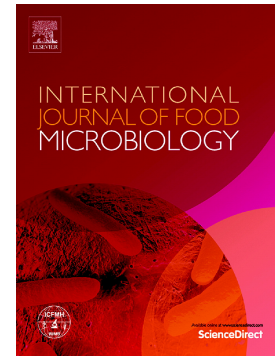


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PII: S0168-1605(21)00162-8

DOI: <https://doi.org/10.1016/j.ijfoodmicro.2021.109203>

Reference: FOOD 109203

To appear in: *International Journal of Food Microbiology*

Received date: 14 February 2020

Revised date: 4 March 2021

Accepted date: 11 April 2021

Please cite this article as: C. Verheecke-Vaessen, E. Garcia-Cela, A. Lopez-Prieto, et al., Water and temperature relations of *Fusarium langsethiae* strains and modelling of growth and T-2 and HT-2 mycotoxin production on oat-based matrices, *International Journal of Food Microbiology* (2021), <https://doi.org/10.1016/j.ijfoodmicro.2021.109203>

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Water and temperature relations of *Fusarium langsethiae* strains and modelling of growth and T-2 and HT-2 mycotoxin production on oat-based matrices

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Carol VERHEECKE-VAESSEN: Resources, Conceptualisation, Methodology, Formal analysis, Writing Original and review, supervision, **Esther GARCIA-CELA:** Methodology, Modelisation, Writing Original and review, supervision, **Alejandro LOPEZ-PIETRO:** Conceptualisation, data collection, **Inga Osk JONSDOTTIR:** Methodology, Modelisation, **Angel MEDINA and Naresh MAGAN:** Writing- Reviewing and Editing, Supervision, Funding acquisition.

ABSTRACT

In the UK and Northern Europe, ripening oats can become contaminated with T-2 and HT-2 mycotoxins, produced mainly by *Fusarium langsethiae*. There are indicative levels related to the maximum limits for oat grain for these toxins. The objectives of this study were to examine the effect of interacting conditions of temperature (10-30°C) and water activity (a_w , 0.995-0.90) on (a) lag times prior to growth, (b) growth and (c) T-2 and HT-2 toxins by two strains of *F. langsethiae* isolated from oats in the UK and compare this with the type strain (Fl201059) which has been genomically sequenced, and (d) develop (and validated with published data) a probabilistic models for impacts of temperature x a_w on growth and toxin production.

All three strains had an optimum a_w range and temperature of 0.995-0.98 and 25°C for growth. For T-2+HT-2 production these were 0.995 a_w and 20°C. Overall, the type strain produced higher amounts of T-2+HT-2 with a HT-2/T-2 ratio of up to 76. Using this study data sets and those from the literature, probabilistic models were developed and validated for growth and T-2+HT-2 toxin production in relation to temperature x a_w conditions. These models, when applied in stored oats, will be beneficial in determining the conditions on the relative level of risk of contamination with these two toxins in the context of the EU indicative maximum levels.

KEYWORDS: Water activity; temperature; mycotoxins; boundary conditions; *Fusarium*; probabilistic models

1. INTRODUCTION

Oat production has steadily increased in Northern Europe, including the UK, because oats are considered superior to wheat and barley for dietary fibre, and the richest sources of soluble fibre beta-glucan, rich in essential amino acids, and contain a range of unique antioxidants and vitamin E compounds (Emmons and Peterson, 2001). Thus, they have become a cereal of social and economic importance.

However, there have been concerns in the EU that oats can become contaminated with type A trichothecene mycotoxins (T-2 and HT-2) produced mainly by *Fusarium langsethiae*, *F. poae* and *F. sporotrichioides* (Thrane et al., 2004; Torp and Nirenberg, 2004). A survey showed that 93% of European oats are contaminated with T-2+HT-2 ($> 5 \mu\text{g.kg}^{-1}$) (Pettersen et al., 2011). T-2+HT-2 are mainly produced pre-harvest during the grain filling period. During the milky ripe and soft dough stages (GS 70-85) the water availability is >0.98 and then >0.95 water activity (a_w) which represent good conditions for colonisation by *Fusaria* and *Alternaria* in temperate cereals (Aldred and Magan, 2004; Magan and Lacey, 1985). In addition, as oats are often harvested in the late autumn any delay in harvesting or drying can lead to conducive conditions for *F. langsethiae* colonisation and additional toxin contamination (Hjelkrem et al., 2018; Medina and Magan, 2011).

This has resulted in EU recommendations on indicative contamination levels for T-2+HT-2 (European Commission 2013/165/EU, 2013) contamination of oats. The present indicative maximum levels are $1000 \mu\text{g kg}^{-1}$ for T-2+HT-2 in unprocessed oats and $200 \mu\text{g kg}^{-1}$ in oats for direct human consumption. The development of minimisation strategies has been difficult because these fungi produce no visible symptoms, and thus, contamination with toxins is challenging to discern. Currently, mitigation strategies to prevent T-2+ HT-2

contamination in oats have been focused on pre-harvest agronomic practices (Bernhoft et al., 2012) and potential of inhibitory crop protection compounds post-harvest (Ferruz et al., 2016; Kawtharani et al., 2020).

Thus, more detailed information is required on the impact of the relationship between key abiotic factors such as a_w and temperature on both colonisation and production of T-2+HT-2 toxins. Such data would help to develop probabilistic models which can be used to predict potential levels of risk of contamination of oats pre- and post-harvest.

Overall, in the UK, *F. langsethiae* is the most predominant species, although in most Nordic countries and Central Europe this species and sometimes *F. sporotrichioides* are important (Imathiu et al., 2010; Torp and Adler, 2004). Thus, understanding the ecology of *F. langsethiae* strains is important to develop effective minimisation strategies. Previous studies comparing different strains of *F. langsethiae* from Northern European countries identified probable optimum conditions for growth at 0.98-0.995 a_w and 25°C, with T-2+HT-2 toxin production being highest when water was freely available at 20-25°C (Medina et al., 2010; Medina and Magan, 2011; Verheecke-Vaessen et al., 2019). These optimum T-2+HT-2 conditions were correlated with increased dry matter losses previously in stored oats (Mylona and Magan, 2011).

There is now data available on the genome of *F. langsethiae* (Lysøe et al., 2016) and comparisons of the ecology of this Norwegian type strain and others have not previously been made. In addition, there have been no studies carried out to develop probabilistic models of the impact that a_w x temperature effects have on growth and T-2+HT-2 toxin production or indeed a validation of such models using published data. This would help in developing appropriate and more accurate probabilistic modelling of relative conditions representing a high or low risk.

The objectives of this study were to examine the impact of interacting conditions of temperature x a_w on (a) lag phases prior to growth, (b) relative growth rates, (c) production of T-2+HT-2 toxins including the ratio of HT-2/T-2 toxins and (d) to develop probabilistic models for both growth and toxin production in the context of the EU indicative maximum recommended levels.

MATERIAL AND METHODS

2.1 Strains

Two *F. langsethiae* strains from Worcestershire (Fe2391, strain 1) and Oxfordshire (Fe2392, strain 2) were isolated from oats in the UK. For comparison, the type strain Fl201059 (IBT 9951, BBA 70945, ITEM 3602) on which genomic data is published was used for comparison (Lysøe et al., 2016; Torp and Nirenberg, 2004).

2.2 Oat-based media and inoculation

Whole oats harvested in 2016 from Northamptonshire were milled in a Waring Laboratory blender (Model 7009G; Waring Laboratory Science, CT, USA) for 5 min at maximum speed. 2 % (w/v) oat flour and 2 % (w/v) agar (Technical agar No. 2, Oxoid) were added to 100 ml water to obtain the basic medium. The water availability (water activity, a_w) of the media was modified by adding mixtures of water/glycerol instead of water to obtain target a_w treatments of 0.995, 0.98, 0.95, 0.93 and 0.90 before autoclaving for 15 min at 121°C. The molten cooled media were vigorously shaken before pouring into 9 cm Petri plates (20 ml per plate), cooled and stored at 4°C in sealed bags prior to use. The target a_w levels were checked with an Aqualab TE4 (Labcell Ltd, Alton, UK) and found to be within ± 0.003 of the target values.

The different treatments were inoculated centrally with sterile 4 mm agar plugs taken from the margin of 7-day old colonies of *F. langsethiae* grown on potato dextrose agar at 25°C. For all temperatures (10, 15, 20, 25, 30°C) and a_w (0.995-0.90) treatments, three replicates per strain were used (a total of 9 plates per treatment), and the experiment repeated once. The replicates of each a_w treatment were enclosed in separate sealed containers and incubated at each temperature for periods of 10 days. This period

corresponds to the relatively high water availability conditions in ripening oats during grain filling and the maximum length of time allowable before drying to a safe moisture content level that needs to be implemented (HGCA, 2011).

2.3 Measurements of lag phases prior to growth and mycelial growth

The colony extension (diameter) was measured daily for each of the three replicates for each strain. The diameters were measured in two directions at right angles to each other. Subsequently, the colony radius was plotted against time and a linear regression model used to calculate the relative growth rates using the linear parts of the growth curves (Medina and Magan, 2010). The lag phases (λ) prior to growth (in days) were calculated from the growth curves considering the inoculum size as 4 mm and applying the equation $\lambda = (4-b)/a$ with a and b being the calculated factors of the equation $y = ax + b$ from the growth rate curves.

2.4 Quantification of T-2+HT-2 mycotoxin production

The methodology used was as described in Verheecke-Vaessen *et al.* (2019). In summary, agar plugs were taken across the colonies and mixed with 1 ml of methanol:water (80:20, v:v). After 90 min at 300 g at 25°C in the dark, the sample was centrifuged at 13,000 g for 15 min. 750 μ l of the supernatant was removed before the agar plugs were re-extracted again. Extracts were then combined and dried for seven hours and re-suspended in acetonitrile:water (50:50 v:v).

The samples were injected into a HPLC coupled with a UV diode-array detector (200nm). The column used was a Poroshell® 120 EC C₁₈ 100 mm x 4.6 mm (Agilent Technologies, Palo Alto, CA, USA) and separation and analyses were performed using the gradient mode at 1.2 ml min⁻¹ with an injection volume of 25 μ l. T-2 and HT-2 standards

were supplied by Cambridge Bioscience (Cambridge Bioscience Ltd, UK). Signals were processed by an Agilent Chem-Station software Ver. B Rev: 03.01 [317] (Agilent Technologies, Palo Alto, CA, USA). The limit of detection and quantification were 100 and 150 ng g⁻¹ for both toxins.

2.5 Probabilistic modelling of growth and toxin data

A logistic regression model using the type strain (3 replicates) was used to calculate the probabilities for growth and T-2+HT-2 toxin production in relation to a_w x temperature interactions. In addition, two data sets were used to validate the model (see Table 1). Firstly, a data set was obtained using both strains studied in the present work (strains 1 and 2; three replicates of each). A second data set was taken from previous results published by Medina and Magan (2010, 2011) where *F. langsethiae* strains from UK (strains 57, 59; three replicates of each) were used to evaluated for variability with UK strain isolated earlier (2004). A total of 15 measures (3 per strain, 5 strains in total), was used to develop the model.

Growth and mycotoxin production data were converted into binary values assigning the value of 1 in the case where visible fungal growth or T-2+HT-2 were detected which were above the thresholds. In this case, as an example, the EU indicative levels set for oats (processed and unprocessed) and 0 in the case of an absence of growth or no detectable T-2+HT-2. To estimate the probabilities of growth/toxin production, experimental data were fitted using the same methodology as the logistic regression model described previously in *Aspergillus* species (García-Cela et al., 2014).

$$\text{Logit}P = \ln\left(\frac{P}{1-P}\right) = b_0 + b_1T - b_{11}T^2 - b_{12}a_wT - b_2a_w^2 + b_{22}a_w$$

where

P: the probability of growth/toxin production.

b_i : the coefficients to be estimated.

2.6 Statistical analysis and profiling

Statistical analyses were performed using the package JMP® 14 (SAS Institute Inc., 2016. Cary, NC, USA). In the absence of any growth after ten days, the data were removed from the statistical analysis. The data for growth and T-2+HT-2 production were tested for normality using the Shapiro-Wilk test. Due to non-normality of the data, a Kruskal-Wallis analysis was performed for single-factor analysis and an effect test was performed for multiple factors analysis. Statgraphics 18® Centurion version 17.7.17 was used to analyse the probabilities of growth and mycotoxin production.

3. RESULTS

3.1 Effect of temperature x water activity interactions on lag phases prior to growth and growth rates

Generally, the lag phases (in days) prior to growth increased at marginal temperatures and under a_w stress treatments (Table 2). At a_w levels ≥ 0.95 there were no significant differences in the lag times. At 0.93 a_w , which was more marginal for growth, the lag time was increased by up to 4 days at 10-15°C. At 0.90 a_w , no growth occurred over the time frame of the experiment. Thus, only the data of one of the UK strains (strain 1) and the type strain are represented in Table 2, while the lag time of strain 2 is provided in Table S1.

Statistically, temperature had a significant impact on the lag phases ($p < 0.0001$; Table 3) except between 20-25°C ($p = 0.0539$) and 10-15°C ($p = 0.0723$). Generally, a_w also had a significant impact on the lag phases prior to growth ($p < 0.0001$).

3.2 Comparison of contour maps for growth of the different strains of *F. langsethiae* examined

Figure 1 shows that contour maps showing the isopleths for different growth rates at different a_w x temperature conditions. This showed that maximum growth rates occurred at 0.995-0.98 a_w and 25°C for the UK strains and 20-25°C for the type strain. At 30°C, maximum growth occurred at 0.98 a_w followed by 0.995 a_w for the three strains. The growth rate of strain 2 was optimal at 0.98 a_w and 25°C.

Significant differences ($p < 0.0001$) were found at all a_w levels except for 0.995-0.98 a_w . For temperature, growth rates were significantly different at between 10-20°C; 10-30°C and 15-30°C ($p < 0.0001$). Both individual factors, temperature ($p < 0.0001$) and a_w ($p < 0.0001$) and the interactions between temperature x a_w ($p < 0.0001$) had significant

effects on growth on the oat-based media. There was also a significant difference between the origin of the strains (one-way ANOVA test) with 7.4, 5.4 and 5.4 mm radial growth rates (mm.day^{-1}) for strains 1, 2 and the type strain, respectively (see Table 2).

3.3 Effect of temperature and water activity on T-2+HT-2 production by the different strains

The contour maps for the effect of these interacting abiotic factors on T-2+HT-2 production appeared to be more complex (Figure 2). The optimum a_w x temperature conditions for T-2+HT-2 production occurred at 0.995-0.98 and 20-30°C for all three strains tested. There was a significant effect of a_w ($p < 0.0001$) and temperature x a_w ($p < 0.0001$) on T-2+HT-2 production. Overall, there were no statistically significant differences between the three strains examined. However, the relative HT-2 production was significantly impacted, depending on strain origin x temperature x a_w ($p < 0.0001$).

The statistical analysis revealed that the effect of temperature was significantly different between 15°C and all the other temperatures examined. Focusing on T-2, the effect of a_w was statistically significant for all the a_w levels tested. For temperature effects, these were statistically different between 10-15°C and 20-15°C. For HT-2, the effect of a_w was statistically significant except for 0.995-0.93 and 0.95-0.93.

The type strain and strain 1 produced much higher titres of T-2+HT-2 ($> 50 \mu\text{g g}^{-1}$) than strain 2, especially under optimum temperature x a_w conditions.

3.4 Effect of water activity x temperature on the ratio of HT-2/T-2 toxins

The ratio of HT-2/T-2 was calculated using the approach of Medina *et al.* (2011). Both a_w and temperature had a significant effect on the HT-2/T-2 ratio. The ratios were

significantly different at all the a_w levels tested except for 0.98-0.995 and 0.93-0.95. Under water stress conditions, ($<0.95 a_w$) the mean ratios were 16.3 and 13.9 for 0.93 and 0.95, respectively. With more available water, there was a switch in this ratio with values of 0.2 and 0.1 at 0.995 and 0.98 a_w , respectively. The temperature did not appear to significantly impact the ratio except at 15°C, where the ratio was the highest (average of 19.1). Interestingly, the type strain showed the highest HT-2/T-2 ratio of all the three strains with up to 75.2 and 59.5 at 0.93 at 15°C and 25°C, respectively.

3.5 Development of probabilistic models for growth and T-2+HT-2 production by the *F. langsethiae* type strain

For the modelling of growth and mycotoxin production, the strains and conditions are shown in Table 1. Initially, a full second-order logistic regression model for the *F. langsethiae* type strain including all the linear, quadratic and interaction terms of temperature and a_w was generated for both fungal growth and T-2+HT-2 production (Table 4; Figures 3 and 4). Backward stepwise selection eliminated some linear and quadratic terms in the models as some were not statistically significant ($p>0.05$).

Plots of the probability of growth and T-2+HT-2 production above the indicative limits in oats for unprocessed oats (1000 ng g⁻¹) and direct human consumption (200 ng g⁻¹) in relation to temperature and a_w for the type strain were made (see Figures 3 and 4). Growth and toxin production probabilities at the same temperature increased when a_w was increased. Prediction of 0.5 probability between 17-24°C occurred at 0.90, 0.93 and 0.95 a_w for growth and toxin production >1000 ng g⁻¹ and >200 ng g⁻¹, respectively. Validation was performed for growth and toxin production using two sets of data, an independent set created in this study (data sets for strains 1 and 2) as well as data sets from the literature

(strain 57 and 59; Medina and Magan, 2010). In the initial growth logistic model, there were some false-positive and one false-negative predictions out of 25 (Table 5). In addition, two false-positive predictions were based on the second data set. Thus, the only unsafe predictions occurred at 25°C/0.90 a_w .

Data sets for the T-2+HT-2 logistical model gave three and two false-positive predictions for the two threshold levels respectively ($>200 \text{ ng g}^{-1}$ and $>1000 \text{ ng g}^{-1}$) in the case of the internal data set validation. There was more disagreement with data sets from the literature, mainly in relation to the 1000 ng g^{-1} because other strains used in the literature did not produce these levels of the toxins in any of the environmental conditions tested. However, as for growth, only one inaccurate prediction occurred as a false-negative at 30°C/0.95 a_w .

4. DISCUSSION

This study investigated the effect of interacting abiotic factors (temperature and a_w) on growth and T-2+HT-2 production by three strains of *F. langsethiae*; 2 strains isolated in the UK and the type strain Fl201059, on oat-based media. These data were used to develop probabilistic models on fungal growth and T-2+HT-2 toxin production in the context of conditions which may exceed/not exceed the recommended maximum limits set by the EU for these toxins in food and feed.

The main window of opportunity for pre-harvest colonisation and mycotoxin contamination is during the grain filling period when the a_w conditions ($>0.93 a_w$) and temperatures are conducive. Subsequently, the damp cool conditions at harvest and poor drying can allow further growth and mycotoxin production. This can lead to an increase in post-harvest contamination with such type A trichothecenes (Mylona and Magan, 2011).

The present study showed significant impacts of the interacting environmental factors on lag phases (λ) prior to growth for the three strains of *F. langsethiae* studied. The strains had shorter lag phases prior to growth under optimal temperature x a_w levels (<1 day) while under more marginal conditions (e.g., 10-15°C, 0.93 a_w) this was extended to 3-4 days prior to growth. This suggests relatively high adaptability of *F. langsethiae* strains to both water and temperature conditions than previously reported when comparing strains from Northern Europe (Medina and Magan, 2010). The previous studies showed no growth at 10-15°C and 0.93 a_w for the strains examined.

The effect of a_w x temperature on fungal growth showed optimum growth rate at 0.995-0.98 a_w at 20-25°C. These are similar to those observed previously for *F. langsethiae* and *F. sporotrichioides* (Kokkonen et al., 2012; Medina and Magan, 2010). However, in the present study, there were significant differences in growth rates under optimum a_w x

temperature conditions between the three strains, with strain 1 having a higher growth rate (7.4 mm.day^{-1}) when compared to the two other strains studied. Previously, growth rates of *F. langsethiae* suggested relatively slow colonisation rates ($3\text{-}4.5 \text{ mm day}^{-1}$) and thus less ability to compete with other phyllosphere *Fusarium* species (e.g., *F. poae*) colonising oats (Imathiu *et al.*, 2013; Kokkonen *et al.*, 2012; Mateo *et al.*, 2013). However, some studies have shown that in the presence of fungicides, *F. langsethiae* is more tolerant than other *Fusarium* species (e.g., *F. sporotrichioides*) and thus may have a competitive advantage depending on the fungicides used (Kokkonen *et al.*, 2014; Mateo *et al.*, 2011). Further studies are needed to understand better the interactions between *F. langsethiae* and other mycobiota and the outcome of such competition on the dominance in the oat phyllosphere and how this influences toxin production.

The present study also showed that the optimum a_w x temperature conditions for T-2+HT-2 production on oat-based medium was at 25°C and $0.995\text{-}0.98 a_w$ for all the three strains tested. Interestingly, one of the UK strains (strain 2) produced less T-2+HT-2 when compared to other two tested. Previous studies showed significantly less T-2+HT-2 toxin production by UK strains than others from Scandinavia. However, the strain 1 from the UK was able to produce amounts of toxin ($>50 \mu\text{g g}^{-1}$) similar to the Scandinavian one under optimum a_w x temperature conditions.

The type strain had significantly higher ratios of HT-2/T-2 at 15 and 25°C when compared to the UK strains. Previously, the ratios found ranged from $0.00\text{-}0.98$ depending on a_w x temperature x strain studied (Kokkonen *et al.*, 2012, 2010; Medina and Magan, 2011). The present study showed higher ratios at lowered a_w levels with up to 76 , suggesting an increase in the conversion of T-2 toxin to HT-2 under water stress conditions on oat-based media.

Our data set obtained for the type strain and two validation sets, one from this study (2 strains, 3 replicates) and one from previously published data (Medina and Magan, 2010), were used to develop the probabilistic models for both growth and toxin production in relation to a_w x temperature conditions. The contour maps for growth showed a wide range of temperatures as well as the marginal and optimum conditions. The data sets for T-2+HT-2 toxins were used to develop contour maps relative to the EU recommended levels for unprocessed and direct consumption. This showed that conditions for optimum production changed significantly depending on the prevailing a_w and temperature conditions. Overall, marginal conditions are around 0.93 a_w over most of the temperature range examined for both growth and T-2+HT-2 toxin production. This is in agreement with recent studies showing the requirement for moderate air temperature and high a_w for the development of increased T-2+HT-2 risk in the field prior to harvest (Hjelkrem et al., 2018). However, these studies did not examine the influence that the pre-harvest window of opportunity which exists when the a_w would be conducive to infection between anthesis and harvest.

In the present work, the probability of growth was predicted after 10 days over a wide range of environmental conditions relevant to ripening oat water contents between flowering and maturity at harvest. Validation of the growth probabilistic model in the same 25 conditions were used to create the model based on the use of the type strain and two external data sets of two strains each of the species with three replicates per strain. This showed a higher level of concordance with the model (92%). In total, 3 replicates for each of the 5 *F. langsethiae* strains were used to develop and validate this model. The number of replicates was chosen to take into account the intra-species variability. Although our replication was relatively limited, by validating with sets of external data we believe that this does provide some useful temperature x a_w conditions which represent relatively low or

high risk in the context of the recommended maximum levels for food and feed. Previously, Samapundo et al. (2005) used 20 replicates of one strain to study *F. verticillioides* and *F. proliferatum* and 15 of a strain of *Aspergillus* section *Flavi* for modelling growth (Samapundo et al., 2007, 2005). Garcia-Cela et al. (2014) used 5 replicates per isolate for species of the *Aspergillus* section *Nigri* species. In the present study, despite the limitations in terms of replication, we had a 92% concordance with the model. Further validation in oat grain needs to be carried out to increase the relative confidence in the model for determining relative risks of growth.

A lower level of concordance was achieved in the case of the toxin logistic model (64-82%). Thus, while relative growth rates between strains were consistent, there was more interspecific variability in terms of toxin production by different strains. Nazari *et al.* (2014) assessed the effect of temperature (5-40°C) on the growth of *F. langsethiae* in wheat-based media without any a_w modification (approx. 0.995 a_w). In contrast to the present results, they found some growth at temperatures <10°C. However, their studies were carried out for periods of 21 days. In the present study we used 10 days. For sporulation, a non-linear regression model was used which suggested optimum temperature for this phase was 24-25°C, and coincided with optimum conditions for fungal growth (20-25°C) (Nazari et al., 2016).

Predictive models based on logistic regression have previously been developed for *Aspergillus* species (Astoreca et al., 2012; Battey et al., 2001; García-Cela et al., 2014; Garcia et al., 2011; Marin et al., 2011, 2012; Tassou et al., 2009). However, this is the first time that this approach has been applied to *F. langsethiae*. Validation steps are critical to defining the reliability of the model. However, different approaches have been applied in

previous studies. The first question to address is how many strains should be included to create the model? Which matrix was used to create and validate the model? Or how many conditions or replicates are needed to validate the model? Most of the cited studies constructed their probability models with only one strain. However, Tassou et al. (2009) combined the growth of two *A. carbonarius* strains and created probabilistic growth models for the two strains. There were similarities for growth but significant differences in terms of toxin production. Astoreca et al. (2012) created different probabilistic models for three *A. flavus* strains in defined artificial, and in maize-based media. Interestingly, the probability for growth of the three strains was similar in the same medium, but narrower probabilistic profiles were observed on the maize-based medium. Thus, they only validated this model. Few studies have used external data sets to validate the models. However, Tassou et al. (2009) validated the probability models using data sets from the literature carried out on similar growth media. The percentage agreement achieved was up to 100% for *A. carbonarius* growth. External sets of data on maize were used by Astoreca et al. (2012) to validate a probabilistic model constructed on maize-based media.

The question arises whether the models developed on *in vitro* oat-based media can be extrapolated to use in predicting growth and T-2+HT-2 toxin production in oats. Some previous studies suggest that these are useful as a good guide to colonisation and toxin contamination (Garcia et al., 2011). However, accurate boundary conditions in the food matrices may be slightly different from that predicted in the oat-based media. Further studies are now necessary to examine in more detail the colonisation and toxin contamination of oats by *F. langsethiae* over a range of a_w x temperature conditions for improving the validation and accuracy of the models. An example of studies could include the use of higher replication numbers at or close to the boundary conditions to increase the

accuracy of the models under the necessary a_w x temperature conditions so that it can be utilised by the oats industry in evaluating risks of colonisation and perhaps T2+HT-2 contamination.

In conclusion, this study has shown that there is high adaptability of *F. langsethiae* to a range of a_w levels with marginal limiting conditions at 0.93 a_w for both mycelial growth and mycotoxin production. This study also showed some strain variability in terms of type A trichothecene production with the type strain producing much higher levels of T-2+HT-2 than the other two examined and reported previously (Kokkonen et al., 2010; Medina et al., 2010; Medina and Magan, 2011). Some other studies have also suggested that dry matter losses due to *F. langsethiae* colonisation of oat grains could be modelled against T-2/HT-2 toxin production relative to the EU recommended maximum contamination levels (Mylona and Magan, 2011). The models generated in the present study could be useful for an indication of relative risks of colonisation of oats and T-2+HT-2 toxin contamination. This would help in the development of decision support system for farmers to identify pre-harvest contamination risks, and also harvesting and drying practices to minimise the post-harvest risks of contamination.

5. ACKNOWLEDGEMENTS

This research is supported by a BBSRC-SFI research grant (BB/P001432/1) between the Applied Mycology Group at Cranfield University and the School of Biology and Environmental Science, University College Dublin, Ireland. We are grateful to Prof. S. Edwards (Harper Adams University) and Dr P. Jennings (FERA Science Ltd.) and Dr. Ingerd Skow Hofgaard (NIBIO, Ås, Norway) for provision of strains used in this study.

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Tables Legends:**Table 1:**

List of the *F. langsethiae* strains used to create the growth and T-2+HT-2 models on oat-based media.

Table 2:

Mean lag time (λ) for *F. langsethiae* strain 1 and the type strain in relation with temperature and a_w expressed in (λ) $\pm$ standard deviation (SD) in days.

Table 3:

Statistical significance of the effect of strain and its origin, water activity x temperature conditions on lag times, growth, T-2+HT-2 and HT-2/T-2 ratio by *Fusarium langsethiae* strains.

Table 4:

Estimated parameters from logistic regression models and maximum r^2 and adjusted r^2 for growth and T-2+HT-2 accumulation for the *F. langsethiae* Type strain. Only significant parameters have been included in the table. Estimates value $\pm$ standard error. T: temperature, a_w : water activity, r^2 displayed are in percentages.

Table 5:

Validation of the probability model on *F. langsethiae* Type strain on growth data and T2+HT-2 accumulation from a) two strains studied in the present work (strain 1 and 2) and b) two strain previously tested by Medina et al., 2010, 2011 (57 and 59).

Table S1:

Mean lag times to detection (λ) for *F. langsethiae* strain 2, 57 and 59 in relation with temperature and a_w expressed in $(\lambda) \pm$ standard deviation (SD) in days.

Figure Legends:**Figure 1:**

Contour maps for *F. langsethiae* from UK (strain 1 and 2) and the type strain in relation with temperature and a_w . Numbers on the isopleths represent growth rates (mm day^{-1}).

Figure 2:

Contour maps of T-2+HT-2 average production (in $\mu\text{g g}^{-1}$ agar) by *F. langsethiae* from UK (strain 1, 2) and the type strain in relation to temperature and water activity. The isopleth ($0.2 \mu\text{g g}^{-1}$) represents the EU indicative level for processed oats.

Figure 3:

Effect of temperature and water activity on the predicted probability of growth.

Figure 4:

Predicted models for T-2+HT-2 production after 10 days incubation of the type strain *F. langsethiae* on an oat-based medium relative to the EU recommendations for (a) processed and (b) unprocessed oats.

Table 1:

List of the *F. langsethiae* strains used to create the growth and T-2+HT-2 models on oat-based media.

Strain	Origin	Isolated from	Year of isolation	Tested conditions			References
				a_w	T (°C)	t (days)	
Type strain	Norway	Oats	1998	0.90, 0.95, 0.995	0.93, 0.98, 10, 15, 20, 25, 30	10	Present study
Strains 1 and 2	UK	Oats	2015	0.90, 0.95, 0.995	0.93, 0.98, 10, 15, 20, 25, 30	10	Present study
57 and 59	UK	Oats	2004	0.88, 0.90, 0.93, 0.95, 0.98, 0.995	10, 15, 20, 25, 30	10	Medina et al. (2010, 2011)

Table 2:

Mean lag times to detection (λ) for *F. langsethiae* strain 1 and the type strain in relation with temperature and a_w expressed in (λ) $\pm$ standard deviation (S.D.) in days.

	a_w	Temperature				
		30 °C	25 °C	20 °C	15 °C	10 °C
		$\lambda \pm$ S.D.	$\lambda \pm$ S.D.	$\lambda \pm$ S.D.	$\lambda \pm$ S.D.	$\lambda \pm$ S.D.
Strain 1	0.995	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.8 $\pm$ 0.2	1.2 $\pm$ 0.1
	0.98	0.4 $\pm$ 0.1	0.8 $\pm$ 0.0	0.5 $\pm$ 0.1	1.0 $\pm$ 0.1	0.8 $\pm$ 0.4
	0.95	<0.1	0.8 $\pm$ 0.0	0.4 $\pm$ 0.1	1.5 $\pm$ 0.4	1.3 $\pm$ 0.2
	0.93	<0.1	1.1 $\pm$ 0.3	0.7 $\pm$ 0.4	3.0 $\pm$ 0.9	3.0 $\pm$ 0.1
	0.9	>10	>10	>10	>10	>10
type strain	0.995	<0.1	0.3 $\pm$ 0.0	0.7 $\pm$ 0.0	1.0 $\pm$ 0.1	1.9 $\pm$ 0.3
	0.98	0.2 $\pm$ 0.2	0.6 $\pm$ 0.2	0.6 $\pm$ 0.1	1.1 $\pm$ 0.1	1.8 $\pm$ 0.2
	0.95	0.3 $\pm$ 0.1	0.7 $\pm$ 0.1	0.8 $\pm$ 0.1	1.1 $\pm$ 0.5	1.5 $\pm$ 0.1
	0.93	0.4 $\pm$ 0.3	1.3 $\pm$ 0.3	0.5 $\pm$ 0.3	3.1 $\pm$ 0.3	4.8 $\pm$ 0.0
	0.9	>10	>10	>10	>10	>10

<0.1 No Lag time calculated; S.D.: standard deviation; >10: No growth observed after 10 days.

Table 3:

Statistical significance of the effect of strain and its origin, water activity x temperature conditions on lag times, growth, T-2+HT-2 and HT-2/T-2 ratio by *Fusarium langsethiae* strains.

Factors	DF	p-value				
		Lag time	Growth rate	HT-2	T-2	HT-2+T-2
Origin	1	0.74	0.0098*	0.030*	0.13	0.050*
Strain	2	0.0007*	0.80	0.09	0.96	0.21
a _w	4	<0.0001*	<0.0001*	0.0041*	0.0001*	<0.0001*
Temperature	4	<0.0001*	<0.0001*	0.10	0.017*	0.06
Origin x a _w	4	0.51	<0.0001*	<0.0001*	0.16	0.26
Temperature x a _w	16	<0.0001*	<0.0001*	<0.0001*	0.0001*	<0.0001*
Temperature x Origin	4	0.93	0.96	<0.0001*	0.79	0.84
Origin x Temperature x a _w	16	0.80	<0.0001*	<0.0001*	0.94	0.90

*: values are statistically significant.

Table 4:

Estimated parameters from logistic regression models and maximum r^2 and adjusted r^2 for growth and T-2+HT-2 accumulation for the *F. langsethiae* Type strain. Only significant parameters have been included in the table. Estimates value $\pm$ standard error. T: temperature, a_w : water activity, r^2 displayed are in percentage.

	Growth	T-2+HT-2 > 200 ng g ⁻¹	T-2+HT-2 > 1000 ng g ⁻¹
Intercept	-2100 $\pm$ 740	-1200 $\pm$ 470	-830 $\pm$ 410
T	25 $\pm$ 19		
T ²	-0.62 $\pm$ 0.47	-0.030 $\pm$ 0.01	-0.030 $\pm$ 0.01
T*a _w		1.0 $\pm$ 0.5	1.3 $\pm$ 0.5
a _w	2100 $\pm$ 700	2400 $\pm$ 1000	1700 $\pm$ 860
a _w ²		-1200 $\pm$ 520	-860 $\pm$ 460
r ²	100	61	54
r ² adjusted	88	50	43

Table 5: Validation of the probability model on *F. langsethiae* Type strain on growth data and T2+HT-2 accumulation from a) two strains studied in the present work (strain 1 and 2) and b) two strain previously tested by Medina and Magan, 2010, 2011 (57 and 59).

T	a _w	Growth			Toxin >200 ng·g ⁻¹			Toxin >1000 ng·g ⁻¹		
		Pred icted F. 2010 59	Observe d Fe2391+ Fe2392	Obse rved 57+5 9	Pred icted F. 2010 59	Observe d Fe2391+ Fe2392	Obse rved 57+5 9	Pred icted F. 2010 59	Observe d Fe2391+ Fe2392	Obse rved 57+5 9
1 0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1 0 3	0.9 3	1.0	1.0	0.0	0.7	0.3	0.0	0.4	0.3	0.0
1 0 5	0.9 5	1.0	1.0	1.0	0.9	0.7	1.0	0.8	0.7	0.0
1 0 8	0.9 8	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	0.0
1 0 95	0.9 95	1.0	1.0	1.0	1.0	1.0	0.5	0.9	1.0	0.0
1 5	0.9	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0
1 5 3	0.9 3	1.0	1.0	0.0	0.9	0.5	0.0	0.8	0.5	0.0
1 5 5	0.9 5	1.0	1.0	1.0	1.0	0.0	1.0	1.0	0.0	0.0
1 5 8	0.9 8	1.0	1.0	1.0	1.0	0.8	1.0	1.0	0.8	0.0
1 5 95	0.9 95	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
2 0	0.9	1.0	0.5	0.0	0.1	0.0	0.0	0.1	0.0	0.0
2 0 3	0.9 3	1.0	1.0	1.0	0.9	0.0	0.0	0.9	0.0	0.0
2 0 5	0.9 5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
2 0 8	0.9 8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
2 0 95	0.9 95	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
2 5	0.9	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2 5 3	0.9 3	1.0	1.0	1.0	0.7	0.0	0.0	0.7	0.0	0.0

2	0.9									
5	5	1.0	1.0	1.0	0.9	<i>0.0</i>	1.0	0.9	<i>0.0</i>	<i>0.0</i>
2	0.9									
5	8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	0.0
2	0.9									
5	95	1.0	1.0	1.0	1.0	1.0	<i>0.5</i>	1.0	1.0	0.0
3										
0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>0.0</i>
3	0.9									
0	3	1.0	1.0	1.0	0.1	0.0	1.0	0.1	0.0	<i>0.0</i>
3	0.9									
0	5	1.0	1.0	1.0	0.5	0.2	<i>1.0</i>	0.4	0.2	0.0
3	0.9									
0	8	1.0	1.0	1.0	0.8	1.0	1.0	0.8	0.8	0.0
3	0.9									
0	95	1.0	1.0	1.0	0.8	1.0	1.0	0.8	1.0	0.0

Italics cells no concordance between observed and predicted values (bold).

Conflict of Interest statement

No conflicts of interest to declare

Highlights

- *F. langsethiae* strains grew optimally at 0.995-0.98 a_w and 20/25°C.
- Marginal conditions for growth and T-2/HT-2 toxin production was 0.93 a_w /15-25°C.
- Probabilistic model for fungal growth for *F. langsethiae* developed for the first time.
- Model for marginal and maximum T-2/HT-2 toxin production by this species developed.
- Potential for use of these models for evaluating relative risk of toxin contamination in oats.



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Water and temperature relations of *Fusarium langsethiae* strains and modelling of growth and T-2 and HT-2 mycotoxin production on oat-based matrices

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2021-04-23

Elsevier

<https://doi.org/10.1016/j.ijfoodmicro.2021.109203>

Verheecke-Vaessen C, Garcia-Cela E, Lopez-Prieto A, et al., (2021) Water and temperature relations of *Fusarium langsethiae* strains and modelling of growth and T-2 and HT-2 mycotoxin production on oat-based matrices. *International Journal of Food Microbiology*, Volume 348, June 2021, Article number 109203