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A cardinal model to describe the effect of water activity on the growth of moulds

L. Rosso*, T.P. Robinson

Food Safety Centre of Expertise (Pôle de Compétence en Sécurité des Aliments), DANONE Inc., CREALIS, Z.I. le Teinchurier, F-19100 BRIVE-LA-GAILLARDE, Maisons-Alfort, France

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Abstract

A simple model was proposed to describe the effect of water activity (*Aw*) on the radial growth rate of moulds. This model is deduced from the cardinal model family proposed by Rosso in 1995, which is only defined from cardinal values of environmental factors (minimum, optimum and maximum values), the growth rate observed at the optimal value of the environmental factors, and *n*, a shape parameter. For *Aw*, a simple form of cardinal model is proposed. This form is obtained for $n = 2$ and $Aw_{max} = 1.0$ (pure water). The final model is so defined from only three parameters: Aw_{min}, Aw_{opt}, and optimal radial growth rate (RGR_{opt}). This model was successfully fitted on a data set of *Aspergillus flavus*, *Aspergillus nomius*, *Aspergillus oryzae*, *Aspergillus parasiticus*, *Aspergillus candidus*, *Aspergillus sydowii*, *Eurotium amstelodami*, *Eurotium chevalieri*, and *Xeromyces bisporus*. The same quality of fit was obtained for different solutes used to control the *Aw* (NaCl, glucose/fructose mixture, glycerol), and at different pH values. From this model and using cardinal values extracted from the literature, theoretical evolutions of the *RGR* of *enicillium roqueforti*, and *Paecilomyces variotii*, were proposed and superimposed on data published in the literature. The results showed a good concordance between the predicted and the observed values for these species. The use of this model in Predictive Microbiology is discussed.

Keywords: Moulds; Water activity; Growth; *Aspergillus*; *Penicillium*; *Paecilomyces*; *Eurotium*; *Xeromyces*; Predictive microbiology

1. Introduction

Moulds are of concern to the food industry as potential spoilage organisms (Samson, 1989), mycotoxin producers (Smith and Moss, 1985) or as part of a fermentation or ripening process (Campbell-Platt and Cook, 1989). For this reason, it is important to understand the growth kinetics of these organisms in the food context, in order to control the quality of the product from formulation to storage, especially for long shelf life products (cakes, biscuits, soft beverages, cheese, bakery goods etc).

Generally, mould spoilage is associated with low water activity foods (dried and intermediate moisture foods; $Aw = 0.60–0.95$) where the water activity is

*Corresponding author. Fax: +33-1-4977-9005.
E-mail address: l.rosso@dg.afssa.fr (L. Rosso).

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controlled either by NaCl, sucrose or glucose/fructose. Mould growth in these products depends closely on the water activity which is directly a function of the product formulation, the solutes used (Silliker et al., 1980) and the temperature.

Some authors have proposed models to describe the effect of water activity (Gibson et al., 1994; Valik et al., 1999) or the effect of salt (Cuppers et al., 1997) on the growth of moulds. These models are in agreement with the observed data evolution, but they are totally or partially defined from parameters which they have non biological interpretation. This obliges the user to fit them on fresh and complete data sets to obtain parameter values. Moreover, if one wants to use this model in a predictive way, for related strains, other species or other products, this may be difficult and hazardous, because as the parameters have no biological interpretation.

For these reasons we propose a simple model based on the same criteria described by Rosso et al. (1995), i.e. a model with a minimum of parameters that are biologically meaningful and have no structural correlation.

2. Materials and methods

Data

Radial growth rates (*RGRs*) of *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus parasiticus*, and *Aspergillus nomius* obtained at 25°C, 30°C, and 37°C on solidified laboratory medium, were extracted from the paper of Pitt and Miscamble (1995). The *RGRs* were estimated for different water activity values controlled by adjusting the solute concentration with equal weights of glucose and fructose.

The *RGRs* of *Eurotium chevalieri* and *Xeromyces bisporus* obtained in laboratory medium at 25°C, pH 4.0 or 6.5, and for different water activity values controlled by NaCl, glycerol, or glucose/fructose mixture, were extracted from the paper of Pitt and Hocking (1977).

The *RGRs* of *Aspergillus candidus*, *Aspergillus sydowii*, *Eurotium amstelodami*, and *Paecilomyces variotii*, obtained in laboratory medium at 25°C, for different water activity values controlled by NaCl, or glucose/fructose mixture, were extracted from the paper of Wheeler and Hocking (1988).

Diameter growth rates of *Penicillium roqueforti* PR3 obtained in Sabouraud agar at 25°C for different water activities controlled by NaCl, were extracted from the paper of Valik et al. (1999) and transformed to *RGR* values by halving them.

2.1. Models

The model used to describe the *Aw* effect on *RGR* of mould colonies, is derived from the Cardinal Model family (Rosso, 1995), CM_n, of which the general equation is:

$$CM_n(x, p_{\min}, p_{\text{opt}}, p_{\max}) = \begin{cases} x \leq p_{\min}, & 0.0 \\ p_{\min} < x < p_{\max}, & \dfrac{(x - p_{\min})^n (x - p_{\max})}{(p_{\text{opt}} - p_{\min})^{n-1}\{(p_{\text{opt}} - p_{\min})(x - p_{\text{opt}}) - (p_{\text{opt}} - p_{\max})[(n-1)p_{\text{opt}} + p_{\min} - nx]\}} \\ x \geq p_{\max}, & 0.0 \end{cases} \quad (1)$$

$$r = r_{\text{opt}} CM_n(x, p_{\min}, p_{\text{opt}}, p_{\max}) \quad (2)$$

Where x is the environmental variable; $p_{\min}$, p_{opt} and $p_{\max}$, are respectively the minimum, the optimum and the maximum growth value for this variable; n is a shape parameter; r the growth rate (specific growth rate, radial growth rate etc); and r_{opt} the optimal value of r in the product considering the environmental factors studied.

For the water activity effect on the mould radial growth rate (*RGR*), the model proposed is defined as follows:

$$RGR = RGR_{\text{opt}} CM_2(Aw, Aw_{\min}, Aw_{\text{opt}}, 1.0) \quad (3)$$

In order to test the simplest form, and because few data are available between Aw_{opt} and $Aw_{\max}$, the maximum water activity for growth was considered equal to 1.0 the value observed in pure water.

Table 1
Parameter values used to predict the effect of *Aw* on growth of *P. roqueforti* and *Pa.variotii* in laboratory medium. The *Aw* values were controlled with NaCl

Strain	Parameter	Reference
P. roqueforti	Aw_{min} (NaCl) = 0.83	Lacey (1989)
	Aw_{opt} = 0.997 (lab. medium)	Lacey (1989)
	$RGR_{opt} \approx 270$ μm h^{-1}	Valik et al. (1999)
Pa. variotii	Aw_{min} (glucose/fructose) = 0.793	Wheeler and Hocking (1988)
	$Aw_{opt} \approx 0.985$	Wheeler and Hocking (1988)
	RGR_{opt}(glucose/fructose) ≈ 308 μm h^{-1}	Wheeler and Hocking (1988)

2.2. Data analysis

Given their quality (number of points, distribution and variability), the data sets of *A. flavus*, *A. oryzae*, *A. parasiticus*, *A. nomius*, *A. candidus*, *A. sydowii*, *E. amstelodami*, *E. chevalieri* and *X. bisporus* were used to fit the model and to test its robustness.

The simple significance of parameters allows a direct estimation of the initial guesses before the computation of the fitted procedure.

The ordinary least square criterion was used to fit model 3 to the data set. The square root transformation of the radial growth rate was used to calculate the sum of the squared residuals, *SSR*.

$$SSR = \sum_{i=1}^{n} \left[\sqrt{RGR_{observed}} - \sqrt{RGR_{theoretical}}\right]^2 \qquad (4)$$

The smaller the *SSR*, the better the fit. The minimum *SSR* values were computed using Mathematica™ 3.0 subroutine NonlinearRegress (Wolfram Research Inc., Champaign, USA) using the classical gradient shift according to the Levenberg-Marquardt method.

The 95% Marginal Confidence Intervals (MCI) of parameter estimations were computed from the same subroutine. These 95% MCI, classically used in a non-linear regression approach, consist in approximating a non-linear regression model with a gaussian linear model having the same number of parameters.

Two data sets were used to test the predictive ability of the model: *P. roqueforti* (*Aw* controlled by NaCl), and *Pa. variotii* (*Aw* controlled by glucose/fructose mixture). To use the model predictively, parameter values were estimated from the literature (Table 1), and predicted curves were superimposed on the experimental values obtained in the medium corresponding to the RGR_{opt} values.

For *P. roqueforti*, the RGR_{opt} was the average Radial Growth Rate estimated from four values from the paper of Valik et al. (1999) in Sabouraud Agar at three different pH values (as shown by the authors, the effect of the pH on the results is not significant).

For *Pa.variotii*, Aw_{opt} was deduced from the *RGR* values obtained by Wheeler and Hocking (1988) in medium adjusted with NaCl, to an *Aw* of between 0.96 and 0.99. The RGR_{opt} was the value observed in laboratory medium controlled by glucose/fructose mixture at *Aw* close to $Aw_{opt} = 0.985$.

3. Results and discussion

3.1. Fit of the model on data sets

As shown by the fits of the model on data for different mould species, different pH and different control solutes (Figs. 1–11), the model has an

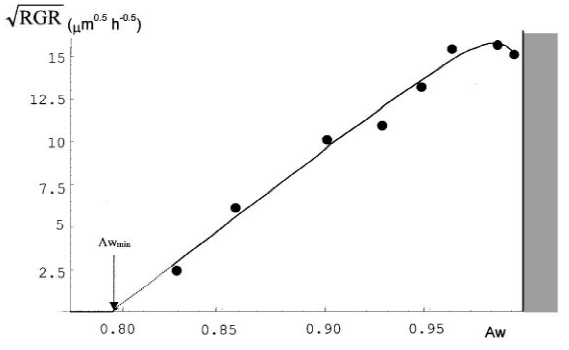

Fig. 1. Fit of model 3 on data sets of *Aspergillus flavus* (Pitt and Miscamble, 1995) obtained at 25°C in medium in which *Aw* was controlled by glucose/fructose mixture.

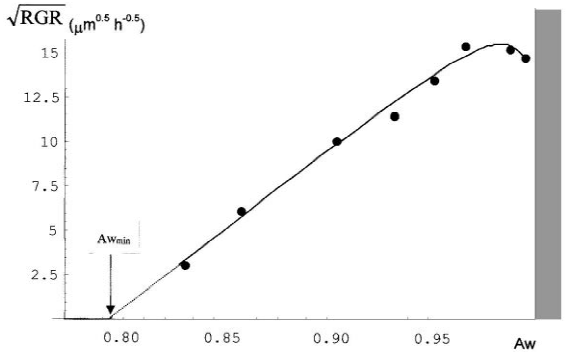

Fig. 2. Fit of model 3 on data sets of *Aspergillus nomius* (Pitt and Miscamble, 1995) obtained at 25°C in medium in which *Aw* was controlled by glucose/fructose mixture.

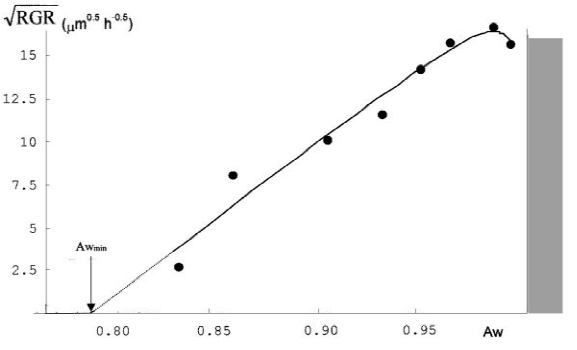

Fig. 3. Fit of model 3 on data sets of *Aspergillus oryzae* (Pitt and Miscamble, 1995) obtained at 25°C in medium in which *Aw* was controlled by glucose/fructose mixture.

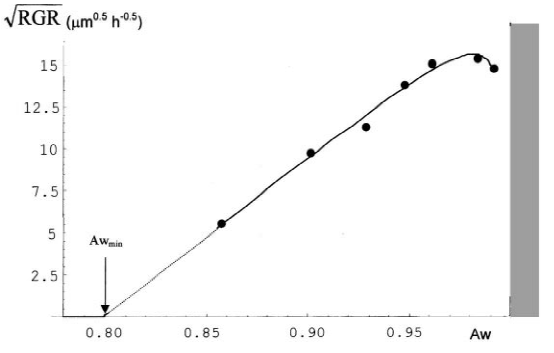

Fig. 4. Fit of model 3 on data sets of *Aspergillus parasiticus* (Pitt and Miscamble, 1995) obtained at 25°C in medium in which *Aw* was controlled by glucose/fructose mixture.

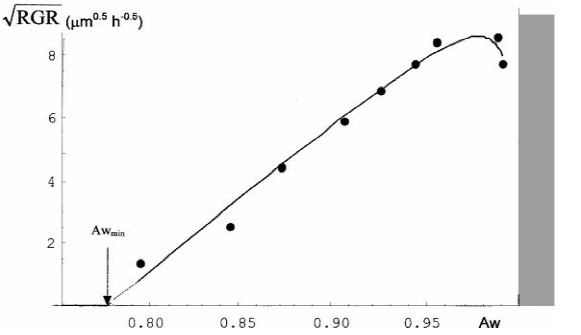

Fig. 5. Fit of model 3 on data sets of *Aspergillus candidus* (Wheeler and Hocking, 1988) obtained at 25°C in medium in which *Aw* was controlled by glucose/fructose mixture.

excellent descriptive ability. Table 2 presents the estimations of the parameter values and their MCIs for all cases tested. A systematic deviation should be identified in Figs. 1–4 at $Aw = 0.925$, but this observation has to be considered with caution given the precision of the corresponding experimental points.

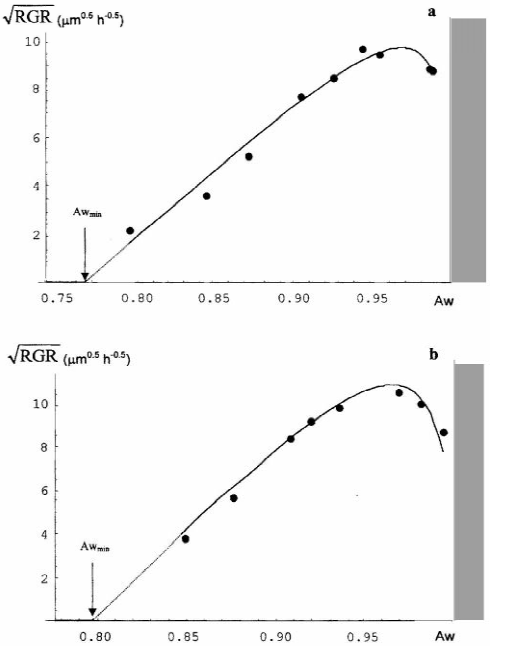

Fig. 6. Fit of model 3 on data sets of *Aspergillus sydowii* (Wheeler and Hocking, 1988) obtained at 25°C in medium in which *Aw* was controlled by NaCl (a) and glucose/fructose mixture (b).

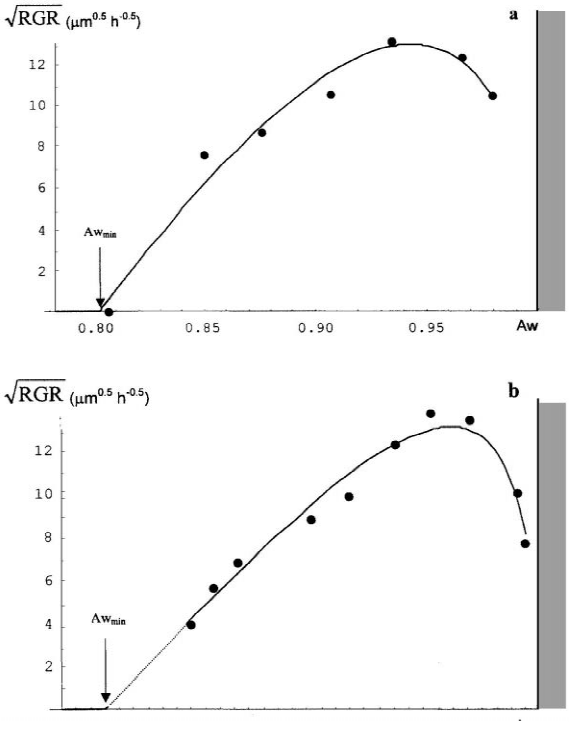

Fig. 7. Fit of model 3 on data sets of *Eurotium amstelodami* (Wheeler and Hocking, 1988) obtained at 25°C in medium in which *Aw* was controlled by NaCl (a) and by glucose/fructose mixture (b).

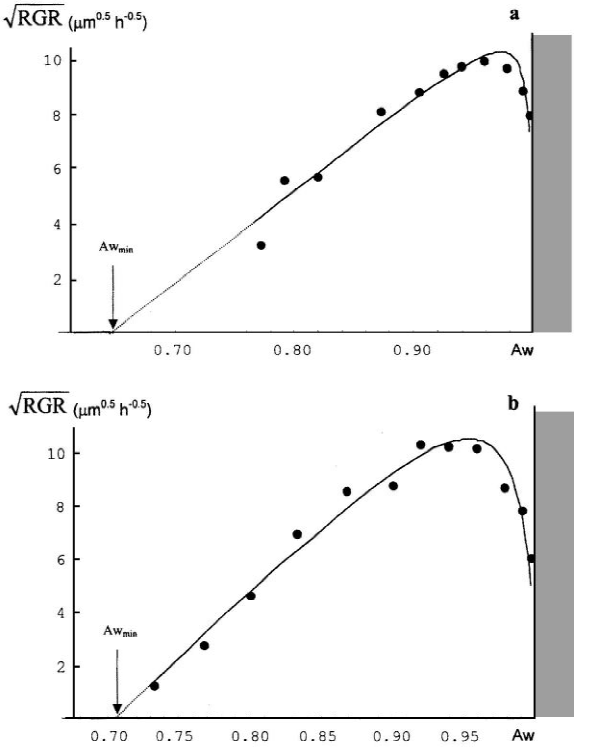

Fig. 8. Fit of model 3 on data sets of *Eurotium chevalieri* (Pitt and Hocking, 1977) obtained at 25°C in medium at pH 4.0 (a) and at pH 6.5 (b) in which *Aw* was controlled by glycerol.

For *A. flavus*, *A. oryzae*, *A. parasiticus*, and *A. nomius* an excellent quality of fit was obtained at 25°C (Fig. 1 to 4), but also at 30°C and 37°C (data not shown). For these three temperatures, no significant difference between cardinal *Aw* (Aw_{min} and Aw_{opt}) was observed taking into account the MCI of each estimation (Table 2). This is perhaps not surprising as the temperatures for which data was available were in the region of the optimal growth temperature. A difference might have been observed at temperatures approaching the minimum or maximum for growth. In medium in which *Aw* was controlled by glucose/fructose mixture, an average of Aw_{min} and Aw_{opt} was respectively estimated as 0.783, and 0.985 for *A. flavus*, 0.786 and 0.981 for *A. nomius*, 0.779 and 0.985 for *A. oryzae*, and 0.794 and 0.980 for *A. parasiticus*. Contrarily to the cardinal values, for each species, the RGR_{opt} value at 25°C was significantly different from the values estimated at 30°C and 37°C.

The cardinal parameters estimated for *A. candidus* in a medium in which the *Aw* was controlled by glucose/fructose mixture, are close to the values obtained for the other *Aspergillus* species.

The study of the fit on data sets *A. sydowii*, *E. amstelodami*, and *E. chevalieri*, shows a significant effect of solute type on Aw_{min} but not on Aw_{opt} (see Table 2), a phenomenon noted by Scott (1957). Moreover, for each solute and each parameter the difference between the estimated values at pH 4.0 and pH 6.5 is not systematically significant: Only Aw_{min}(NaCl), Aw_{min}(glycerol), Aw_{opt}(glycerol), and Aw_{opt}(glucose/fructose) of *E. chevalieri* seem to be significantly influenced by these difference of pH.

Finally, the model also gave a good quality fit for the extreme xerophile *X. bisporus*, the *RGR* evolution of which is very different to the other species (Fig. 11). In this case, the particular behaviour of this strain, may be well described by the model.

All these results show a good robustness of the model, considering the variety of strains, solutes, temperatures (only around optimal) and pH tested.

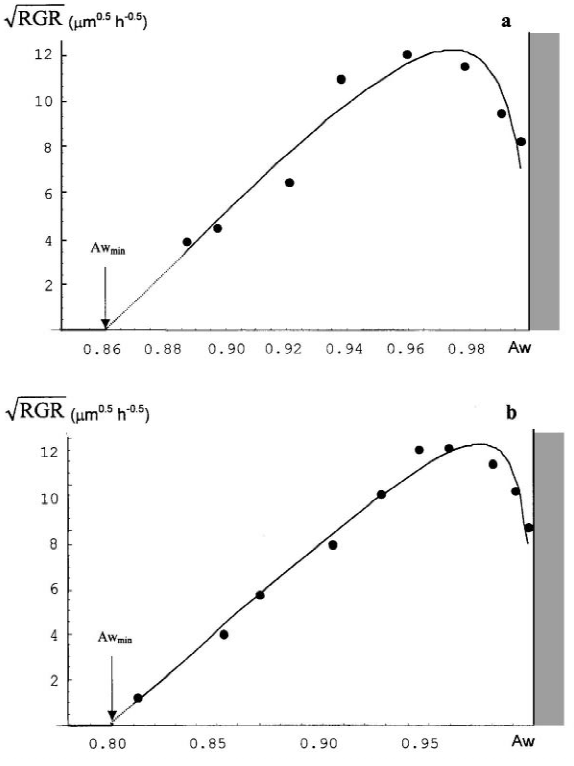

Fig. 9. Fit of model 3 on data sets of *Eurotium chevalieri* (Pitt and Hocking, 1977) obtained at 25°C in medium at pH 4 (a) and pH 6.5 (b) in which *Aw* was controlled by NaCl.

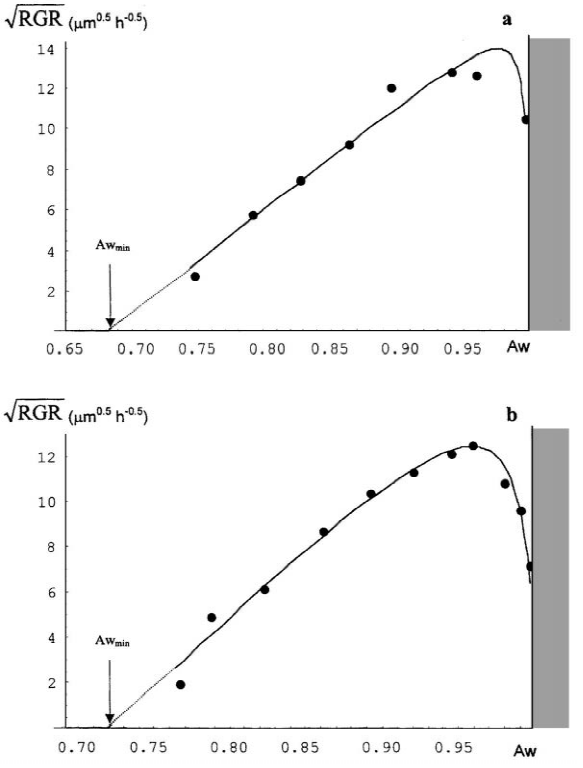

Fig. 10. Fit of model 3 on data sets of *Eurotium chevalieri* (Pitt and Hocking, 1977) obtained at 25°C in medium at pH 4 (a) and at pH 6.5 (b) in which *Aw* was controlled by glucose/fructose mixture.

Nevertheless, some divergence was observed for *E. chevalieri* in the neighbourhood of the optimal water activity (see Fig. 8a, Fig. 9a and Fig. 10a). This divergence, observed at low pH and with glycerol and glucose/fructose mixture, is due to the constraint imposed on the model through the $Aw_{max} = 1.0$.

Similarly the fit for *X. bisporus* has the $Aw_{max} = 1.0$. For the data range plotted, ($Aw = 0.68–0.96$) this does not cause a bad fit. However, for this extreme xerophile, if the data was available to extend this plot towards $Aw = 1.0$, it would be seen that there would be increasing divergence between the prediction and the data, due to *X. bisporus* having a maximum *Aw* significantly less than 1.0 (Pitt and Hocking, 1977; Pitt, 1989).

In fact, in both cases this divergence concerns only the $Aw_{opt}–Aw_{max}$ range and, given the lack of an important structural correlation between parameters of the cardinal model family (Rosso, 1995; Rosso et al., 1995), this divergence has a low impact on the sub-optimal and minimum *Aw* domain, as demonstrated in Fig. 11. As summarised in Table 3, the fits computed by fixing the Aw_{max} to 0.98, 0.99 and 1.0, gave non significantly different estimations of Aw_{min}, Aw_{opt} and RGR_{opt} considering their associated MCI and the precision of the points. Nevertheless, these results have not to be generalised to all xerotrophic and xerotolerant moulds and it may be possible that it could be necessary to consider the complete form of the model 2 (with Aw_{max} as a parameter) to describe some of these type of moulds.

3.2. Example of prediction of Aw effect on growth of moulds

Model 3 was used to predict the *RGR* values of *P. roqueforti* and *Pa. variotii*, as a function of the water activity. Table 1 shows the cardinal values (Aw_{min} and Aw_{opt}) extracted from the literature that were

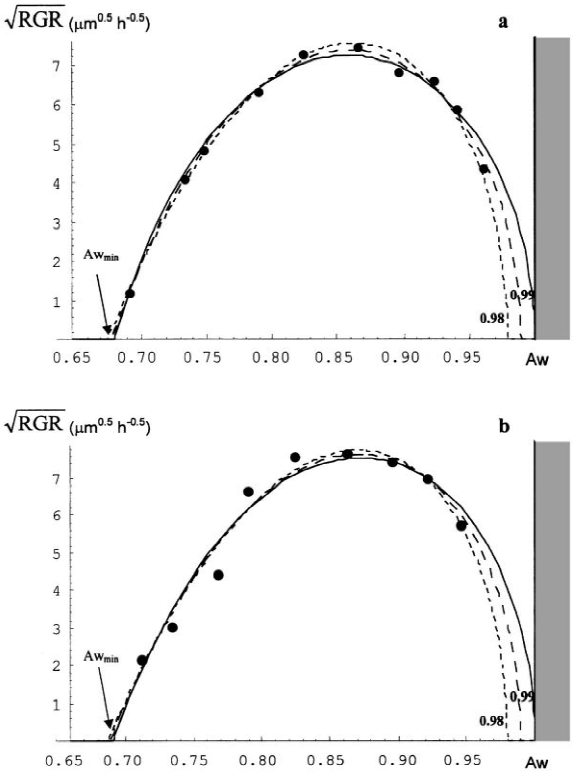

Fig. 11. Fit of model 3 on data sets of *Xeromyces bisporus* (Pitt and Hocking, 1977) obtained at 25°C in medium at pH 4 (a) and at pH 6.5 (b) in which *Aw* was controlled by glucose/fructose mixture, with Aw_{max} constrained to 1.000, 0.990 and 0.980.

used for each strain to produce this prediction. The RGR_{opt}, specifying the growth abilities of the moulds in the medium studied for prediction, were extracted from data sets used to validate the prediction. For that, the points used to estimate RGR_{opt} were distinguished from the other points, because of their different status.

As shown in Figs. 12 and 13, the predicted curves are in agreement with the superimposed experimental points.

In conclusion, this paper has shown that a simple candidate model, defined from the cardinal model's family, may be used to describe and to predict the effect of water activity on radial growth rate of moulds. At this stage of development, it is important to extend the validation of the model by fitting it on larger data sets, to confirm its robustness, already demonstrated here, on 9 species. Given the simplicity of use for prediction, and given the first successful prediction obtained for two new strains in laboratory medium, it would be interesting to compare predicted and observed *RGR* values for mould growth in foods. For that it will be important to take into account the dependency of the cardinal values, especially Aw_{min}, on the solutes used to control the *Aw*.

Moreover, it would be important to test this model with bacterial and yeast specific growth rates. If it is able to describe the influence of *Aw* on μ_{max} of these

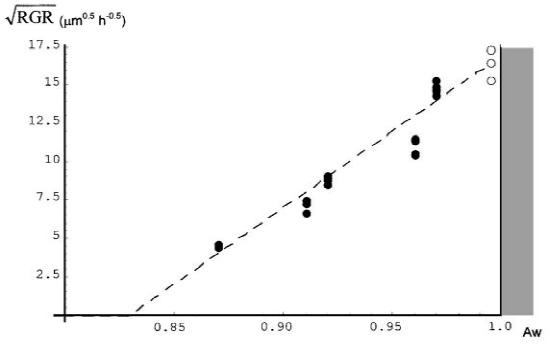

Fig. 12. Prediction of the effect of *Aw* on the radial growth rate (*RGR*) of *Penicillium roqueforti* in growth medium in which *Aw* was controlled by NaCl. Dashed line represents the prediction curve obtained with model 3 and parameter values extracted from the literature. RGR_{opt} was estimated from Valik et al. (1999) in the same medium at 0.995 (white points). The other experimental points observed by Valik et al. (1999) in the same medium at different pH values (black points), are superimposed on the curve.

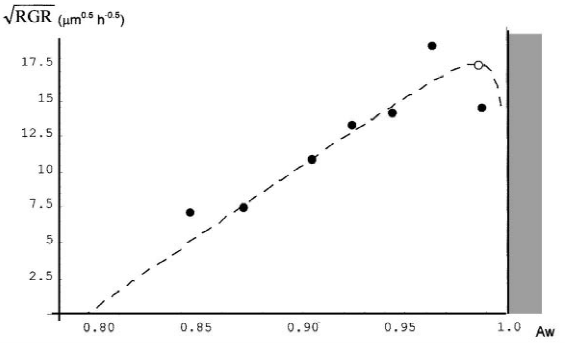

Fig. 13. Prediction of the effect of *Aw* on the radial growth rate (*RGR*) of *Paecilomyces variotii* in growth medium in which *Aw* was controlled by glucose/fructose mixture. Dashed line represents the prediction curve obtained with model 3 and parameter values extracted from literature. Point used to estimate RGR_{opt} (white point) is distinguished from the other experimental points observed by the authors (black points) and superimposed to the curve.

Table 2
Estimations of Aw_{min}, Aw_{opt} and RGR_{opt} obtained by fit of model 3 on data sets of different moulds. Values in brackets: MC Interval values

Strain	T (°C)	pH	Solutes	Aw_{min}	Aw_{opt}	RGR_{opt} (μm h^{-1})
Aspergillus flavus	25	6.5	Glu.-fru[b].	0.797 *(0.777, 0.818)*	0.982 *(0.973, 0.991)*	248 *(217, 278)*
	30	6.5	Glu.-fru.	0.778 *(0.753, 0.803)*	0.990 *(0.980, 0.999)*	403 *(356, 450)*
	37	6.5	Glu.-fru.	0.773 *(0.757, 0.788)*	0.984 *(0.979, 0.990)*	429 (*393, 465)*
Aspergillus nomius	25	6.5	Glu.-fru.	0.793 *(0.778, 0.808)*	0.980 *(0.975, 0.986)*	242 *(222, 262)*
	30	6.5	Glu.-fru.	0.783 *(0.750, 0.816)*	0.987 *(0.976, 0.998)*	375 *(316, 433)*
	37	6.5	Glu.-fru.	0.782 *(0.762, 0.803)*	0.977 *(0.970, 0.984)*	322 *(283, 361)*
Aspergillus oryzae	25	6.5	Glu.-fru.	0.786 *(0.751, 0.822)*	0.983 *(0.969, 0.997)*	267 *(218, 315)*
	30	6.5	Glu.-fru.	0.778 *(0.754, 0.801)*	0.992 *(0.981, 1.000)*	368 *(324, 412)*
	37	6.5	Glu.-fru.	0.772 *(0.749, 0.795)*	0.979 *(0.971, 0.986)*	378 *(332, 423)*
Aspergillus parasiticus	25	6.5	Glu.-fru.	0.800 *(0.781, 0.819)*	0.981 *(0.975, 0.986)*	243 *(225, 262)*
	30	6.5	Glu.-fru.	0.792 *(0.771, 0.814)*	0.985 *(0.977, 0.992)*	377 *(333, 421)*
	37	6.5	Glu.-fru.	0.790 *(0.773, 0.807)*	0.975 *(0.968, 0.982)*	322 *(286, 359)*
Aspergillus candidus	25	*NC*[a]	Glu.-fru.	0.776 *(0.756, 0.795)*	0.977 *(0.969, 0.985)*	74 *(65.6, 82.4)*
Aspergillus sydowii	25	*NC*	NaCl	0.797 *(0.769, 0.825)*	0.965 *(0.957, 0.973)*	119 *(103, 135)*
	25	*NC*	Glu.-fru.	0.765 *(0.743, 0.788)*	0.968 *(0.960, 0.976)*	94 *(83.0, 104)*
Eurotium amstelodami	25	*NC*	NaCl	0.802 *(0.783, 0.821)*	0.944 *(0.929, 0.959)*	165 *(130, 200)*
	25	*NC*	Glu.-fru.	0.700 *(0.674, 0.727)*	0.940 *(0.930, 0.950)*	171 *(151, 191)*
Eurotium chevalieri	25	4	Glycerol	0.647 *(0.595, 0.698)*	0.974 *(0.967, 0.981)*	105 *(92.3, 117)*
	25	6.5	Glycerol	0.705 *(0.681, 0.729)*	0.955 *(0.946, 0.964)*	111 *(98.1, 124)*
	25	4	NaCl	0.860 *(0.832, 0.888)*	0.975 *(0.965, 0.985)*	147 *(107, 186)*
	25	6.5	NaCl	0.799 *(0.783, 0.815)*	0.975 *(0.970, 0.980)*	152 *(135, 169)*
	25	4	Glu.-fru.	0.682 *(0.644, 0.721)*	0.977 *(0.969, 0.984)*	193 *(162, 224)*
	25	6.5	Glu.-fru.	0.720 *(0.698, 0.742)*	0.960 *(0.954, 0.967)*	155 *(140, 171)*
Xeromyces bisporus	25	4	Glu.-fru.	0.680 *(0.671, 0.690)*	0.858 *(0.849, 0.867)*	52 *(47.8, 55.7)*
	25	6.5	Glu.-fru.	0.691 *(0.666, 0.716)*	0.872 *(0.851, 0.893)*	57 *(48.2, 65.9)*

[a] Non communicated.
[b] Glucose/fructose mixture.

Table 3
Estimations of Aw_{min}, Aw_{opt} and RGR_{opt} obtained by fit of model 2 with Aw_{max} fixed to three different values, on data sets of *X. bisporus*

pH	Aw_{max} value	Estimated parameters		
	fixed in model 2	Aw_{min} (MCI)	Aw_{opt} (MCI)	RGR_{opt} (MCI)
4	1.000	0.680 (0.671, 0.690)	0.858 (0.849, 0.867)	52.0 (47.8, 55.7)
	0.990	0.678 (0.672, 0.685)	0.859 (0.830, 0.864)	53.9 (51.4, 56.5)
	0.980	0.675 (0.666, 0.683)	0.862 (0.855, 0.868)	56.6 (53.4, 59.8)
6.5	1.000	0.691 (0.666, 0.716)	0.872 (0.851, 0.893)	57.0 (48.2, 65.9)
	0.990	0.690 (0.666, 0.712)	0.871 (0.853, 0.890)	58.5 (50.4, 66.7)
	0.980	0.687 (0.666, 0.710)	0.871 (0.855, 0.887)	60.3 (52.6, 68.0)

other microorganisms, then the biological significance of its parameters will readily allow the modeller to exploit the knowledge available in the literature.

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