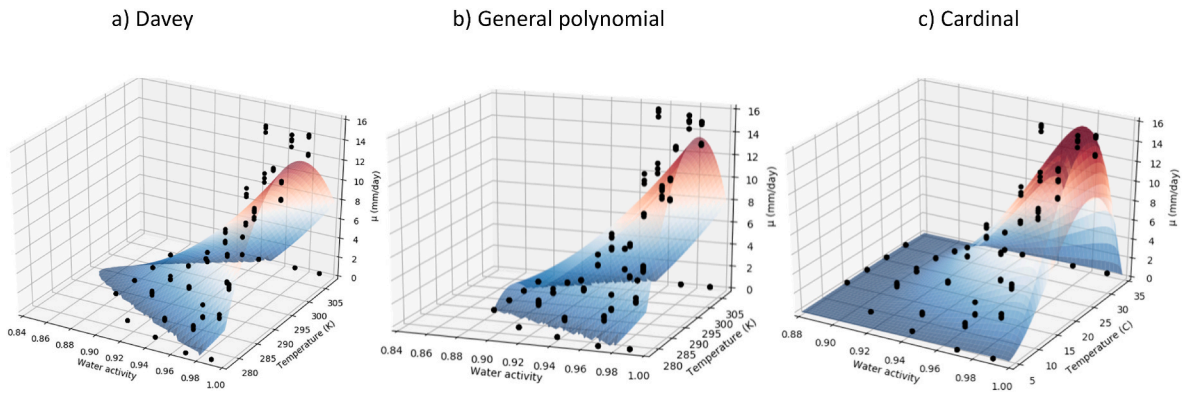

Fig. 2. Surface plots showing the fitting to observed growth rate values of *F. graminearum* on wheat-based media a) Davey, b) General polynomial and c) Cardinal.

Table 2
Secondary kinetic models performance.

		ME	MAE	RMSE	r	P-value	Bias Factor	Accuracy Factor
Davey	No transform rowhead	0.00	1.86	2.45	0.88	0.00	27.42	28.04
	sqrt(μ) rowhead	−0.34	1.14	1.93	0.94	0.00	33.32	37.61
	sqrt(μ) sqrt(aw) rowhead	−0.34	1.14	1.93	0.94	0.00	33.32	37.61
	sqrt(aw) rowhead	0.00	1.86	2.45	0.88	0.00	27.42	28.04
	sqrt(μ) sqrt(1-aw) rowhead	−0.34	1.14	1.93	0.94	0.00	33.32	37.61
	sqrt(1-aw) rowhead	0.00	1.88	2.45	0.88	0.00	22.78	23.40
Polynomial	No transform rowhead	0.00	1.71	2.37	0.89	0.00	27.43	27.99
	sqrt(μ) rowhead	−0.34	1.15	1.96	0.93	0.00	33.28	37.56
	sqrt(μ) sqrt(aw) rowhead	−0.34	1.15	1.96	0.93	0.00	33.28	37.56
	sqrt(aw) rowhead	0.00	1.71	2.37	0.89	0.00	27.43	27.99
	sqrt(μ) sqrt(1-aw) (Gibson) rowhead	−0.34	1.15	1.94	0.94	0.00	33.31	37.59
	sqrt(1-aw) (μ) rowhead	0.00	1.68	2.37	0.89	0.00	27.39	27.90
Cardinal Models	Original model rowhead	0.94	1.57	2.66	0.87	0.00	7.78	9.07
	Progressive model rowhead	1.04	1.61	2.75	0.87	0.00	7.83	9.09
	sqrt(μ) Original model rowhead	0.05	0.33	0.45	0.94	0.00	7.63	8.38
	sqrt(μ) Progressive model rowhead	2.44	2.56	4.13	0.89	0.00	9.27	10.06

ME: Margin of Error, MAE: Mean Absolute Error, RMSE: Root Mean Square Error.

distributions, or heteroscedasticity, which can compromise the model's effectiveness and the validity of inference statistics. By applying transformations, such as square root, non-linear relationships can be linearized, making the patterns in the data more easily identifiable.

Table 2 shows the results of the different models and the applied transforms. The results for the Davey and polynomial models are largely similar. The main difference being the addition of an interaction term between temperature and a_w in the polynomial model. A common feature across these models is that the transformation of the growth rate improves prediction accuracy when considering MAE, RMSE and r, however the ME is non-zero indicating a bias in the predictive model. Transforming the a_w parameter as suggested by Gibson et al. (1994) shows a marginal improvement in the summary statistics.

Likewise, for the cardinal models, the square root transform of growth rate leads to the model with the highest accuracy. Although the correlation is similar to the other models, the MAE and RMSE are significantly smaller. Interestingly, the progressive model with growth rate transform leads to the poorest results.

3.3. Probability models

Coefficient estimates of the developed logistic regression model for the growth of *F. graminearum* 08/091 and 08/111 are presented in Supplementary Table 1. The model included a_W, temperature and time observations; considering the full matrix the model predicted correctly 95 % of the cases in both strains. After 10 days, incorrect predictions were observed at 6 °C/0.97 a_w predicted (0.38–0.49) and observed 1, and 20, 25 and 30 °C/0.91a_W where between 0.27 and 0.70 and 0.38 and 0.71 for Fg08/091 and Fg08/111 respectively, while the observed values were 1. At the end of the experiment (30 days) the models predicted the growth was possible at 0.88 a_W at 20, 25 and 30 °C and also at 35 °C, although growth was never observed in the wheat-based media (Fig. 3). As illustrated in the plots, the probability of growth increases with time and with an increase in water activity (a_w) level. As observed in the figures, probabilities of growth for *F. graminearum* exceeding 0.80 were predicted to be in the range of 0.90–0.95 a_w at temperatures between 16 and 34 °C after 30 days. Thus, for safe wheat storage, maintaining an aw below 0.89 and avoiding temperatures in the range of 18–31 °C ($P < 0.5$) are recommended. Conversely, probabilities below 0.10 were observed at temperatures below 15 °C when the a_w is under 0.89.

3.4. Validation of the models in wheat kernels

3.4.1. Validation of growth models

Validation was performed using two independent data sets of *F. graminearum* on irradiated wheat of kernels. The first set of data produced in this work (Fg. 08/111) was located at the boundaries of the domain of the model. Maximum radial growth rate (μ_{max}) and lag phase (λ) were estimated through Baranyi's primary model as previously done in wheat based media (Table 3). Interestingly fungal growth occurred between 15 and 35 °C and under $a_W \geq 0.92$,in a different range of environmental conditions compared with the growth in media (6–30 °C).

The second data set was from ecophysiology studies performed in wheat from two *F. graminearum* strains (RC17 and RC22) isolated from

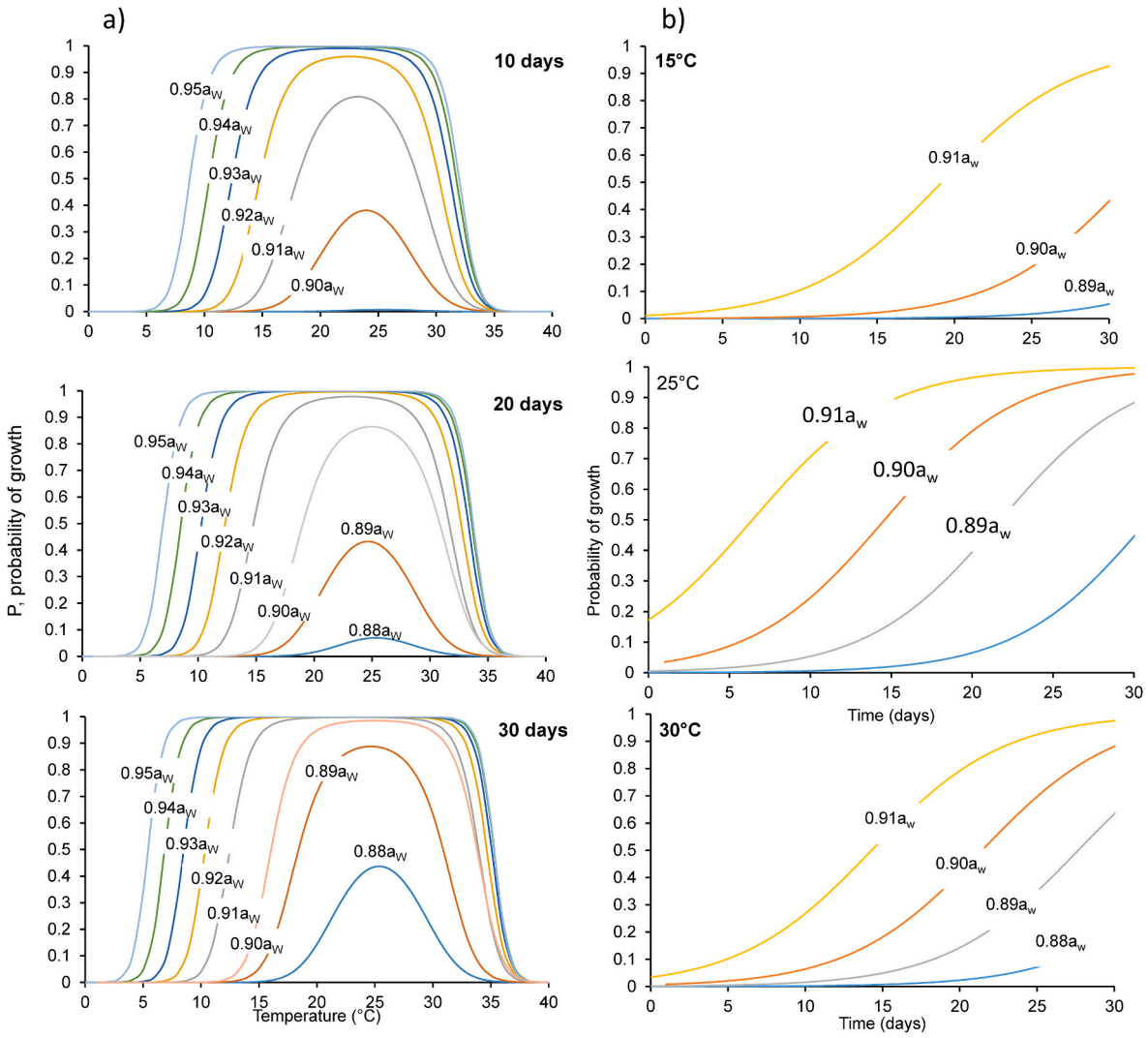

Fig. 3. Predicted effect of temperature (T) and water activity (aw) on probability of *F. graminearum* (Fg 08/091) growth in wheat-based media (3 %) incubated for 10, 20 and 30 days (a), and at 15, 23 and 30 °C (b).

Argentina including boundary and optimal conditions (Ramirez et al., 2006).

3.4.2. *Validation of growth probabilistic models*

Predictions made using the probabilistic model, developed from the data from Fg 08/111, were compared against actual observations in wheat grain. This comparison revealed a 65 % accuracy, as detailed in Table 4. The model exhibited errors in 10 specific combinations of a_W and temperature. Notably, most prediction errors were false positives, indicating an overly cautious prediction trend, apart from a significant false negative error at 35 °C and $a_W > 0.92$. Table 5 also presents predicted growth responses derived from the literature, specifically the study by Ramirez et al. (2006). Improved prediction accuracy was noted with a second dataset, focusing on two strains of the fungus, achieving an 83 % concordance rate. However, there was a single instance of a false negative prediction at a condition of 0.90 a_W and 15 °C.

3.5. *Zearalenone accumulation*

The production of ZEN at day 10, 20, and 30 on wheat-based media is presented in Supplementary Fig. 2. The impact of T on ZEN production followed a discernible pattern, with the optimal temperature range falling between 15 and 25 °C. The optimal temperature for ZEN production was observed between 25 and 30 °C. Additionally, ZEN accumulation increased in the media over time, except at 30 °C. Regarding the impact of a_w, no clear trend was observed. However, overall, higher ZEN accumulation was quantified at lower a_w levels. Interestingly, in most cases, the lowest ZEN production was quantified at 0.99 a_w.

Coefficient estimates from the logistic regression model developed for ZEN production by *F. graminearum* 08/091 and 08/111, as well as a combined model for both strains, are presented in Supplementary Table 2. A lower percentage of prediction accuracy was observed compared with the growth models (>86 %). Contrary to the growth obtained, the logistic regression equations from both strains exhibited significant differences in terms of parameter estimates and significant terms. Fig. 4 depicts the probability of accumulating ZEN in wheat-based media inoculated with two different *F. graminearum* strains. It appears that ZEN production by strain Fg 08/111 is more influenced by the a_w compared to strain Fg 08/091. For both strains, there is a high probability of risk for ZEN accumulation at low a_w levels (0.91 a_w) across a wider range of temperatures (15–30 °C).

3.5.1. *Validation of ZEN probabilistic models*

The optimal conditions for ZEN accumulation were observed at 0.95 a_w and 30 °C in wheat, resembling those observed in media at 25–30 °C. Conversely, higher a_w levels were necessary compared to the observation in wheat-based media, where the lowest a_w conducive to ZEN

Table 3
Estimated maximum growth rates (μmax), time to visible growth (λ) for *Fusarium graminearum* isolate and zearalenone production on wheat grain at different temperature (T) and water activity levels (aw).

T(°C)	aw	Fg08/111		
		μ_{max} (mm/day) ± SD	λ (day) ± SD	ZEA (ng/g) ± SD
5	0.88	–	–	–
5	0.9	–	–	–
5	0.92	–	–	–
5	0.95	–	–	–
10	0.88	–	–	–
10	0.9	–	–	–
10	0.92	–	–	–
10	0.95	–	–	–
15	0.88	–	–	–
15	0.9	–	–	–
15	0.92	3.75 ± 0.11	4.74 ± 0.15	<LOD
15	0.95	3.74 ± 0.24	1.85 ± 0.06	<LOD
20	0.88	–	–	–
20	0.9	–	–	–
20	0.92	5.44 ± 0.47	3.61 ± 0.14	34.24 ± 20.75
20	0.95	10.90 ± 0.75	2.88 ± 0.43	8.70 ± 1.66
25	0.88	–	–	–
25	0.9	–	–	–
25	0.92	3.74 ± 0.51	1.21 ± 0.73	213.60±[a]
25	0.95	21.00 ± 0.78	2.55 ± 0.17	993.23 ± 629.10
30	0.88	–	–	–
30	0.9	–	–	–
30	0.92	6.01 ± 0.35	1.96 ± 0.78	248.27 ± 129.66
30	0.95	24.75 ± 0.35	2.58 ± 0.08	4147 ± 469.52
35	0.88	–	–	–
35	0.9	–	–	–
35	0.92	2.06 ± 0.00	1.74 ± 0.00	<LOD
35	0.95	4.17 ± 0.30	0.87 ± 0.11	2.86±[a]

- No growth was observed for 30 days.
Average of three plates. SD: standard deviation.
[a] Only one replicate.

Table 4
Comparison of predicted and observed *F. graminearum* Fg 08/111 growth responses at 30 days in wheat kernels. Characters in bold highlight no concordance between observed and predicted values.

T (°C)	aw	time	Predicted probability of growth	Observed outcomes in wheat[a]
5	0.88	30	0.00	0
5	0.9	30	0.00	0
5	0.92	30	0.00	0
5	0.95	30	0.34	0
10	0.88	30	0.00	0
10	0.9	30	0.00	0
10	0.92	30	0.39	0
10	0.95	30	1.00	0
15	0.88	30	0.00	0
15	0.9	30	0.49	0
15	0.92	30	0.99	1
15	0.95	30	1.00	1
20	0.88	30	0.14	0
20	0.9	30	0.97	0
20	0.92	30	1.00	1
20	0.95	30	1.00	1
25	0.88	30	0.44	0
25	0.9	30	0.98	0
25	0.92	30	1.00	1
25	0.95	30	1.00	1
30	0.88	30	0.20	0
30	0.9	30	0.90	0
30	0.92	30	0.99	1
30	0.95	30	1.00	1
35	0.88	30	0.01	0
35	0.9	30	0.09	0
35	0.92	30	0.39	1
35	0.95	30	0.61	1

[a] Number of observed wheat Petri plates at given aW out of the 3 inoculates plates after 30 days of incubation at the given temperature.

Table 5
Comparison of predicted and observed *F. graminearum* (RC 17-2 and RC 22-2) growth responses data on wheat of Ramirez et al., 2006). Characters in bold highlight no concordance between observed and predicted values.

T (°C)	a_w	time	Predicted probability of growth	Observed outcomes in wheat[a]
5	0.9	30	0.00	0
5	0.93	30	0.00	0
5	0.95	30	0.34	0
5	0.97	30	**0.96**	**0**
5	0.99	30	**1.00**	**0**
15	0.9	30	**0.49**	**1**
15	0.93	30	1.00	1
15	0.95	30	1.00	1
15	0.97	30	1.00	1
15	0.99	30	1.00	1
25	0.9	30	0.98	1
25	0.93	30	1.00	1
25	0.95	30	1.00	1
25	0.97	30	1.00	1
25	0.99	30	1.00	1
30	0.9	30	**0.90**	**0**
30	0.93	30	1.00	1
30	0.95	30	1.00	1
30	0.97	30	1.00	1
30	0.99	30	1.00	1
37	0.9	30	0.01	0
37	0.95	30	0.05	0
37	0.99	30	0.00	0

[a] Number of observed wheat Petri plates at given a_W out of the 2 inoculates plates after 49 days of incubation at the given temperature.

accumulation was 0.92 a_w between 20 and 30 °C. ZEN values obtained from our experiment and in wheat, mixed cereals, or ensilages containing wheat inoculated with *F. graminearum* were utilised to evaluate the accuracy of the probability models derived from production in wheat-based media. Tables 6 and 7 depict the relationship between the predictions provided for both single and combined models. Experimental conditions in previous publications typically ranged from 10 to 30 °C, with only four experiments conducted at 0.90 a_w, closer to the boundary condition of *F. graminearum* growth. Consequently, ZEN was detected in most of the publications. Most incorrect predictions occurred at lower temperatures (10–15 °C) (see Table 8).

4. Discussion

This study evaluated the marginal and optimal conditions for *F. graminearum* growth in a wheat-based medium, comparing it with growth in wheat and analysing the ZEN profile of two *F. graminearum* strains in wheat-based media.

Interestingly, we observed a shift of 5 °C in the *F. graminearum* growth profile between minimum (10 °C), optimum (25 °C), and maximum (30 °C) temperatures when comparing growth in the medium and in wheat. Additionally, higher a_w was required to promote fungal growth on kernels compared to the wheat-based medium, likely due to the nutrient availability differences between the two substrates. The maximum fungal growth recorded in wheat grains (24 mm/day) exceeded that in the medium (17 mm/day), possibly because fungal hyphae grow rapidly over the grain surface to access more areas, emphasising the need to validate models in food matrices, especially in boundary condition regions (Garcia et al., 2011). In our study maximum fungal growth occurred at 25 °C at 0.99 a_w. Similarly, Brennan et al. (2003) found that the growth of *F. graminearum*, *Fusarium culmorum* and *Fusarium poae*, isolated from infected wheat seed and grown on potato dextrose agar, was stimulated when temperatures increased from 10 to 25 °C, peaking at 25 °C. In another study, maximum growth also occurred at 25 °C (10 mm/day), with higher growth observed at increased a_w levels and at 15 °C in solid agar medium GYEP (glucose–yeast extract–peptone media). However, contrary to our findings,

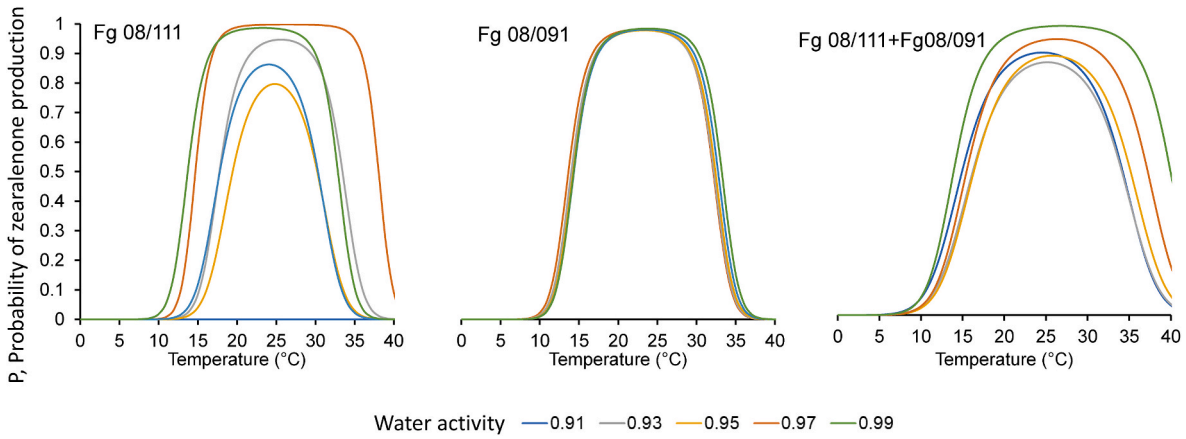

Fig. 4. Predicted effect of temperature (T) and water activity (aw) on probability of *F. graminearum* single strain (a, Fg 08/111 and b, Fg 08/091) and combined data from both strains (c, Fg 08/091 + Fg 08/111) zearalenone production in wheat-based media (3 %) after 30 days, and at 15, 25 and 30 °C.

Table 6
Zearalenone (μg/g) accumulation in wheat-based media (3 %) at different temperatures (T) and water activity levels (a_w) after 10, 20 and 30 days.

		Temperature (°C)							
		aw	6	8.5	15	20	25	30	35
F. graminearum 08/091	*Day 10*	0.88							
		0.91							
		0.94				0.02 ± 0.02	1.77 ± 1.09	4.88 ± 3.63	
		0.97				0.15 ± 0.03	7.61 ± 0.46	91.78 ± 86.28	
		0.99			0.01 ± 0.01		0.11 ± 0.02	1.49 ± 0.56	
	Day 20	0.88							
		0.91				48.31 ± 10.87	3.05 ± 0.73		
		0.94				2.24 ± 1.18	17.42 ± 12.62	251.11 ± 51.73	
		0.97			2.32 ± 1.08	14.84 ± 4.92	27.51 ± 10.68	181.32 ± 193.20	
		0.99			0.22 ± 0.22		0.28 ± 0.15	0.52 ± 0.08	
	Day 30	0.88							
		0.91			52.28 ± 18.60	59.98 ± 18.16	136.14 ± 48.68		
		0.94			0.05 ± 0.07	16.08 ± 14.68	99.12 ± 50.44		
		0.97			2.37 ± 0.33	4.24 ± 4.92	427.10 ± 101.18	0.41 ± 0.16	
		0.99			0.04 ± 0.03	0.13 ± 0.09	0.13 ± 0.09	2.44 ± 2.77	
F. graminearum 08/111	*Day 10*	0.88							
		0.91							
		0.94					0.55 ± 0.64	2.77 ± 0.63	
		0.97					0.22 ± 0.15	9.13 ± 2.31	
		0.99			0.07 ± 0.02	0.17 ± 0.11	0.04 ± 0.01	4.66 ± 2.33	
	Day 20	0.88							
		0.91				44.45 ± 44.67	10.04 ± 7.11		
		0.94					1.60 ± 1.12	47.76 ± 17.44	
		0.97			0.01 ± 0.01	0.04 ± 0.01	1.19 ± 0.07	8.16 ± 6.90	
		0.99			0.19 ± 0.20	0.37 ± 0.42	0.30 ± 0.37	3.66 ± 0.14	
	Day 30	0.88							
		0.91			13.91 ± 9.32	150.39 ± 32.23	113.84 ± 93.94		
		0.94				0.85 ± 0.47	13.13 ± 7.73		
		0.97				2.86 ± 1.40	169.35 ± 212.28	0.12 ± 0.18	
		0.99			0.16 ± 0.09	0.03 ± 0.03	0.68 ± 0.93	3.58 ± 2.04	

No toxin was detected at 6 and 8 °C. No growth at 35 °C. Limit of detection 0.01 μg/g. SD: standard deviation. Only positive samples are included.

F. graminearum was reported to grow in the medium at 35 °C (Marín et al., 2010). Previous ecophysiological studies on irradiated wheat layers showed that 25 °C was also the optimum temperature confirming the findings in media (Ramirez et al., 2006; Hope et al., 2005). However, contrasting behaviour was noted in the spectrum of environmental conditions conducive to *F. graminearum* growth in irradiated wheat. Our study observed growth from 15 to 35 °C, whereas Ramirez et al. (2006), reported growth from 10 to 30 °C. This discrepancy suggests that strains isolated from different areas may exhibit different resilience.

In this study, the maximum fungal growth rate was estimated using the Baranyi and Roberts model. Previous authors have indicated that the model is the most suitable for describing *F. graminearum*'s growth (Cambaza et al., 2019). Furthermore, probabilistic models have been developed to predict fungal growth in cereals. This approach has recently been employed to characterise the growth of other *Fusarium* species in oat and wheat-based media (Verheecke-Vaessen et al., 2021; Garcia-Cela et al., 2022). Logistic regression model validated in food matrix showed concordant data at range of 64–83 %. Although most of the incorrect predictions occurred under safe conditions, an exception was at 35 °C where *Fusarium graminearum* growth was observed in wheat but not in wheat-based media. Thus, for safe wheat storage, an a_w<0.89 should be maintained and temperatures in the range 18–31 °C should be avoided ($P < 0.5$). A higher level of concordance (92 %) was found for *F. langsethiae*, but it's important to note that the model was developed

Table 7
Comparison of predicted and observed Zearalenone accumulation (at 30 days) in wheat inoculated with the strain Fg 08/111. Characters in bold highlight no concordance between observed and predicted values.

T (°C)	a_w	time	Predicted probability of growth Fg 08/111	Predicted probability of growth Fg 08/091	Predicted probability of growth Fg08/111 + Fg 08/091	Observed outcomes in wheat
15	0.9	30	**0.92**	**0.78**	**0.61**	0
15	0.92	30	0.22	**0.71**	0.41	0
15	0.95	30	0.04	**0.64**	0.34	0
20	0.9	30	1.00	0.98	0.88	1
20	0.92	30	0.94	0.97	0.79	1
20	0.95	30	**0.56**	0.96	0.77	1
25	0.9	30	1.00	0.98	0.92	0
25	0.92	30	0.99	0.98	0.87	1
25	0.95	30	0.79	0.98	0.88	1
30	0.92	30	0.97	0.83	0.78	1
30	0.95	30	0.50	0.85	0.83	1
35	0.92	30	**0.61**	0.06	0.38	0
35	0.95	30	0.03	0.08	0.50	1

Table 8
Comparison of predicted and observed Zearalenone accumulation (at 30 days) in wheat inoculated with the strain Fg 08/111. Characters in bold highlight no concordance between observed and predicted values.

T (°C)	aw	time	Predicted probability of growth Fg 08/111	Predicted probability of growth Fg 08/091	Predicted probability of growth Fg08/111 + Fg 08/091	Observed outcomes in wheat
15	0.9	30	0.92	0.78	0.61	0
15	0.92	30	0.22	0.71	0.41	0
15	0.95	30	0.04	0.64	0.34	0
20	0.9	30	1.00	0.98	0.88	1
20	0.92	30	0.94	0.97	0.79	1
20	0.95	30	0.56	0.96	0.77	1
25	0.9	30	1.00	0.98	0.92	0
25	0.92	30	0.99	0.98	0.87	1
25	0.95	30	0.79	0.98	0.88	1
30	0.92	30	0.97	0.83	0.78	1
30	0.95	30	0.50	0.85	0.83	1
35	0.92	30	0.61	0.06	0.38	0
35	0.95	30	0.03	0.08	0.50	1

using oat-based media, as well as the external dataset used for validation (Verheecke-Vaessen et al., 2021). To minimise fungal spoilage, prevention of fungal growth is crucial. Nonetheless, given the potential for growth to occur, it is essential to understand the risk of ZEN contamination in terms of T and a_w.

ZEN production exhibited temperature dependence, with optimal production occurring between 20 and 30 °C. Interestingly, ZEN production in wheat-based media was feasible at very low a_w levels, such as 0.94 a_w at 20–30 °C and even 0.91 a_w at 20–25 °C after 20 days. Previous research also documented ZEN production in wheat media at 0.93 a_w within the same temperature range (20–30 °C); either no or very low concentrations were produced at a high a_w (>0.98) by *F. asiaticum* (Garcia-Cela et al., 2022; Cervini et al., 2024). In irradiated wheat after 15 days, optimal production of ZEN, alpha-zearalenol, and beta-zearalenol were observed at 0.90 a_w/25 °C, with no testing conducted at 30 °C. However, ZEN and its metabolites did not show production at 0.90 a_w, except at 10 °C (Garcia-Cela et al., 2018). Conversely, in irradiated maize, higher production was observed at 0.98 a_w, according to Velluti et al. (2001). In malted barley flour, bran, and germ inoculated with *F. graminearum*, ZEN production was higher at 20 °C than 30 °C. Interestingly, levels of a_w and incubation time did not correlate with maximum ZEN accumulation. For instance, while high contamination was observed at 0.95 a_w/30 °C, contamination was higher at 0.98 a_w/20 °C in flour after 34 days of incubation (Habschied et al., 2011). Different ZEN production profiles were observed in three *Fusarium incarnatum* isolates inoculated in sterile sorghum, showing that at each temperature, the maximum level of ZEN was observed at different values of a_w and after different incubation period from one isolate (Lahouar et al., 2017). Therefore, the role of a_w in the production of ZEN is not clear.

To produce a useful tool for wheat under storage conditions, a probabilistic model for ZEN production was developed. Comparison with results from previous publications was limited due to limited data about ZEN contamination in wheat correlated with the environmental factors (T and a_w).

5. Conclusion

This study assessed the fundamental growth patterns of *F. graminearum* in wheat-based media and evaluated the performance of various kinetic and probabilistic models. Minimal variability in growth among different *F. graminearum* strains was observed, suggesting that these models could be applicable for controlling colonisation in wheat. Integrating these models into decision support systems could assist farmers in identifying pre-harvest contamination risks and in optimising harvesting and drying practices to minimise post-harvest contamination. Additionally, this study is the first to assess ZEN accumulation in wheat-based media across an extensive range of temperatures and water activities. We established a probabilistic model for ZEN production; however, determining general conclusions about the optimal and minimal conditions for ZEN production across various fungal strains proved elusive due to insufficient data for model validation. Therefore, the development of accurate prediction models for ZEN contamination within the food chain continues to present significant challenges. However, preventing *Fusarium* growth will also limit ZEN contamination. According to our models, water activity should be maintained below 0.89, particularly when storage temperatures range between 18 °C and 31 °C.

CRediT authorship contribution statement

B. Ingram: Visualization, Validation, Formal analysis, Writing – original draft. **S. Marin:** Visualization, Methodology, Data curation, Conceptualization. **E. Kiaitsi:** Investigation, Formal analysis. **N. Magan:** Supervision, Resources, Project administration, Funding acquisition. **C. Verheecke-Vaessen:** Writing – review & editing. **C. Cervini:** Writing – review & editing, Investigation. **F. Rubio-Lopez:** Writing – original draft, Resources, Project administration, Investigation. **E. Garcia-Cela:** Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors have no conflicts of interest to declare.

All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2025.101572.

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