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Predictive modelling of temperature and water activity (solutes) on the *in vitro* radial growth of *Botrytis cinerea* Pers

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Abstract

The objective of this work was to develop validated models predicting the 'in vitro' effect of a_w and temperature on the radial growth of *Botrytis cinerea*. The growth rate (g, mm d^{-1}) of *B. cinerea* was calculated at three incubation temperatures (25 °C, 15 °C, 5 °C) and six water activities (ranging from 0.995 to 0.890). The water activity was adjusted with glucose, NaCl, glycerol, or sorbitol. Statistical analysis showed a significant effect of temperature, solute, a_w, and their two- and three-way interactions on the growth rate. No growth was observed at $a_w = 0.93$ in the presence of NaCl or at 0.89 in the presence of a non-ionic solute. The maximum colony growth rate decreased when the incubation temperature and water activity was lowered. Secondary models, relating the colony growth rate with a_w or a_w and temperature were developed. Optimum a_w values for growth ranged from 0.981 to 0.987 in glycerol-, sorbitol-, or glucose-modified medium and were close to 1 in NaCl-modified medium. A quadratic polynomial equation was used to describe the combined effects of temperature and a_w on g (mm d^{-1}) in the presence of each solute. The highest and lowest radial growth rates were observed in models based on glucose and NaCl respectively, whatever the incubation temperature. All models prove to be good predictors of the growth rates of *B. cinerea* within the limits of experiments. The quadratic polynomial equation has bias factors of 0.957, 1.036, 0.950, and 0.860 and accuracy factors of 1.089, 1.070, 1.120 and 1.260 in media supplemented with glucose, NaCl, glycerol and sorbitol respectively. The results from modelling confirm the general finding that a_w has a greater influence on fungal growth than temperature.
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Keywords: Solute; Temperature; Growth rate; Water activity; *B. cinerea*; Predictive modelling

1. Introduction

Botrytis cinerea Persoon: Fries (known as "grey mould") causes serious pre- and post-harvest diseases in at least 235 plant species (Jarvis, 1977; Agrios, 1988), including a range of agronomically important crops such as grapes, apples, pears, tomatoes, strawberries, cucumbers, bulb flowers, and ornamental plants. *B. cinerea* control strategy mainly relies on chemical treatments. Nevertheless alternative strategies, such as integrated pest management, including a combination of several control methods (farming practices, chemical and biological control) are under development (Köhl et al., 1995; Elad, 1996; Jijakli and Lepoivre, 1998). Indeed, effective integrated pest management control of this pathogenic fungus relies on good knowledge of infection cycle in order to reduce the number of unnecessary spray applications when *B. cinerea* is not threatening and to improve the timing of spray applications when conditions are favourable for disease development (Ellison et al., 1998a). An expert system for management of *B. cinerea* was developed and validated in Australian vineyards (Ellison et al., 1998a,b). Information given by this system led to a reduction of the number of chemical spray as compared to classical chemical approach.

Because the optimal temperature for mycelial growth, sporulation, and conidial germination ranges from 18 to 23 °C, *B. cinerea* is essentially present in temperate and subtropical regions (Bondoux, 1992). Nevertheless, the pathogen is also active at lower temperatures since conidium germination and mycelial growth can occur at temperatures as low as 0 °C (Jarvis, 1980; Agrios, 1988; Gindro and Pezet, 2001). Under favourable conditions, a complete infection cycle can occur in 3 to 4 days, depending on the type of host tissue attacked.

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After the contact between conidium and the host tissue, a number of factors influence its germination. Free surface water or a high relative humidity (>93% RH) is required for germination and penetration of the host epidermis (Williamson et al., 1995). Moisture helps the pathogen to take up nutrients present on the host epidermis or on pollen grains (Blakeman, 1980). In contrast, when dry conidia are inoculated onto a plant surface and subsequently incubated in the absence of free surface water, the germ tube that would normally affect penetration remains shorter than the length of a conidium (Salinas and Verhoeff, 1995; Williamson et al., 1995; Cole et al., 1996).

There have been few reports on the effects of environmental factors like a_w and temperature and the influence of solutes on *B. cinerea* growth under 'in vitro' conditions (Dantigny et al., 2005a). Beside an extensive study focusing on the effect of wind speed, relative humidity and temperature on aerial mycelium produced by *B. cinerea* (Thomas et al., 1988). The influence of the environment on mycelial infection has been a neglected field of study, although it is known that the process can occur in the absence of free water (Jarvis, 1977).

Mathematical modelling is an efficient tool for assessing how individual or combined environmental factors affect microorganisms that degrade processed foods. Various models have been developed in predictive microbiology for fitting growth curves and estimating biological parameters of food-borne pathogens (McMeekin et al., 1993, 2002). Nevertheless, most of the models derive from models based on bacterial data due to the inherent difficulties in assessment of fungal growth rates and gathering of sufficient suitable and reproducible data (Gibson and Hocking, 1997; Dantigny et al., 2005a). Various workers have developed probability, mechanistic/semi-mechanistic, empirical and thermal death models for a variety of toxigenic and spoilage fungi (Pitt, 1993; Skirdal and Eklund, 1993; Gibson et al., 1994; Marín et al., 1996; Cuppers et al., 1997; El-Halouat and Debevere, 1997; Valík and Piecková, 2001; Sautour et al., 2002). The major objective of the present work was to construct and evaluate models describing the individual and combined effects of water activity and temperature on the radial growth rate of *B. cinerea* for each tested solute.

2. Materials and methods

2.1. Fungi

B. cinerea strain V was isolated from rotting strawberry (Plant Pathology Unit, FUSAGx, Belgium). For long-term storage, the strain was placed at −70 °C in tubes containing 25% glycerol. During experiments the initial conidial inoculum was taken from Petri-dish cultures on Potato Dextrose Agar (PDA, Merck, Darmstadt, Germany) medium, preserved at 4 °C for no more than 6 months.

2.2. Media

PDA was used as the basal medium (a_w=0.995). The a_w of the medium was modified by replacing some of the water with an equal weight of glucose (200 g/l; 313.33 g/l; 583.33 g/l; 803.33 g/l and 933.33 g/l), NaCl (20 g/l; 47.56 g/l; 105 g/l; 136.66 g/l and 170 g/l), glycerol (40 ml/l; 181.66 ml/l; 265 ml/l; 346.66 ml/l and 406.66 ml/l) or sorbitol (176.66 g/l; 243.33 g/l; 561.66 g/l; 660 g/l and 800 g/l) to obtain a_w levels of 0.980, 0.960, 0.930, 0.910, and 0.890 at 25, 15, and 5 °C (Lahlali et al., 2005). An AquaLab CX2T (Decagon Devices, 950 NE Nelson Court Pullman, Washington 99163, USA) was used to determine water activities in the adjusted media with an accuracy of 0.003. The final media were autoclaved at 120 °C for 20 min.

2.3. Inoculation and incubation of B. cinerea

B. cinerea strain was grown on PDA (pH 5.6) at 25 °C. A conidial suspension adjusted to 1×10^6 spores/ml with a Bürker cell was prepared from a 10±1-day-old colony culture in sterile distilled water containing 0.05% Tween 20. Ten-microlitre aliquots of this suspension were inoculated at the centre of Petri dishes containing a test medium. After inoculation, the Petri plates were sealed in polyethylene bags to prevent water loss and incubated at 5, 15, or 25 °C for a maximum of 25 days. The preservation of water content in the media were checked by measuring the a_w of inoculated Petri dishes after 25 days at each temperature and no change in the a_w of any tested medium was detected. Each experiment was carried out in triplicate for each solute–a_w–temperature combination.

2.4. Experimental design

A fully factorial design run in triplicate was used to generate the growth rate of *B. cinerea* in media modified with glucose, NaCl, glycerol, and sorbitol at three temperatures and six a_w levels.

2.5. Growth measurement

The average diameter of each growing mycelial colony was measured daily in two perpendicular directions (Marín et al., 1996; Lahlali et al., 2005) without opening the Petri dishes.

Growth rates (g, mm d^{-1}) were calculated for each a_w–solute–temperature combination by linear regression from the linear phase of the growth curve. Simultaneously, the time required (t_v, days) to form a visible colony was evaluated for each solute and each a_w and each temperature (Patriarca et al., 2001).

2.6. Mathematical and statistical methods

2.6.1. Influence of a_w, temperature, and solute

The influence of a_w, solute, temperature, and their interactions on the radial growth rate was analysed by applying the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Statistical significance was judged at the $P<0.05$ level. When analysis revealed statistically significant differences, Duncan's multiple-range mean separation test was performed.

2.6.2. Secondary modelling

2.6.2.1. Effect of a_w. A mathematical model proposed by Gibson et al. (1994) was applied to the radial growth rates. This model involves a transformation to the natural logarithm to stabilize the variance of the radial growth rate values. Furthermore, a new transformation of a_w to b_w was made to fit the data to a curve with a simpler shape. The transformation of a_w to b_w was done according to the following formula:

$$b_w = \sqrt{(1-a_w)}. \quad (1)$$

The use of lng-versus-b_w curves was more appropriate for parabolic adaptation. The following model (Eq. (2)) was fitted separately for each solute at three temperatures (25, 15, and 5 °C):

$$\ln g = C_0 + C_1 b_w + C_2 b_w^2 \quad (2)$$

where the coefficients C_0, C_1, and C_2 were calculated by quadratic regression. The optimum a_w values and the radial colony growth rates at optimum a_w were calculated in each solute model and for each incubation temperature. This was done as follows:

$$a_w(\text{opt}) = 1-\left(\frac{C_1}{2C_2}\right)^2 \quad (3)$$

$$g(\text{opt}) = \exp\left(C_0-\frac{C_1}{4C_2}\right)^2. \quad (4)$$

The time required (t_v) for a colony to become visible was also calculated by applying the same modelling approach. The t_v model is:

$$\ln t_v = D_0 + D_1 b_w + D_2 b_w^2 \quad (5)$$

where coefficients D_0, D_1, and D_2 are also estimated by quadratic regression.

2.6.2.2. Combined influence of a_w and temperature. A second-order polynomial model (Eq. (6)) was fitted to the radial growth rate data for each solute:

$$g = C_0 + C_1 b_w + C_2 b_w^2 + C_3 T + C_4 T^2 + C_5 b_w T \quad (6)$$

with six coefficients (C_0, intercept; C_1, C_3, linear coefficients; C_5, interaction coefficient; C_2, C_4, squared coefficients) by means of the statistical software package 'DESIGN-EXPERT® version 6.0., (StatEase, Inc., Minneapolis, USA).

2.6.2.3. Mathematical and statistical validation. To evaluate the performance of the predictive models, i.e. their ability to describe the observed experimental data adequately, we calculated the following mathematical and statistical indices: root mean square error (RMSE), F-value, regression coefficient (r^2), bias factor, and accuracy factor (Ross, 1996; te Giffel and Zwietering, 1999; Dantigny et al., 2005b; Samapundo et al., 2005). F-values were calculated and compared with tabulated F-values. The RMSE and the bias and accuracy factors were calculated as follows:

$$\text{RMSE} = \sqrt{\frac{\text{RSS}}{df}} = \sqrt{\frac{\sum(\mu_{\text{observed}}-\mu_{\text{predicted}})^2}{df}} \quad (7)$$

$$\text{Bias factor} = 10^{\left[\sum \log(\mu_{\text{observed}}/\mu_{\text{predicted}})/n\right]} \quad (8)$$

$$\text{Accuracy factor} = 10^{\left[\sum |\log(\mu_{\text{observed}}/\mu_{\text{predicted}})|/n\right]}. \quad (9)$$

3. Results and discussion

3.1. Influence of a_w, temperature, and solute

The effect of a_w, temperature, and solute and their two- and three-way interactions was studied on the radial growth rate of *B. cinerea*. The results in Table 1 revealed significant effect of each single factor and interaction. These results are in agreement with those reported by Lahlali et al. (2005) for the radial growth rate of *P. expansum* on similarly modified Potato Dextrose Agar medium. Teixidõ et al. (1998) have observed similar effects on the growth rate of *Candida sake* in nutrient yeast dextrose broth. In our experiment, *B. cinerea* was unable to grow at $a_w \leq 0.89$ in glucose-, sorbitol-, or glycerol-modified medium. When NaCl was used to adjust the a_w, no growth was observed at $a_w = 0.93$.

Duncan's multiple-range mean separation test was performed according to each factor (Fig. 1). The results show three statistically homogenous groups, one for each temperature (Fig. 1a). For the a_w factor, similar test distinguished six homogenous groups, one for each a_w value (Fig. 1c). Lastly, the test revealed four homogenous groups for the influence of solute, one for each solute treatment (Fig. 1b). The growth rate was found to decrease with decreasing a_w and temperature and was highest on unmodified medium, followed by the modified media in the order: glucose-, sorbitol-, glycerol-, and NaCl-modified medium (Fig. 1b).

A previous study carried out on the grape berry surface showed inhibition of *B. cinerea* growth in an 'in vitro' experiment conducted at $a_w < 0.93$ (KCl, $CaCl_2$), but when the

Table 1
Variance analysis of the effects of water activity (a_w), temperature (T), and solute (Sol) and their two- and three-way interactions on the radial growth rate of *B. cinerea* in unmodified and modified PDA medium

Source	*df*	Mean square	F-value	Pr>F
T	2	61.7569954	9298.05	0.0001*
a_w	4	134.9000508	20310.4	0.0001*
Sol	3	13.2964639	2001.90	0.0001*
$T \times a_w$	8	7.9291381	1193.80	0.0001*
$T \times$ Sol	6	0.939674	141.44	0.0001*
Sol $\times a_w$	12	2.0478495	308.32	0.0001*
$T \times a_w \times$ Sol	24	0.2360443	35.54	0.0001*

*Highly significant $P < 0.0001$.

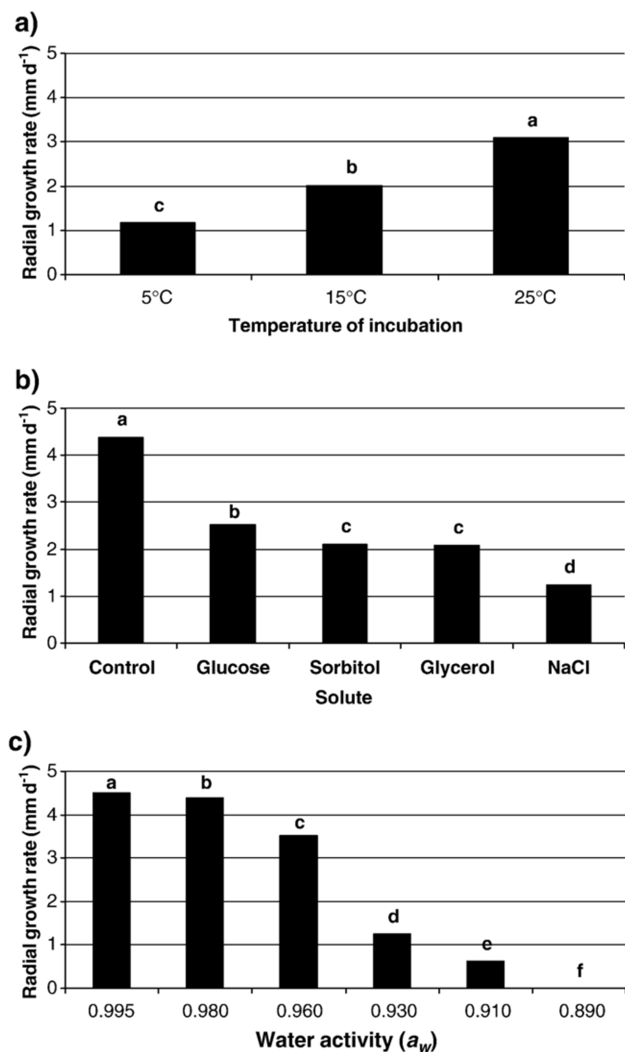

Fig. 1. Mean separation of *B. cinerea* growth rate per incubation temperature (a), solute (b) and water activity level (c), performed by Duncan's multiple-range test. Treatments having the same letters are not significantly different ($P<0.05$).

medium was supplemented with increasing amounts of sucrose, growth was observed at $a_w<0.93$ (Rousseau and Deneche, 2001). The choice of the solute used to modify the a_w of the PDA medium has a significant impact on the growth rate of *P. expansum* (Lahlali et al., 2005). Abadias et al. (2000) report similar results for the antagonistic yeast *C. sake*. Beuchat and Pitt (1990) also studied the influence of glucose, sorbitol, and NaCl on fungal growth. They looked at solute effects on colony formation by sublethally heat-stressed *Wallemia sebi* in a basal enumeration medium at water activities ranging from 0.82 to 0.97. They report that over this a_w range, glucose and sorbitol had similar effects on the recovery of cells. NaCl, in contrast, had an inhibitory effect over an a_w range of 0.82 to 0.92. Colony diameters were generally largest on media containing sorbitol and glucose and smallest on media supplemented with NaCl. Our work shows likewise that the non-ionic solutes glycerol, sorbitol, and glucose have a lesser effect on the growth rate of *B. cinerea* than the ionic solute NaCl. Lahlali et al. (2006) proposed that diffusion occurring in both directions across the cell membrane might be the major explanation for the lesser growth observed with NaCl, as Na^+ and Cl^- ions should diffuse more readily than non-ionic solutes from a higher-concentration area to a lower-concentration area, i.e. from PDA medium into the pathogen cells, causing a greater loss of water. Jennings (1983) underlined a weaker growth of fungi in media containing increasing sodium concentration and associated this reduction with a decrease of internal potassium concentration.

3.2. Effect of a_w

Modelling of *B. cinerea* growth was carried out on the basis of the growth data obtained on PDA medium at pH 5.6 under 6 water activities ranging from 0.995 to 0.890, reached by adding increasing amounts of either a non-ionic solute (glycerol, sorbitol, or glucose) or the ionic solute NaCl. The growth curves, based on colony diameter, were typical of fungal growth, with a germination period followed by an acceleration phase, a linear phase, and finally a slowing towards an upper asymptote (data not shown). Valík and Piecková (2001) obtained similar results with the fungi *Byssochlamys fulva*, *Neosartorya fischeri*, and *Talaromyces avellaneus* at 25 °C, at water activities ranging from 0.85 to 0.995. Our model (Eq. (2)) was applied to the radial colony growth rates recorded at a_w values ranging from 0.91 to 0.995 for non-ionic solutes and from 0.96 to 0.995 for NaCl. In an empirical modelling approach the effect of a_w on the growth area of *B. cinerea* and the radial growth rate (g), expressed as the increase in colony radius per day, were estimated for each combination of a_w and solute at the three studied temperatures. For each humectant these values were fitted as a function of the b_w parameter by quadratic linear regression. The use of lng led to homogenisation of the variance for parabolic adjustment. Coefficients C_0, C_1, and C_2 used to calculate optimum a_w values for *B. cinerea* growth under various stress conditions are listed in Table 2. The colony growth rates predicted by the secondary models pertaining to the different solutes are shown in Fig. 2.

The results obtained by applying secondary model (Eq. (2)) are in agreement with the experimental observation that temperature has little influence on the a_w optimum for growth. At any temperature and whatever the solute, the *B. cinerea* growth rate appears to increase with the a_w. Patriarca et al. (2001) reported similar findings for *W. sebi* at 25 and 30 °C, and Samapundo et al. (2005), Cahagnier et al. (1995), and Marín et al. (1995, 1999a,b) made similar observations on *F. verticilloides* and *F. proliferatum*.

At 25 °C, the optimum a_w for growth was 0.987 in the glucose model, 0.990 in the NaCl model, and 0.985 in the glycerol and sorbitol models. The corresponding radial growth rates were 7.18, 6.75, 7.36, and 7.60 mm d^{-1} respectively. At 15 °C, the growth rates were lower, the highest being 5.34 mm d^{-1} (sorbitol, $a_w=0.984$) and the lowest being 4.27 mm d^{-1} (NaCl, $a_w=0.994$).

The optimum a_w for growth remained unchanged at 5 °C in all models except the NaCl model, where it dropped to 0.989. The growth rates were drastically reduced, the highest rate being observed in the sorbitol model (3.16 mm d^{-1}, $a_w=0.983$).

Table 2
Coefficients and some predictions of the radial growth rate (Eq. (2)) and t_v models (Eq. (5)) for *B. cinerea* in modified PDA media with different solutes at different temperatures

	Growth rate model					t_v model				
	Coefficients			Characteristics		Coefficients			Characteristics	
	C_0	C_1	C_2	$a_w(\mathrm{opt})_1$ [a]	$g(\mathrm{opt})$ [b]	D_0	D_1	D_2	$a_w(\mathrm{opt})_2$ [c]	$t_v(\mathrm{opt})$ [d]
25 °C										
Glu	1.290	10.848	−42.715	0.987	7.18	2.457	−17.218	56.080	0.976	3.12
Na	1.260	13.333	−68.315	0.990	6.75	1.635	−7.580	45.514	0.993	3.74
Gly	1.223	12.931	−53.975	0.985	7.36	2.425	−16.843	55.479	0.976	3.14
Sorb	1.045	15.928	−64.508	0.985	7.60	2.214	−14.135	50.032	0.980	3.37
15 °C										
Glu	0.741	13.092	−48.370	0.981	5.09	2.214	−14.135	50.032	0.976	3.40
Na	1.247	5.678	−39.250	0.994	4.27	1.399	−3.948	31.104	0.996	3.57
Gly	0.856	13.410	−60.550	0.987	4.94	2.192	−14.205	52.031	0.981	3.39
Sorb	0.333	21.590	−86.785	0.984	5.34	2.111	−13.123	49.658	0.982	3.47
5 °C										
Glu	−0.153	18.297	−65.019	0.980	3.10	2.329	−7.372	29.930	0.984	6.52
Na	+0.019	17.736	−84.415	0.989	2.58	2.250	−7.540	37.240	0.989	6.48
Gly	+0.266	12.262	−54.928	0.987	2.58	2.311	−7.428	31.545	0.986	6.51
Sorb	−0.385	23.609	−90.671	0.983	3.16	2.315	−7.410	31.220	0.986	6.52

Glu: glucose, Na: NaCl, Gly: glycerol and Sorb: sorbitol.
[a] Predicted optimum a_w for the radial growth rate.
[b] Predicted colony growth rate (mm day^{-1}) at the optimum a_w.
[c] Predicted a_w for the shortest time required to form a visible colony.
[d] Predicted time (days) required to form a visible colony at a_w (opt)$_2$.

Another fungal growth parameter having high practical utility is the time required for a fungus to form a 2-mm colony (Horner and Anagnostopoulous, 1973) or a 3-mm colony (Gibson et al., 1994). The latter parameter, called t_3, appears to be favoured in the predictive microbiology literature.

In this study, a third parameter, the time of visibility (t_v) described by Patriarca et al. (2001) as the time required to form a visible colony from the initial inoculum, was chosen. This parameter does not imply a colony size because when a colony becomes visible, its diameter usually exceeds 3 mm. Product

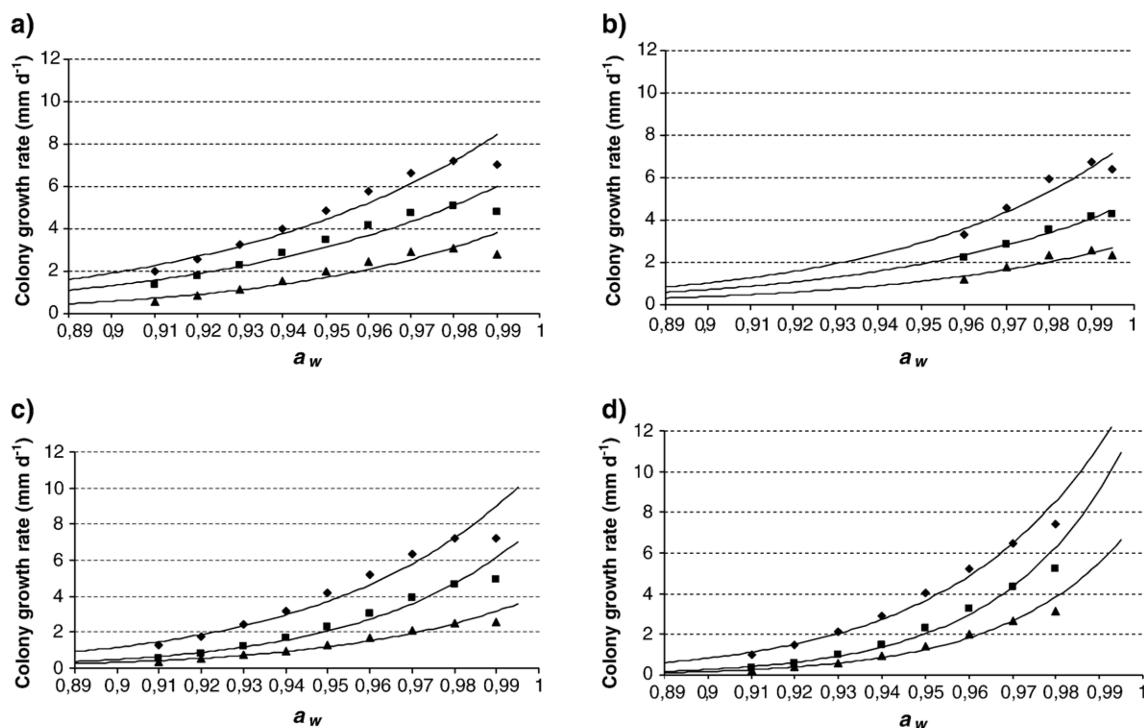

Fig. 2. Plots of colony growth rates, g (mm d^{-1}), versus a_w for *B. cinerea* at 25 (◆), 15 (■) and 5 °C (▲). Values of a_w were adjusted with glucose (a), NaCl (b), glycerol (c), and sorbitol (d). The continuous line indicates the fitted g vs. a_w function, where $g=\exp(C_0+C_1 b_w+C_2 b_w^2)$ and $b_w=\sqrt{(1-a_w)}$.

presenting such as colony is generally considered as spoiled and is rejected by the market. We applied our modelling approach to the t_v data, using the above-mentioned transformation (Eq. (5)). The *Di* coefficients were calculated and the a_w (opt)$_2$ and t_v (opt) values are shown for three incubation temperatures (5 °C, 15 °C, 25 °C) in Table 2. Only slight differences were observed between the predicted a_w (opt)$_1$ values of the radial growth model and the predicted a_w (opt)$_2$ values of the t_v model. The time required to form a visible colony was found to increase with decreasing incubation temperature. Depending on the solute used, this time varied from 3 to 3.5 days at 25 °C, approximately doubling at 5 °C. These results are in accordance with our experimental observations: the fungus needed 4 days to form a visible colony at 25 or 15 °C and 6 to 7 days at 5 °C at water activities ranging from 0.98 to 0.995. At the optimum a_w for growth, a conidial suspension placed on NaCl-modified PDA required more time to form a visible colony than one placed on PDA modified with one of the other solutes. Thomas et al. (1988) found the minimum period required for production of a visible aerial mycelium on vineyards was 2 days at temperatures between 21 and 26 °C and 3 days at 16 °C. Production of aerial mycelium tended to fall as the relative humidity declined below 94% (Thomas et al., 1988), but can still occur at the relative humidity of 60% (Salinas et al., 1989).

3.3. Combined effect of a_w and temperature

Contour plots of radial growth rate as a function of a_w and temperature were produced for each solute (Fig. 3). The plots show, within the range of the experimental data, a growth rate increasing with both a_w and temperature. The influence of a_w is much larger in comparison with the temperature. Whatever the solute model, growth at 25 °C is maximal at a_w values ranging from 0.96 to 0.995 (control). At low water activity and temperature, the glucose model is the one predicting the fastest growth (0.219 mm d^{-1}) and the NaCl model predicts the slowest (0.165 mm d^{-1}). All solute models make illogical predictions at a_w of 0.89 and at temperature of 5 °C; i.e. predicted values are slowly higher than observed values. Samapundo et al. (2005) have stressed that overfitting is typical of polynomial equation

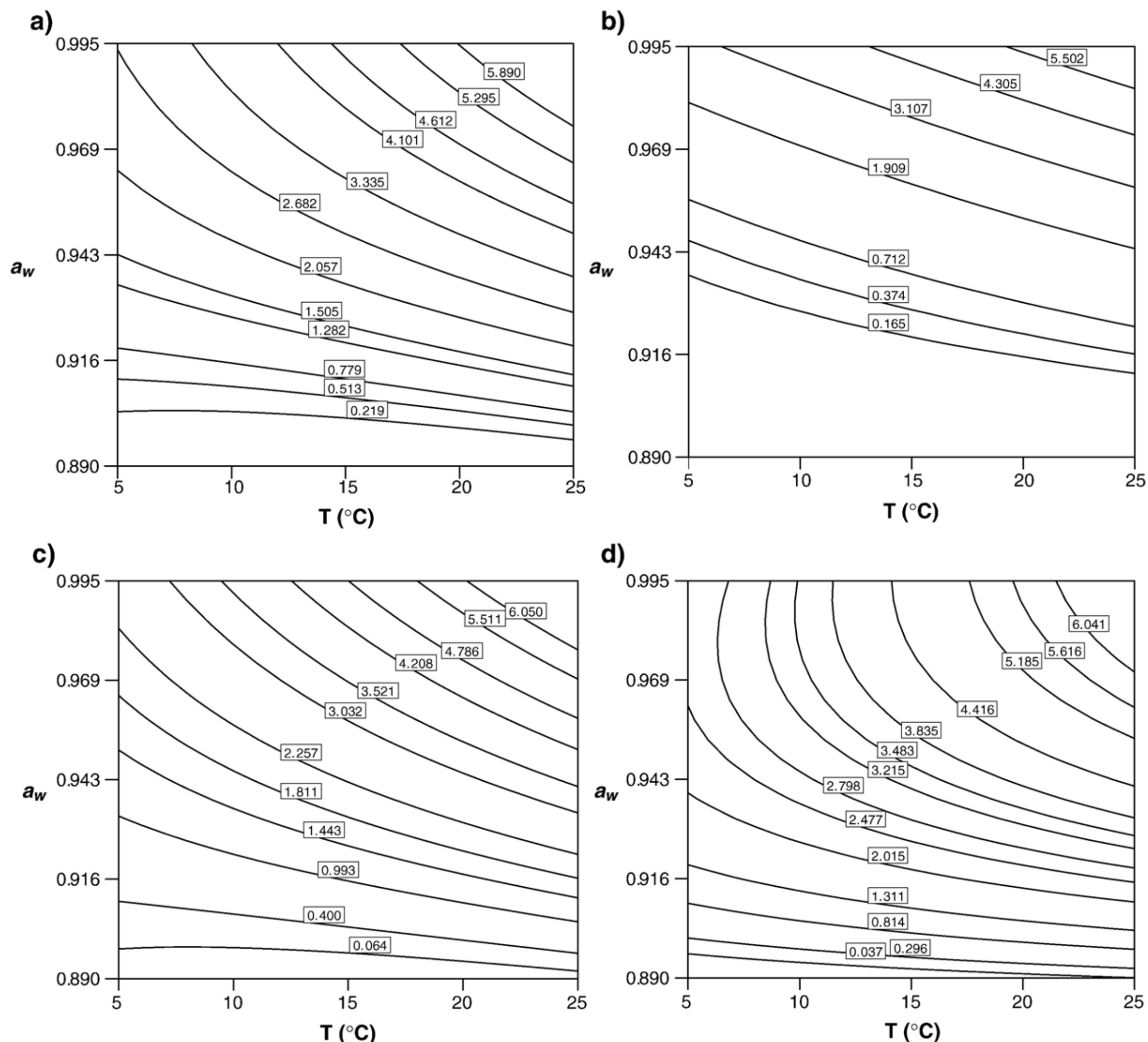

Fig. 3. Contour plots showing the combined effects of a_w and temperature on the radial growth rate ($g=C_0+C_1b_w+C_2b_w^2+C_3T+C_4T^2+C_5b_wT$) of *B. cinerea* in models based on glucose (a), NaCl (b), glycerol (c), and sorbitol (d).

Table 3
Coefficients of growth rate secondary models, ($g = C_0 + C_1 b_w + C_2 b_w^2 + C_3 T + C_4 T^2 + C_5 b_w T$) for different solutes

Factor	Glu	Na	Gly	Sorb
	Coefficients			
C_0	−1.439*	2.053*	−0.125^{ns}	−0.417^{ns}
b_w	40.774*	−6.484^{ns}	22.690*	30.548*
b_w^2	−116.289*	1.210^{ns}	−71.360*	−92.980*
T	0.289*	0.248*	0.267*	0.242*
T^2	−0.0000^{ns}	0.0015^{ns}	0.0015^{ns}	0.0018^{ns}
$b_w T$	−0.049*	−0.917*	−0.877*	−0.849*

*Significant; ^{ns} = not significant; Glu: glucose; Na: NaCl; Gly: glycerol; Sorb: sorbitol.

predictions under limit growth conditions. Table 3 provides the multiple regression coefficients relative to the quadratic polynomial model (Eq. (6)) applicable to *B. cinerea*. In the case of each solute, all coefficients have a significant effect, except coefficient C_0 in the glycerol and sorbitol models, linear and quadratic coefficient (b_w) in the NaCl model, and the quadratic effect of temperature (T^2) in all models. A significant interaction between b_w and temperature is observed in all polynomial models. This coefficient has a negative effect, suggesting a synergistic interaction. The higher the absolute value of a linear coefficient (b_w or T), the greater the influence of the corresponding factor (a_w or temperature) on the growth rate of *B. cinerea*. Thus, in all cases, a_w is more influent than temperature. Applying the same approach with a third polynomial model, Samapundo et al. (2005) found a negative interaction between the effects of b_w and temperature on the growth rates of *F. proliferatum* and *F. verticilloides* on corn.

In summary, our results from quadratic polynomial model (Eq. (6)) and our statistical and graphical analyses confirm the previous findings that the a_w has a greater effect than temperature on radial fungal growth under *in vitro* conditions (Sautour et al., 2002; Samapundo et al., 2005; Lahlali et al., 2005, 2006). Haasum and Nielsen (1998) have likewise observed that the radial growth rate of *B. cinerea* is more affected by a_w than temperature, confirming our particular data on the same fungus.

3.4. Secondary models validation

In predictive microbiology, several mathematical and statistical indices are used to evaluate the ability of predictive models to describe experimental data adequately: regression coefficient, F-value, root mean square error (RMSE), and bias and accuracy factors (Ross, 1996; te Giffel and Zwietering, 1999; Dantigny et al., 2005b). The r^2 coefficient of a model measures the fraction of the variation explained by the model. The closer the r^2 value is to 1.00, the better the data are predicted by the model. In our fungal study, the value of this coefficient was consistently very high for secondary model (Eq. (2)), exceeding 97% whatever the solute and incubation temperature (Table 4). The RMSE was reported to be the most simple and most informative measure of goodness of fit of model. The smaller the RMSE value, the better the performance of the model (Dantigny et al., 2005b). All models displayed a small RMSE value, ranging between 0.03 and 0.09. On the basis of the same modelling approach, Valík and Piecková (2001), reported similar results for three fungal heat-resistant strains. The lack of fit test of the model was also considered. If $f_{value} < F_{table}$, the model is accurate enough to describe the experimental data. The lack of fit test revealed no significant difference between observed and predicted values for secondary models (Eq. (2)), after growth in glucose-, sorbitol- or glycerol-modified medium. This test was inapplicable to the NaCl model because the number of variables was equal to the number of experiments.

Ross (1996) and te Giffel and Zwietering (1999) reported that a model should be considered 'fail safe' when the bias factor is below 1, whereas a model yielding a bias factor larger than 1.1 should be considered as 'fail dangerous' because the difference between observed and predicted growth rates would exceed 10%. However, a bias factor of 0.5 indicates a poor model that is overly conservative as it predicts growth rates that are on average, twice as large as the observed values. The accuracy factor gives an indication of the average difference between the predictions and the observations (Ross, 1996). Table 4 shows for our secondary model (Eq. (2)) a bias factors ranging from 0.999 to 1.00 and an accuracy factor ranging from 1.072 to 1.17. At the growth temperature (25 °C), the average difference between observed and predicted *B. cinerea* colony growth rates was 6.9% for the glucose model, 4.6% for the NaCl model, 2.8% for the glycerol model, and 7.2% for the sorbitol model. The results for a_w and temperature effects are compatible with those determined for other fungi. Samapundo et al. (2005) modelled at four temperatures (15, 22, 25, and 30 °C) the effect of a_w on the growth rates of *F. verticilloides* and *F. proliferatum*. The bias factor ranged from 0.84 to 1.08 and the accuracy factor from 1.075 to 1.218. At 25 °C, Valík and

Table 4
Evaluation of the performance the $g = \exp(C_0 + C_1 b_w + C_2 b_w^2)$ models for *B. cinerea* placed at different temperatures in medium adjusted to the desired a_w with different solutes

	25 °C				15 °C				5 °C			
	Glu	Na	Gly	Sorb	Glu	Na	Gly	Sorb	Glu	Na	Gly	Sorb
r^2	0.97	0.99	0.99	0.98	0.96	0.99	0.99	0.96	0.97	0.98	0.99	0.97
RSME	0.09	0.10	0.03	0.09	0.09	0.20	0.09	0.2	0.16	0.30	0.04	0.17
f_{value}	0.61	_	1.15	2.67	1.97	_	1.89	2.02	0.89	_	2.25	2.40
F_{table} (95%)	4.10	4.46	4.10	4.10	4.10	4.46	4.10	4.10	4.10	4.46	4.10	4.10
Bias factor	0.999	1.005	1.000	0.999	1.000	1.004	0.999	1.000	1.000	1.000	0.999	1.000
Accuracy factor	1.069	1.046	1.028	1.072	1.081	1.041	0.999	1.170	1.080	1.031	1.033	1.140

Glu: glucose, Na: NaCl, Gly: glycerol and Sorb: sorbitol. _: No 'lack of fit' detected.

Table 5
Statistical and mathematical performance evaluation of secondary models (Eq. (6)) for *B. cinerea* growth in the presence of different solutes

	Glu	Na	Gly	Sorb
r^2	98.6	93.9	95.4	94.2
RSME	0.26	0.54	0.50	0.57
f_{value}	1.07	2.30	1.57	1.94
F_{table} (95%)	2.04	2.04	2.04	2.04
Bias factor	0.957	1.036	0.950	0.860
Accuracy factor	1.089	1.070	1.120	1.260

Glu: glucose; Na: NaCl; Gly: glycerol; Sorb: sorbitol.

Piecková (2001) reported a bias factor ranging from 1.007 to 1.014 and an accuracy factor from 1.070 to 1.106.

In our quadratic polynomial models (Eq. (6)), the percentage variation explainable by the model is 95.4, 94.2, 98.6 and 93.9% for the glycerol, sorbitol, glucose, and NaCl models respectively (Table 5). The smallest RSME value is observed with the glucose model (0.26), followed by the glycerol, NaCl, and sorbitol models with 0.50, 0.54, and 0.57 respectively. The lack of fit is significant only in the case of the NaCl model. The bias and accuracy factors for the glycerol, glucose, and NaCl are close to 1, indicating that the models are good predictors of the true mean *B. cinerea* colony growth rate. In the case of the sorbitol model, a bias factor below 1 would suggest that the model is fail-safe for *B. cinerea*, yet this is not so, since the average difference between predicted and real growth rates is about 26%. This difference is much lower in the case of the glucose, glycerol, and NaCl models (9%, 12%, and 7% respectively) (Table 5). Using another polynomial model, Samapundo et al. (2005) reported, for both *Fusarium* isolates studied, a difference of 25.4% between predicted and observed colony growth rates in the case of glycerol used as humectant.

4. Conclusion

We described here a first model predicting *B. cinerea* growth rate according to environmental factors such as temperature and a_w. Models developed here are based on 'in vitro' experiments carried out with glycerol, sorbitol, glucose and NaCl-modified medium. The results show a significant effect of water activity and temperature on the radial growth rate of *B. cinerea* highlighting that the fungal growth decreases when water activity of the medium and temperature are low.

Secondary models (Eqs. (2) and (6)) described here can both predict, within the limits of our experiments, the *B. cinerea* growth rate and the time required for this fungus to form a visible colony. Yet we have noted growth rate overfitting in the polynomial models used (Eq. (6)) in case of low water activity and temperature. Secondary polynomial models should thus be used with caution when assessing the relationship between environmental factors and fungal growth. In contrast, our secondary models (Eq. (2)) offer good accuracy and predict even the slowest growth observed in our experiments. Beside secondary models used here to predict the growth of grey mould under 'in vitro' conditions, literature describes other models such as the linear Arrhenius–Davey equation (Davey, 1989) and the cardinal modelling approach (Dantigny, 1998; Sautour et al., 2001, 2002). These models could also be applied in the same way to predict the radial growth of *B. cinerea*.

Our t_v model (Eq. (5)) addresses an additional aspect of what is likely to happen in a *B. cinerea*-infected commodity characterized by a given water activity. Nevertheless it is important to bear in mind that such predictive growth rate and t_v models are designed to fit 'in vitro' data. Most likely, the predicted growth will be faster than growth under natural conditions, because the artificial medium is probably richer in nutrients (Lahlali et al., 2005). Furthermore, any extrapolation to natural conditions remains hazardous, because other factors such as interactions between microflora and the oxygen or carbon dioxide level may be involved. All results developed here could be applied in practice to various commodities susceptible to grey mould infection, as we have used multiple solutes to adjust the water activity. Implementation of these results should contribute to elaborating a more efficient control strategy against grey mould on commodities in field and storage conditions.

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