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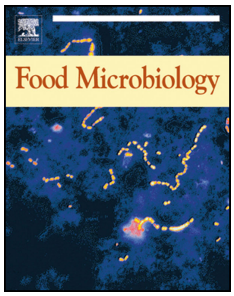

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Temperature and water activity effects on production of T-2 and HT-2 by *Fusarium langsethiae* strains from north European countries

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Abstract

This study has examined the effect of ecophysiological factors, water activity ($a_{w,}$ 0.995-0.90) and temperature (10-37^{o}C), on the T-2 and HT-2 toxins production by *Fusarium langsethiae*. Two dimensional profiles for optimum and marginal conditions have been built for two strains from each of four northern European countries (UK, Norway, Sweden, Finland) on an oat-based medium. This showed that the optimum a_w and temperature conditions for T-2 + HT-2 production was between 0.98-0.995, and 20-30ºC respectively. Kruskal-Wallis analysis of ranks showed a statistically significant differences between the different a_w levels examined (P<0.001) but no significant effect of the temperatures examined. The ratio of HT-2/T-2 was investigated and non-uniform distribution of HT-2 toxin was found under different ecological conditions. No statistically significant differences were found for the mean toxin production between strains from the different countries. Intra-strain differences in toxin production was only found for those from Finland (P-value=0.0247). The growth/no growth and toxin/no toxin conditions in relation to a_w x temperature have been constructed for the first time. This knowledge will be useful in developing prevention strategies to minimise T-2 and HT-2 toxin contamination by strains of *F. langsethiae* on important small grain cereals.

Key words: Type A trichothecenes, Fusarium, mycotoxins, ecological conditions

1. Introduction

Fusarium langsethiae has been isolated from infected oats, wheat and barley in central and northern Europe (Torp & Adler, 2004; Torp & Niremberg; 2004). This species has been implicated in the production of high levels of T-2 and HT-2 mycotoxins in cereals in Norway (Langseth & Rundberget, 1999; Torp and Langseth, 1999) and in oats in the UK (Edwards, 2007). *F. langsethiae* has been isolated from infected symptomless oat and wheat grains which makes detection of contamination often very difficult. Its pathogenicity on these cereals has been recently demonstrated (Imathiu *et al*., 2009).

T-2 and HT-2 toxins are type-A trichothecenes produced by different *Fusarium* species such as *F. acuminatum*, *F. sporotrichioides*, *F. poae*, and the recently described species *F. langsethiae* (Bottalico, 1998; Torp & Adler, 2004; Torp & Niremberg; 2004). T-2 toxin is produced by *Fusarium* species and is rapidly metabolized to HT-2 toxin which is also the main metabolite *in vivo* (Eriksen & Alexander, 1998; Visconti, 2001). Studies on the metabolism of T-2 (Matsumoto *et al.*, 1978) suggested that the liver is the major organ for its metabolism, although other tissues are capable of metabolic modification of this toxin. Hepatic carboxylesterases have been shown to be responsible for the specific deacetylation of T-2, resulting in HT-2 as the major metabolite (Matsumoto *et al.*, 1978; Johnsen *et al*., 1988). T-2 toxin, the most toxic Type A trichothecene, is a potent inhibitor of DNA, RNA, protein synthesis and mitochondrial function, and shows immunosuppressive and cytotoxic effects both *in vivo* and *in vitro* (Visconti *et al.*, 1991; Canady *et al.*, 2001; Visconti, 2001). A recent survey conducted in order to evaluate the risk of dietary exposure to *Fusarium* toxins by the population of EU member states, showed that T-2 and HT-2 toxins are quite common contaminants in cereals in the EU (Schothorst & van Egmond, 2004).

This resulted in special attention being paid to the toxic effects of T-2 in the Joint FAO/WHO Expert Committee on Food Additives (JECFA) where the safety of certain mycotoxins in food was evaluated (WHO/FAO, 2001). JECFA concluded that the toxic effects of

T-2 and its metabolite HT-2 could not be differentiated, and that the *in vivo* toxicity of T-2 might be due partly to toxic effects of HT-2. Therefore, the provisional maximum tolerable daily intake (PMTDI) for these toxins was fixed at 60 ng/kg body weight per day, including intake of T-2 and HT-2, alone or in combination (WHO/FAO, 2001). Recently, the European Food Safety Authority (EFSA) published a report on the toxicity of these trichothecenes and they concluded that the toxicity of T-2 toxin *in vivo* is considered to include that of HT-2 toxin and the results of studies with T-2 toxin are used to approximate the effects of HT-2 toxin (Schuhmacher-Wolz et al., 2010). The European Commission (EC) has established, with Regulations No. 856/2005 and No. 1881/2006, admissible levels of several *Fusarium* toxins in cereals and cereal-based products which became effective from 1 July, 2006. Maximum admissible levels for T-2 and HT-2 toxins in unprocessed cereals and cereal products are currently under discussion (Commission Regulation (EC) No 856/2005; Commission Regulation (EC) No 1881/2006).

It is now accepted that mycotoxin production is predominantly dependent on nutritional and ecological factors. Of the abiotic ecological factors, the water availability (water activity; a_w) and temperature are important factors which impact on both growth and mycotoxin production (Magan & Lacey, 1984; Sanchis & Magan, 2004; Magan & Aldred, 2007; Magan et al., 2010). While information is available on the relationship between these factors and profiles for growth and deoxynivalenol production by *F. culmorum* and *F. graminearum*, there is practically no information for *F. langsethiae* (Hope *et al.*, 2005). As this species has become very important in northern Europe in a range of small grains it is important to understand the ecology of this species and whether any intra- or inter-strain differences may exist. Recently, we reported on the effect of a_w x temperature on growth of 8 strains, two each from the U.K., Norway, Sweden and Finland on an oat-based medium (Medina & Magan, 2010). This showed that there were no statistical differences in terms of a_w and temperature tolerances of the strains in terms of growth. The a_w and temperature optima were 0.98-0.995

and 25ºC and growth limits were established at 0.92-0.93 a_w and 37ºC and 5ºC, respectively. There is no comparable information on how these interacting factors may affect T-2 and HT-2 production and whether intra- or inter-strain differences exist. This is critical in developing models to predict environmental conditions which represent a high risk and those that represent a low risk for contamination with these two mycotoxins.

The objectives of this study were thus to (a) determine the effect of a_w x temperature interactions on T-2, HT-2 and totals for two strains from four different northern European countries (U.K., Norway, Sweden, Finland) on an oat-based medium, (b) to evaluate any intra- or inter-strain differences and (c) develop contour maps of the optimum and marginal conditions for the production of these two mycotoxins and compare these with limits for growth reported recently (Medina & Magan, 2010).

2. Materials and methods

2.1.Strains.

Eight *F. langsethiae* strains from different northern European countries were examined. The isolates were from the UK (2004/57, 2004/59); Norway (44P, 88E); Sweden (560, 562) and Finland (05010, 05014).

2.2.Medium preparation and fungal culture.

Milled oats were prepared by homogenisation for 5 mins in a Waring laboratory science homogeniser model 7009G (Waring Laboratory Science, CT, USA). Mixtures of 2% (w/v) oat flour in water were prepared and 2% (w/v) agar added. Water used to prepare the medium was modified with glycerol to the required water activity levels (a_w; 0.995, 0.98, 0.95, 0.93, 0.90 and 0.88). The culture media were prepared by autoclaving for 20 minutes at 121ºC. The medium was vigorously shaken and poured into 9 cm diameter Petri dishes.

The eight strains of *F. langsethiae* were inoculated using 7 day old cultures by taking agar discs (4 mm diameter) with a cork borer from the growing margin of the colonies and inoculating the treatment plates centrally. The a_w treatments were incubated at different temperatures (35, 30, 25, 20, 15 and 10 ºC) for 10 days. All experiences were carried out with three replicates per treatments, and in some cases repeated twice.

2.3.Reagents and standards.

Trichothecene standards, including T-2 and HT-2 were supplied by Sigma (Sigma-Aldrich, UK). T-2 standard was dissolved in acetonitrile at a concentration of 2.0 mg mL^{-1} and stored at -26 ºC in a sealed vial until use. HT-2 standard solution in acetonitrile was purchased at a concentration of 100.2 µg mL^{-1}. Working standards (50, 20, 10, 5, 2.5, 1, 0.5 and 0.2 µg mL^{-1}) were prepared by appropriate dilution of known volumes of the stock solutions with acetonitrile and used to obtain calibration curves for LC-DAD analysis. Acetonitrile and methanol were purchased from Fisher Scientific (Fisher Scientific UK Ltd., UK). All solvents were HPLC grade. Pure water was obtained from a Milli-*R*/Q water system (Millipore, Billerica, MA, USA) and used when water was required.

2.4.Sample preparation.

Using a cork borer, six-seven discs of agar weighing approx. 0.75 grams were removed from the fungal cultures and placed in previously weighed 2 mL volume safe-lock Eppendorf tubes. A total of 3 replicates per treatment were collected, weighed, and immediately frozen at -20ºC and stored.

2.5.Extraction procedure.

Extraction was made using the methodology described by Medina et al. (2010). Briefly, samples were thawed and extracted by mixing the agar plugs with a one ml mixture of

methanol:water (80:20 v/v). The tubes were shaken at 150 rev min^{-1} at 25ºC in the dark in an orbital shaker for 90 min. They were then centrifuged at 1150 g for 15 min and 750 µL of supernatant transferred to a 2 mL chromatography silanized amber vial. Extracts were completely dried in a stream of nitrogen. Dry extracts were redissolved in 300 µl of acetonitrile:water (50:50 v/v).

2.6.HPLC-DAD analysis.

The HPLC equipment consisted of an Agilent 1100 Series HPLC system equipped with a UV diode-array detector set at 200 nm (Agilent Technologies, Palo Alto, CA, USA). The column was a Phenomenex® Gemini C_{18}, 150 mm × 4.6 mm, 3 µm (Agilent Technologies, Palo Alto, CA, USA) preceded by a Phenomenex® Gemini C_{18} 3 mm, 3 µm guard cartridge. Signals were processed by Agilent ChemStation software Ver. B Rev: 03.01 [317] (Agilent Technologies, Palo Alto, CA, USA).

Analyses were performed in the gradient mode. Solvent A was water and solvent B acetonitrile. Gradient conditions were initiated by holding for the first 3 minutes with 30% B, after this the conditions were changed linearly to 55% B for 18 min. The composition was then changed to 99% B in one minute and maintained for 5 min as a cleaning step in order to improve the results. After cleaning, conditions were returned to the initial 30% B. The flow rate of the mobile phase was 1 mL min^{-1} and injection volume was 50 µL.

2.7.Statistical analysis and profiling.

Statistical analysis was performed using the package JMP® 8 (SAS Institute Inc., 2008. Cary, NC, USA) and package STATISTICA 8 (StatSoft® Inc., 2007. Tulsa, OK, USA).

Data on toxin production were tested for normality using the Shapiro-Wilk test. Due to non-normality of the toxin concentration data, analysis was performed using non-parametric tests for testing whether distributions across factor levels were centered at the same location.

Differences between independent groups, using temperature and water activity as factors, were examined by the Kruskal-Wallis analysis of ranks. Analysis was performed for all data sets, including the 8 strains, and also for each strain individually. In order to compare strains from the same origin, t-tests were used.

Profiling graphs were performed using Sigma Plot v.10.0 (Systat Software Inc. Hounslow, London, UK).

3. Results

3.1. Effects of temperature and water activity on T-2 and HT-2 production.

Effect of a_w in the production of both mycotoxins individually is shown in Figure 1. This shows that reducing a_w from 0.995 to 0.95 produced a decrease in the mean production of both T-2 and HT-2 by a factor of approx. 17 and 20 respectively. No toxin production was found when the a_w treatment was <0.93. The effect of the a_w was studied for the whole data set by Kruskal-Wallis analysis of ranks and this showed a statistically significant differences between the different a_w levels examined ($p<0.001$). Statistical analysis of each strain gave the same results, showing the influence of water activity on all strains examined. In this case the p-values ranged from 0.0014 to 0.0078.

An example of the effect of temperature on T-2 and HT-2 production is shown in Figure 2. There was an up to 50% reduction in production of both the toxins at 10°C when compared with that at 20°C and 0.995 a_w. The Swedish strains appeared to be somewhat different as HT-2 production was higher at 10 than 20°C. The effect of the temperature was studied for the whole data set, including the 8 strains, by Kruskal-Wallis analysis of ranks and no significant differences were found. Strain by strain analysis did not show any significance of temperature as a factor with all the p-values >0.6078. Despite this lack of statistical differences, there was a general trend in the behavior of *F. langesthiae* strains. Thus beyond the maximum production point a decrease of temperature resulted in a decrease in toxin production.

Response surfaces representing mean total mycotoxin (T-2 + HT-2) production for two strains in relation to a_w (from 0.93 to 0.995) x temperature (10-30ºC) are shown for each country in Figure 3. Generally, the means of the two English and Finnish strains showed very similar contour maps for production which represent conditions of similar production levels. Maximum production were reached when the temperature was 25ºC and the a_w was 0.98, and when the temperature was 20ºC and the a_w 0.995 respectively. Also, in both cases, high mycotoxin levels were observed at 30ºC at a slightly reduced a_w of 0.98.

Swedish strains appeared to produce higher concentrations of both T-2 and HT-2. In this case maximum production occurred when a_w and temperatures were 0.995 and 25ºC respectively. Furthermore an increase was observed at 30ºC at 0.98 a_w. The Norwegian strains produced the lowest concentrations of both mycotoxins with maximum production at 20ºC and 0.98 a_w.

The representation of growth/no growth and toxin/no toxin (T-2 + HT-2) boundaries of *F. langsethiae* for different water availabilities and temperatures are shown in Figure 4. This demonstrates that toxin production by strains of *F. langsethiae* occurred over a narrower range of environmental conditions. While growth occurred at 0.90 a_w under the best temperature conditions, toxin production was only possible at 0.93 a_w. At marginal temperatures the difference between growth and toxin production was reduced.

3.2.Examination of HT-2/T-2 ratios.

The ratio of HT-2 against T-2 toxin in each treatment condition was calculated using the formula:

$$R = HT\text{-}2 / T\text{-}2$$

Results obtained showed a non-uniform distribution of HT-2 toxin at all the ecological conditions. Overall, a substantial increase in R was observed in all strains studied under different ecological conditions.

In experiments where the temperatures where higher (20, 25 and 30ºC) the R increased when a_w was decreased to 0.95 or 0.93. When temperatures where lower (10 and 15ºC) the R was higher when a_w levels were 0.98 and 0.995. An example of this behaviour is shown in Figure 5. Thus, under marginal conditions HT-2 toxin increased in *F. langsethiae* strains although growth rates were reduced by between 0.5 and 2.5 mm/day.

3.3. Intra-strain differences based on source of strains.

Homoscedasticity was checked using the Levene's and Brown-Forsythe's tests. Since variances were not equal in some pairs, two different t-tests, one assuming equal and the other unequal variances, were applied where appropriate to find possible differences in T-2 and HT-2 production in strains from the same country of origin.

Significant differences regarding toxin production were found between strains from Finland (*P*-value=0.0247). No differences were observed between the strains from the other three countries.

3.4. Inter-strain differences between countries of origin.

Kruskal-Wallis analysis of ranks using the country of origin as a factor was applied to the data in order to find possible differences among countries. Since the p-value was 0.3487, no significant differences were found regarding the mean toxin production between strains from different countries of origin.

4. Discussion

This is the first study to examine the effect of ecological factors on the production of T-2 and HT-2 and the T-2/HT-2 ratio by *F. langsethiae* strains from a range of countries. This has provided new data which suggests that the variation between strains may be quite small, although there are some exceptions.

Changes in water availability appear to be a major controlling factor in affecting T-2 and HT-2 production. Generally maximum toxin production occurred where mycelia growth was under either a slight a_w or temperature stress. These results agree with data from other studies which have compared growth and toxin production by *Fusarium* species (Magan & Lacey, 1984; Magan et al., 2002; Sanchis & Magan, 2004).

Overall strains from England, Finland and Norway exhibited similar tolerances over a_w conditions of 0.995-0.93 for toxin production. Swedish strains showed a narrower toxin production window with less produced at 0.98 a_w, and practically almost none produced at 0.95 a_w. Evidences of genetic variability has been reported in this species (Yli-Mattila *et al*., 2004), supporting the possible existence of variability in ecophysiological performance at an intraspecific level.

Interestingly, for changes in temperature, no statistically significant differences were found. These results showed that *F. langsethiae* strains can produce T-2 and HT-2 toxins over a wide temperature range of 10 to 30ºC, which were tested in this study. Some production may occur at 5ºC with much longer incubation times but this was not tested.

Our results show that the optimum a_w and temperature conditions for T-2 + HT-2 production was between 0.98-0.995, and 20-30ºC respectively. The optimum conditions for Type A trichothecenes have been previously described in different substrates for related species such as *F. sporotrichioides* and *F. poae.* Other authors have studied the production of type A trichothecenes by these species and concluded that moderate rather than warm temperatures were optima for these toxins. Also the optimum production conditions varied depending on the substrate and toxic metabolite. As an example, *F. sporotrichioides*-infected maize, wheat and rice grains contained more type A trichothecenes when a_w was 0.99 and were incubated at 20ºC (Miller, 1994; Mateo *et al*., 2002). Recently Kokkonen *et al.* (2010) have studied toxin production of *F. langsethiae* on a mixed cereal medium under 3 environmental regimes (0.996, 0.96 aw) and described maximum production of T-2 and HT-2

269 at a_w 0.996 and 15°C. However, they did not include 0.98 aw which we found to be optimum
270 for production.

271 In their reports, JECFA and EFSA concluded that the toxicity of T-2 toxin *in vivo* is
272 considered to include that of HT-2 toxin and the results of studies with T-2 toxin are used to
273 approximate the effects of HT-2 toxin (WHO/FAO, 2001; Schuhmacher-Wolz et al., 2010).
274 Because of this, there has been interest in the total sum of both toxins. However, we have also
275 examined the ratio of these two toxins. This suggested that this ratio may change as water
276 stress conditions are imposed. Thus, we observed that more HT-2 toxin was produced when *F.*
277 *langsethiae* was under intermediate stress conditions, where some reduction in growth occurs,
278 but represent conditions which still allow toxin production. More investigation is needed to
279 evaluate whether HT-2 toxin is directly produced by the strains, or, whether under ecological
280 stress the fungus itself may degrade T-2, the main toxin produced, to HT-2. There are no
281 studies of the metabolic pathways of HT-2 in *F. langsethiae* but for *F. sporotrichioides*, a close
282 related species, a clear pathway has been proposed for the production of T-2 and also HT-2
283 (Meek *et al.*, 2003). On the other hand, degradation of T-2 to HT-2 has been described in *F.*
284 *graminearum*, *F. nivale*, *F. solani*, *F. sporotrichioides* and *Calonectria nivalis* (Vlastimil *et al.*,
285 2008) suggesting the possibility of similar metabolic pathways in *F. langsethiae*.

286 The data obtained in this study on an oat-based nutritional matrix show that *F.*
287 *langsethiae* produces mainly T-2 toxin. This finding is in contrast to other studies that describe
288 a higher amount of natural samples contaminated with HT-2 and also higher concentrations
289 (Langseth & Runberget, 1999; Edwards, 2009). However, these studies never examined the
290 effect of water or temperature stress.

291 In oat-based culture medium HT-2 was observed in small quantities. Our findings
292 suggest that the predominant toxin produced may be T-2 under most environmental
293 conditions. The amount of HT-2 could increase because of the imposed ecological stress
294 increasing the HT-2/T-2 ratio. Recent data supports our findings describing a switch from T-2 to

HT-2 as the major toxin produced depending on the environmental conditions (Kokkonen *et al.*, 2010). Partially, this may be due to T-2 being rapidly transformed to HT-2 by other microorganisms, by the fungus itself or by the cereal. This possibility has been suggested by Lattanzio et al. (2009). These authors studied the natural bio-transformation of T-2 into HT-2. Forty two per cent of the initial T-2 level was transformed naturally by the effect of carboxilesterase (CXE) enzymatic activity after 120 minutes. Different transformation speeds have been observed in different cereals; pointing out that the difference between them could be the amount of CXE or the expression of isoenzymes having different affinities for T-2. This enzymatic activity is also present in animal liver and is responsible for degradation *in vivo* (Matsumoto *et al.*, 1978). This enzymatic effect could explain why in natural cereal samples the toxin that is generally found is HT-2 instead of T-2. These differences regarding enzymatic activities could be on the basis of differences that have been suggested in some studies pointing out higher contamination with *F. langsethiae* and T-2/HT-2 toxin in conventional than organically produced oats (Edwards, 2009).

Differences in toxin contamination of cereals depending on the country of origin have also been found. Scudamore et al. (2009) in a recent four year study of contamination of oats with T-2/HT-2 found highest contamination in samples from the UK and Ireland while levels from Scandinavia were usually lower. No differences were found regarding the toxin production ability of strains of different origin. This supports the findings in the present study that there is very little difference in ecology and toxin production by *F. langsethiae* strains regardless of country of origin. This needs to be further tested by examining strains which have also been found in Germany and France.

Recently Parikka et al. (2007) found differences on the percentage of infected kernels depending on the cultivation practices. In this case Finnish cereals cultivated using direct drilling had higher *F. langsethiae* contamination while tilled plots were less contaminated.

Combining all this it is clear that different mycotoxin levels in grains would depend on different infection rates by *F. langsethiae* in the plots and prevailing weather conditions.

In summary, the present study has detailed, for the first time, information on the influence of interacting ecophysiological factors on T-2 and HT-2 toxin by strains of *F. langsethiae*. Water availability appears to be very important in determining contamination levels with these toxins. In contrast, they are produced over a wide temperature range. Often oats are harvested late in the season when conditions are wet. Thus inefficient drying may allow *F. langsethiae* to continue to colonise and increase contamination post-harvest. The contour maps of the growth/no growth and toxin/no toxin boundaries are useful for determining whether a high risk of contamination might occur post-harvest, and perhaps also in the field during ripening, in small grain cereals generally, especially oats. Thus, this study provides useful base line data on the conditions which represent a high and low risk for contamination by these mycotoxins which are becoming of growing importance in Europe.

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444 **FIGURE CAPTIONS.**

445

446 **Figure 1.** Effect of two water activity conditions (a_w; 0.995 and 0.95) on the production of T-2
447 and HT-2 toxins by *F. langsethiae* strains after 10-days of incubation at 25 ºC on an agar oat-
448 based medium.

449

450 **Figure 2.** Effect of temperature (20 and 10ºC) on the production of T-2 and HT-2 toxins in *F.*
451 *langsethiae* strains after 10-days incubation at 0.995 a_w on an agar oat-based medium.

452

453 **Figure 3.** Two dimensional contour maps of T-2 + HT-2 toxin production profiles of *F.*
454 *langsethiae* from different countries of origin (A: England, B: Finland, C: Norway and D:
455 Sweden) in relation to temperature and water activity. The numbers on the isopleths are for
456 the same mg of toxin/Kg of agar.

457

458 **Figure 4.** Two dimensional contour graph showing *F. langsethiae* mean boundary conditions
459 for growth (growth 0.1 mm radium/day) vs. mean boundary conditions for T-2 + HT-2
460 production (0.5 mg of toxin/Kg). The graph has been obtained using average values obtained
461 from all strains.

462

463 **Figure 5.** Effect of temperature (20 and 15ºC) and a_w (0.995 and 0.98) on the HT-2/T-2 ratio (R)
464 in *F. langsethiae* cultures after 10-days incubation on an agar oat-based medium.

Research highlights

Ms. Ref. No.: FM-D-10-00483
Title: Water availability and temperature affects production of T-2 and HT-2 by *Fusarium langsethiae* strains from north European countries.

Fusarium langsethiae is an important mycotoxigenic species and has become very important in northern Europe in a range of small grains that appeared to be contaminated with high amounts of these toxins. Thus it was very important to understand the ecology and how control of these toxins can be achieved.

- In the present study we have detailed, for the first time, information on the influence of interacting ecophysiological factors on T-2 and HT-2 toxin by strains of *F. langsethiae*.

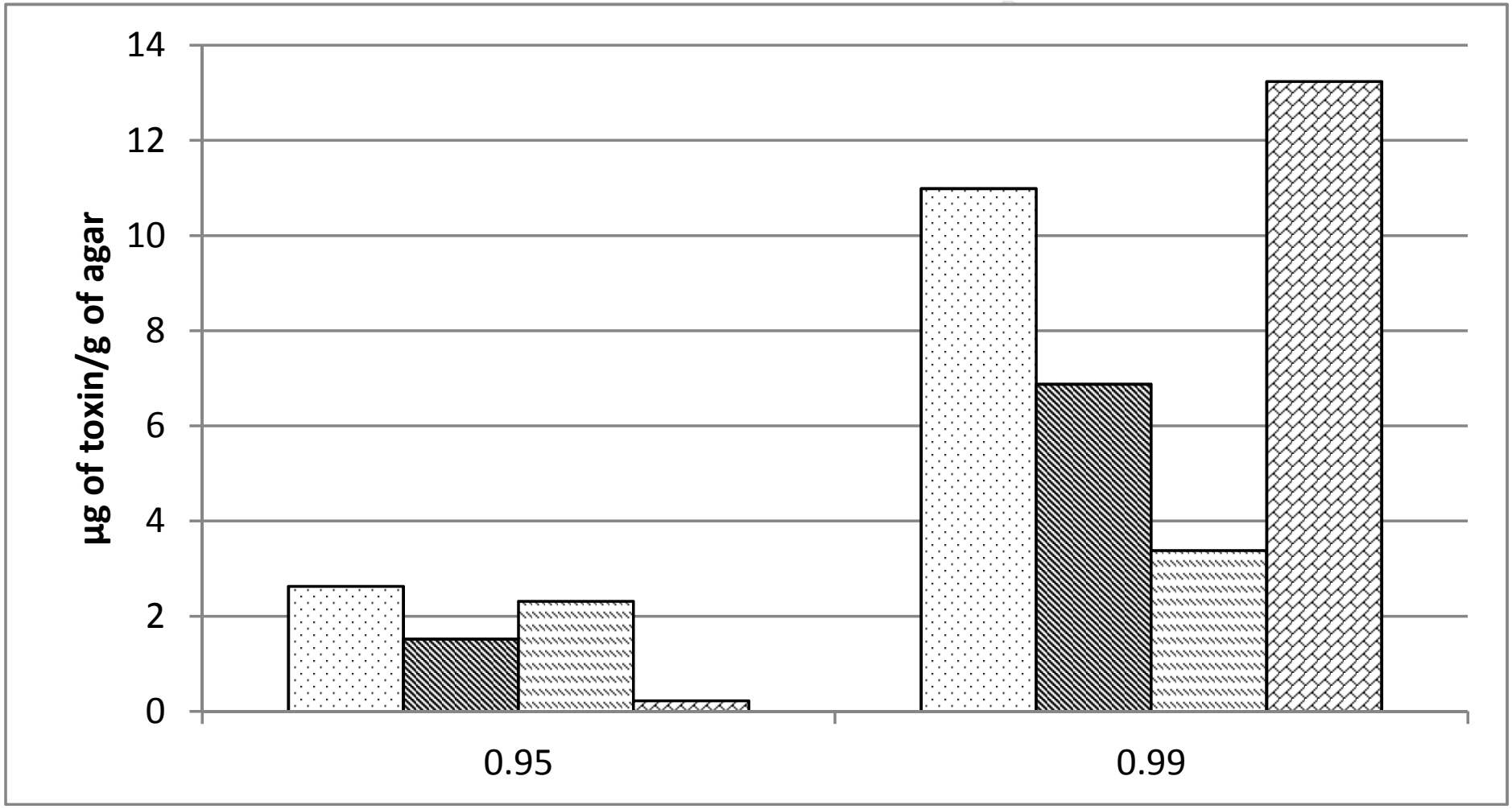
  -Water availability appears to be very important in determining contamination levels with these toxins. In contrast, they are produced over a wide temperature range.

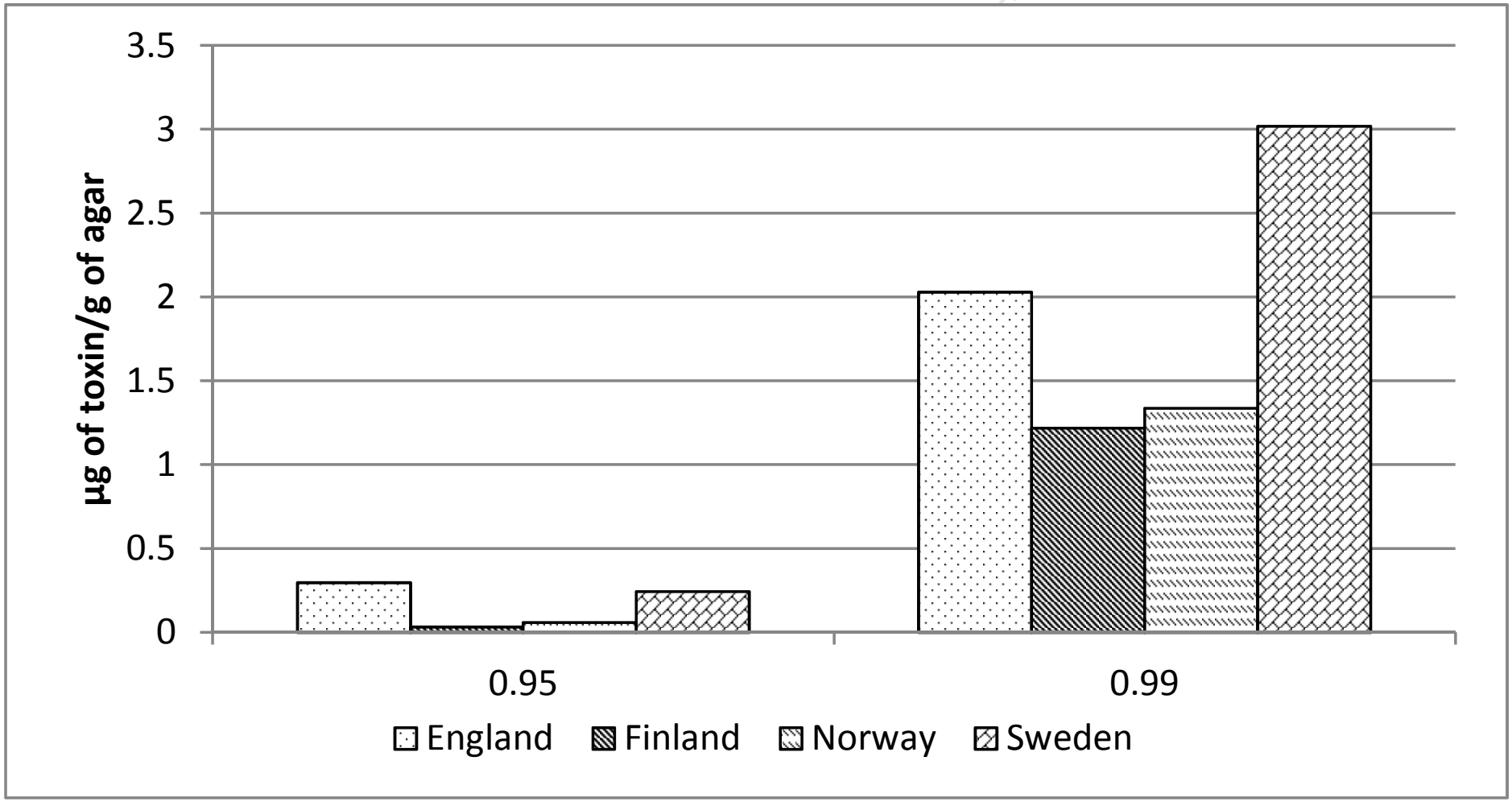
- Two dimensional profiles for optimum and marginal toxin production conditions have been built for two strains from each of four northern European countries (UK, Norway, Sweden and Finland) on an oat-based medium.
- It is the first time that, for this new species, contour map of the growth/no growth and toxin/no toxin boundaries have been built and will be published.

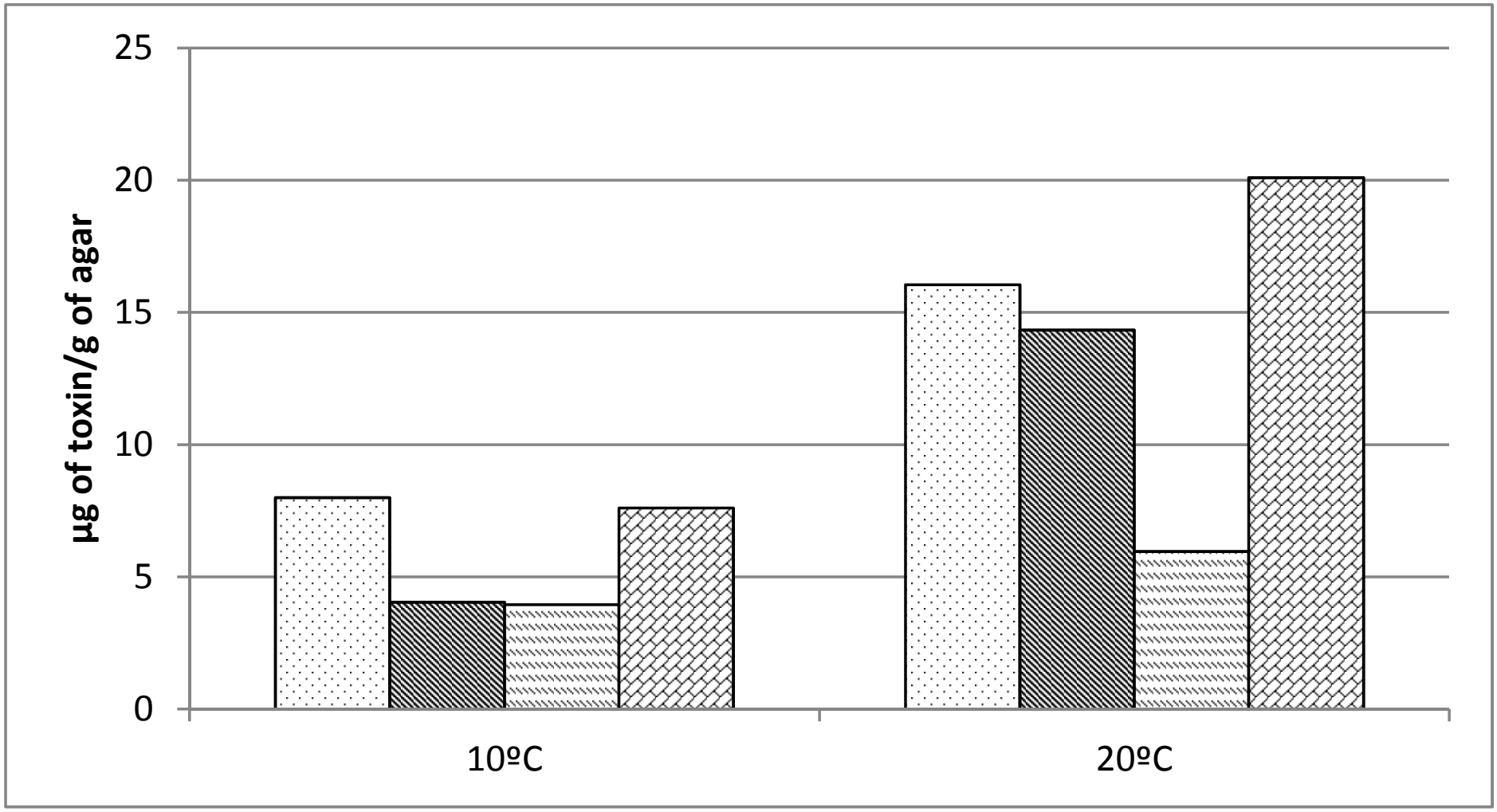
  -These maps will be useful for determining whether a high risk of contamination might occur post-harvest, and perhaps also in the field during ripening, in small grain cereals generally, especially oats.

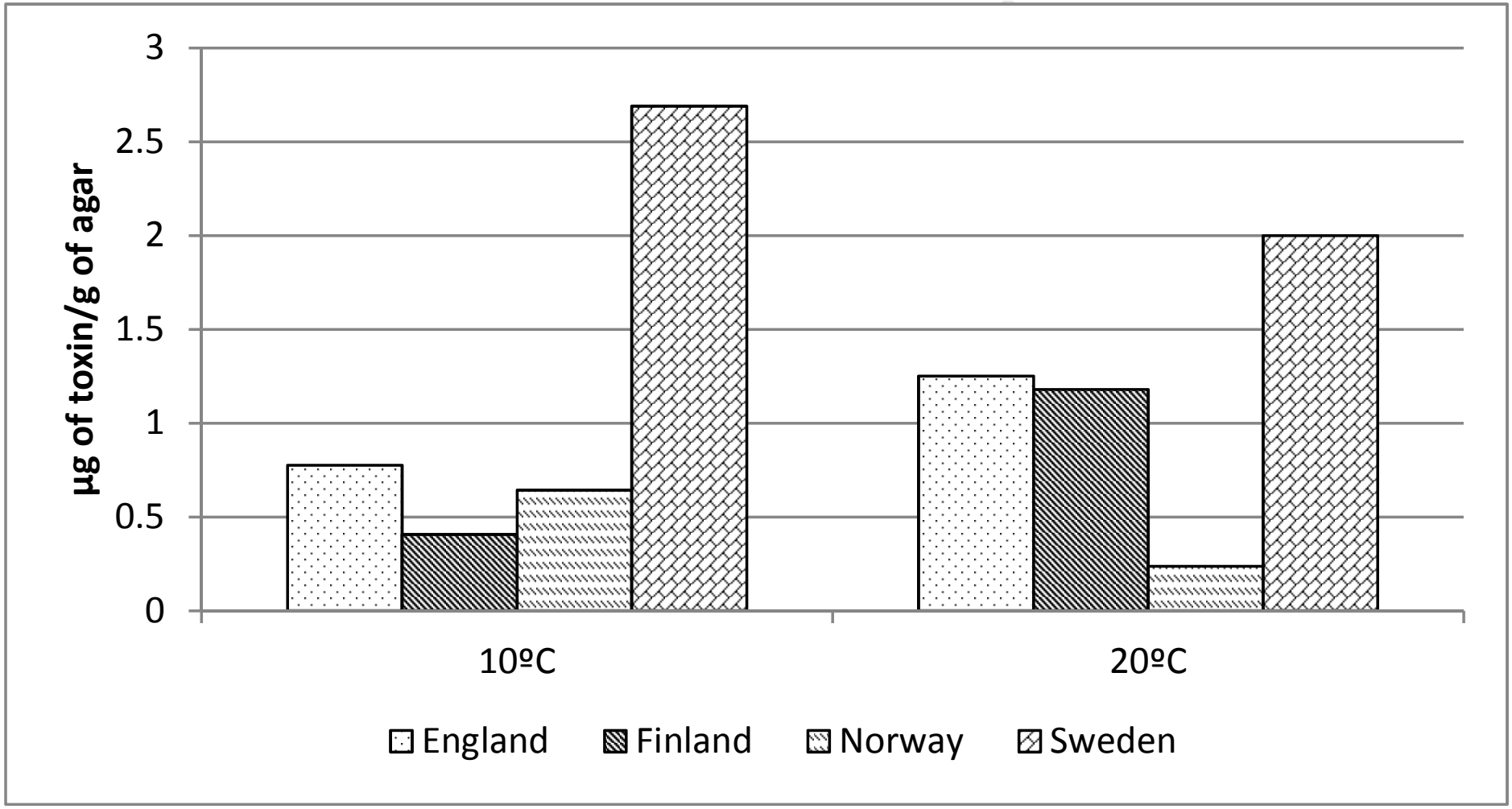
- For the first time the ratio T-2/HT-2 ratio has been studied for *F. langsethiae*. We have described the effect that environmental conditions have on this ratio increasing the production of HT-2 under stress regimes.
  - This is the first time that the production of T-2 toxin as main toxins and the different possibilities for its transformation to HT-2 have been discussed.

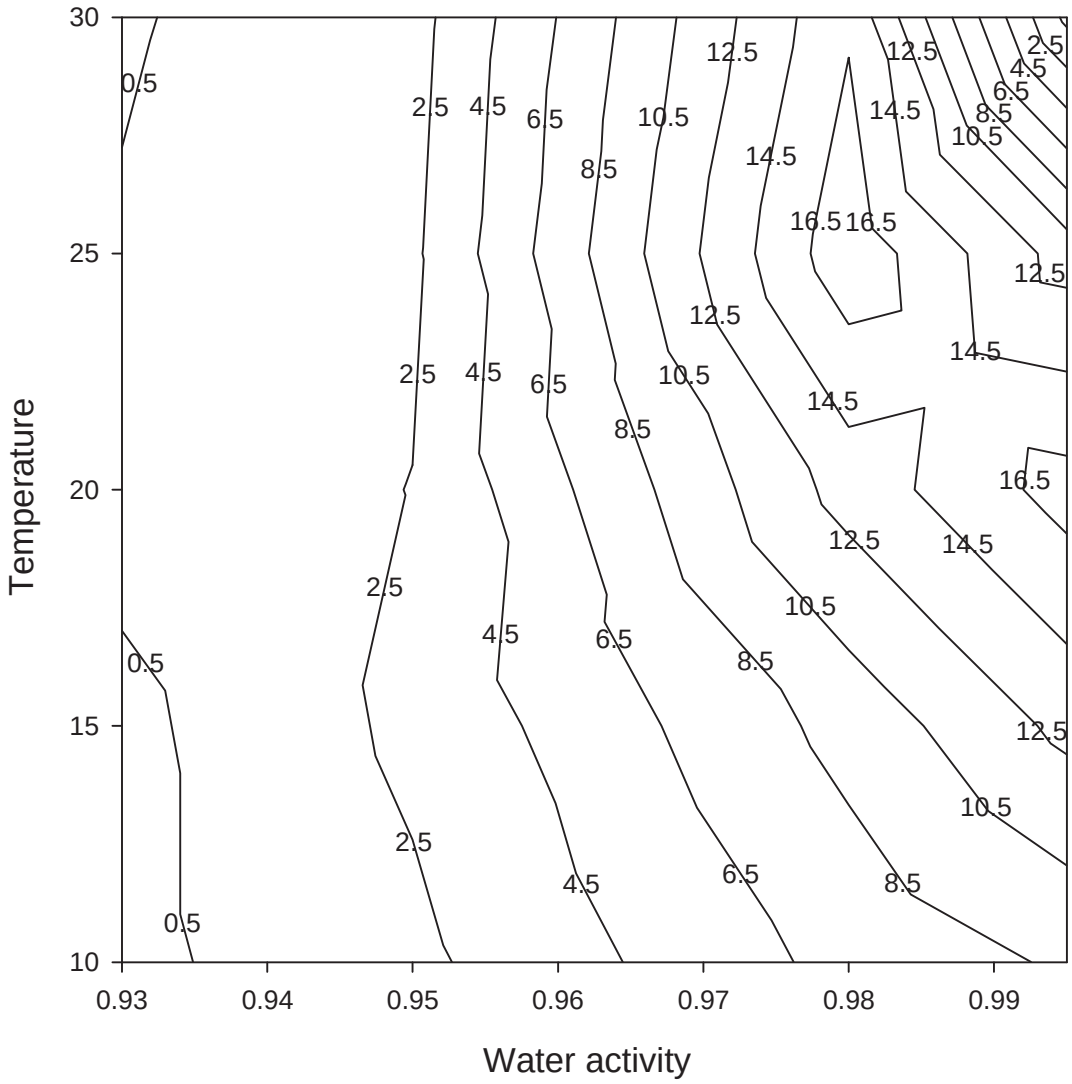
Thus this study provides useful base line data on the conditions which represent a high and low risk for contamination by these mycotoxins which are becoming of growing importance in Europe. Also we provide interesting information that will enable further research to be carried out on this species.

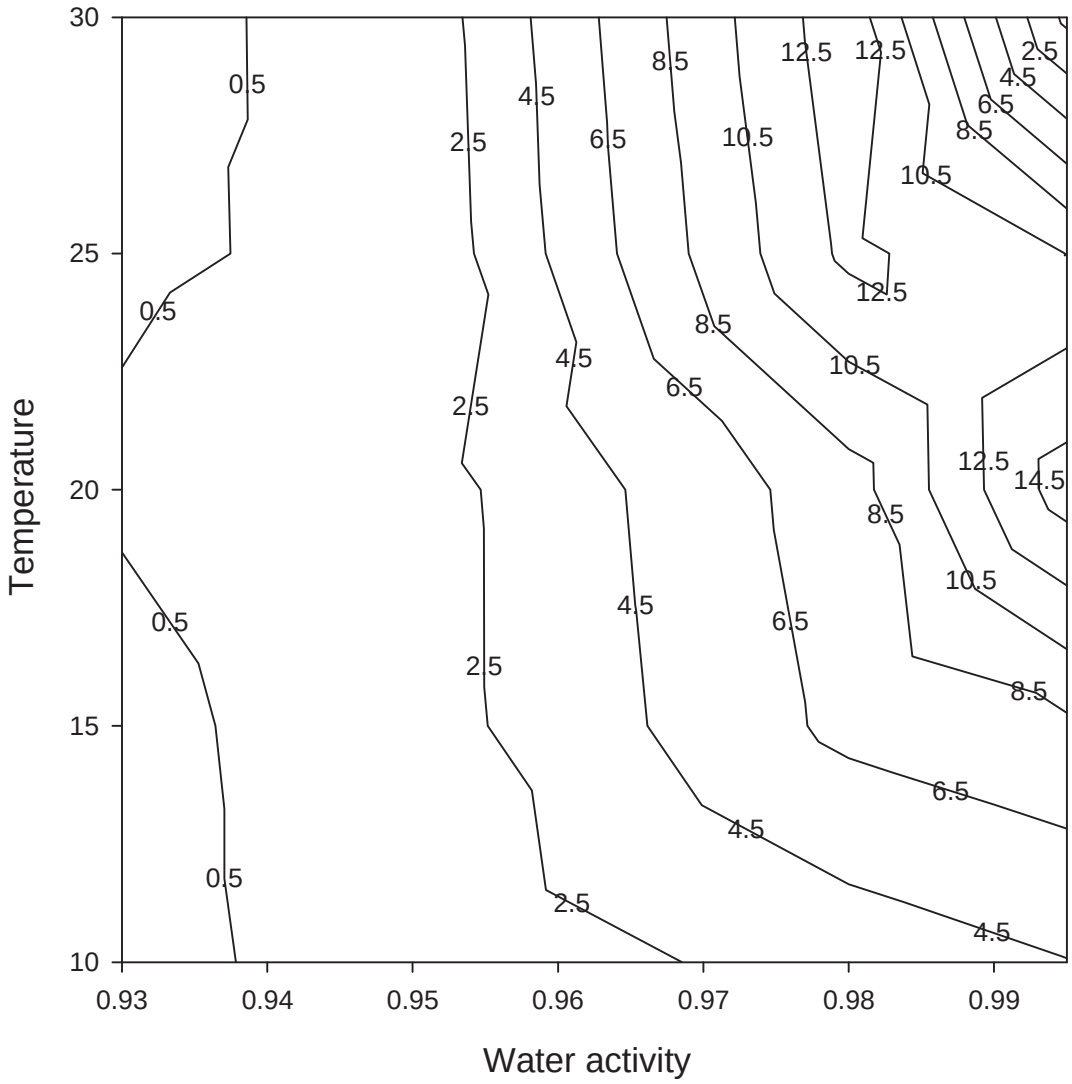

µg of toxin/g of agar
14
12
10
8
6
4
2
0
0.95
0.99

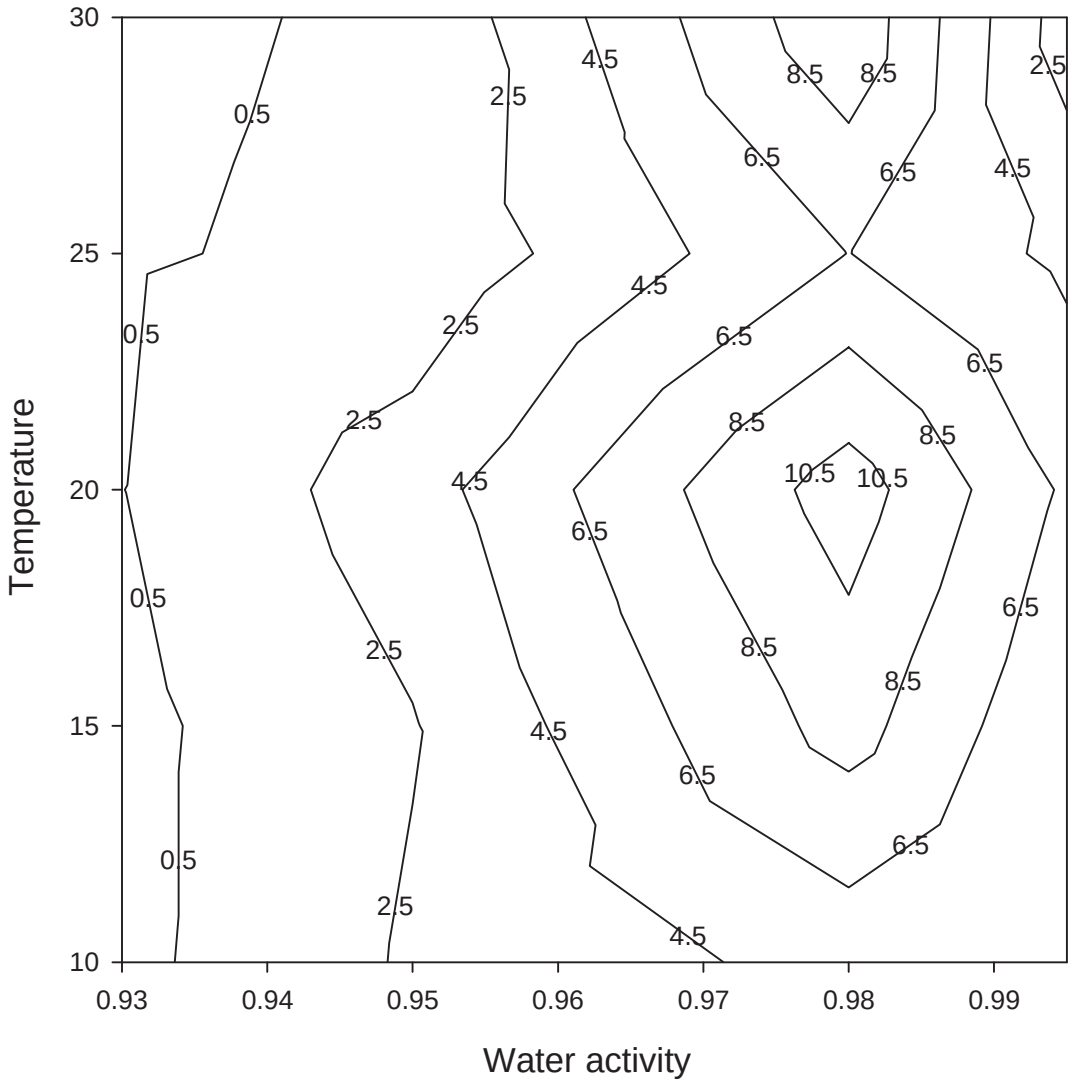





µg of toxin/g of agar
3
2.5
2
1.5
1
0.5
0
10ºC
20ºC
England
Finland
Norway
Sweden


Temperature
Water activity
30
25
20
15
10
0.93
0.94
0.95
0.96
0.97
0.98
0.99
0.5
2.5
4.5
6.5
8.5
10.5
12.5
14.5
16.5


30
25
20
15
10
Temperature
0.93
0.94
0.95
0.96
0.97
0.98
0.99
Water activity
0.5
2.5
4.5
6.5
8.5
10.5
12.5
14.5


30
25
20
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Temperature
0.93
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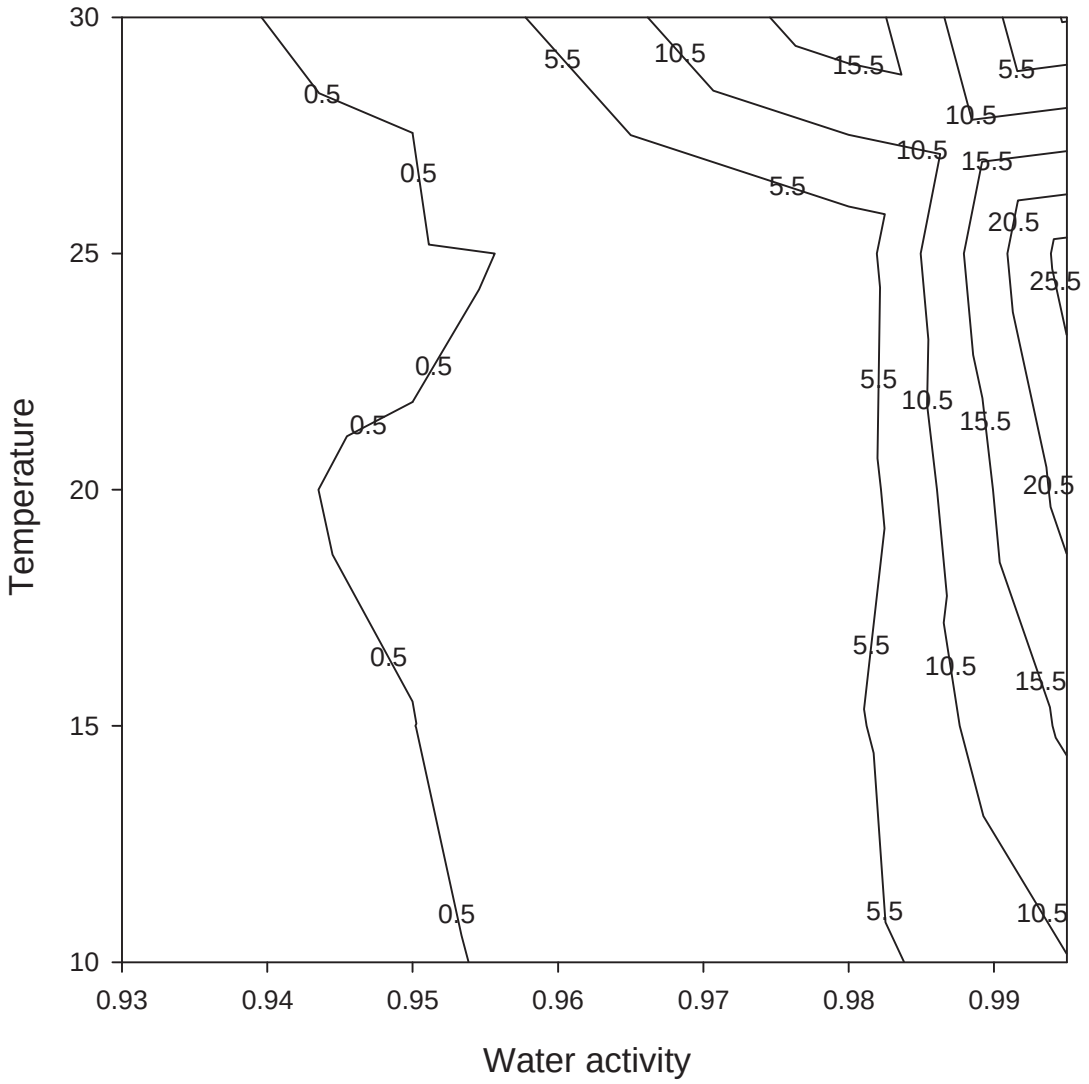
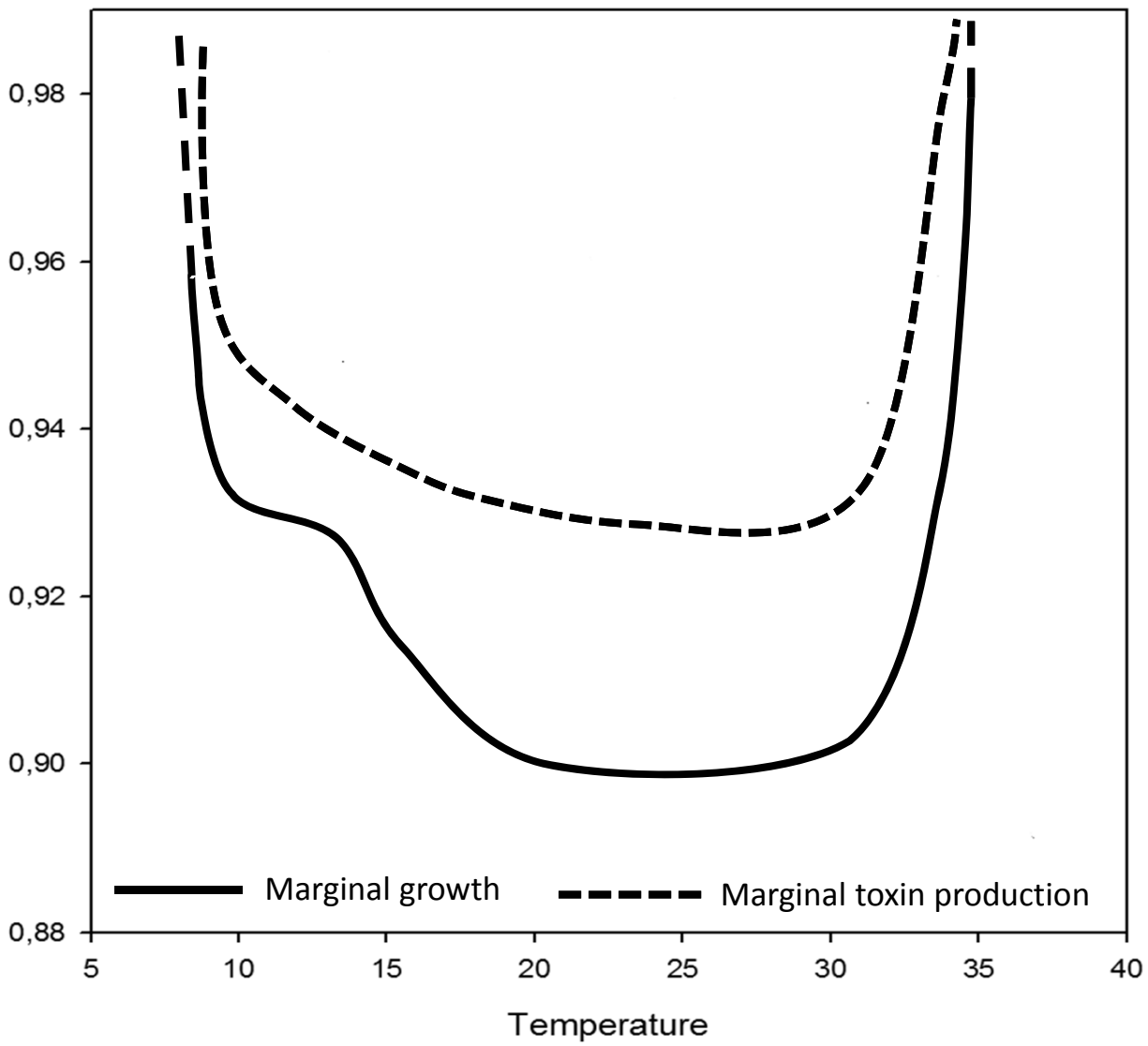
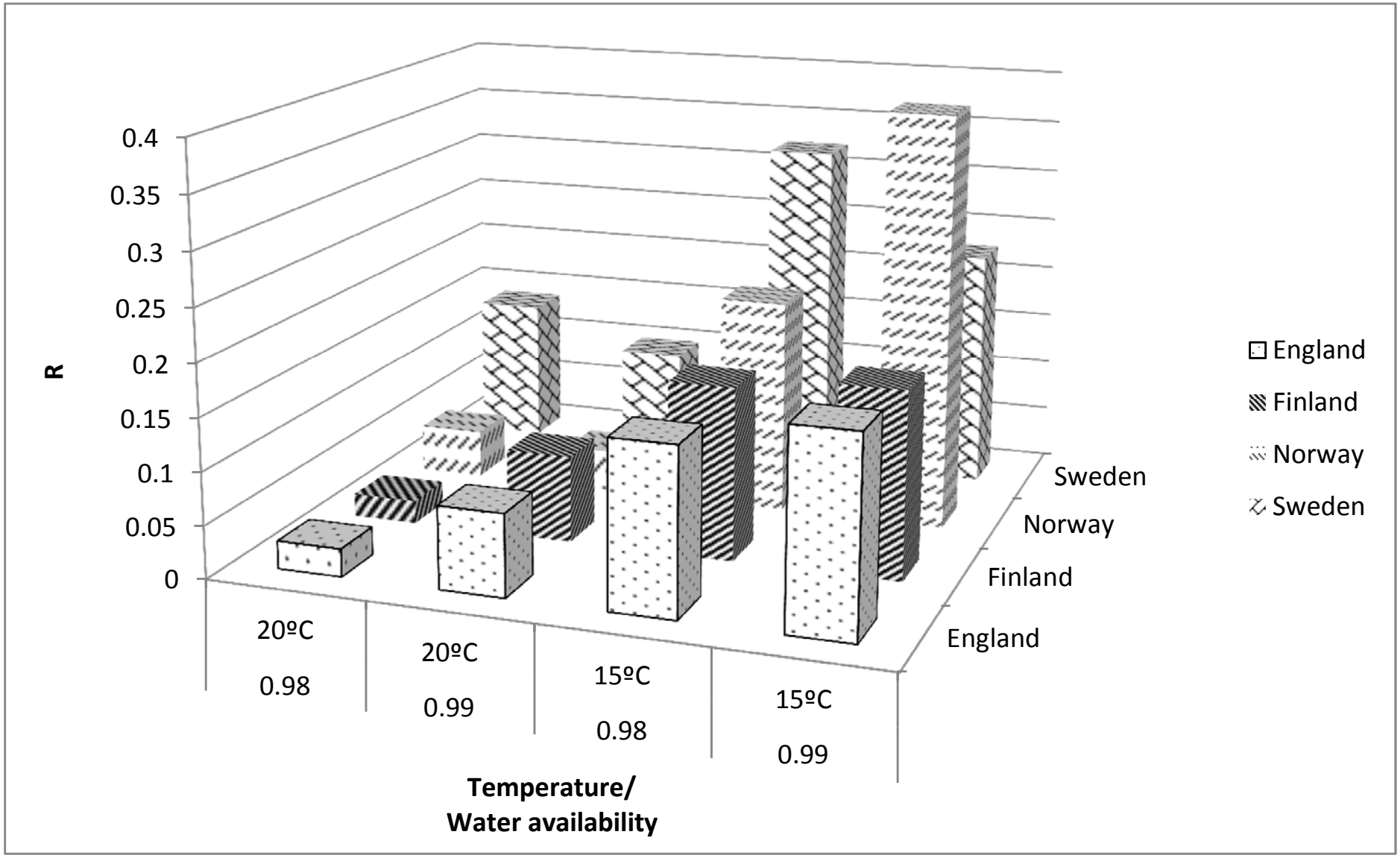
Water activity
0.5
2.5
4.5
6.5
8.5
10.5


30
25
20
15
10
Temperature
0.93
0.94
0.95
0.96
0.97
0.98
0.99
Water activity
0.5
5.5
10.5
15.5
20.5
25.5


0,98
0,96
0,94
0,92
0,90
0,88
5
10
15
20
25
30
35
40
Temperature
Marginal growth
Marginal toxin production


R
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
20ºC
0.98
20ºC
0.99
15ºC
0.98
15ºC
0.99
Temperature/
Water availability
Sweden
Norway
Finland
England
England
Finland
Norway
Sweden