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Title: Water and temperature relations of F. langsethiae strains and modelling of growth and T-2 and HT-2 mycotoxin production on oat-based matrices

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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 (aw, 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 probabilistic models for impacts of temperature x aw on growth and toxin production.
All three strains had optimum aws and temperatures of 0.995-0.98 and 25°C for growth. For T-2+HT-2 production these were 0.995 and 20°C. Overall, the type strain produced higher amounts of T-2+HT-2 with a HT-2/T-2 ratios of up to 76. Using both these data sets and those from the literature probabilistic models were developed for growth and T-2+HT-2 toxin production in relation to temperature x aw conditions. These models will be beneficial in determining the conditions, both pre- and post-harvest which would represent a high risk for contamination with these two toxins with regard to the EU indicative levels.

Cover Letter

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Dr. Marcel H Zwietering,
Editor
International Journal of Food Microbiology

Dear Prof. Zwietering,

Re: Revision of FOOD-D-20-00164

We have revised the above manuscript entitled "Water and temperature relations of *F. langsethiae* strains and modelling of growth and T-2 and HT-2 mycotoxin production on oat-based matrices".

We have addressed the reviewers' comments below and the changes made have all been highlighted in blue text. We hope these are satisfactory. If any additional minor changes are required we would be happy to make these.

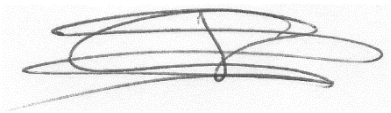
All authors have approved the modified manuscript and agree with its submission to International Journal of Food Microbiology.

Please address any correspondence to c.verheecke@cranfield.ac.uk.

We look forward to hearing from you in due course.

With best regards

Dr. Carol Verheecke-Vaessen



Below we have answered the referees comments

Reviewer 1:

Question: Part 2.2 ln 17 ...within 0.003.. what does this mean ?
Answer: It is the measurement of the variation of treatment a_w levels. A ± sign has been added for clarity.

Question: 3.1 ln 10 Where are these values shown in table 2?
Answer: Table 2 has been replaced with Table 1.

Question: Ln 14 delete "was"
Answer: The second "was" has been removed.

Question: Ln 17-18: please mention table 2 and "For temperature, growth rate were significantly different at between 10-20ºC; 10-30ºC 18 and 15-30ºC ($p<0.0001$)." where is this shown in Table 2?
Answer: This is the result of the statistical analysis that was done, the results are not displayed in the tables.

Question: Table 1 presents the Mean lag time (<lambda>) for F. langsethiae strain 1. What about strain 2 is it similar? It should be mentioned
Answer: a phrase " thus only the data of one of the UK strains (strain 1) and the type strain are represented in Table 2" has been added after the validation, and that no inter-strain differences were found.

Question: in Fig 1 for strain 2 and type strain the line of 15oC should be light grey
Answer: Well spotted. This has been modified as required.

Question: Part 4. Ln 7 typo "the" ln 14 brackets not needed (3-4 days) Pg 15 ln 17 toxin (>50 ug g-1) is it <mu>g?
Answer: these typos has all been amended.

Reviewer 2:

Q: The highlights: "First probabilistic model developed for Fusarium spp."
"First probabilistic model on mycotoxins production ..."
These highlights are very general. Please be more precise. The present model was developed for *F. langsethiae* and it cannot be generalized for all *Fusarium spp*. The model was developed for T-2 and HT-2 not for all mycotoxins. Besides that, there are already predictive models for growth and toxin production for several *Fusarium* species in different types of grains, at field and laboratory levels.
A: This is the first model using a probabilistic approach in *Fusarium*. It can be used as a first report for *Fusarium* spp producing trichothecenes. *F. langsethiae*.

Q: Introduction Page 3, lines 9-11: What is the occurrence of T-2 and HT-2 in oat samples? At which stage are these toxins formed in oats? More data on these topics are needed.
A: Data were added as requested: "A survey showed that 93% of European oats are contaminated by T-2+HT-2 (> 5 µg.kg-1) (Petterson et al., 2011). T-2 and HT-2 production usually occurs pre-harvest, especially during anthesis to harvest. However, there are indications that as oats are the last cereals to be harvested in the late summer/autumn that

poor drying may lead to increased contamination subsequently in storage (Hjelkrem et al., 2018; Medina and Magan, 2011).”

Q: Page 3, lines 17-21 "Thus more detailed information is required on the relationship between key abiotic factors such as water activity (aw) and temperature may have 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."
Fusarium toxin is usually produced in cereals at pre-harvest and not at post-harvest. Are there any data suggesting the production of T-2 and HT-2 at post-harvest?
A: Although pre-harvest remains one of the essential sources of T-2 and HT-2 in oats, many studies have already highlighted the risk of T-2 and HT-2 production post-harvest if the oats are not dried or managed properly during stored. The relative equivalent moisture contents of the a_w levels used can help in ensuring that the risk is reduced at different temperature regimes.

2. Material and Methods
Q: Page 5, item 2.2 Oat-based media: The culture medium (2% of oat flour and 2% of agar in 100 ml of water/glycerol) is fine for measuring the growth rate, but for mycotoxin production it is an artificial condition and different from oat cereal. It is understood that to have different water activity levels it is more precise to use culture media. However, in parallel the isolates could be inoculated on oats at different water activities and temperatures. This would give a more real conditions of toxin production in oats. Why were these tests not carried out together?
A: The extensive work developed in the present study will provide a good base-line for potential impacts on application to oats. Previous studies suggest that the main difference may be related to the relative amounts of T-2 and its conversion to HT-2 toxin being the predominant difference (Medina and Magan, 2011).

Q: Page 5 line 20: Please specify which temperatures were tested.
A: This has been added.

Q: Page 5 line 23: What were the criteria to incubate for periods of 10 days?
A: Previous data using both molecular analyses of T-2/HT-2 toxin biosynthetic genes and production of the two toxins suggested that day 10 was preferable for obtaining the necessary data on both these two toxins.

Q: Page 6, item 2.4 - Quantification of T-2+HT-2 mycotoxin production : What was the limit of detection and quantification of T-2 and HT-2 methods?
A: The LOD and LOQ values have been added.

Results
Q: Page 11-12, item 3.5- Development of probabilistic models for growth and production of T-2+HT-2 for the *F. langsethiae* type strain:
…Plots of the probability 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 aw for the type strain are shown in Figures 4 and 5.
These data have limited use since all the experiments were performed in vitro using an oat-based culture medium. These data cannot be related to the EU recommendations for

unprocessed oats and processed oats. It is expected that oat-based culture medium at optimum temperature and aw have much better conditions for growth and toxin production than oats.
A: While this is true, we believe that it still provides useful information which can help in identifying the optimum and marginal conditions for growth and toxin production, We have related to these limits more or context and can highlight that our experiments should help focus on what conditions should represent the highest risks in oats pre-harvest during ripening (especially the milky ripe, early dough stages) or post-harvest.

Discussion
Q: Page 16 lines 19-22: "The models generated in the present study could be effectively used in developing mitigation strategies to minimize F. langsethiae growth and T-2+HT-2 production, both pre- and post-harvest stages in the oats production chain"
Please give some examples of mitigation strategies to minimize F. langsethiae growth and T-2+HT-2 production, both pre- and post-harvest stages in the oats production chain.
A: We have tried to put this in context and have discussed some aspects of mitigation strategies including fungicides (see p15 - Line 3-7).

Q: Remember that Fusarium species are hydrophilic which produce their toxins in plants while they grow in the fields not during the storage period. It is possible to control the water activity in oats after being harvested but how do you control it in the field? How can the temperatures and aw of oat plants be controlled?
A: There is a window of opportunity for infection of ripening oats which is determined by the stage of maturity of oat grains. This window (>0.93-0.95 aw and 15-20oC) is very short, about 10-14 days maximum. After this, the oats are too dry for infection unless established already at the hard dough stage. Thus our information does provide useful information on the range of conditions which may allow infection to occur pre-harvest. Thus these models will be very useful for this application and for post-harvest management of risk due to inefficient drying or poor storage.

Conclusions:
Q: The work was well designed and well controlled on the growth and T-2 and HT-2 production by F. langsethiae under laboratory conditions. All the experiments were performed in oat-based culture media with 2% of oat, which does not represent real oats. Fusarium growth and toxin formation occur in oats in the field. It is not possible to mitigate T-2 and HT-2 contamination in oats controlling temperature and water activity in the field.
The results of this work can help in the construction of models for F. langsethiae growth and T-2+HT-2 production, but they should be considered as preliminary with limited application.
A: We certainly understand that there are limitations to these models and further validation will be required, especially in oat grains. We have added in the text on p17, L.20 the words “after validation in vivo”. We hope that this will be satisfactory.

Reviewer 3:

Question / Answer.

Q: The article would be improved by including a critical discussion on the number of replicates required for probability modelling and the model validation carried out.

A: A paragraph has been added which discusses validation of models and the use of replicates. We hope that this will suffice. in other papers 1 to 10 replicates were used for those analysis.

Q: Please describe why the 'type' strain is considered as a reference.

A: The "type" strain is considered as reference because it was one of the first described by Torp and Nirenberg (2004) when *F. langsethiae* was discovered and it is the first strain with the genome published (P6, Ln 4-6).

Q: Pag 2, line 12 'ratio'/ Pag 3, line 16 'has'/ Pag 3, lines 17-21, please rephrase/
Pag 4, lines 1-3, this information is repeated/ Pag 6, line 11, please refer to g/ Page 7, line 14, 'from'/ Page 9, line 6, 'was'/ Page 9, line 14, delete 'was'/ Page 9, line 17, 'rates'.

A: These changes have all been made.

Q: Pag 6, line 5-6, please describe how lag phases were calculated

A: Additional information has been added to explain this parameter**.**

Q: Page 6, line 13, was the supernatant re-extracted?

A: Only the agar plugs were re-extracted, and this had been added for clarity now.

Q: Page 7, line 16-17, if only 2 replicates were carried out, conversion to binary codes led to just 0, 0.5 and 1 probability values, thus the approach is quite limited.

A: The data used were based on the three replicates for each of the strains from this work and the three replicates form UK strains (2 different ones) used in an earlier paper. In total, 12 values were used in total (Pg 7, Ln 12 to Pg 8 Ln 2).

Q: Page 7, lines 20-21, did the authors use that model or just the same methodology?

A: The same methodology was used. This has been was added in the text.

Q: Page 10, line 1, figure 2 does not add much information to what presented in figure 1.

A: Figure 1 has now been removed.

Q: Page 10, lines 9-10, I cannot confirm this from figure 1

A: This Section has been removed.

Q: Page 10, line 14, instead of figure 3, a table or simpler figure would be more informative. Moreover, whole section 3.3 is based on results of Kruskal-Wallis tests which confirms that figure 3 is not useful for interpretation of results

A: The Kruskal Wallis tests are test used when either no normality and/or no homoscedasticity is validated for the dataset being analysed. However, if the Kruskal-Wallis test shows significant differences, this means that some significant differences exist. It is a valid statistical test used in many scientific papers when dealing with non-parametric dataset.

Q: Page 11, line 3, is this relevant with just one type strain and 2 others?

A: We think this is adequate for ecological studies of the type reported here. We have however, highlighted that that there were some significant inter-strain variations.

Q: Page 14, lines 19-20, the number of strains is too small for conclusions to be relevant
A: We are just highlighting differences between strains. This is not a molecular taxonomic study where a wide range of strains would be necessary.

Q: Page 15, line 23, 10 days
A: The paper discussed (Nazari et al., 2014; 2016) were both carried out for 21 days.

Q: Figures 4 and 5, legends, no validation result can be observed in these figures
A: The legends for these figures are listed in the "Figures legends" list. This is as per author guidelines.

Q: Table 3 should be table 1 in M&M
A: Table 3 is referred to later as it includes the compilation of the results presented in Sections 3.1-3.4 with the other strain data that were used to create the model.

Q: Table S1 should be moved to the manuscript and fully described in the results section
A: Table S1 has now been included as Table 5 and is referred to in the Results section.

Q: Figure 1, please revise markers, they are wrong
A: Figure 1 has been removed.

Editor comments:

Q: KEYWORDS: temperature; mycotoxins; Keywords that are already in the title are not effective (see instructions)

A: These Keywords were replaced by environmental conditions; toxic secondary metabolites.

Q: 3:3: health benefits ? This type of strong claims cannot be made and should not be made. This is a scientific microbiology journal, not a marketing channel. Marshall is not a decent source for this information and Thies does not give strong enough evidence for a health benefit. There is some association but this is no proof of a health benefit. So this type of statement should be deleted. I am very allergic to this type of statement since literature information is slightly changed and nuance in an initial publication is lost in a bold statement.

A: The statement has been removed.

Q: 6:5 it is not really a lag phase but a time to detection

A: the calculation of the lag phase has been added to explain.

Q: 6:7 until 7:7 almost literal copy of Verheecke-Vaessen 2019: refer and shorten.

A: This has been modified appropriately.

Q: 18:21 Plant

A: This has been changed.

Q: 19:14: link does not work to document

A: This reference has now been removed.

Q: 23:10 watch out for non-breaking hyphen

A: This has been modified**.**

Q: Table 2 would it not be suitable to have two significant digits for lag in days (meaning that 0.6 gets a digit more but 1.1 does NOT)

A: The data were mathematically calculated and the values obtained are significant in accordance with previous work.

Q: Table 1 again look at reasonable number of digits 0.0007 is OK but 0.7404 or 0.9019 is largely overdone

A: The numbers have been rounded up.

Q: Table 4 also here get rid of a standard windows setting of number of digits after a decimal point to a realistic informative number 2142.90 is largely overdone 0.03 might have more significance. 0.03*T^2 or 0.034*T^2 has a big impact !

A: The Reviewer is correct. The complete set of decimals have been included in the Table.

Q: figure 4 0.91 aw/ also figure 5

A: This has been modified.

HIGHLIGHTS

- *F. langsethiae* higher producer of T-2+HT-2 than previously reported.
- High ratio of HT-2/T-2 reported for the first time.
- First probabilistic model developed for *Fusarium* spp.
- First probabilistic model on mycotoxins production versus EU indicative levels.

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

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Sample CRediT author statement:

Carol VERHEECKE-VAESSEN: Resources, Conceptualization, 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 probabilistic models for impacts of temperature x a_w on growth and toxin production.

All three strains had optimum a_ws and temperatures of 0.995-0.98 and 25°C for growth. For T-2+HT-2 production these were 0.995 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 both these data sets and those from the literature, probabilistic models were developed for growth and T-2+HT-2 toxin production in relation to temperature x a_w conditions. These models will be beneficial in determining the conditions, both pre- and post-harvest, 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 fiber, 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* 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 $\mu g.kg^{-1}$) (Petterson et al., 2011). T-2 and HT-2 production usually occur pre-harvest between anthesis and harvest and can also be produced when drying is delayed or poor storage conditions prevail (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. Currently, the indicative maximum levels are 1000 $\mu g\ kg^{-1}$ for T-2+HT-2 in unprocessed oats and 200 $\mu g\ kg^{-1}$ 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 difficult to discern. Thus, more detailed information is required on the impact of the relationship between key abiotic factors such as water activity (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 25ºC, with T-2+HT-2 toxin production being highest when water was freely available at 20-25ºC (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 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 HT-2/T-2 ratio and (d) the development of probabilistic models for both growth and toxin production and validation using published data.

2. 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) prior to autoclaving for 15 min at 121ºC. The molten cooled media were vigorously shaken before pouring into 9 cm Petri plates (20 ml per plate), cooled and stored at 4ºC in sealed bags where necessary 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 25ºC. For all temperature (10, 15, 20, 25, 30ºC) and a_w (0.995-0.90) treatments, three replicates were used 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.

2.3 Measurements of lag phases prior to growth and mycelial growth

The colony extension (diameter) was measured daily. 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 25ºC in the dark, the sample were centrifuged at 13,000 g for 15 min. 750 µl of the supernatant was removed before the agar plugs were re-extracted again. Extracts were then combined and dried for 7 h 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 C_{18} 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 µL. 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 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 3). Firstly, a data set was obtained using both strains studied in the present work (strains 1 and 2). A second data set was obtained for previous results published by Medina and Magan (2010, 2011) where other strains of *F. langsethiae* were used. Data from other UK strains (57, 59) were used to evaluated for variability with UK strain isolated earlier (2004).

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 EU indicative levels set for oats (processed and unprocessed) and 0 in the case of absence of growth or no detectable T-2+HT-2. To estimate the probabilities of growth/toxin production, experimental data was fitted using the same methodology as the logistic regression model described previously (García-Cela et al., 2014).

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

where

P: 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 10 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) increased at marginal temperatures and under a_w stress treatments (Table 1). At a_w levels $\geq$ 0.95 there was no significant differences in the lag times. At 0.93 a_w, which was marginal for growth, the lag time was increased by up to 4 days at 10-15ºC. At 0.90 a_w, no growth occurred over the time frame of the experiment. There was also no inter-strain differences found. Thus only the data of one of the UK strains (strain 1) and the type strain are represented in this table (Table 1).

Statistically, temperature had a significant impact on the lag phases ($p<0.0001$; Table 2) except between 20-25ºC ($p=0.0539$) and 10-15ºC ($p=0.0723$). Generally, a_w also had a significant impact on the lag phase ($p<0.0001$).

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 differnet growth rates at different a_w x temperature conditions. This showed that maximum growth rates occurred at 0.995-0.98 a_w and 25ºC for the UK strains and 20-25ºC for the type strain. At 30ºC, maximum growth occurred at 0.98 a_w with a decrease at 0.995 a_w for the three strains. The growth rate of strain 2 was optimum at 0.98 a_w and 25ºC.

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 10-20ºC; 10-30ºC and 15-30ºC ($p<0.0001$). Both temperature ($p<0.0001$), a_w ($p<0.0001$) independently and

temperature x a_w ($p<0.0001$) factors had significant effects on growth on oat-based media. There were some significant differences between the strains under optimum conditions. There was a significant difference (one-way ANOVA test) with 7.4, 5.4 and 5.4 mm radial growth rate ($mm.day^{-1}$) for strains 1, 2 and the type strain, respectively.

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 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 was no statistically significant differences between the three strains examined. Although the relative HT-2 production was significantly impacted, depending on strain origin x temperature x a_w ($p<0.0001$).

The effect of temperature was significantly different between 15°C and all the other temperatures tested (Table 2). 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. Overall, there was a significant difference between 15°C and all the temperatures tested.

The type strain produced much higher titres of T-2+HT-2 (>50 µg g^{-1}) than the UK strains, especially under optimum conditions. This strain was also able to produce >1 µg g^{-1} at very marginal conditions of 10°C/0.995 a_w.

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

The ratio of HT-2/T-2 was calculated using the approach of Medina *et al.* (2011). Both a_w and temperature had a significantly effect on the HT-2/T-2 ratio. The ratios were significantly different between all the a_w levels tested except for 0.98-0.995 and 0.93-0.95. At low a_w levels the mean ratios were 16.3 and 13.9 for 0.93 and 0.95, respectively. At higher a_w levels, a switch in this ratio occurred with values of 0.2 and 0.1 for 0.995 and 0.98 a_w, respectively. Temperature did not appear to significantly impact on the ratio except at 15ºC, 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 production of T-2+HT-2 for the *F. langsethiae* type strain

For the modelling of growth and mycotoxin production the strains and conditions are shown in Table 3. 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 (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 >1,000 ng g^{-1} and >200 ng g^{-1}, respectively. In terms of temperature, the toxin production profile was more restricted than that for growth. Validation was performed for growth and toxin production using two sets of data, an independent set created in this study as well as data sets from the literature. 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 ng g^{-1} and >1000 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/0.95a_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 help develop probabilistic models on fungal growth and T-2+HT-2 toxin production in the context of conditions which may allow or not allow contamination to exceed the recommended maximum limits set by the EU for these toxins in food and feed. This type of data is useful for helping to determine relative risk levels of exceeding these contamination levels.

The present study showed significant impacts of environmental factors on lag phases (λ) prior to growth for the three strains of *F. langsethiae* studied. The present data showed that the strains have short lag phases prior to growth under optimal conditions of 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 a 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, 20-25ºC. 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 (3-4.5 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 better understand 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 0.995-0.98 a_w, for all the 3 strains tested. Interestingly, the Norwegian strain was able to produce higher amount of T-2+HT-2 compared to those isolated from UK oats. This difference in production pattern was especially highlighted under marginal boundary a_w x temperature conditions (10°C/0.995; 15°C/0.93 a_w). Previous studies showed significantly less T-2+HT-2 toxin production by UK strains than others from Scandinavia. However, the type strain used in the present study was able to produce significantly higher amounts of toxin (>50 µg g^{-1}) than found previously under optimum a_w x temperature conditions.

These differences were reflected in the relative ratio of HT-2 to T-2 toxin. The type strain had significantly higher ratios (HT-2/T-2) at 15 and 25°C when compared to the UK strains. Previously the ratios found ranged from 0.00-0.98 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.

The data sets from the present study and those previously published were used to develop probabilistic models for both growth and toxin production in relation to a_w x temperature conditions. The contour maps for growth showed the 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 measure the changes in a_w in ripening oats.

In the present work, the probability of growth was predicted after 10 days over a wide range of environmental conditions relevant to oats water contents between flowering and maturity of oats at harvest. Validation of the growth probabilistic model in the same 25 conditions were used to create the model with two external data sets four strains of the species with three replicates per strain showed a higher level of concordance with the model (92%). However, a lower level of concordance was achieved in the case of the toxin logistic model (64-82%). These results highlighted that the growth pattern of the different strains was consistent even in the boundary conditions region. However, there may be some interspecific variability in terms of toxin production by different strains. Nazari *et al.* (2014) assessed the effect of temperature (5-40°C) on growth of *F. langsethieae* in wheat-based media without any a_w modification (probably around 0.995 a_w). In contrast to the present results they found growth at temperatures <10°C. However, their studies were

carried out for periods of 21 days. We used 10 days in the present study. For sporulation, a non-linear regression model was used suggesting optimum temperature for this phase was 24-25°C, and coincided with optimum 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 define 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 need to validate the model? Most of the studies aforementioned cited constructed the probability models with only one strain. However, Tassou et al. (2009) combained the growth of two *A. carbonarius* strains and created probablistics 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* 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 media with external literature data obtained in similar 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 condition in the food matrices may be slightly dfferent 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 under a range of aw x temperature conditions for improving the validation and accuracy of the models.

In conclusion, this study has shown that there is a 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. langsethaie* 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, need to be extended and combined with studies on colonisation of oats which would then potentially provide more accurate information for the prediction of risks of infection of oats by this pathogen and of T-2+HT-2 toxin containation. This would help in the development of appropriate mitigation strategies to reduce the contamination of oats with these toxins pre- and post-harvest.

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6.REFERENCES

Aldars-García, L., Ramos, A.J., Sanchis, V., Marín, S., 2016. Modeling postharvest mycotoxins in foods: recent research. Curr. Opin. Food Sci. 72, 166-175.

Battey, A.S., Duffy, S., Schaffner, D.W., 2001. Modelling mould spoilage in cold-filled ready-to-drink beverages by *Aspergillus niger* and *Penicillium spinulosum*. Food Microbiol. 18, 521–259.

Emmons, C.L., Peterson, D.M., 2001. Antioxidant activity and phenolic content of oat as affected by cultivar and location. Crop Sci. 41, 1676–1681.

European Commission 2013/165/EU, 2013. Commission Recommendation (2013/165/EU) on the presence of T-2 and HT-2 toxin in cereals and cereal products. Off. J. Eur. Union 2013 91, 12–15.

García-Cela, E., Crespo-Sempere, A., Ramos, A.J., Sanchis, V., Marin, S., 2014. Ecophysiological characterization of *Aspergillus carbonarius, Aspergillus tubingensis* and *Aspergillus niger* isolated from grapes in Spanish vineyards. Int. J. Food Microbiol. 173, 89–98.

Garcia, D., Ramos, A.J., Sanchis, V., Marín, S., 2011. Modelling the effect of temperature and water activity in the growth boundaries of *Aspergillus ochraceus* and *Aspergillus parasiticus*. Food Microbiol. 28, 406–417.

Hjelkrem, A.-G.R., Aamot, H.U., Brodal, G., Strand, E.C., Torp, T., Edwards, S.G., Dill-Macky, R., Hofgaard, I.S., 2018. HT-2 and T-2 toxins in Norwegian oat grains related to weather conditions at different growth stages. Eur. J. Plant Pathol. 151, 501–514.

Imathiu, S.M., Edwards, S.G., Ray, R. V., Back, M.A., 2013. *Fusarium langsethiae* - a HT-2 and T-2 Toxins Producer that Needs More Attention. J. Phytopathol. 161, 1–10.

Imathiu, S.M., Hare, M.C., Ray, R. V, Back, M., Edwards, S.G., 2010. Evaluation of

pathogenicity and aggressiveness of *Fusarium langsethiae* on oat and wheat seedlings relative to known seedling blight pathogens. Eur. J. Plant Pathol. 126, 203–216.

Kokkonen, M., Magan, N., Medina, A., 2014. Comparative effects of fungicides and environmental factors on growth and T-2 + HT-2 toxin production by *Fusarium sporotrichioides* and *Fusarium langsethiae* strains on an oat-based matrix. World Mycotoxin J. 7, 177–186.

Kokkonen, M., Medina, A., Magan, N., 2012. Comparative study of water and temperature relations of growth and T-2/HT-2 toxin production by strains of *Fusarium sporotrichioides* and *Fusarium langsethiae*. World Mycotoxin J. 5, 365–372.

Kokkonen, M., Ojala, L., Parikka, P., Jestoi, M., 2010. Mycotoxin production of selected *Fusarium* species at different culture conditions. Int. J. Food Microbiol. 143, 17–25.

Lysøe, E., Frandsen, R.J.N., Divon, H.H., Terzi, V., Orrù, L., Lamontanara, A., Kolseth, A.-K., Nielsen, K.F., Thrane, U., 2016. Draft genome sequence and chemical profiling of *Fusarium langsethiae*, an emerging producer of type A trichothecenes. Int. J. Food Microbiol. 221, 29–36.

Marín, S., Hodžić, I., Ramos, A.J., Sanchis, V., 2008. Predicting the growth/no-growth boundary and ochratoxin A production by *Aspergillus carbonarius* in pistachio nuts. Food Microbiol. 25, 683–689.

Marín, S., Ramos, A.J., Sanchis, V., 2012. Modelling *Aspergillus flavus* growth and aflatoxins production in pistachio nuts. Food Microbiol. 32, 378–388.

Mateo, E.M., Valle-Algarra, F.M., Jiménez, M., Magan, N., 2013. Impact of three sterol-biosynthesis inhibitors on growth of *Fusarium langsethiae* and on T-2 and HT-2 toxin production in oat grain under different ecological conditions. Food Control 34, 521–529.

Mateo, E.M., Valle-Algarra, F.M., Mateo, R., Jiménez, M., Magan, N., 2011. Effect of fenpropimorph, prochloraz and tebuconazole on growth and production of T-2 and HT-2 toxins by *Fusarium langsethiae* in oat-based medium. Int. J. Food Microbiol. 151, 289–298.

Medina, A., Magan, N., 2011. Temperature and water activity effects on production of T-2 and HT-2 by *Fusarium langsethiae* strains from north European countries. Food Microbiol. 28, 392–398.

Medina, A., Magan, N., 2010. Comparisons of water activity and temperature impacts on growth of {Fusarium} langsethiae strains from northern Europe on oat-based media. Int. J. Food Microbiol. 142, 365–369.

Medina, A., Valle-Algarra, F.M., Jiménez, M., Magan, N., 2010. Different sample treatment approaches for the analysis of T-2 and HT-2 toxins from oats-based media. J. Chromatogr. B 878, 2145–2149.

Mylona, K., Magan, N., 2011. *Fusarium langsethiae*: Storage environment influences dry matter losses and T2 and HT-2 toxin contamination of oats. J. Stored Prod. Res. 47, 321–327.

Nazari, L., Manstretta, V., Rossi, V., 2016. A non-linear model for temperature-dependent sporulation and T-2 and HT-2 production of *Fusarium langsethiae* and *Fusarium sporotrichioides*. Fungal Biol. 120, 562–571.

Pettersson, H., Brown, C., Hauk, J., Hoth, S., Meyer, J., Wessels, D. 2011. Survey of T-2 and HT-2 toxins by LC–MS/MS in oats and oat products from European oat mills in 2005–2009. Food Addit. Contam. Part B, 4, 110–115.

Tassou, C.C., Natskoulis, P.I., Magan, N., Panagou, E.Z., 2009. Effect of temperature and water activity on growth and ochratoxin A production boundaries of two *Aspergillus*

carbonarius isolates on a simulated grape juice medium. J. Appl. Microbiol. 107, 257–268.

Thrane, U., Adler, A., Clasen, P.-E., Galvano, F., Langseth, W., Lew, H., Logrieco, A., Nielsen, K.F., Ritieni, A., 2004. Diversity in metabolite production by *Fusarium langsethiae*, *Fusarium poae*, and *Fusarium sporotrichioides*. Int. J. Food Microbiol. 95, 257–266.

Torp, M., Adler, A., 2004. The European Sporotrichiella project: a polyphasic approach to the biology of a new *Fusarium* species. Int. J. Food Microbiol. 95, 241–245.

Torp, M., Nirenberg, H.I., 2004. *Fusarium langsethiae* sp. nov. on cereals in Europe. Int. J. Food Microbiol. 95, 247–256.

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.

Tables Legends:

Table 1:

Mean lag time (λ) for *F. langsethiae* strain 1 and the type strain in relation with temperature and a_w expressed in (λ) ± standard deviation (S.D.) in days.

Table 2:

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 3:

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

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.

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).

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 production (in μg 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 μg g^{-1}) represents the EU indicative level for processed oats).

Figure 3:

Effect of temperature and water activity on the predicted probability of growth based on data from the present study and using published data for validation.

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 unprocessed oats and processed oats using data from the present study and validation with published data.

Table 1:

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) ± standard deviation (S.D.) in days.

		Temperature				
		30 ºC	25 ºC	20 ºC	15 ºC	10 ºC
	a_w	λ ± S.D.	λ ± S.D.	λ ± S.D.	λ ± S.D.	λ ± S.D.
Strain 1	0.995	0.6±0.1	0.5±0.1	0.4±0.1	0.8±0.2	1.2±0.1
	0.98	0.4±0.1	0.8±0.0	0.5±0.1	1.0±0.1	0.8±0.4
	0.95	<0.1	0.8±0.0	0.4±0.1	1.5±0.4	1.3±0.2
	0.93	<0.1	1.1±0.3	0.7±0.4	3.0±0.9	3.0±0.1
	0.9	>10	>10	>10	>10	>10
type strain	0.995	<0.1	0.3±0.0	0.7±0.0	1.0±0.1	1.9±0.3
	0.98	0.2±0.2	0.6±0.2	0.6±0.1	1.1±0.1	1.8±0.2
	0.95	0.3±0.1	0.7±0.1	0.8±0.1	1.1±0.5	1.5±0.1
	0.93	0.4±0.3	1.3±0.3	0.5±0.3	3.1±0.3	4.8±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 2:

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.

		p-value				
Factors	DF	Lag time	Growth rate	HT-2	T-2	HT-2+T-2
Origin	1	0.74	0.0098*	0.0301*	0.13	0.0495*
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	0.0174*	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 3:

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, 0.93, 0.95, 0.98, 0.995	10, 15, 20, 25, 30	10	Present study
Strain 1 and 2	UK	Oats	2015	0.90, 0.93, 0.95, 0.98, 0.995	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 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*.

	Growth			T-2+HT-2 > 200 ng g^{-1}			T-2+HT-2 >1000 ng g^{-1}		
Intercept	-2142.90	±	739.91	-1157.09	±	474.37	-826.52	±	411.40
T	24.68	±	18.87						
T^2	-0.62	±	0.47	-0.03	±	0.01	-0.03	±	0.01
$T*a_w$				0.98	±	0.53	1.26	±	0.48
a_w	2115.09	±	696.91	2376.53	±	995.89	1682.03	±	864.86
a_w^2				-1223.82	±	523.37	-863.11	±	455.24
r^2	99.96			60.56			53.79		
r^2 adjusted	87.86			49.75			43.31		

langsethiae Type strain.

Only significant parameters have been included in the table. Estimates value ±standard error.
T: temperature, a_w: water activity.

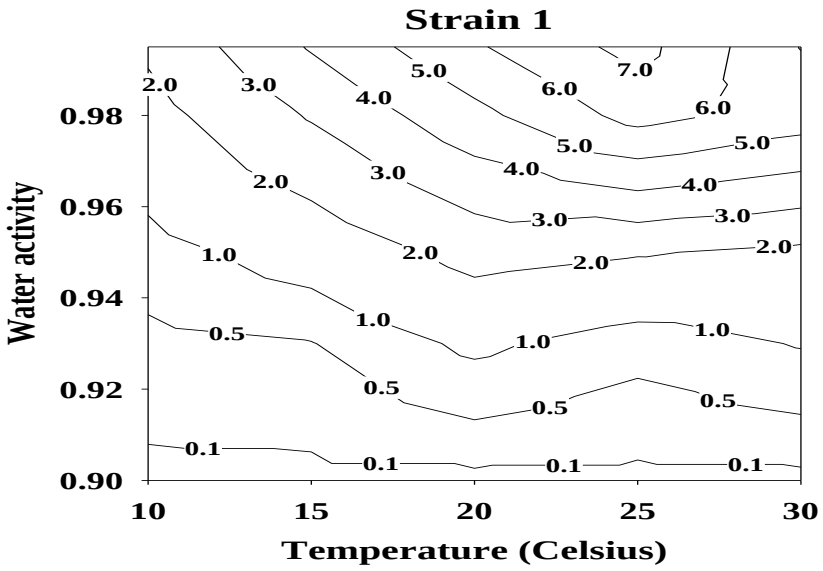
Table 5: Validation of the probability model on *F. langsethiae* Type strain on growth data and T2+HT-2 accumulation from a) two strains studied in the present work (strain 1 and 2) and b) two strain previously tested by Medina and Magan, 2010, 2011 (57 and 59).

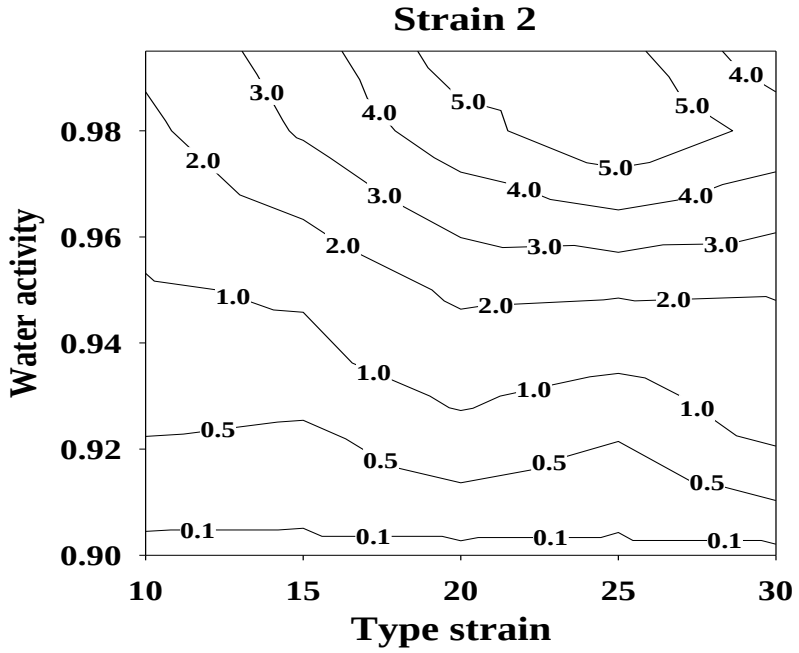
		Growth			**Toxin >200 ng·g^{-1}**			**Toxin >1000 ng·g^{-1}**		
T	**a_w**	**Predicted F. 201059**	**Observed Fe2391+ Fe2392**	**Observed 57+59**	**Predicted F. 201059**	**Observed Fe2391+ Fe2392**	**Observed 57+59**	**Predicted F. 201059**	**Observed Fe2391+ Fe2392**	**Observed 57+59**
10	**0.9**	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0
10	**0.93**	**1.0**	1.0	***0.0***	**0.7**	0.3	0.0	**0.4**	0.3	0.0
10	**0.95**	**1.0**	1.0	1.0	**0.9**	0.7	1.0	**0.8**	0.7	0.0
10	**0.98**	**1.0**	1.0	1.0	**1.0**	1.0	1.0	**0.9**	1.0	0.0
10	**0.995**	**1.0**	1.0	1.0	**1.0**	1.0	***0.5***	**0.9**	1.0	***0.0***
15	**0.9**	**0.0**	0.0	0.0	**0.1**	0.0	0.0	**0.1**	0.0	***0.0***
15	**0.93**	**1.0**	1.0	0.0	**0.9**	0.5	0.0	**0.8**	0.5	0.0
15	**0.95**	**1.0**	1.0	1.0	**1.0**	0.0	1.0	**1.0**	0.0	0.0
15	**0.98**	**1.0**	1.0	1.0	**1.0**	0.8	1.0	**1.0**	0.8	***0.0***
15	**0.995**	**1.0**	1.0	1.0	**1.0**	1.0	1.0	**1.0**	1.0	0.0
20	**0.9**	**1.0**	***0.5***	***0.0***	**0.1**	0.0	0.0	**0.1**	0.0	***0.0***
20	**0.93**	**1.0**	1.0	1.0	**0.9**	***0.0***	0.0	**0.9**	*0.0*	0.0
20	**0.95**	**1.0**	1.0	1.0	**1.0**	1.0	1.0	**1.0**	1.0	0.0
20	**0.98**	**1.0**	1.0	1.0	**1.0**	1.0	1.0	**1.0**	1.0	0.0
20	**0.995**	**1.0**	1.0	1.0	**1.0**	1.0	1.0	**1.0**	1.0	***0.0***
25	**0.9**	**0.0**	***0.5***	0.0	**0.0**	0.0	0.0	**0.0**	0.0	***0.0***
25	**0.93**	**1.0**	1.0	1.0	**0.7**	***0.0***	***0.0***	**0.7**	***0.0***	***0.0***
25	**0.95**	**1.0**	1.0	1.0	**0.9**	***0.0***	1.0	**0.9**	***0.0***	***0.0***
25	**0.98**	**1.0**	1.0	1.0	**1.0**	1.0	1.0	**1.0**	0.8	0.0
25	**0.995**	**1.0**	1.0	1.0	**1.0**	1.0	***0.5***	**1.0**	1.0	0.0
30	**0.9**	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	***0.0***
30	**0.93**	**1.0**	1.0	1.0	**0.1**	0.0	1.0	**0.1**	0.0	***0.0***
30	**0.95**	**1.0**	1.0	1.0	**0.5**	0.2	***1.0***	**0.4**	0.2	0.0
30	**0.98**	**1.0**	1.0	1.0	**0.8**	1.0	1.0	**0.8**	0.8	0.0
30	**0.995**	**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).

Figure

Figure 1





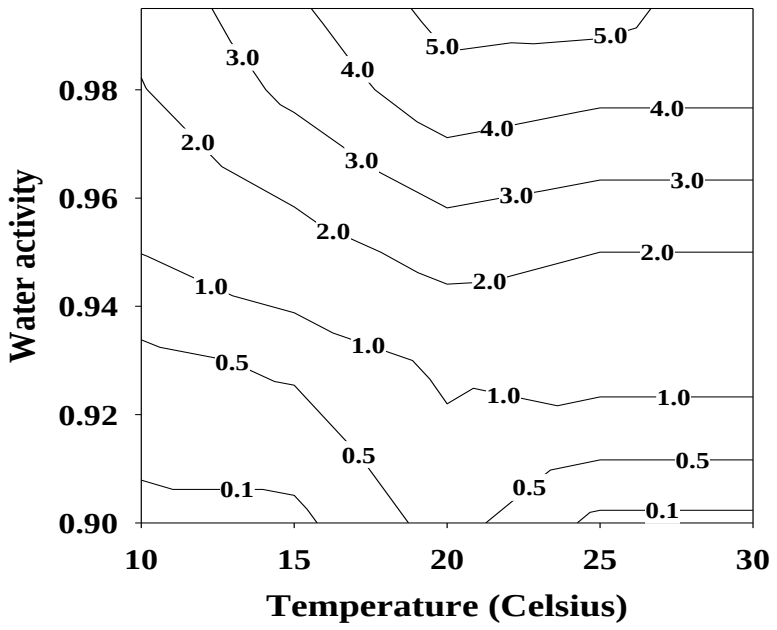


Figure 2

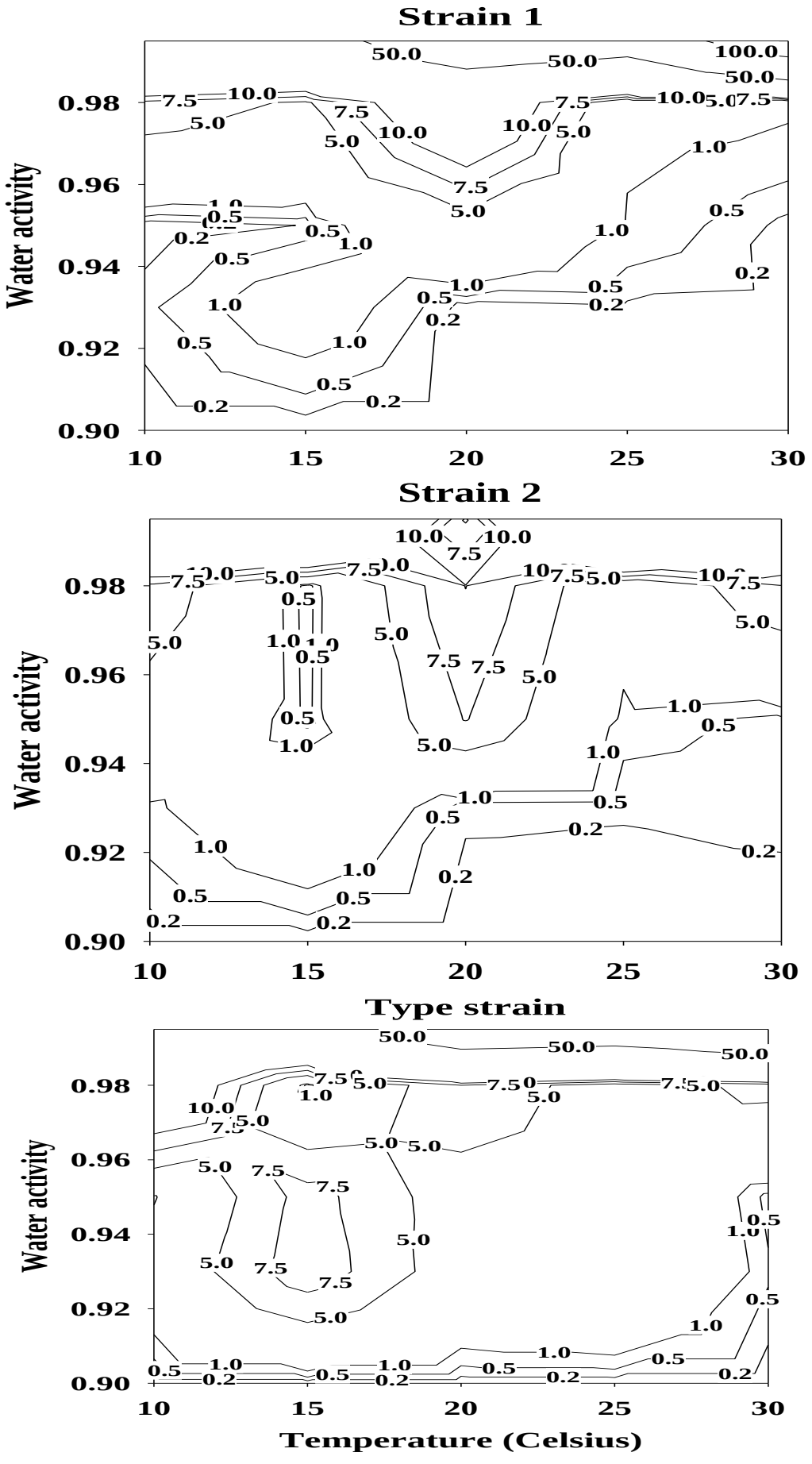

Strain 1
Water activity
0.98
0.96
0.94
0.92
0.90
10
15
20
25
30
Strain 2
Water activity
Type strain
Water activity
Temperature (Celsius)

Figure 3

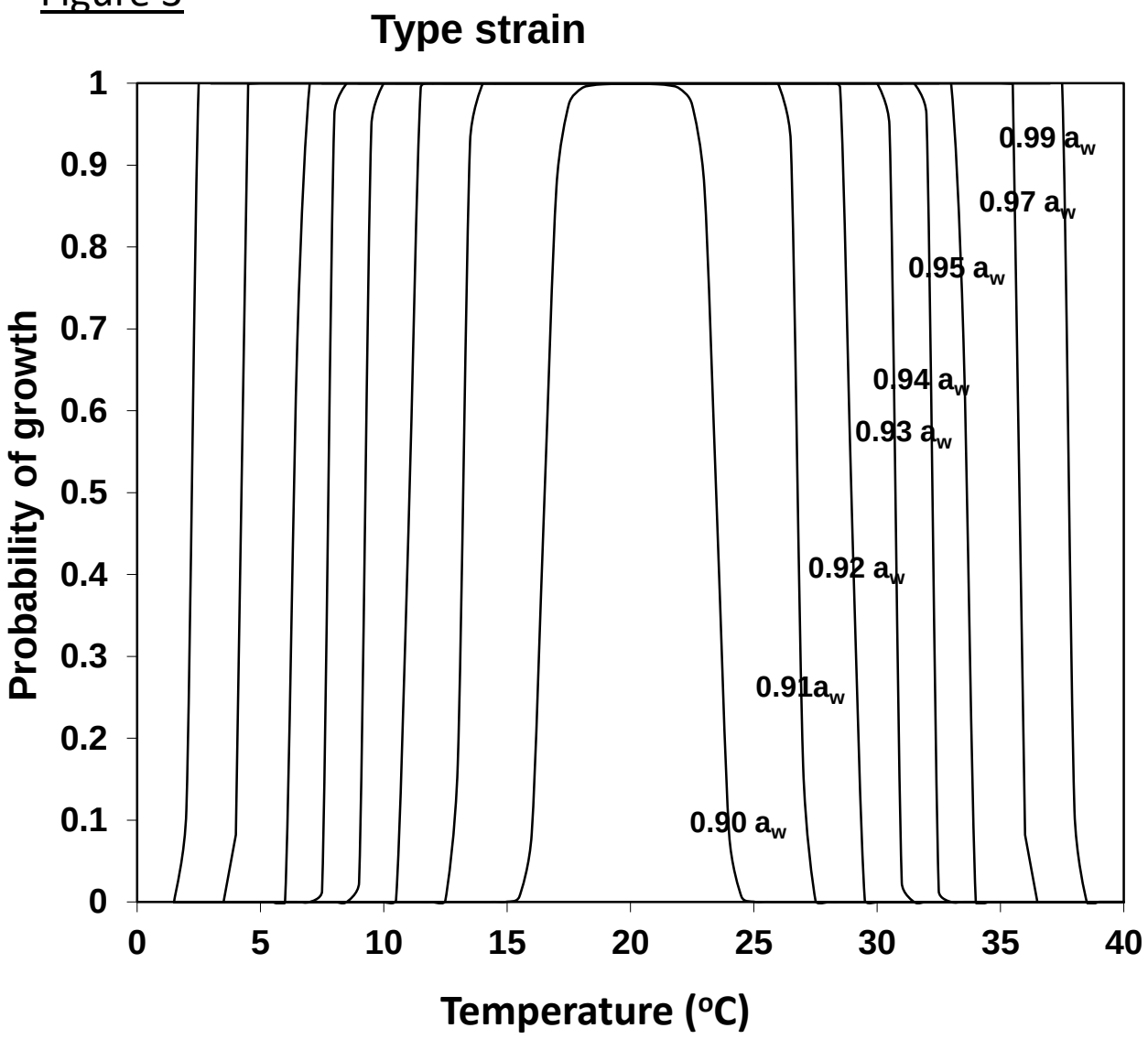
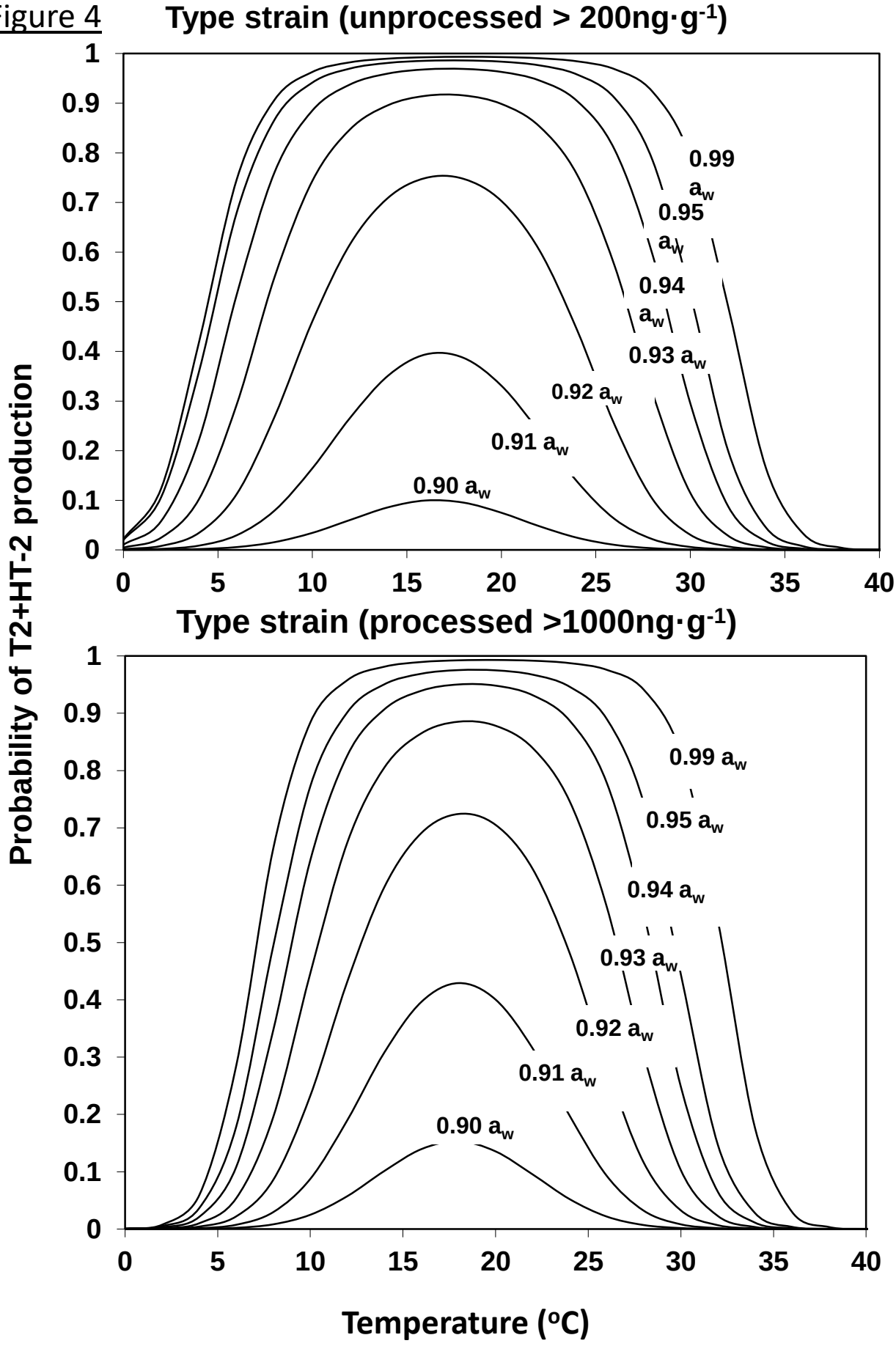

Type strain
Probability of growth
Temperature (°C)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
5
10
15
20
25
30
35
40
0.99 a_w
0.97 a_w
0.95 a_w
0.94 a_w
0.93 a_w
0.92 a_w
0.91a_w
0.90 a_w

Figure 4


Type strain (unprocessed > 200ng·g-1)
Type strain (processed >1000ng·g-1)
Probability of T2+HT-2 production
Temperature (oC)
0.99 aw
0.95 aw
0.94 aw
0.93 aw
0.92 aw
0.91 aw
0.90 aw
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
5
10
15
20
25
30
35
40