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ORIGINAL ARTICLE

In vitro effects of water activity, temperature and solutes on the growth rate of *P. italicum* Wehmer and *P. digitatum* Sacc.

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Penicillium digitatum, *Penicillium italicum*, predictive model, solute, temperature, water activity.

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Abstract

Aims: To evaluate the effect of water activity (a_w 0·98–0·89, adjusted with glycerol, sorbitol, glucose, or NaCl) and temperature (5–25°C) on the lag phase and radial growth rate (mm day^{-1}) of the important citrus spoilage fungi, such as *Penicillium italicum* and *Penicillium digitatum* grown in potato dextrose agar (PDA) medium. To select, among models based on the use of different solutes, a model fitting accurately the growth of these species in relation to a_w and temperature.

Methods and Results: Extensive data analyses showed for both *Penicillium* species a highly significant effect of a_w, temperature, solutes and their interactions on radial growth rate ($P < 0·0001$). Radial growth rate was inhibited and the lag phase (i.e. the time required for growth) lengthened as the a_w of the medium decreased. NaCl appeared to causes the greatest stress on growth when compared with other nonionic solutes. *Penicillium italicum* stopped growing at 0·96 a_w and *P. digitatum* at 0·93 a_w. Under the dry conditions where growth was observed, *P. italicum* grew faster than *P. digitatum* at low temperature and *P. digitatum* remained more active at ambient temperature. Multiple regression analysis applied to the square roots of the growth rates observed in the presence of each solute showed that both the 'glycerol model' and the 'sorbitol model' yielded a good prediction of *P. italicum* growth and the 'sorbitol model' gave an accurate fit for *P. digitatum* growth, offering high-quality prediction within the experimental limits described.

Conclusions: Mathematical models describing and predicting, as a function of a_w and temperature, the square root of the radial growth rate of the agents responsible for blue and green decays are important tools for understanding the behaviour of these fungi under natural conditions and for predicting citrus fruit spoilage.

Significance and Impact of the Study: Implementation of these results should contribute towards a more rational control strategy against citrus spoilage fungi.

Introduction

Citrus fruits are generally stored in temperatures ranging from 0 to 7°C and relative humidities from 85% to 90%. During their storage, fruits are affected by several decays (Brown and Miller 1999). *Penicillium digitatum* Wehmer and *P. italicum* Sacc. are the main postharvest pathogens of citrus fruits, responsible, respectively, for green and blue decay. In turn massive spore production by these two pathogens ensures their presence wherever fruit is produced, handled and stored. Contamination takes place only through wounds where nutriments are available to

stimulate spore germination. Fruit decay thus begins at the site of injury. Blue decay develops less rapidly than green decay under ambient conditions. Green decay is often observed in mixed infections. Blue decay is more common in fruit held in cold storage during the summer; it can spread through packed cartons more easily than green decay, producing a 'nest' of decayed fruit (Tuset 1987; Ismail and Zhang 2004).

The importance of *Penicillium* spp. contamination of oranges during storage makes it necessary to learn more about the ecological behaviour of these two pathogens, and particularly about their interactions with the microflora present on the fruit surface (Lacey 1989), in order to develop more rational control strategies. Water activity (a_w) and temperature are the most important abiotic factors influencing the potential for spore germination and growth of propagules on the fruit surface (Magan and Lacey 1988; Plaza *et al.* 2003).

Little information is available on how water activity, temperature and solutes affect the germination and growth of major postharvest pathogens of citrus fruit. Investigators have observed maximal *in vitro* growth of *P. digitatum* at 1·00 a_w at temperatures ranging from 20 to 25°C, detecting no growth at water activities below 0·90 a_w (Hocking and Pitt 1979; Lacey 1989). Wyatt and Parish (1995) reported that *P. italicum* can germinate at lower temperature (0°C) on orange juice serum agar (OJSA) medium. In a recent investigation, Plaza *et al.* (2003) found *P. italicum* to germinate and grow faster than *P. digitatum*, particularly at 0·95 a_w. *Penicillium italicum* can also grow under dry conditions (0·87 a_w). Other studies with common cereal fungi have shown the influence of different abiotic factors, such as temperature and a_w on the activity of *Fusarium* spp., some *Aspergillus* spp. and *Penicillium* spp. during spoilage of feeds and foods (Marín *et al.* 1996, 1998). The data obtained were used to develop predictive models based on these factors in order to control grain spoilage.

In the general literature, predictive microbiology has focused on modelling bacterial growth more than fungal growth (McMeekin *et al.* 1993, 2002). This may be due to the complexity of fungal growth quantification (Gibson and Hocking 1997).

Two approaches are used to model the growth rate of spoilage micro-organisms. The first is based on response surface methods (RSM) using different factorial designs. The second is empirical and requires the logarithmic transformation of dependent variables and square root transformation of the a_w factor. Parra and Magan (2004) have developed a model describing the combined effect of temperature and a_w on the growth rate of *Aspergillus niger*, based on a square root model showing the relationship between temperature and bacterial growth rate, developed by Ratkowsky *et al.* (1983), and on the parabolic relationship between the logarithm of the growth rate and a_w, developed by Gibson *et al.* (1994). Polynomial models based on RSM usually involve simultaneous determination of the effects of several factors on microbial behaviour. They provide a precise experimental model of the studied system. On the other hand, empirical models, such as a square root or Arrhenius model for temperature are usually based on the sequential determination of the effects of individual factors on the growth rate (McMeekin *et al.* 2002).

As no predictive model considering the effect of abiotic factors, such as temperature and a_w or the effect of solutes on the radial growth rate of postharvest citrus pathogens has been developed. The main objective of the present study was thus to determine the effect of water activity, temperature and solute on the lag phase and radial growth rate of both *Penicillium* species responsible for citrus decays. A second step was to use quadratic models to use these effects in relation to solute.

Materials and methods

Isolates

Strains of *P. digitatum* PDRBM1 and *P. italicum* PIRBM1 were isolated from decayed 'Clementine' fruits harvested from Tadla plain in Morocco by the Laboratory of Plant Pathology (ENA-Meknes). The strains were stored on potato dextrose agar (PDA; Merck, Darmstadt, Germany) medium at 4°C in the dark.

Medium

The water activity of the basic PDA medium (0·995) was modified with calculated amounts of a nonionic solute (glycerol, sorbitol, glucose) or an ionic solute (NaCl; Teixidõ *et al.* 1998; Lahlali *et al.* 2005) to obtain a_w levels of 0·98, 0·96, 0·93, 0·91 and 0·89 at 25, 15 and 5°C. The a_w of all media were measured with an AquaLab series 3 instrument (Decagon, 950 NE Nelson Court Pullman, WA 99163, USA) with an accuracy of 0·002–0·003.

Radial growth rate assessment

A conidial suspension was prepared from 9 ± 2-day-old fungal cultures by scraping the surface of colonies and recovering conidia in Tween 20 solution (0·05% v/v). Spore suspensions were adjusted to 1×10^6 spores ml^{-1} with sterile distilled water by means of a Bürker cell. A 10-μl aliquot of this suspension was 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 of a_w of media were detected. Each experiment was carried out in triplicate for each solute-a_w-temperature combination.

The radial mycelial growth of each growing mycelial colony was measured daily in two perpendicular directions, without opening the Petri dishes, until the plates were completely colonized (Marín *et al.* 1996; Parra and Magan 2004). The radial mycelial growth was plotted and radial growth rates (μ, mm day^{-1}) were evaluated for each experimental combination from linear regression slopes of the temporal growth curves. The time required for growth (lag phase) was also recorded in each experiment for each treatment.

Statistical treatment of results

Growth rates (mm day^{-1}) were statistically analysed with a general linear model (GLM) available as SAS software (SAS Institute, version 8·2, Cary, NC, USA). Statistical significance was judged at the $P < 0{\cdot}05$ level. Whenever analysis revealed statistically significant differences, Duncan's multiple range test for separation of mean values was performed. To correct the homogeneity of variance, the Box-Cox transformation was used to transform the radial growth rate data to the square root. For both *P. digitatum* and *P. italicum*, the square root of the radial growth rate (μ) was modelled by means of the full factorial design including three levels of T (25, 15 and 5°C) and a_w (0·98, 0·93 and 0·89; Lahlali *et al.* 2005). This design was applied to triplicate combinations in a single block. A second-order polynomial equation was used to fit the square root of the radial growth rate for each solute ($\sqrt{\mu} = \beta_0 + \beta_1 T + \beta_2 a_w + \beta_{11} T^2 + \beta_{22} a_w^2 + \beta_{12} T a_w$) with six coefficients (β_0, intercept; β_1, β_2, linear coefficients; β_{12}, interaction coefficient; β_{11}, β_{22}, squared coefficients) by means of the statistical software package DESIGN-EXPERT®, version 6·0 (StatEase, Inc., Minneapolis, MN, USA). The parameters used for statistical comparison of the solute models were regression coefficient significance, the model's 'Lack-of-Fit of f_{value}', and the root mean square error (RMSE) given by the following equation:

$$\mathrm{RMSE} = \sqrt{\frac{\mathrm{RSS}}{\mathrm{d.f.}}} = \sqrt{\frac{\sum(\mu_{\mathrm{observed}} - \mu_{\mathrm{predicted}})^2}{\mathrm{d.f.}}},$$

where RSS is the residual sum of squares and d.f. is the number of degrees of freedom.

Results

Effect of water activity (a_w), temperature and solutes on the lag phase and growth rate of Penicillium species

The *in vitro* effects of a_w and solute on the radial growth rates of *P. digitatum* (Fig. 1) and *P. italicum* (Fig. 2) were studied at three incubation temperatures (25, 15 and 5°C). Figure 1 illustrates that the radial growth rate of *P. digitatum* was markedly reduced at low a_w, whatever the incubation temperature and solute used. The radial growth rate was highest at 0·98 a_w in all cases. In the presence of increasing amounts of sorbitol, glycerol, or glucose (lower a_w values), *P. digitatum* was able to grow as long as the a_w remained ≥0·89. With the ionic solute NaCl, its growth stopped at 0·93 a_w, whatever the incubation temperature.

The duration of the *P. digitatum* lag phase was also recorded for each temperature-a_w-solute combination

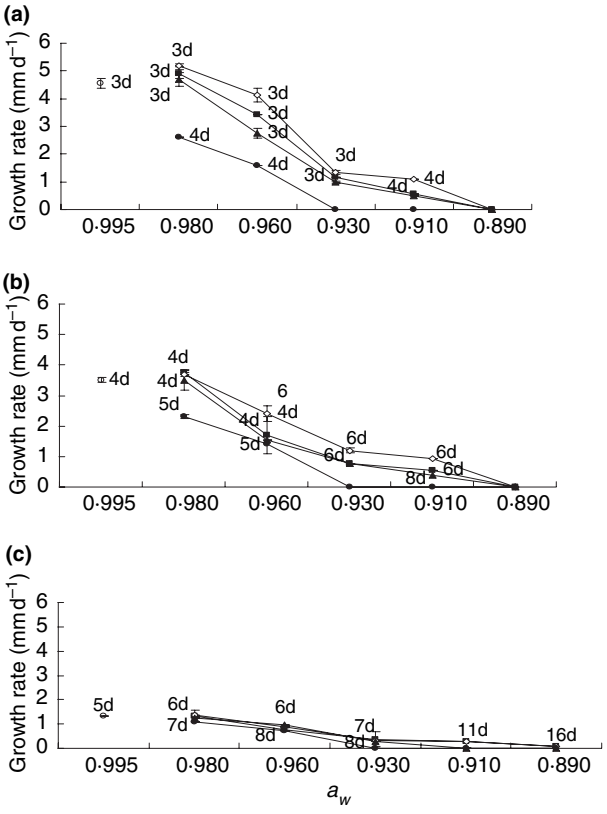

Figure 1 Effect of water activity on lag phase and growth rate of *Penicillium digitatum* in modified medium with, glycerol (■), sorbitol (◇), glucose (▲) and NaCl (●) and unmodified medium 0.995 (○) at 25°C (a), 15°C (b), 5°C (c). The number of days (d) for initiation of growth is shown. Bars represent the standard error of the mean values. Where the bars are not shown, they are smaller than the symbol size.

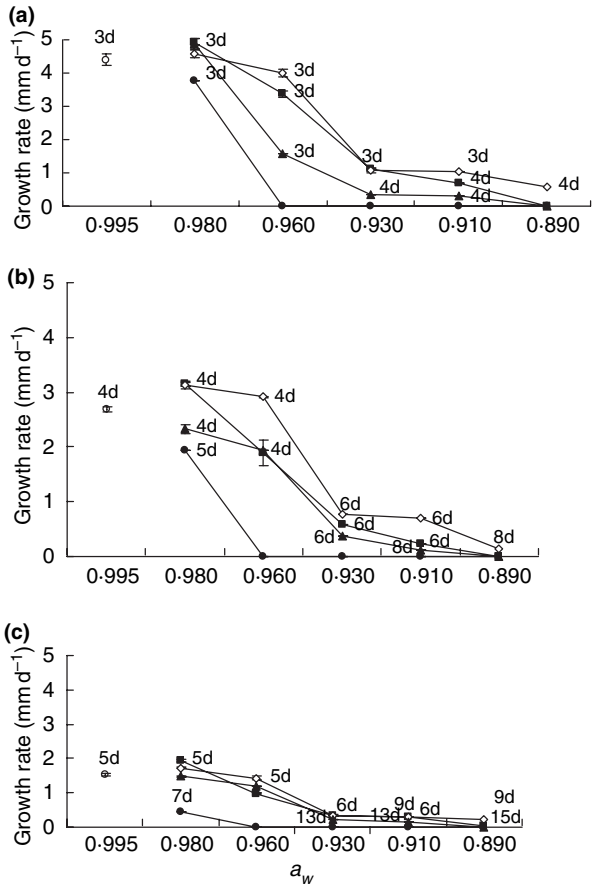

Figure 2 Effect of water activity on lag phase and growth rate of *Penicillium italicum* in modified medium with, glycerol (■), sorbitol (◇), glucose (▲) and NaCl (●) and unmodified medium 0.995 (○) at 25°C (a), 15°C (b), 5°C (c). The number of days (d) for initiation of growth is shown. Bars represent the standard error of the mean values. Where the bars are not shown, they are smaller than the symbol size.

(Fig. 1). At 25°C (Fig. 1a), *P. digitatum* began to grow on unmodified medium (a_w: 0·995) after 3 days. Except when the added solute was NaCl, the lag phase was similar in the range of 0·93–0·98 a_w. At 0·91 a_w, growth at 25°C on glucose-, sorbitol-, or glycerol-supplemented medium began 1 day later.

The lag phase of *P. digitatum* was consistently longer at 15°C than at 25°C (Fig. 1b). On unmodified medium and medium adjusted to 0·98 a_w, it was 1 day longer. At 0·93 a_w (achieved with a nonionic solute), it was 3 days longer. At 0·91 a_w, it was 2 days (sorbitol, glycerol) to 4 days (glucose) longer.

At 5°C, growth was much slower than at 25 and 15°C on all media (Fig. 1c). At a_w 0·96–0·98, the lag phase was 2–4 days longer at 5°C than at 25°C; below 0·96 a_w it was 4–7 days longer.

Statistical analysis shows a significant effect of a_w, incubation temperature, solute and their interactions (data not shown) on the growth rate of *P. digitatum*. For the a_w factor, Duncan's multiple range test revealed six statistically homogenous groups, one for each a_w value. Growth was increased faster at 0·98 a_w. With regard to the solute, a similar test distinguished five groups, one for each solute. Finally, the test revealed three homogenous groups for the influence of incubation temperature. Growth was faster at 25°C than at 15 and 5°C.

The response of *P. italicum* to solute addition was similar to that of *P. digitatum*. Figure 2 again illustrates a radial growth rate decreasing with decreasing a_w and a lag phase increasing with water stress. Growth was faster in medium supplemented with sorbitol or glycerol than in medium supplemented with glucose or NaCl. In sorbitol-supplemented medium, *P. italicum* was able to grow at $a_w < 0{\cdot}89$ whatever the temperature. This contrasts with its behaviour in NaCl-supplemented medium, where no growth was detected below 0·96 a_w at any temperature.

As with *P. digitatum*, GLM data analysis revealed significant effects of all three studied factors (temperature, a_w and solute) and of their two- and three-way interactions (data not shown). For the a_w factor, Duncan's multiple range test distinguished five homogenous groups, with 0·995 and 0·98 a_w belonging to the same group displaying the highest growth rate. For the solute and temperature effects, the results were similar to those reported for *P. digitatum*, with one group for each solute or temperature. Growth was much faster at 25°C than at 15 and 5°C.

Modelling the growth rate of these *Penicillium* species

Before using the data relative to each solute and each *Penicillium* species for modelling, we subjected them to Box-Cox transformation. The aim was to determine the best transformation leading to the most convenient regression model. We selected square root transformation of the radial growth rate. A value of 0·5 was obtained for the λ-transformation parameter (results not shown). For each *Penicillium* species and each solute, a complete three-level factorial design was applied to the square roots of the radial growth rates.

Models of square root of growth rate *vs* a_w and temperature were obtained for *P. digitatum* (Fig. 3) and *P. italicum* (Fig. 4). Table 1 provides, for each solute, the multiple regression coefficients relative to the model applicable to *P. digitatum*. In the case of each solute, all coefficients have a significant effect, except coefficient β_0 in the NaCl model, quadratic coefficient (β_{11}) of temperature in the sorbitol model, and quadratic coefficient (β_{22}) of a_w in the glycerol, sorbitol and glucose models. Only in the sorbitol model does this coefficient have a negative effect. A similar effect was observed in all models for quadratic coefficient β_{11} of temperature.

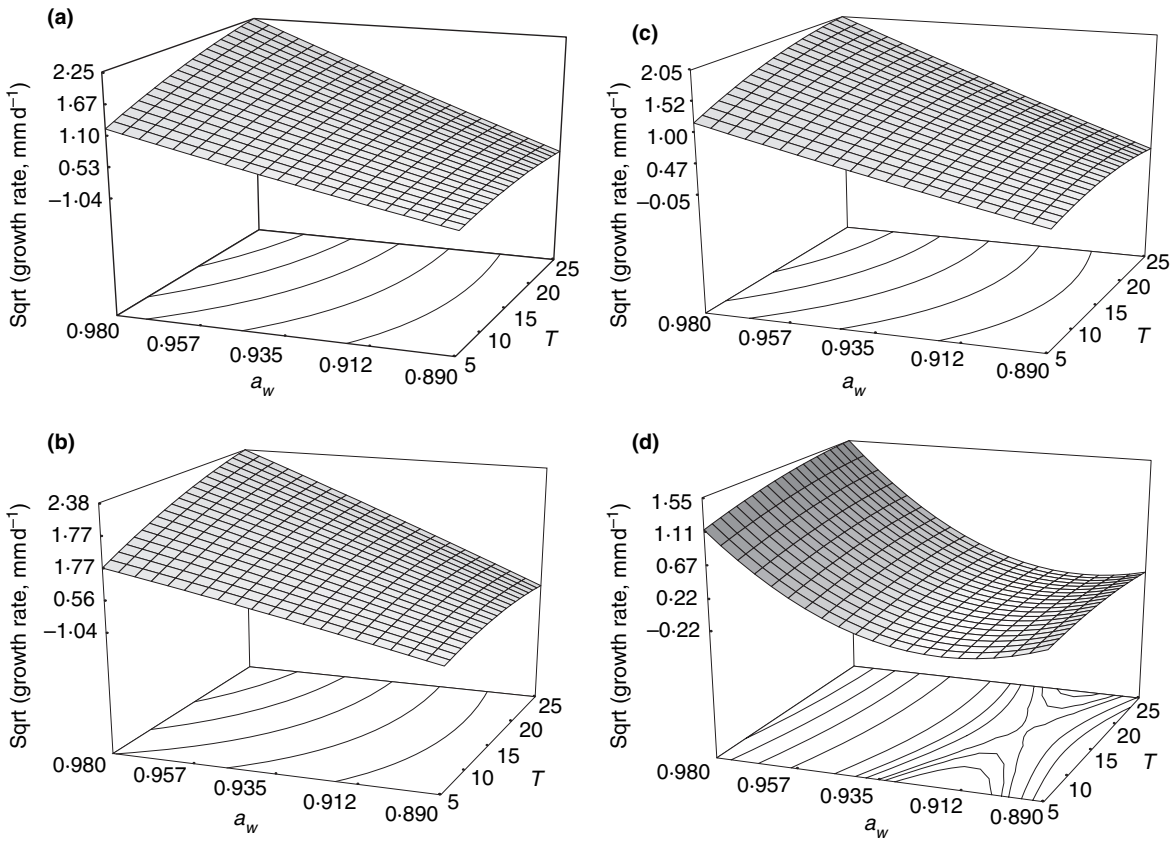

Figure 3 Response surface contour plots showing the effect of a_w and temperature on square root of growth rates (Sqrt, mm day^{-1}) of *Penicillium digitatum* in models based on glycerol (a), sorbitol (b), glucose (c) and NaCl (d).

High R^2 coefficients (ranging from 98·91% to 99·54%) express the proportion of the variation of the square root of the radial growth rate that is explainable by a_w and temperature. In all constructed models, a_w has a greater effect than temperature.

For *P. italicum*, the models show a significant effect of all coefficients except β_0 in the NaCl model and the quadratic coefficient of temperature in all models (Table 2). The latter coefficient has a negative effect in the NaCl and glycerol models and a positive effect in the glucose and sorbitol models. The R^2 value reaches 97·59%, 98·90%, 98·42%, and 96·70%, respectively, in the glycerol, sorbitol, glucose and NaCl models. Differences between these values are very less.

Model fit

After estimating the regression coefficients of all models, an important step in analysis is to evaluate the goodness-of-fit of all obtained models. For this the most appropriate indicator is the RMSE, expressing the standard deviation of residuals (Panagou *et al.* 2003; Ratkowsky 2003; Dantigny *et al.* 2005). Results for both fungi are summarized in Table 3. The smaller the RMSE value, the better the performance of the model. In the case of *P. digitatum*, the smallest value of this coefficient is found in the sorbitol model, followed by the glycerol, glucose, and NaCl models respectively. Here, the RMSE values obtained with the different models are too close to allow selection of a 'best model'. In the case of *P. italicum*, two groups can be distinguished: on the one hand, the glycerol, glucose and NaCl models with RMSE values between 0·10 and 0·12, and on the other hand, the sorbitol model, with an RMSE of 0·07 (Table 3).

A second statistical test (Lack-of-Fit of f_{value}) was applied in order to select the most appropriate model (Table 3). For each model pertaining to each pathogen, the f_{value} was superior to F_{table}, indicating that the test was significant in each case. The Lack-of-Fit of f_{value} was lowest in the glycerol model for *P. italicum* and in the sorbitol model for *P. digitatum*.

Discussion

The results obtained in this study highlight a significant effect of a_w, temperature, solutes and the interactions of

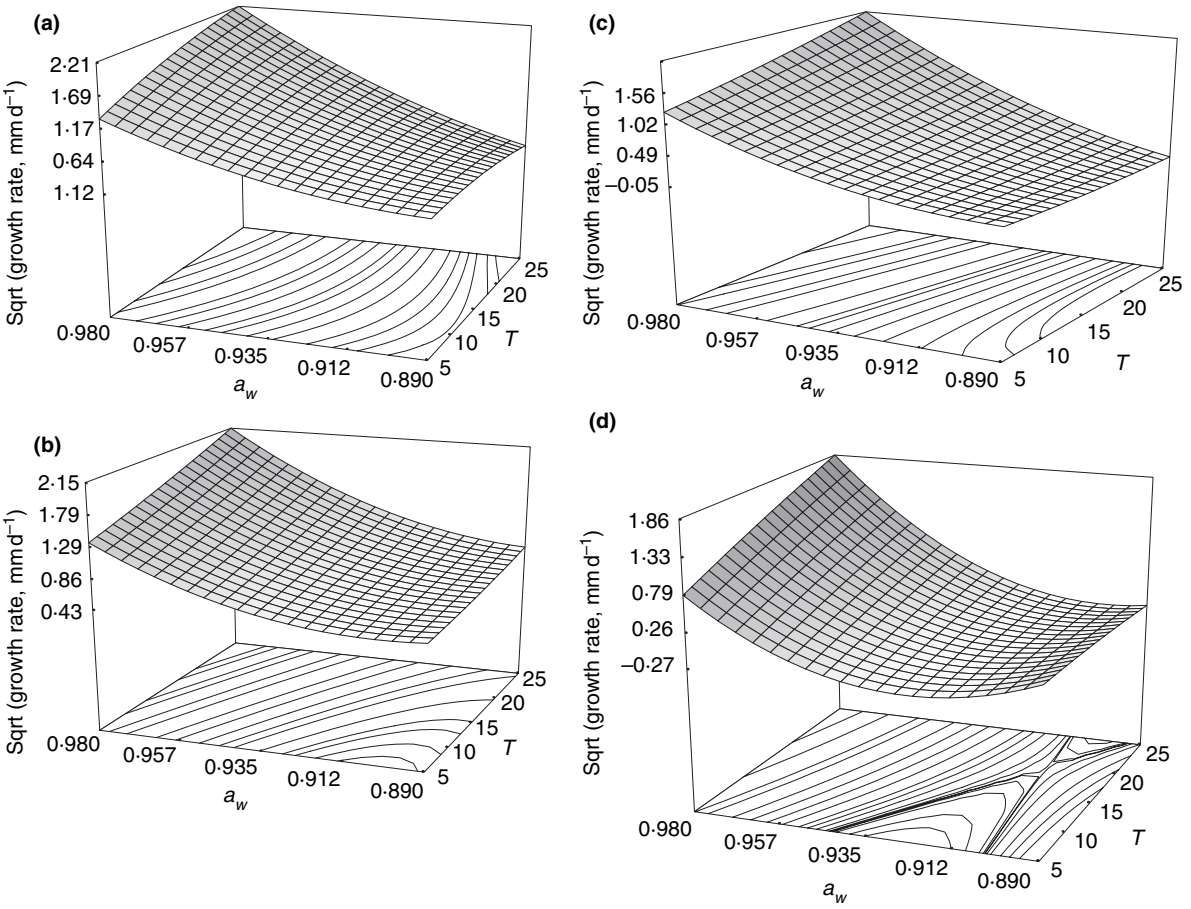

Figure 4 Response surface contour plots showing the effect of a_w and temperature on square root of growth rates (Sqrt, mm day^{-1}) of *Penicillium italicum* in models based on glycerol (a), sorbitol (b), glucose (c) and NaCl (d).

Table 1 Model coefficients and their significant effects on root square radial growth rate of *Penicillium digitatum* obtained for nonionic solute glycerol, sorbitol, glucose and ionic solute NaCl

	Coefficients	Glycerol model	Sorbitol model	Glucose model	NaCl model
R^2		99·47	99·54	99·14	98·91
Response mean	β_0	0·92**	1·08**	0·90**	0·049^{ns}
T	β_1	0·25**	0·30**	0·22**	0·083**
a_w	β_2	0·88**	0·91**	0·83**	0·68**
T^2	β_{11}	−0·097**	−0·13^{ns}	−0·14*	−0·074*
a_w^2	β_{22}	0·027^{ns}	−0·03^{ns}	0·028^{ns}	0·68**
$T \times a_w$	β_{12}	0·26**	0·30**	0·22**	0·13**

*Significant ($P < 0·05$); **highly significant.
ns, not significant.

Table 2 Model coefficients and their significant effects on root square radial growth rate of *Penicillium italicum* obtained for nonionic solute glycerol, sorbitol, glucose and ionic solute NaCl

	Coefficients	Glycerol model	Sorbitol model	Glucose model	NaCl model
R^2		97·59	98·90	98·42	96·70
Response mean	β_0	0·86**	0·82**	0·53**	0·019^{ns}
T	β_1	0·18**	0·25**	0·18**	0·21**
a_w	β_2	0·78**	0·59**	0·83**	0·67**
T^2	β_{11}	−0·088^{ns}	0·027^{ns}	0·036^{ns}	−0·029^{ns}
a_w^2	β_{22}	0·21**	0·31**	0·27**	0·67**
$T \times a_w$	β_{12}	0·26**	0·14**	0·25**	0·32**

*Significant ($P < 0·05$); **highly significant.
ns, not significant.

these factors on the lag phase and the radial growth of both *Penicillium* species tested. The two species occupy slightly different ecological niches, reflected in their growth capacities. *Penicillium italicum* grows more quickly than *P. digitatum* at 5°C, but more slowly at 25°C. At 0·89 a_w, no growth was observed for *P. digitatum* at any temperature and regardless of the solute used. *Penicillium italicum*, in contrast, was found to germinate at this water activity under some conditions. These results

Table 3 Validation indices (RMSE, f_{value}) for the performance of the root square radical growth rate models ($\sqrt{4} = \beta_0 + \beta_1 T + \beta_2 a_w + \beta_{11} T^2 + \beta_{22} a_w^2 + \beta_{12} T a_w$) of *Penicillium italicum* and *P. digitatum*

	P. italicum		*P. digitatum*	
	RMSE	f_{value}	RMSE	f_{value}
Glycerol	0·12	**31·73**	0·06	402·97
Sorbitol	**0·07**	61·91	**0·06**	**13·39**
Glucose	0·10	340·58	0·08	33·96
NaCl	0·14	94·88	0·08	1785·73

RMSE, root mean square error; f_{value}, Lack-of-Fit of f_{value}; $F_{table}(3,18)_{0·05} = 3·16$ for all models. Bold values indicate selected models.

are in accordance with those of Plaza *et al.* (2003), showing germination and growth of *P. italicum* at 0·87 a_w and failure of *P. digitatum* to grow at 0·90 a_w. These authors also found *P. italicum* to grow faster than *P. digitatum* at low temperature and *P. digitatum* to be more active at high temperature.

In the present study, both *Penicillium* species grew fastest at 25°C, as previously reported by Plaza *et al.* (2003) and Lacey (1989). These authors demonstrated that *P. digitatum* and *P. italicum* could germinate and grow at temperatures ranging from 4 to 30°C; they detected no growth or germination of either species at 37°C.

On the basis of our results and those of Plaza *et al.* (2003) regarding the influence of temperature on the growth capacity of these pathogens, it is possible to explain the observation that green decay is 'favoured' in citrus fruit stored under ambient conditions and blue decay is more likely under cold storage conditions.

We have also examined the influence of water activity on the growth of *P. digitatum* and *P. italicum*. Consistently the growth rate was found to decrease as the a_w was lowered from 0·98 to 0·89. When a nonionic solute was used to adjust the a_w, the lag phase prior to the start of growth was increased as the a_w decreased.

It is worth stressing at this point that previous investigations of the effects of a_w and temperature on the germination and growth of major citrus fruit spoilage organisms (Lacey 1989; Plaza *et al.* 2003) have focused solely on a_w and temperature, without considering the effect of the solute used to adjust the a_w of the medium. Here, we have taken this aspect into account.

In addition to the ionic solute NaCl, investigators have used various nonionic solutes to adjust the a_w of a medium, particularly glycerol (Parra and Magan 2004), sucrose (Sparringa *et al.* 2002), sorbitol and glycerol (Sautour *et al.* 2001b). One advantage of nonionic solutes is that it is possible to obtain lower a_w values than with NaCl. Hocking and Pitt (1979), furthermore, observed less dramatic effects with nonionic solutes than with NaCl. Two other observations are worth mentioning in this context. At first, the minimum predicted a_w allowing growth of *Penicillium expansum* depends on the solutes used (Lahlali *et al.* 2005). Secondly, glycerol can sometimes be a source of carbon. Here, we have compared and analysed the growth effects observed with three nonionic solutes (glycerol, sorbitol and glucose) and the ionic solute NaCl. Beuchat and Pitt (1990) likewise reported that NaCl mediated a greater reduction in *Wallemia sebi* growth compared with nonionic solutes (sorbitol, glucose). In our view, a diffusion phenomenon occurring in both directions across the cell membrane could be the major explanation for the reduced fungal growth capacity observed with NaCl. The Na^+ and Cl^- ions diffuse from area of high concentration (PDA medium) to an area of lower concentration (fungal cells). This phenomenon leads to a greater loss of water from cell and is more marked in case of ionic solutes than nonionic solutes. Sodium toxicity is another explanation that could be advanced. 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.

Our second major undertaking was to model the effect of a_w and temperature on the square root of the radial growth rate observed with each solute. Several studies highlight the importance of predictive microbiology as a tool for developing mathematical food spoilage models. Such models help to predict microbial outbreaks in relation to abiotic factors and point to measures that should be taken to avoid their spread (Buchanan 1993; McMeekin *et al.* 1993, 2002). Among the mathematical approaches available for this purpose, we selected RSM. A full three-level factorial design was used for each solute in order to model the behaviour of the square root of the radial growth rate of each citrus spoilage fungus according to the temperature and a_w of medium. A quadratic polynomial equation was used to fit the square roots of the growth rates. The model's R^2 coefficient measures how much of the variability of the observed response value is attributable to the experimental factors and their interactions (Box and Draper 1987). The closer the R^2 value is to 1·00, the stronger the model and the greater its ability to predict the response (Haaland 1989). In our study, the value of this coefficient was markedly and consistently high, exceeding 96% for both pathogens. Use of the RMSE and of the Lack-of-Fit test enabled us to evaluate the quality of all models and to determine which model provides a very good fit with actual growth. The Lack-of-Fit of f_{value} proved significant for all constructed models. The results obtained lead to the conclusion that both models based on glycerol and sorbitol supplementation are most appropriate for accurate prediction of

P. italicum growth and the model based on sorbitol supplementation is best for predicting *P. digitatum* growth under the conditions used. These polynomial models might help to predict satisfactorily the spread of citrus decay caused by *Penicillium*. The difference observed between the sorbitol and glycerol models was slight, in accordance with the results of Sautour *et al.* (2001b) who found no significant difference between a glycerol and a sorbitol model based on germination of *Penicillium chrysogenum* and showed that a_w has a greater effect on germination than temperature. The RSM has been applied to other food spoilage fungi, such as *Rhizopus oligosporus* NRR2720 (Sparringa *et al.* 2002) and *P. chrysogenum* (El-Halouat and Debevere 1997; Sautour *et al.* 2001a,b). In addition for *Aspergillus*, Parra and Magan (2004) obtained results similar to ours in the temperature range of 25–35°C and the 0·90–0·99 a_w when glycerol was used as the humectant.

The models employed here were developed as tools for interpreting square root of radial growth data of *P. italicum* and *P. digitatum*. Within the a_w and temperature ranges specified the selected models can accurately predict *Penicillium* growth rates (mm day^{-1}). This constitutes the originality of the present study with respect to previous reports (Lacey 1989; Plaza *et al.* 2003) on the same *Penicillium* species. Yet it is crucial to emphasize that the selected models, i.e. the glycerol and sorbitol models for *P. italicum* and the sorbitol for *P. digitatum*, are based on data obtained under *in vitro* conditions. Consequently, the predicted growth could be faster than growth under natural conditions, because the artificial medium is probably richer in nutriments. Furthermore, extrapolation to natural conditions remains hazardous because factors other than those studied here may be involved, such as the interaction between existing microflora and the pH of citrus fruit. Therefore, it may be necessary to develop models based on *in vivo* conditions, taking into account factors, such as relative humidity, temperature and CO_2.

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