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A new model for germination of fungi

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ABSTRACT

The objective of this study was to design a germination model dedicated to fungi. The percentage of germinated spores, P(%), depended on the maximum percentage of germination P_{max} (%), the germination time, τ (h) and a design parameter, d (-) according to : $P = P_{max}\left[1-\frac{1}{1+\left(\frac{t}{\tau}\right)^{d}}\right]$. The model was capable to fit satisfactorily either apparent symmetric and asymmetric shapes of germination curves. The accuracy of τ determined by using the logistic or the present model was at least twice that obtained by the Gompertz equation. In contrast to the logistic model, the new model is by essence asymmetric. Therefore, its use is consistent with skewed distributions of the individual germination times that were observed experimentally in many cases.

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1. Introduction

For fungi germination can be considered as the main step to focus on, because a product is spoiled as soon as visible hyphae can be observed (Dantigny et al., 2005a). A spore is considered to have germinated when the length of the longest germ tube is greater than or equal to the greatest dimension of the swollen spore (Dantigny et al., 2006). But since spores do not germinate at the same time, the distribution of the germination time among a population of spores should be considered. From the cumulative frequency distributions, germination curves can be drawn (Nguyen et al., 2010). The shape of the germination curves, percentage of germinated spores *versus* time, would therefore depend on these distributions. Right skewed distributions were observed experimentally, thus leading to asymmetric germination curves (Judet et al., 2008).

Many mycologists agreed to define the germination time, t_g (h), of a population of spores as the time required to obtain a percentage of germinated spores equal to 50% of the viable spores (Huang et al., 2001). Even assuming that the maximum percentage of viable spores equals 100%, the probability of examining the population of spores when exactly 50% of the spores had germinated is close to zero. Therefore the percentage of germinated spores, P(%), is plotted against time and kinetic models are used to estimate t_g.

Models were developed for the germination of fungi (Bosch et al., 1995; Gervais et al., 1988), but two models are widely used in predictive mycology. The Gompertz and the logistic equation that were used originally to fit the growth of bacteria (Zwietering et al., 1991) and to determine the time of growth and toxin production by *Clostridium botulinum* (Whiting and Call, 1993), respectively. Later the Gompertz and the logistic equations were used to fit the germination data of *Fusarium moniliforme* and *Fusarium proliferatum* (Marín et al., 1996) and *Mucor racemosus* (Dantigny et al., 2002), respectively.

Both models were tested against many data sets available in the literature (Dantigny et al., 2007). Based on RMSE values, it was impossible to determine which one of the models performed better than the other one. The Gompertz model is asymmetrical but the germination time cannot be determined directly thus leading to inaccuracy of this parameter, in addition to erroneous determination of the maximum percentage of germinated spores, P_{max} (%), in some cases. In contrast, the logistic equation provided accurate estimations of P_{max} and t_g, but concerns were raised because attempting to fit the symmetrical logistic function to actual asymmetrical data sets seemed inappropriate.

Therefore the objective of the present study was to design an asymmetric model based on the maximum percentage of germination and the germination time. Firstly, the characteristics of the asymmetric model were detailed. Secondly, the asymmetric model was tested against the Gompertz and the logistic equations on germination data that exhibited significant overestimates of P_{max} (Dantigny et al., 2005b) and skewed distributions of the individual germination times (Judet et al., 2008). Thirdly, the asymmetric model was fit to the germination data obtained from the literature for different species.

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2. Material and methods

2.1. Logistic model

The logistic function is:

$$P = \frac{Pmax}{1 + \exp[k(\tau - t)]} \quad (1)$$

where P_{max} (%) is the asymptotic P value at $t \to +\infty$, τ (h) is the inflection point where P equals half of the P_{max}, t is the time (h) and k (h^{-1}) is related to the slope of the tangent line through the inflection point. The slope of the tangent line at τ, is equal to $k \cdot P_{max}/4$ (Dantigny et al., 2007). The germination time t_g is equal to τ.

2.2. Gompertz model

The modified Gompertz equation is:

$$P = A.\exp.\left(-\exp\left[\frac{\mu_m.e(1)}{A}(\delta - t) + 1\right]\right) \quad (2)$$

where A (%) is the asymptotic P value at $t \to +\infty$, μ_m ($\%h^{-1}$) is the slope term of the tangent line through the inflection point (t_i) as defined further, δ (h) is the t-axis intercept of the tangent through the inflection point and t is the time (h).
The inflection point is $t_i = \delta + A/(\mu_m e(1))$ (Dantigny et al., 2003).

The germination time t_g (h) can be determined for $P = A/2$ as follows:

$$\exp\left(-\exp\left[\frac{\mu_m e(1)}{A}\right](\delta - t_g) + 1\right) = 0.5 \quad (3)$$

$$\left(-\exp\left[\frac{\mu_m e(1)}{A}\right](\delta - t_g) + 1\right) = \ln(0.5) \quad (4)$$

$$\left[\frac{\mu_m e(1)}{A}\right](\delta - t_g) = \ln(-\ln(0.5)) - 1 \quad (5)$$

$$(\delta - t_g) = \frac{[\ln(-\ln(0.5)) - 1]A}{\mu_m e(1)} \quad (6)$$

Eventually,

$$t_g = \delta - \frac{[\ln(-\ln(0.5)) - 1]A}{\mu_m e(1)}. \quad (7)$$

The relative accuracy of t_g can also be deduced from Eq. (6) using the differential form of the logarithm.

$$\frac{d(\delta - t_g)}{(\delta - t_g)} = \frac{d(\ln(-\ln(0.5)) - 1)}{\ln(-\ln(0.5)) - 1} + \frac{dA}{A} - \frac{d\mu_m}{\mu_m} - \frac{de(1)}{e(1)} \quad (8)$$

The constant terms can be omitted, then:

$$\frac{d\delta}{(\delta - t_g)} - \frac{dt_g}{(\delta - t_g)} = \frac{dA}{A} - \frac{d\mu_m}{\mu_m} \quad (9)$$

$$\frac{dt_g}{t_g} = \left[\frac{d\delta}{(\delta - t_g)} - \frac{dA}{A} + \frac{d\mu_m}{\mu_m}\right]\frac{(\delta - t_g)}{t_g}. \quad (10)$$

Eventually,

$$\frac{\Delta t_g}{t_g} = \left[\frac{\Delta\delta}{(\delta - t_g)} + \frac{\Delta A}{A} + \frac{\Delta\mu_m}{\mu_m}\right]\frac{(\delta - t_g)}{t_g}. \quad (11)$$

By comparison, for the logistic model and the asymmetric model (see below), $\frac{\Delta t_g}{t_g} = \frac{\Delta\tau}{\tau}$.

2.3. Asymmetric model

The asymmetric model:

$$P = P_{max}\left[1 - \frac{1}{1 + \left(\frac{t}{\tau}\right)^d}\right] \quad (12)$$

is derived from the non competitive inhibition model described by Yano and Koya (1973). P_{max} is the asymptotic P value at $t \to +\infty$, τ(h) the point where P equals half of the P_{max}. The germination time t_g is equal to τ. The different shapes of the curves for different values of the design parameter $d > 0$ are shown in Fig. 1. For $d = 1$ the shape of the curve differed from the S-shaped germination curve. For $d = 2$, the percentage of germinated spores increases shortly after the origin. This is contradictory to the observation of a swelling period before the germinating tube can be formed and the spore germinated. Therefore, in practice the asymmetric model is a candidate for fitting the germination data for d values greater than 2 only.

It is shown in Fig. 1, that if the curve is symmetric, symmetry would be observed with respect to the point defined by $t/\tau = 1$ and $P/P_{max} = 0.5$. Assuming $0 \le x = t/\tau < 1$.

For x, the percentage of germinated spores is:

$$\frac{P_x}{P_{max}} = 1 - \frac{1}{1 + x^d}. \quad (13)$$

With respect to $x = 1$, the symmetric of x is $(2 - x)$.

For $(2 - x)$, the percentage of germinated spore is:

$$\frac{P_{2-x}}{P_{max}} = 1 - \frac{1}{1 + (2-x)^d}. \quad (14)$$

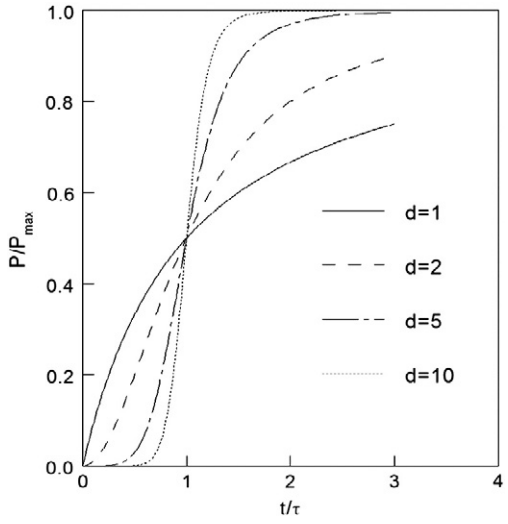

Fig. 1. Effect of the design parameter d on the shape of the asymmetric model.

Table 1
Estimates of the percentage of viable conidia of *Penicillium chrysogenum* exposed to vapors generated by 2.5% (v/v) ethanol (data from Dantigny et al., 2005b) determined by three germination models for three replicates (runs 1 to 3).

Run	Model					
	Logistic		Gompertz		Asymmetric	
	P_{max} (%)	t-value	A (%)	t-value	P_{max} (%)	t-value
1	98.2 [91.2; 105.2]*	34.3	105.2 [98.8; 111.5]	40.5	100.9 [93.6; 108.1]	33.9
2	99.0 [90.5; 107.4]	28.8	107.7 [95.1; 120.3]	20.9	102.2 [92.4; 112.0]	25.4
3	100.5 [94.1; 106.8]	38.8	108.2 [101.1; 115.3]	37.3	103.3 [96.6; 110.1]	37.4

* 95% confidence intervals in brackets.

If the model is symmetric, it should be demonstrated that

$$\frac{P_x}{P_{max}} + \frac{P_{2-x}}{P_{max}} = \left[1-\frac{1}{1+x^d}\right] + \left[1-\frac{1}{1+(2-x)^d}\right] = 1 \quad (15)$$

$$1 = \frac{1}{1+x^d} + \frac{1}{1+(2-x)^d} \quad (16)$$

$$1 = \frac{\left(1+x^d\right)+\left(1+(2-x)^d\right)}{(1+x^d)(1+(2-x)^d)} \quad (17)$$

Developing and simplifying,

$$[(2-x)x]^d = 1. \quad (18)$$

The equation has no solution because x differed from 1, therefore the model is asymmetric.

2.4. Data sets

The raw germination data, percentage germination P (%) *versus* time t (h), were obtained for *Aspergillus carbonarius* (Mitchell, 2006), *Aspergillus ochraceus* (Pardo et al., 2004), *Fusarium verticillioides* and *Fusarium proliferatum* (Marín et al., 1996), *Gibberella zeae* (Beyer et al., 2005), *M. racemosus* (Dantigny et al., 2002), *Penicillium chrysogenum* (Dantigny et al., 2005b; Judet et al., 2008) and *Penicillium verrucosum* (Pardo et al., 2006).

2.5. Model fitting

Non linear regressions were performed by using SlideWrite 5.0 (Advanced Graphics Software, Inc., Carlsbad, CA, USA). This software was based upon the Levenberg–Marquardt Algorithm. The goodness of fit was evaluated by means of the root mean square error, RMSE, (Ratkowsky, 2004) and extracted from ANOVA tables.

3. Results

3.1. Estimation of the percentage of viable spores

The estimates of the percentage of viable spores are reported for three replicates (run 1 to 3) assessing the effect of ethanol vapors generated by 2.5% (v/v) solutions on the germination of *P. chrysogenum*, Table 1. An overestimation of P_{max} was shown for the Gompertz and the asymmetric models for runs 1 and 2, but the 100% value remained within the 95% confidence intervals. A clear overestimation of P_{max} was demonstrated for the Gompertz model for run 3. In contrast, the asymmetric model did not provide any erroneous value for P_{max}. It was also shown that the confidence interval for the asymmetric model was not wider than that for the Gompertz model as demonstrated by almost identical t-values, Table 1. Therefore the fact that P_{max} did not differ significantly from 100% with the asymmetric model was not due to a wider confidence interval than that calculated for the Gompertz model. For all the other data sets tested in the present study, no significant overestimation of P_{max} was noticed for any of the three models (data not shown).

3.2. Accuracy of the models

Based on RMSE values, it was shown that the asymmetric model performed better than the logistic one, for fitting the germination curves obtained from skewed distributions, experiments (a) and (d), Table 2. However, the Gompertz and the asymmetric model exhibited the lowest RMSE value for experiment (a) and (d), respectively. Surprisingly, the logistic model did not perform better than the two other models for fitting germination curves obtained from un-skewed distributions, experiments (b) and (c). For the other data sets tested in the present study, the logistic, the Gompertz and the asymmetric model performed better in 14, 12 and 8 cases, respectively (Table 3). When the asymmetric model did not perform better, it was ranked second in 22 cases out of 26, third in only 4 cases.

3.3. Estimation of the germination time

For experiments (b) and (c), the germination times obtained with the logistic and the asymmetric models differed, almost non-

Table 2
Estimates of the germination time of *Penicillium chrysogenum* (data from Judet et al., 2008) determined by three germination models for different distributions of the individual germination times and estimates of the design parameter for the asymmetric model.

Distribution of individual germination times	Model						
	Logistic		Gompertz		Asymmetric		
	τ (h)	RMSE	t_g (h)	RMSE	τ (h)	RMSE	d (-)
Skewed (a)	33.5 [33.1; 33.9]*	1.691	33.4 [32.5; 34.3]	1.055	33.5 [33.1; 33.9]	1.451	13.7 [11.9; 15.6]
Un-skewed (b)	44.8 [44.5; 45.1]	1.257	44.4 [43.6; 45.2]	1.037	44.9 [44.6; 45.1]	1.074	15.7 [14.5; 16.9]
Un-skewed (c)	8.73 [8.66; 8.80]	0.771	8.94 [8.46; 9.42]	1.323	8.72 [8.71; 8.73]	0.100	17.3 [17.0; 17.5]
Skewed (d)	10.6 [10.5; 10.7]	1.741	10.6 [10.3; 10.9]	2.181	10.6 [10.5; 10.7]	1.474	20.7 [17.4; 24.1]

(a) $S = 0.987$, $p < 0.001$; (b) $S = 0.298$, $p = 0.306$; (c) $S = 0.426$, $p = 0.131$; (d) $S = 0.585$, $p = 0.026$. S: skewness.
* 95% confidence intervals in brackets.

Table 3
Determination of RMSE values for different set of germination data taken from the literature adjusted to the models. *Aspergillus carbonarius* (Mitchell, 2006), *A. ochraceus* (Pardo et al., 2004), *Fusarium proliferatum* (Marín et al., 1996), *Gibberella zeae* (Beyer et al., 2005), *Mucor racemosus* (Dantigny et al., 2002), *Penicillium verrucosum* (Pardo et al., 2006).

Fungus	*Aspergillus carbonarius*						
Factor	Water activity/Temperature (°C)	0.90/25	0.90/30	0.90/35	0.90/40	0.93/25	
Model	Logistic/Gompertz/Asymmetric	4.457/**4.005**/4.235	**3.680**/3.919/3.729	4.660/**4.170**/4.243	6.927/8.583/**6.888**	**4.026**/5.092/4.840	
Factor	Water activity/Temperature (°C)	0.95/15	0.95/20	0.95/25	0.95/30	0.95/35	0.95/40
Model	Logistic/Gompertz/Asymmetric	5.015/**4.559**/4.678	6.065/**5.302**/5.466	**4.405**/4.909/4.810	3.955/4.105/**3.793**	**6.192**/7.697/8.122	8.433/**8.252**/8.436
Factor	Water activity/Temperature (°C)	0.987/15	0.987/20	0.987/25	0.987/30	0.987/40	
Model	Logistic/Gompertz/Asymmetric	9.485/**8.254**/8.292	8.034/6.554/**6.064**	**6.249**/6.762/7.128	8.335/**8.322**/8.642	**4.636**/5.661/5.043	
Fungus	*Aspergillus ochraceus*						
Factor	Water activity	0.85	0.90	0.95	0.99		
Model	Logistic/Gompertz/Asymmetric	5.765/**5.312**/5.424	4.952/4.855/**4.834**	**5.140**/5.921/5.288	4.816/3.530/**2.858**		
Mold	*Fusarium proliferatum*						
Factor	Water activity	0.88	0.90	0.92	0.94	0.96	0.98
Model	Logistic/Gompertz/Asymmetric	**4.157**/4.944/4.508	**4.933**/5.255/5.038	**6.506**/7.477/6.843	**4.908**/5.791/5.236	4.280/**3.658**/3.824	2.158/2.378/**2.095**
Fungus	*Gibberella zeae*						
Factor	Kind of spores	Macroconidia	Ascospores				
Model	Logistic/Gompertz/Asymmetric	6.071/**5.707**/5.764	6.567/**5.867**/6.076				
Fungus	*Mucor racemosus*						
Factor	Temperature (°C)	15	25				
Model	Logistic/Gompertz/Asymmetric	7.463/7.453/**7.404**	**8.098**/8.592/8.134				
Fungus	*Penicillium verrucosum*						
Factor	Water activity	0.85	0.90	0.95	0.99		
Model	Logistic/Gompertz/Asymmetric	14.73/28.54/**14.59**	6.638/**6.107**/6.298	**6.290**/7.483/6.696	**3.604**/5.167/3.856		

Bold values indicated the best goodness of fit.

significantly, from that estimated with the Gompertz model, Table 2. For these experiments, the difference between the estimated germination differed from less than 0.1 h between the logistic and the asymmetric model. For experiments (a) and (d), no difference was shown between the estimates obtained by these models. The difference between the germination time obtained by the Gompertz model was not significantly different (ca. less than 0.1 h). In contrast, the confidence intervals for the germination time obtained by the Gompertz model were more than 2 fold, up to 6.9 fold for experiment (c), those obtained by the other models.

3.4. Estimation of the design parameter

The values of the design parameter d were significantly less for the experiments (a) and (b), than those obtained for the other experiments, Table 2. The smaller values of d (13.7 and 15.7) were obtained for shallow slopes of the germination curves, Fig. 2. The greatest values of d (17.3 and 20.7) were obtained for steeper slopes. In the latter conditions, a visual inspection cannot determine whether the germination curves were symmetrical or not.

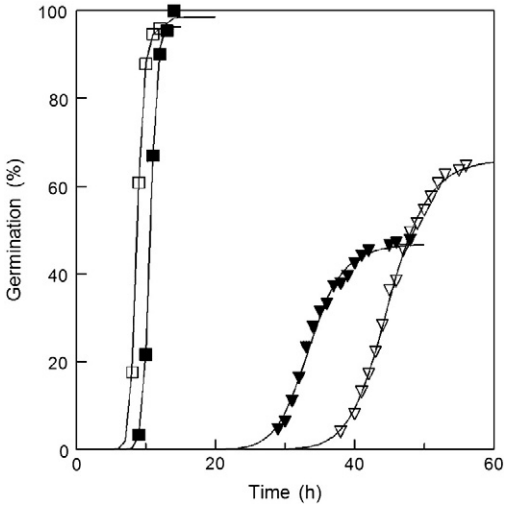

Fig. 2. Germination curves obtained for *Penicillium chrysogenum* by the asymmetric model, (■, ▼) skewed distributions and (□, ▽) un-skewed distribution. Data from Judet et al. (2008).

For the data set obtained for *F. verticillioides*, the values of d were significantly less, in the range 4.27–6.26, Table 4. The values of d did not depend on the water activity, whereas the slopes of the germination curves were clearly steeper at higher a_w, Fig. 3. The effect of d on the shape of the germination curves was shown in a dimensionless form, (i.e., independent from the value of τ), Fig. 1. Therefore the effect of d of the shape of the germination curves cannot be compared on Fig. 3, because the germination time depended on the water activity. However, for the same germination time and also the same percentage of viable spores, the slope of the curve increased with increasing the value of d. For example, the slope of the germination curve obtained for *P. chrysogenum* (experiment c) was greater than that of *F. verticillioides* (0.96 a_w). Although, this cannot be seen because the time-scales were different in Figs. 2 and 3.

The values of the design parameter depended on the experimental conditions. A value of d as low as 2.75 was obtained for *A. carbonarius* at 0.987 a_w/30 °C (Table 5). But, for this species, a value of d = 16.3 was obtained at 0.90 a_w/40 °C. There was no clear relationship between the environmental conditions and the value of the design parameter. However, for *P. verrucosum*, a trend in an increase of d with increasing a_w was noticeable. The values of d depended also on the fungi. Some species such as *F. proliferatum* and *G. zeae* exhibited d-values less than 10. This was also the case for *A. carbonarius* in 9 cases out of 16, Table 5. In contrast, *Mucor racemosus* was characterized by d-values greater than 10.

Table 4
Parameter estimates obtained by fitting the germination data of Fusarium verticillioides (Marín et al., 1996) to the asymmetric model.

Experimental conditions	Parameter estimates			
	P_{max} (%)	τ (h)	d (-)	RMSE
0.88 a_w	97.6 [86.9; 108.3]*	121 [110; 131]	4.27 [2.79; 5.76]	7.235
0.90 a_w	107 [98.4; 116]	54.3 [51.5; 57.1]	4.71 [3.76; 5.65]	3.842
0.92 a_w	105 [96.5; 114]	26.3 [24.7; 28.0]	4.46 [3.80; 5.12]	3.223
0.94 a_w	110 [96.7; 123]	13.3 [12.5; 14.0]	6.26 [4.83; 7.69]	4.535
0.96 a_w	114 [91.1; 138]	8.58 [7.82; 9.34]	6.00 [4.22; 7.78]	5.046
0.98 a_w	116 [94.6; 138]	6.64 [6.04; 7.23]	5.69 [4.08; 7.30]	5.128

* 95% confidence intervals in brackets.

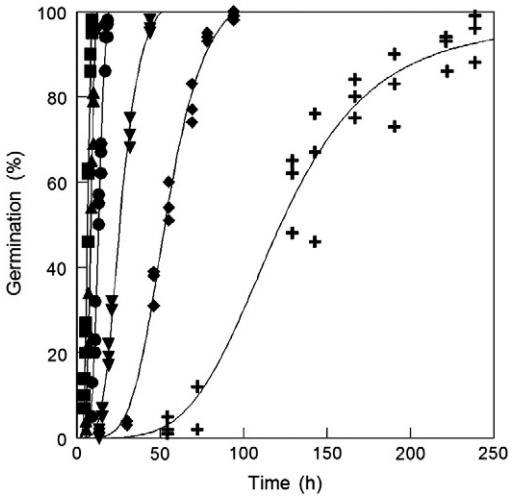

Fig. 3. Germination curves obtained for *Fusarium verticillioides* by the asymmetric model (■) 0.98 a_w, (▲) 0.96 a_w, (●) 0.94 a_w, (▼) 0.92 a_w, (◆) 0.90 a_w, (+) 0.88 a_w. Data from Marín et al. (1996).

4. Discussion

The goodness of fit of a model is often evaluated by the coefficient of determination or more accurately by the root mean square error, RMSE, for non-linear models (Ratkowsky, 2004). In order to determine which model fitted germination data sets better, two models (i.e., Gompertz and logistic) were compared previously (Dantigny et al., 2007). Based on RMSE values, it was impossible to determine which model performed better, because this depended on the fungi and the experimental conditions. This was also observed in the present study when comparing the RMSE values obtained by fitting the asymmetric and the two other models to data taken from the literature.

It should be reminded that one of the major roles of a model is to provide accurate estimations of a parameter that cannot be determined easily. These parameters are usually of some biological significance (i.e., growth rate, lag time, germination time, minimum inhibitory concentration, cardinal values for temperature and water activity, d-values, …). All these parameters are also characterized by definitions widely admitted by the scientific community. The definition of the germination time varied greatly, depending on the objective. For the production of starters or metabolites, a typical value was 90% but in food mycology the value was usually 10% (Magan and Lacey, 1984). A germination time based on a given percentage of inoculated spores depends on the value of the three parameters whatever the model. For example, the time at which 10% of the inoculated spores had germinated is $t_{10\%} = \frac{\tau - \ln\left(\frac{P_{max}}{10} - 1\right)}{k}$ with the logistic model. Whatever the percentage of germination, 10%, 50% or 90%, all models would provide inaccurate estimations of the germination time because none of the model had been designed for determining this parameter.

It is better to base the definition of the germination time on a certain percentage of the viable spores, not the inoculated spores. A practical interest of the definition is whatever the percentage of viable spores, the germination time can be determined. For example, if the germination time is defined as the time required to have 50% of the inoculated spores, this time cannot be determined if the percentage of viable spores is 40% only. In contrast, 50% of the viable spores is synonymous to 20% of the inoculated spores. During the last workshop dedicated to germination, it was recommended to define the germination time of a population of spores as the time required to have 50% of the viable spores germinated (Huang et al., 2001; Dantigny et al., 2006). Among the viable spores, 50% was a good trade-off between 10 and 90%. It was demonstrated in the present study that a more accurate determination of the germination time was obtained when the model (i.e., logistic and asymmetric models) was designed for providing this parameter. It was also shown that the estimation of the germination time did not depend on the model. Therefore there is no penalty in using the asymmetric model for future studies because the germination time can be compared to values determined in previous studies by the other models.

The percentage of viable spores is also an important parameter for assessing germination. In the present study, P_{max} values greater than 100% (although not significant) were obtained by fitting the asymmetric model to germination data of *F. verticillioides*. This effect was also observed with the Gompertz model. It was partly due to the sampled data. Whereas all spores were probably viable, as suggested by the estimates of the maximum percentage of germination, the last recorded values of P were less than 100%. The overestimation can be corrected by collecting data until 100% germination or by setting the maximum percentage of germination to 100% (Marín et al., 1996).

Table 5
Estimation of the design parameter d for different set of germination data taken from the literature. *Aspergillus carbonarius* (Mitchell, 2006), *A. ochraceus* (Pardo et al., 2004), *Fusarium proliferatum* (Marín et al., 1996), *Gibberella zeae* (Beyer et al., 2005), *Mucor racemosus* (Dantigny et al., 2002), *Penicillium verrucosum* (Pardo et al., 2006).

Fungus	*Aspergillus carbonarius*						
Factor	a_w/Temperature (°C)	0.90/25	0.90/30	0.90/35	0.90/40	0.93/25	
d	Estimate [95% CI]	6.50^{ac} [5.16;7.85]	5.74^{ac} [4.92;6.57]	7.66^{a} [6.28;9.03]	16.3^{b} [9.24;23.4]	4.83^{c} [3.91;5.75]	
Factor	a_w/Temperature (°C)	0.95/15	0.95/20	0.95/25	0.95/30	0.95/35	0.95/40
d	Estimate [95% CI]	4.66^{acd} [3.72;5.60]	4.60^{acd} [3.55;5.66]	11.7^{ab} [8.13;15.2]	6.19^{ac} [5.16;7.23]	3.29^{cd} [2.12;4.47]	2.94^{d} [2.10;3.80]
Factor	a_w/Temperature (°C)	0.987/15	0.987/20	0.987/25	0.987/30	0.987/40	
d	Estimate [95% CI]	3.39^{cd} [2.02;4.77]	3.17^{d} [2.48;3.86]	3.59^{cd} [2.74;4.44]	2.75^{d} [1.97;3.53]	7.00^{ac} [5.67;8.32]	
Fungus	*Aspergillus ochraceus*						
Factor	Water activity	0.85	0.90	0.95	0.99		
d	Estimate [95% CI]	9.46^{a} [8.09;10.8]	18.5^{b} [15.5;21.5]	10.8^{a} [8.66;12.9]	10.6^{a} [9.22;12.0]		
Mold	*Fusarium proliferatum*						
Factor	Water activity	0.88	0.90	0.92	0.94	0.96	0.98
d	Estimate [95% CI]	6.57^{ab} [5.25;7.89]	6.13^{ab} [4.53;7.74]	9.25^{b} [6.76;11.7]	8.47^{b} [6.43;10.5]	5.30^{a} [4.25;6.35]	7.00^{ab} [6.33;7.67]
Fungus	*Gibberella zeae*						
Factor	Kind of spores	Macroconidia	Ascospores				
d	Estimate [95% CI]	7.10^{a} [6.10; 8.11]	7.80^{a} [6.25; 9.35]				
Fungus	*Mucor racemosus*						
Factor	Temperature (°C)	15	25				
d	Estimate [95% CI]	22.2^{a} [14.5;29.8]	31.1^{a} [22.6;39.6]				
Fungus	*Penicillium verrucosum*						
Factor	Water activity	0.85	0.90	0.95	0.99		
d	Estimate [95% CI]	5.05^{a} [1.49; 8.60]	5.70^{a} [3.25;8.15]	11.7^{ab} [7.36;16.1]	15.3^{b} [12.9;17.7]		

Different letters for the same species denoted significant differences in the value of d.

Another important but neglected quality of a model is the consistency with the observed experimental responses and the underlying mechanisms of development of the organisms. It was demonstrated that germination curves exhibited different shapes, apparently symmetric or clearly asymmetric. It was already noted for bacteria that attempting to fit the symmetrical logistic function to actual asymmetrical data sets seemed inappropriate (Skinner et al., 1994). The main advantage of the asymmetric model was its versatility. The capability of this model to adjust satisfactorily symmetric and asymmetric shapes of germination curves was highlighted in the present study. This versatility was obtained through the design parameter d. Depending on the species and the environmental conditions, the values of d varied in the range 3–30. The lowest and the greatest values of d were obtained for *A. carbonarius* and *M. racemosus* respectively. Regardless of the germination time, the germination curves appeared steeper and more symmetric as the values of d increased.

Lastly but not least, the facility of convergence is an important criterion for using a model. The convergence is usually ensured if the initial values of the parameters are close to the final estimates (Zwietering, personal communication). The asymmetric model can be used easily by mycologists because it is based on biological parameters. The initial values of the maximum percentage of germination, P_{max} and the germination time, τ can be evaluated by a rough inspection of the data. The design parameter depended on the environmental conditions and on the species. But by using the initial value $d = 10$, the non-linear regression software used in the present study did not fail to converge.

5. Conclusions

In predictive mycology, all the existing models were previously used for bacteria, although not necessarily for the same purpose. Up to our knowledge, this study described the first model dedicated to the germination of fungi. The asymmetric model was based on the definition of the germination time widely accepted by food mycologists and also plant pathologists. The versatility of the model was obtained by the use of a design parameter. For values in the range 3–30, the asymmetric model fitted many germination data sets described in the literature satisfactorily. It was shown that other interesting shapes can be obtained for values of d less than or equal to 2. Therefore, in the present study, only one example of the potential applications of the equation was shown. There are many possibilities that the equation can be used, maybe after some transformations, to model inhibition or inactivation kinetics observed not only in fungi but also in bacteria or other organisms.

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