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Comparison of the effects of temperature and water activity on growth rate of food spoilage moulds

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The influence of temperature (T) and water activity (a_w) on the growth rate (μ) of seven moulds (*Alternaria alternata*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Mucor racemosus*, *Penicillium chrysogenum*, *Rhizopus oryzae* and *Trichoderma harzianum*) was assessed in suboptimal conditions. Firstly, the dependence of fungal growth on temperature, at a_w 0.99, was modelled through an approach described previously for bacteria. A dimensionless growth rate variable: $\mu_{dim\alpha}=\mu/\mu_{opt\alpha}$ depended on the following normalised temperature: $T_{dim}=(T-T_{min})/(T_{opt}-T_{min})$ according to a power function: $\mu_{dim\alpha}=[T_{dim}]^{\alpha}$, where α was an exponent to be estimated. Secondly, the same approach was used to describe the influence of a_w on fungal growth, at the respective optimum temperatures for each mould. Similarly, $\mu_{dim\beta}=\mu/\mu_{opt\beta}$ depended on the following normalised water activity: $a_{wdim}=(a_w-a_{wmin})/(a_{wopt}-a_{wmin})$ according to a power function: $\mu_{dim\beta}=[a_{wdim}]^{\beta}$. Results show: (i) for each mould, the α-value is significantly less than the β-value, confirming that water activity has a greater influence than temperature on fungal development; (ii) the α-values and the β-values depend on the mould; (iii) the α-value is less than 1 for the mesophilic mould *A. flavus*, whereas the other moulds are characterised by higher α-values ranging from 1.10 to 1.54; (iv) the mesophilic *A. flavus* exhibits a low β-value, 1.50, compared to the hydrophilic *T. harzianum*, β=2.44, while β-values are within the range (1.71–2.37) for the other moulds.
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Introduction

In the last 20 years, several mathematical models describing microbial growth rate have been published. Most of these models were used for simulating the effects of different environmental factors on bacterial growth. Predictive modelling of filamentous fungal growth has not received the same attention [12]. Nevertheless, moulds play a specific part in the spoilage of food products by changing their organoleptic properties. Economic losses can be considerable [7]. Two important environmental parameters that determine the ability of moulds to grow on food are water activity (a_w) and temperature (T) [23,25,27,36], the effect of a_w on mould growth being more important than T [19].

Recently, a dimensionless approach based on biological parameters (e.g., μ_{opt}, T_{opt} and T_{min}) has been described for comparing the growth rate dependence on suboptimal temperatures, using normalised variables implemented within a power function: $[\mu_{dim\alpha}]=[T_{dim}]^{\alpha}$ where α is a design parameter to be estimated [8]. This model was originally described by Bělehrádek [4,5] who found that the power differs from one biological reaction to another. Later, it was demonstrated that thermophilic bacteria are characterised by α-values less than those obtained for mesophiles and psychrotrophs [8].

This work aims at assessing, in suboptimal conditions, the effect of T and a_w on growth of various moulds responsible for food spoilage. In order to compare moulds classified into distinct classes by their positions in T and a_w spectra, the normalised variables: $\mu_{dim\alpha}=\mu/\mu_{opt\alpha}$, $T_{dim}=(T-T_{min})/(T_{opt}-T_{min})$ and $\mu_{dim\beta}=\mu/\mu_{opt\beta}$, $a_{wdim}=(a_w-a_{wmin})/(a_{wopt}-a_{wmin})$ were used throughout this study. First, the influence of T on fungal growth, at $0.99a_w$, was examined using the following equation: $[\mu_{dim\alpha}]=[T_{dim}]^{\alpha}$. Second, the influence of a_w, at the respective optimum temperatures for each mould, was assessed using a similar equation: $[\mu_{dim\beta}]=[a_{wdim}]^{\beta}$. The minimum values ($T_{min}$ and a_{wmin}) and the parameters obtained at optimal conditions (e.g., T_{opt}, $\mu_{opt\alpha}$, a_{wopt} and $\mu_{opt\beta}$) were determined experimentally. The main objective of this paper is to assess the relative influence of the environmental variables T and a_w on fungal growth by comparing the estimated α- and β-values.

Materials and methods

Alternaria alternata, *Aspergillus flavus*, *Cladosporium cladosporioides* and *Penicillium chrysogenum* were isolated from spoiled pastry products and identified according to the descriptions of Samson *et al* [36]. *Mucor racemosus*, *Rhizopus oryzae* and *Trichoderma harzianum* were provided by the Department of Mycology, University of Pharmacy (Dijon, France). The moulds were maintained on potato dextrose agar (PDA) medium (BioMérieux, Marcy l'Etoile, France) at room temperature (18–25°C). Spores, obtained from mycelium aged 4 days, were collected by flooding the surface of the plates with ~5 ml of

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sterile saline solution (NaCl, 8.5 g/l water) containing Tween 80 (0.1% vol/vol; Prolabo, MERCK-Eurolab, Lyon, France). Mould growth was observed after inoculation of 10^4 spores (10 μl of a 10^6 spores/ml suspension) at the centre of the plates.

All experiments were performed in triplicate on PDA medium. The initial pH for all experiments, 5.8±0.1, was close to the values considered as optimal for most moulds [22].

Cultivations were carried out on Petri dishes containing about 25 ml of PDA medium at $0.99a_w$. Inoculated Petri dishes were incubated at different temperatures between −6 and 35°C with an accuracy of ±1°C: *Alt. alternata*, −6, −4, −2, 1, 3, 9, 11, 16, 19, 21 and 25°C; *A. flavus*, 3, 9, 12, 15, 19, 21, 25, 28 and 31°C; *C. cladosporioides*, −6, −4, −2, 0, 3, 11, 15, 17, 21 and 25°C; *M. racemosus*, −6, −4, −2, 0, 4, 10, 13, 16, 19, 23 and 25°C; *P. chrysogenum*, −6, −4, −2, 0, 3, 9, 11, 16, 21 and 25°C; *R. oryzae*, −4, −2, 0, 4, 10, 13, 16, 19, 23, 25, 28, 31 and 35°C; *T. harzianum*, −4, −2, 0, 1, 4, 9, 11, 16, 18, 20 and 25°C. In order to determine T_{opt}, experiments at greater temperatures were carried out for each mold, but the results were not used because the model described here is valid at suboptimal temperatures and water activities.

Media were adjusted to various a_w (0.99, 0.985, 0.98 and then in decrements of 0.01 to a minimum of 0.80) by substituting a part of water by glycerol (wt/wt). Water activity measurements were determined using an Aqualab CX2T (Decagon Devices, Pullman, Washington, USA) with an accuracy of ±0.003. The plates were incubated at the optimum temperatures for growth. *Alt. alternata*, *M. racemosus* and *P. chrysogenum*, 25°C; *C. cladosporioides* and *T. harzianum*, 26°C; *A. flavus*, 31°C and *R. oryzae*, 36°C in closed boxes in which relative humidity was controlled by a large volume of a glycerol–water solution.

Growth was evaluated daily by measurement of the average increase of the fungal colony along two perpendicular diameters [11,38]. A radial growth rate (μ, mm/day) was evaluated from the slope of the plot radius *versus* time by means of a linear regression [6,21]. The results obtained in triplicate were averaged.

The minimum temperature, T_{min}, was defined, at $0.99a_w$, as the temperature at which no growth was observable after 6 weeks. The minimum water activity, a_{wmin}, was defined, at the respective optimum temperatures for each mould, as the water activity at which no growth was observed after 6 weeks. The optimum temperature, T_{opt}, was defined as the temperature at which the growth rate, $\mu_{opt\alpha}$, was maximum. The optimum water activity was defined as the water activity at which the growth rate, $\mu_{opt\beta}$, was maximum.

The dimensionless variables T_{dim} and a_{wdim} are described as follows:

$$T_{dim} = \frac{T - T_{min}}{T_{opt} - T_{min}} \quad (1)$$

$$a_{wdim} = \frac{a_w - a_{wmin}}{a_{wopt} - a_{wmin}} \quad (2)$$

Thereafter, these variables were implemented within Bělehrádek-type models:

$$\mu_{dim\alpha} = \mu/\mu_{opt\alpha} = [T_{dim}]^{\alpha} \quad (3)$$

and

$$\mu_{dim\beta} = \mu/\mu_{opt\beta} = [a_{wdim}]^{\beta} \quad (4)$$

In order to stabilise the variance of the growth rate, a logarithmic transformation was used for Eqs. (3) and (4) as suggested previously [1,40]. All coefficients were estimated by means of a nonlinear regression software based upon the Levenberg–Marquardt Algorithm (SlideWrite 5.0; Advanced Graphics Software, Carlsbad, CA, USA) as described previously [8].

Results and discussion

For PDA medium at $0.99a_w$, it appeared that the optimal growth rate ($\mu_{opt\alpha}$) for *Alt. alternata*, *A. flavus*, *C. cladosporioides* and *P. chrysogenum* remained low, ranging from 3 to 6 mm/day, compared to *M. racemosus* and *T. harzianum*, which were characterized by greater growth rates ranging from 10 to 20 mm/day. In contrast, *R. oryzae* presented the highest value of 57.0 mm/day. The differences in the optimal growth rate observed between the moulds can be attributed to the nature of the microorganism. For example, due to the absence of septa in hyphae, which facilitates rapid translocation of nutrients and organelles between sites of growth, mucorales (*M. racemosus* and *R. oryzae*) grow very rapidly [32]. The optimum temperature for growth (T_{opt}) was about 25°C with the exception of *A. flavus* and *R. oryzae*, which exhibited values of 31 and 35°C, respectively. For each mould, the growth rate was almost constant at temperatures close to T_{opt}.

Table 1 shows that a_{wopt} was 0.985 or 0.99, which was the nominal value for PDA medium, except for *A. flavus* (0.970). For this mould, $\mu_{opt\beta}$ was significantly larger than $\mu_{opt\alpha}$, the influence of temperature being carried out at nonoptimum conditions in

Table 1 The selected organisms grown on PDA medium at pH 5.8±0.1

Moulds	T_{opt} (°C)	$\mu_{opt\alpha}$ (mm/day)	a_{wopt}	$\mu_{opt\beta}$ (mm/day)	T_{min} (°C)	a_{wmin}
Alt. alternata	25	4.8	0.985	4.7	−2	0.88
A. flavus	31	5.7	0.970	9.7	12	0.83
C. cladosporioides	25	3.0	0.985	4.4	−4	0.86
M. racemosus	25	11.2	0.985	13.3	−4	0.91
P. chrysogenum	25	3.1	0.985	4.6	−4	0.81
R. oryzae	35	56.7	0.985	56.8	2	0.89
T. harzianum	25	19.6	0.990	15.3	4	0.91

Their optimum temperature (T_{opt}) and water activity (a_{wopt}), the optimal growth rates at $0.99a_w$ ($\mu_{opt\alpha}$) and at the respective optimum temperature for each mould ($\mu_{opt\beta}$).
The minimum temperature (T_{min}) and water activity (a_{wmin}) for growth were experimentally determined after 6 weeks of incubation.

Table 2 Minimum temperature and water activity for some species of fungi as reported in the literature

Moulds	T_{min} (°C)	References	a_{wmin}	References
Alt. alternata	−5, 6.5	[10,17]	0.85	[27]
	0	[27]	0.89	[15]
			0.88	[24]
A. flavus	6–8	[27]	0.78	[3]
	10–12	[10]	0.85	[19,39]
C. cladosporioides	−5	[14]	0.84	[15,20]
			0.88	[24]
M. racemosus	−3, −4	[28]	0.92	[27]
P. chrysogenum	−4	[27]	0.78	[2]
	4	[31]	0.81	[26]
			0.85	[27]
R. oryzae	7–9	[27]	0.88	[18]
T. harzianum	5	[32]	0.91	[16]

terms of a_w. By decreasing a_w from 0.99 to 0.97 at 31°C, the optimum growth rate for *A. flavus* was increased from 5.7 to 9.7 mm/day. For *Alt. alternaria and R. oryzae*, no significant difference between $\mu_{opt\alpha}$ and $\mu_{opt\beta}$ values were observed. Therefore, both of these moulds were characterised by a_{wopt} ranging from 0.985 to 0.990. In contrast, *C. cladosporioides* and *M. racemosus* exhibited greater values for $\mu_{opt\beta}$ than $\mu_{opt\alpha}$, suggesting that the optimum a_w was closer to 0.985–0.990. For some moulds, a slight difference of about 0.005 at a_w close to the optimum may lead to significant differences in growth. This may explain the differences observed between $\mu_{opt\alpha}$ and $\mu_{opt\beta}$ with *T. harzianum*.

The minimum temperatures for growth are reported in Table 1. Most of the moulds exhibited T_{min} close to the freezing point, with the notable exception of *A. flavus*. The minimum temperatures reported in the literature (Table 2) exhibit a wide range of variation for *Alt. alternata* and *P. chrysogenum*. In this study, both moulds grew at temperatures below the freezing point. The experimental determinations of T_{min} for *A. flavus*, *C. cladosporioides*, *M. racemosus* and *T. harzianum* were consistent with literature data. *R. oryzae* grew at a lower temperature, 2°C, than previously described [33]. Comparisons between experimental a_{wmin} and literature data are often difficult because authors used a wide range of temperature and a_w depressors [13]. Despite these facts, our results are in accordance with the literature data shown in Table 2.

The α-values describe the influence of temperature on fungal growth (Table 3). *A. flavus* was characterised by a very low α-value of 0.81. All other moulds were characterised by α-values greater than 1, exhibiting clearly a concave shape of the curve μ_{dim} *versus* T_{dim} (Figure 1). It should be pointed out that *A. flavus* was characterised by a greater T_{min} and by a smaller value of α. The α-values describe the influence of temperature on the growth rate. First, in contrast to the square root model, which is a particular case of the Bělehrádek model [35], the power α is not necessarily equal to 2, but a design parameter to be estimated [8]. Second, with regard to temperature, fungi as well as other microorganisms are divided into psychrophiles, mesophiles and thermophiles [27]. In this study, the majority of fungal species are mesophiles, growing at temperatures within the range 0–35°C with the optimum being 25–30°C. The genus *Aspergillus* is typical, growing readily at temperatures between 15 and 40°C [37]. This was confirmed in this study for *A. flavus*, which grew in the range 12–42°C. This fungus could be considered as upper mesophilic [23] and presented a value of the power α less than for other species. It would be interesting to test other common fungi, such as the thermotolerants (*A. fumigatus*, *M. pusillus*), to strengthen this hypothesis.

Table 3 Estimation of the α- and β values with 95% confidence intervals and regression coefficients for the selected organisms

Molds	α	r^2	β	r^2
Alt. alternata	1.10±0.08	0.950	2.11±0.10	0.965
A. flavus	0.81±0.05	0.972	1.50±0.08	0.985
C. cladosporioides	1.26±0.09	0.956	1.71±0.05	0.978
M. racemosus	1.10±0.04	0.984	1.91±0.14	0.951
P. chrysogenum	1.54±0.12	0.933	2.00±0.08	0.954
R. oryzae	1.40±0.03	0.990	2.37±0.18	0.924
T. harzianum	1.44±0.09	0.966	2.44±0.12	0.971

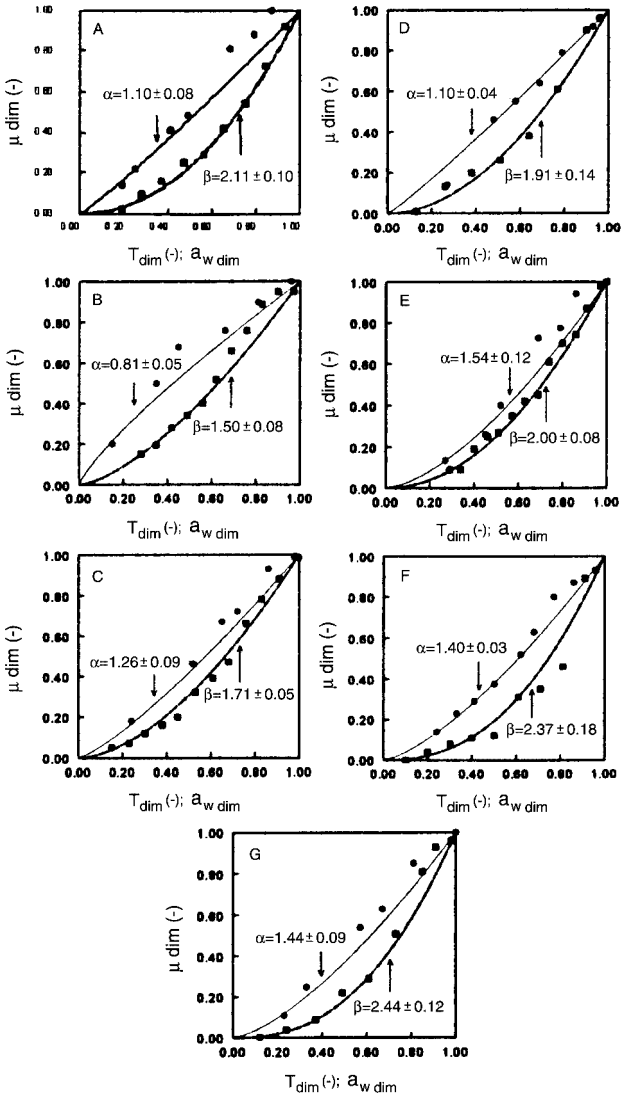

Figure 1 Dimensionless plots, growth rate *versus* temperature (●) and growth rate *versus* water activity (■) for (A) *Alt. alternata*; (B) *A. flavus*; (C) *C. cladosporioides*; (D) *M. racemosus*; (E) *P. chrysogenum*; (F) *R. oryzae*; (G) *T. harzianum* and power function curves.

The β-values describe the influence of water activity on fungal growth. They were significantly different from α-values (Table 3). Again, *A. flavus* presented the lower β-value, whereas *R. oryzae* and *T. harzianum* presented higher values of β. *Alt. alternata*, *M. racemosus* and *P. chrysogenum* were characterized by β-values close to 2, whereas *A. flavus* and *C. cladosporioides* exhibited lower values of β. The β-values describe the influence of water activity on fungal growth. Xerophilic, mesophilic and hygrophilic species are classified depending on whether optimum growth occurred below $0.95a_w$, from 0.95 to $1.00a_w$ or only at $1.00a_w$ [28]. All fungi examined in this study can be considered mesophiles, although care should be taken with these criteria for no mould is capable of growing in pure water ($a_w=1$). According to our results, some moulds *T. harzianum*, 2.44±0.12; *R. oryzae*, 2.37±0.17 and *Alt. alternaria*, 2.11±0.10 grew at the same rate at $0.985a_w$ and $0.99a_w$. In contrast, *C. cladosporioides*, *M. racemosus* and *P. chrysogenum* grew faster at $0.985a_w$ than at 0.99, suggesting that these moulds required less humidity to grow at the optimum rate. Xerophilic fungi are capable of growing at $0.85a_w$ or less [29]. *P. chrysogenum* and *A. flavus* could be considered xerophiles as suggested by Pitt and Hocking [31,32], although *P. chrysogenum* exhibited a high β-value (2.00±0.08). Attempting to classify the moulds according to their β-value is rather difficult. Nevertheless, in this study, moulds growing faster at 0.99 than 0.985 were characterised by β-values greater than 2.

In all cases, r^2 coefficients were greater than or equal to 0.924. The power function cannot represent the S-shaped curves of μ *versus* T and μ *versus* a_w. For example, the S-shaped curve μ_{dim} *versus* a_{wdim} described for *A. flavus* in Figure 1 made it difficult to obtain a regression coefficient greater than 0.972. In a previous study [8], the regression coefficients were generally greater because T_{min} was estimated. The reported T_{min} for *Alt. alternata* and *P. chrysogenum* depends on the literature cited (Table 2). Therefore, it would have been impossible to check the consistency of the exponent α if T_{min} was estimated. Accordingly, T_{min} was experimentally determined, such as T_{opt} and μ_{opt}.

The use of normalised variables allowed the dimensionless plots of growth rate *versus* temperature, and growth rate *versus* a_w to be plotted on the same graphs. It should be pointed out that the former curve was always above the latter one. The relative influence of T and a_w on fungal growth can be interpreted by comparing α- and β-values. After logarithmic transformation of Eqs. (3) and (4), $\ln(\mu_{dim\alpha})$ and $\ln(\mu_{dim\beta})$ can be plotted against $\ln(T_{dim})$ and $\ln(a_{wdim})$, respectively. α and β can be considered as the following derivatives:

$$\alpha = \frac{d(\ln\mu_{dim\alpha})}{d(\ln T_{dim})} \quad (5)$$

and

$$\beta = \frac{d(\ln\mu_{dim\beta})}{d(\ln a_{wdim})}. \quad (6)$$

For the same X-axis deviation, $d(\ln T_{dim})=d(\ln a_{wdim})$, $d(\ln\mu_{dim\alpha})$ and $d(\ln\mu_{dim\beta})$ are proportional to α and β, respectively. In such a condition, if α is less than β, the decrease in the growth rate in logarithmic coordinate due to temperature $d(\ln\mu_{dim\alpha})=\ln\mu-\ln\mu_{opt\alpha}$ is less than the one due to water activity $d(\ln\mu_{dim\beta})=\ln\mu-\ln\mu_{opt\beta}$. For all fungi examined in this paper, the α-value was significantly less than the β-value, confirming that water activity has a relatively greater effect on fungal development than temperature.

Let us consider, for example, *M. racemosus*, at $T_{dim}=(25-T)/(25-(-4))=0.4$ and $a_{wdim}=(0.985-a_w)/(0.985-0.91)=0.4$. In these conditions, the temperature is about 13.4°C for the α curve, and a_w about 0.955. The dimensionless approach predicts that at $0.99a_w$ and 13.4°C, the growth rate is decreasing to $0.4^{1.10}$ (36.5%) of $\mu_{opt\alpha}$ (4.60 mm/day). At 25°C and $0.955a_w$, the growth rate is decreasing to $0.4^{1.91}$ (17.4%) of $\mu_{opt\beta}$ (2.31 mm/day).

Conclusions

The shape of the curves μ *versus* temperature and μ *versus* water activity are similar; therefore, the same structure of equation has been used for describing the effects of T and a_w on fungal growth. The use of normalised variables allowed moulds classified into distinct classes by their position into the T and a_w spectra to be compared. In addition, it was possible to compare α and β for assessing the influence of each variable on the growth rate.

This study also contributes to the improvement of existing models for predicting mould growth in foods. Due to a shortage of models dedicated to fungal growth, there is a tendency to use models that were developed for bacteria. It has been demonstrated that the use of the square root model when α-values are significantly less than 2 leads to an estimation of T_{min} less than the experimental one [9]. Therefore, when α-values are significantly less than 2, the use of square root models [33,34,40] for describing the effect of temperature on fungal growth may lead to erroneous estimation of T_{min}. This point should be examined. Compared to bacteria, a_w is a key parameter for controlling fungal growth; β-values were greater than the α-values for all moulds. The individual effects of T and a_w have been assessed by means of this progressive approach. The combined effects of these factors on fungal growth should also be evaluated. These are the future developments we are currently looking at.

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References

1 Alber SA and DW Schaffner. 1992. Evaluation of data transformations used with the square root and Schoolfield models for predicting bacterial growth rate. *Appl Environ Microbiol* 58: 3337–3342.
2 Armolik N and JG Dobson. 1956. Minimum humidity requirements for germination of conidia of fungi associated with storage of grain. *Phytopathology* 46: 462–465.
3 Ayerst G. 1969. The effects of moisture and temperature on growth and spore germination in some fungi. *J Stored Prod Res* 5: 127–141.
4 Bělehrádek J. 1926. Influence of temperature on biological processes. *Nature (London)* 118: 117–118.
5 Bělehrádek J. 1926. Protoplasmic viscosity as determined by a temperature coefficient of biological reactions. *Nature (London)* 118: 478–480.
6 Brancato FP and NS Golding. 1953. The diameter of the mold colony as a reliable measure of growth. *Mycologia* 45: 848–864.
7 Bullerman LB. 1984. Effects of potassium sorbate on growth and patulin production by *Penicillium patulum* and *Penicillium roquefortii*. *J Food Prot* 47: 312–316.
8 Dantigny P. 1998. Dimensionless analysis of the microbial growth rate dependence on sub-optimal temperatures. *J Ind Microbiol Biot* 21: 215–218.

9 Dantigny P and P Molin. 2000. Influence of the modelling approach on the estimation of the minimum temperature for growth in Bělehrádek-type models. *Food Microbiol* 17: 597–604.
10 Domsch KH, W Gams and TH Anderson. 1980. Compendium of Soil Fungi. 2 volumes. Academic Press, London.
11 Gervais P, M Bensoussan and W Grajek. 1988. Water activity and water content: comparative effects on the growth of *Penicillium roquefortii* on solid substrate. *Appl Microbiol Biot* 27: 389–392.
12 Gibson AM and AD Hocking. 1997. Advances in the predictive modelling of fungal growth in food. *Trends Food Sci Technol* 8: 353–358.
13 Gibson AM, J Baranyi, JI Pitt, MJ Eyles and TA Roberts. 1994. Predicting fungal growth: the effect of water activity on *Aspergillus flavus* and related species. *Int J Food Microbiol* 23: 419–431.
14 Gill CO and PD Lowry. 1982. Growth at sub-zero temperatures of black spot fungi on meat. *J Appl Bacteriol* 52: 245–250.
15 Grant C, CA Hunter, B Flannigan and AF Bravery. 1989. The moisture requirements of molds isolated from domestic dwellings. *Int Biodeterior Biodegrad* 25: 259–284.
16 Griffin DM. 1963. Soil moisture and the ecology of soil fungi. *Biol Rev Cambridge Philos Soc* 38: 141–166.
17 Hasija SK. 1970. Physiological studies of *Alternaria citri* and *A. tenuis*. *Mycologia* 62: 289–295.
18 Hocking AD and BF Miscamble. 1995. Water relations of some Zygomycetes isolated from food. *Mycol Res* 99: 1113–1118.
19 Holmquist GU, HW Walker and HM Stahr. 1983. Influence of temperature, pH, water activity and antifungal agents on growth of *Aspergillus flavus* and *A. parasiticus*. *J Food Sci* 48: 778–782.
20 Hunter CA, C Grant, B Flannigan and AF Bravery. 1988. Moulds in buildings, the air spora of domestic dwellings. *Int Biodeterior Biodegrad* 24: 81–101.
21 Koch AL. 1975. The kinetics of mycelial growth. *J Gen Microbiol* 89: 209–216.
22 Lacey J. 1989. Pre- and post-harvest ecology of fungi causing spoilage of foods and other stored products. In: Moss MO, B Jarvis and FA Skinner (Eds), Filamentous Fungi in Foods and Feeds. The Society for Applied Bacteriology Symposium Series. Blackwell, Oxford, UK, pp. 11–25.
23 Lacey J, ST Hill and MA Edwards. 1980. Micro-organisms in stored grain: their enumeration and significance. *Trop Stored Prod Inf* 39: 19–33.
24 Magan N and J Lacey. 1984. Effect of temperature and pH on water relations of field and storage fungi. *Trans Br Mycol Soc* 82: 71–81.
25 Marín S, V Sanchis, A Teixido, AJ Ramos, I Vinas and N Magan. 1998. Environmental factors, *in vitro* interactions, and niche overlap between *Fusarium monoliforme*, *F. proliferatum*, and *F. graminearum*, *Aspergillus* and *Penicillium* species from maize grain. *Mycol Res* 102: 831–837.
26 Mislivec PB and J Tuite. 1970. Temperature and relative humidity requirements of species of *Penicillium* isolated from yellow dent corn. *Mycologia* 62: 75–88.
27 Panasenko VT. 1967. Ecology of microfungi. *Bot Rev* 33: 189–215.
28 Pelhate J. 1968. Determination of water requirements in grain storage fungi. *Mycopathol Mycol Appl* 36: 117–128.
29 Pitt JI. 1975. Xerophilic fungi and the spoilage of foods of plant origin. In: Duckworth RB (Ed), Water Relations of Food. Academic Press, London, UK, pp. 273–307.
30 Pitt JI. 1979. The Genus *Penicillium* and Its Teleomorphic States *Eupenicillium* and *Talaromyces*. Academic Press, London.
31 Pitt JI and AD Hocking. 1977. Influence of solute and hydrogen ion concentration on the water relations of some xerophilic fungi. *J Gen Microbiol* 101: 35–40.
32 Pitt JI and AD Hocking. 1999. Fungi and Food Spoilage. *Aspen Publishers, Gaithersburg, MD.*
33 Ratkowsky DA, J Olley, TA McMeekin and A Ball. 1982. Relationship between temperature and the growth rate of bacterial cultures. *J Bacteriol* 149: 1–5.
34 Ratkowsky DA, RK Lowry, TA McMeekin, AN Stokes and RE Chandler. 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *J Bacteriol* 154: 1222–1226.
35 Ross T. 1987. Bělehrádek temperature functions and growth of organisms. CSIRO-DSIR Joint Workshop on Seafood Processing, Nelson, New Zealand, April 1986. Occasional Paper 18 CSIRO Tasmanian Regional Laboratory, Hobart, Australia, 16 pp.
36 Samson RA, ES Hoekstra, JC Frisvad and O Filtenborg. 1995. In: Centraalbureau Voor Schimmelcultures (Ed), Introduction to Food-Borne Fungi. 4th edn. Baarn, the Netherlands, 322 pp.
37 Smith JE. 1994. Physiology of *Aspergillus*. In: Aspergillus. Plenum, New York, pp. 23–39.
38 Trinci APJ. 1969. A kinetic study of the growth of *Aspergillus nidulans* and other fungi. *J Gen Microbiol* 57: 11–24.
39 Wheeler KA, AD Hocking and JI Pitt. 1988. Water relations of some *Aspergillus* species isolated from dried fish. *Trans Br Mycol Soc* 91: 631–637.
40 Zwietering MH, HGAM Cuppers, JC de Wit and K van't Riet. 1994. Evaluation of data transformations and validation of a model for the effect of temperature on bacterial growth. *Appl Environ Microbiol* 60: 195–203.