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Growth kinetics of cultures from single spores of *Aspergillus flavus* and *Fusarium verticillioides* on yellow dent corn meal

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Abstract

A dilution protocol originally developed for the isolation of single bacterial cells was modified to suite the specificities of fungal growth. The modified protocol was used to study the growth kinetics of single spores of *Aspergillus flavus* and *Fusarium verticillioides* on yellow dent corn meal. Both a_w and temperature significantly influenced the distributions of the colony growth rates and lag phases and the rate at which individual spores of both isolates completed the lag period. An interaction between a_w and temperature was noted on the spread of the distributions of these growth parameters. The histograms of the single spore colony growth rates and lag phases generally became wider the more compromising the conditions for growth became, indicating a greater variation in growth ability at these conditions. The rate at which the single spores passed through the lag phases generally decreased with decrease in temperature and/or a_w, with an interaction again noted between these two factors on the rate. These results show the potential range and variability in growth of individual fungal spores at the lowest inoculum level possible.

Keywords: *Aspergillus flavus*; *Fusarium verticillioides*; Individual lag phases; Individual colony growth rates; Water activity; Temperature; Individual spores

1. Introduction

Traditionally the growth and ecophysiological characterization of fungal contaminants of foods has been determined using very high inoculum levels in the form of spore suspensions or circular disks cut from the margins of growing colonies. This approach, although sufficient, may over look the significance of fungal behaviour at the individual spore level. The behaviour and spread of the distribution of the growth rates and lag phase duration/germination times of individual spores potentially provides important information with regards to the ability of a single spore to germinate, grow and spoil a food product. It may actually be that contamination at low inoculum levels or by individual spores plays a more important role in fungal infection, spoilage of foods and subsequent production of mycotoxins of public and animal health significance than previously thought. The size of the spore inoculum has been already found to have direct relationship to fumonisin production on corn (Chulze et al., 1999), with an increase in the inoculum size resulting in an increase in the amount of fumonisins produced. The size of the spore inoculum has also been found to have a strong effect on the lag duration of *Penicillium chrysogenum* (Sautour et al., 2003), which was reported to decrease as the size of the inoculum increased.

As part of a larger study on the growth of fungal contaminants of corn and the evaluation of preservation techniques to inhibit their growth and mycotoxin production, an attempt was made to develop a method for the isolation of single spores and for the determination of their ability to grow and variation in their growth on corn as influenced by the most important environmental factors.

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This would importantly also give an idea of the potential of single spores to germinate and spoil food products. Recently, a protocol was developed for the isolation of single bacterial cells of *Lactococcus lactis* by Francois et al. (2003). This method was chosen and modified to isolate single fungal spores on the basis that it was reported to combine a higher chance of having one cell in a microtitre plate with a high yield and its relative simplicity. In addition to validation of the protocol for the isolation of single spores of *Aspergillus flavus* and *Fusarium verticillioides*, the method was then used to investigate the growth kinetics of these two isolates on yellow dent corn meal and the influence of a_w and temperature on the trends observed.

2. Material and methods

2.1. Fungal isolates and preparation of inoculum

The isolates used in the study, *F. verticillioides* Sheldon (25 N) and *A. flavus* (IITAB 139), were obtained courtesy of the Food Technology Department Culture Collection of the University of Lleida (Spain) and the International Institute of Tropical Agricultural (Cotonou, Benin), respectively. The isolates were sub-cultured on potato dextrose agar (PDA) (Oxoid, Basington, UK). To prepare the inoculum, centrally inoculated PDA plates were incubated at 30 °C for 7 d to enable significant sporulation to take place. The plates were then transferred to an incubator at 20 or 30 °C for a further day, which enabled the spores to adapt to their final incubation temperature. After incubation 5 ml of wetting agent (3 g Tween-20/l distilled H_2O) was aseptically added to each plate. A sterile plastic inoculation loop was then used to loosen the colonies (spores and mycelia) from the PDA plates. The suspension created containing the spores and mycelia was then filtered through sterile glass wool into to a sterile 50 ml capacity Falcon tube (Meus, Piove di Sacco, Italy). This process was repeated a further three times. The spores were then separated from any remaining debris (mostly mycelia) by centrifuging in a Sigma 4K15 centrifuge (Sigma, Göttingen, Germany) at 10,000 rpm for 15 min. The wetting agent was then carefully decanted from the pellet of spores and replaced by 20 ml of sterile phosphate buffered saline with Tween-20, pH 7.4 (PBS) (Sigma-Aldrich, Steinheim, Germany). The number of spores per ml of spore suspension was then determined using a Bürker counting chamber (Superior Mareinfeld, Lauda-Könisghofen, Germany). A Carl Zeiss Axio Imager A1 microscope (Göttingen, Germany) was used to visualize the spores on 20 different cells on the counting chamber. After determination of the average of number of spores per ml of spore suspension, the suspension was then serially diluted to a concentration of $\approx 1 \times 10^3$ spores/ml.

2.2. Protocol evaluation

The protocol developed by Francois et al. (2003) for the isolation of single bacterial cells was evaluated for its ability to isolate single fungal spores. The evaluation was performed on PDA. In brief, 200 µl of PBS were placed in all of the wells of a 96-well microtitre plate using a micropipette. Then, 200 µl of the spore suspension, prepared on the same day as described in Section 2.1 and adjusted to a concentration of $\approx 10^3$ spores/ml, were added to the wells in the first column resulting in approximately 200 spores in each of these wells. A $\frac{1}{2}$ dilution series was then prepared sequentially, by thoroughly mixing the contents in these wells before transferring 200 µl to the next well, until the last column of the microtitre plate was reached. This was repeated on six microtitre plates, giving rise to 48 dilution series per isolate. The contents of the wells were then transferred and spread aseptically on PDA plates which were incubated at 30 °C for 3–5 d before enumeration. The performance of the method was determined by comparison of the mean number of spores per column with the theoretically expected means. A comparison was also made with the results obtained for bacterial growth by Francois et al. (2003). According to Francois et al. (2003), each cell theoretically has 50% probability of staying in the well and an equal probability of going to the next well. This would ideally result in 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625, 0.1953125, 0.09765625 and 0.09765625 cells in 12 consecutive wells.

2.3. Growth kinetics of single spores of *A. flavus* and *F. verticillioides* on yellow dent corn meal

2.3.1. Experimental design

The growth kinetics and effect of a_w and temperature on the distribution of the colony growth rates and lag phase for growth of single spores of *A. flavus* and *F. verticillioides* on yellow dent corn meal was determined at two a_w and temperatures values. For *A. flavus*, a_w values of 0.88 and 0.98 were evaluated, whereas values of 0.92 and 0.98 were evaluated for *F. verticillioides*. As *A. flavus* grows over a wider a_w range than *F. verticillioides*, a larger interval in the experimental a_w limits was used to better reveal any effect a_w may have on growth at the single spore level. The temperatures evaluated for both isolates were 20 and 30 °C. For each condition the colony growth rates and lag phases from about 100 individual spores were determined.

2.3.2. Preparation of growth substrate

Fumonisin free yellow dent corn supplied by Aveve NV (Belgium) was used as the growth substrate. The initial water content of the corn was 12.79 ± 0.45 kg/100 kg on a dry matter basis, which corresponded to a a_w value of 0.698 ± 0.015. The corn was sterilized by means of 25 kGy of γ-irradiation at IBA Mediris (Fleurus, Belgium) ensuring

Table 1
$\frac{1}{2}$ dilution series of *A. flavus*, including the 95% confidence limits and the theoretically expected values of the mean numbers of spores in each column

Column	1	2	3	4	5	6	7	8	9	10	11	12
Trial no.												
1	91	44	28	17	10	7	3	2	1	1	1	0
2	102	56	32	11	9	5	1	1	1	0	1	1
3	85	42	29	15	9	4	2	3	0	1	0	0
4	86	43	20	16	12	6	3	0	1	0	1	1
5	92	49	29	12	8	6	3	1	1	1	0	0
6	105	51	22	20	12	5	2	0	2	0	1	0
7	82	45	36	15	7	4	4	1	1	0	0	0
8	84	49	23	10	6	6	2	0	0	0	0	0
…48												
Mean	95.42	48.38	25.46	13.00	8.10	4.79	2.25	1.06	0.67	0.40	0.31	0.19
Upper 95% CL*	97.19	49.94	26.80	13.81	8.76	5.28	2.53	1.33	0.87	0.54	0.45	0.30
Lower 95% CL	93.64	46.81	24.11	12.19	7.45	4.30	1.97	0.79	0.46	0.26	0.18	0.08
Theoretical mean values	100	50	25	12.5	6.25	3.125	1.5625	0.78125	0.39062	0.19531	0.09765	0.09765

The first 8 of 48 trials are shown.
*CL: confidence limit.

Table 2
$\frac{1}{2}$ dilution series for *F. verticillioides*, including the 95% confidence limits and the theoretically expected values of the mean numbers of spores in each column

Column	1	2	3	4	5	6	7	8	9	10	11	12
Trial no.												
1	98	52	26	16	8	4	2	2	1	0	1	0
2	95	43	30	12	7	4	2	1	1	0	1	0
3	105	40	25	12	9	4	1	3	0	1	0	0
4	94	51	27	15	10	5	2	0	1	0	0	1
5	90	50	32	13	7	3	3	1	1	1	0	0
6	93	46	24	13	7	4	3	0	3	1	0	0
7	104	39	21	14	8	3	1	1	1	0	0	0
8	102	48	30	15	6	3	1	0	0	0	0	0
…48												
Mean	97.27	47.52	25.46	12.42	6.48	3.98	2.23	1.10	0.69	0.40	0.17	0.13
Upper 95% CL*	98.66	48.79	26.44	12.97	6.92	4.31	2.48	1.39	0.89	0.54	0.27	0.22
Lower 95% CL	95.89	46.25	24.47	11.86	6.04	3.65	1.98	0.82	0.48	0.26	0.06	0.03
Theoretical mean values	100	50	25	12.5	6.25	3.125	1.5625	0.78125	0.39062	0.19531	0.09765	0.09765

The first 8 of 48 trials are shown.
*CL: confidence limit.

that the decontaminated corn retained its germinative capacity. Upon reception, the corn was kept in a storage room at 7 °C to prevent fungal contamination. Adsorption isotherms developed for the corn (Samapundo et al., 2006) were used to determine the amount of sterile distilled water that had to be aseptically added to the corn to reproducibly achieve the desired a_w value. The rehydrated corn was then equilibrated over 2 d at 4 °C before it was aseptically ground to a fine meal using a Waring blender (model 8010E, Torpington, CT, USA). The corn meal was then incubated for a further day at the final incubation temperature (20 or 30 °C) to enable it to attain the incubation temperature. The exact a_w of the corn meal was then determined by the Novasina Thermoconstanter TH200 (Axair Ltd. Systems, Pfapfikkon, Switzerland).

2.3.3. Inoculation, assessment and mathematical analysis of the growth

Approximately 22 g of the corn meal was aseptically transferred into Petri dishes to form a uniform layer. The spore inoculum was prepared and a $\frac{1}{2}$ dilution series was performed as described in Sections 2.1 and 2.2, respectively. However, only the contents of the last five columns were transferred to the centre of the plates with the corn meal as single spore inoculum. To maintain a constant relative humidity during the incubation period, Petri plates with treated grain of the same a_w were placed in plastic buckets containing glycerol/water solutions of the same a_w. During incubation, growth was assessed regularly by measurement of the perpendicular diameters of each growing colony. Considering the known linearity of the

radial extension of fungal colonies after the lag for growth (Samapundo et al., 2005a), it was decided that at least five or six data points be collected per condition during the linear phase of growth. This was strictly adhered to as Dantigny et al. (2005) observed that early measurements of diameter of the colony improved the accuracy of the

Table 3
Mean colony growth rates (g, mm d^{-1}) and lag phase durations (λ, d) of *A. flavus* and *F. verticillioides* on yellow dent corn meal

Temperature (°C)	a_w 0.98				a_w 0.88			
	g (mm d^{-1})	Std. dev.[a]	λ (d)	Std. dev.	g (mm d^{-1})	Std. dev	λ (d)	Std. dev.
A. flavus								
30	11.03	1.55	0.69	0.48	7.89	2.35	1.31	0.58
20	9.13	1.62	2.65	0.58	5.43	1.50	2.98	0.73
F. verticillioides								
	a_w 0.98				a_w 0.92			
30	8.69	1.89	1.45	0.64	6.81	1.84	1.62	0.72
20	6.67	1.57	3.44	0.86	3.45	0.79	4.95	1.04

[a]Standard deviation.

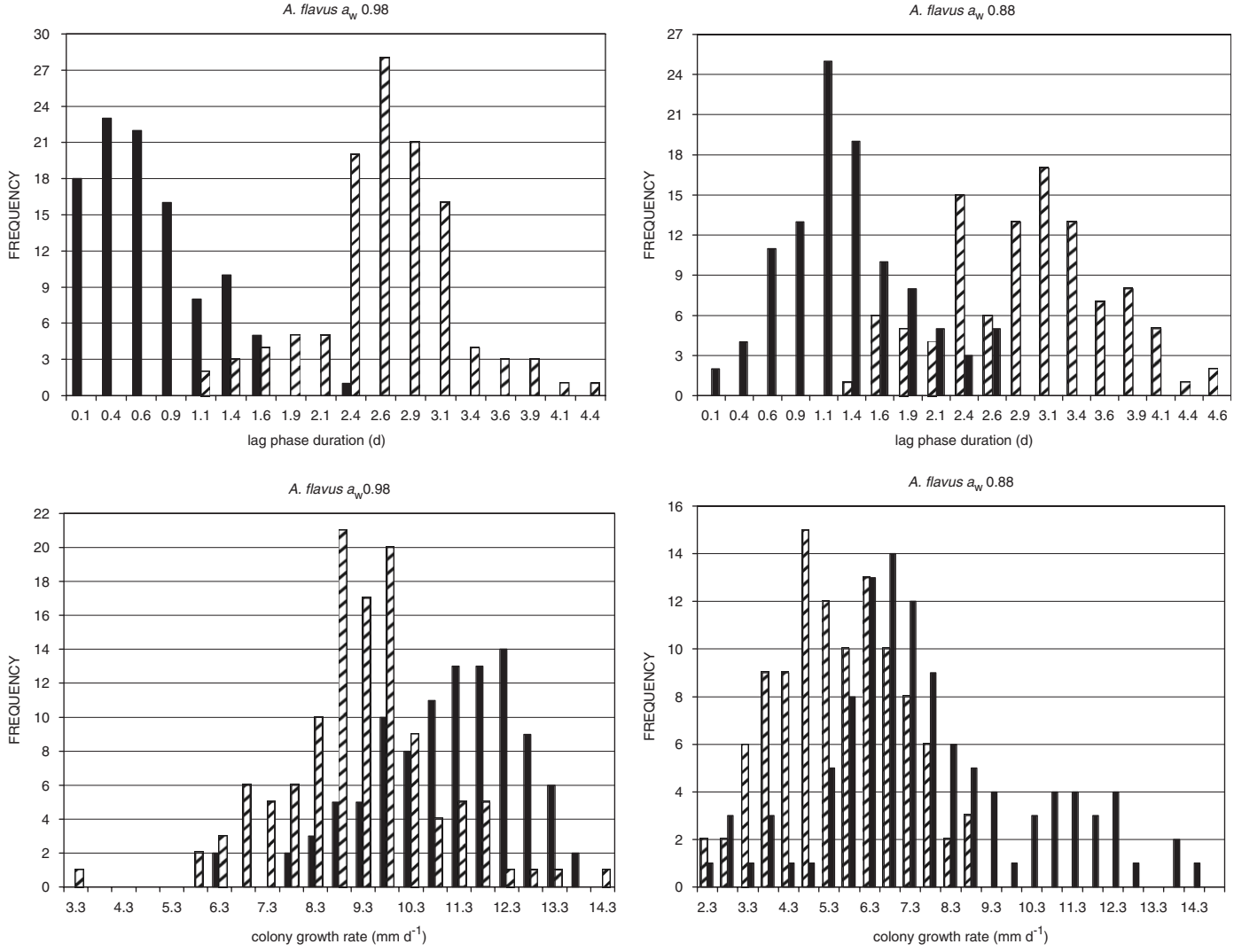

Fig. 1. Influence of temperature on the distribution of the lag phases and colony growth rates of individual spores of *A. flavus* at 20 (black and white columns) and 30 °C (black columns).

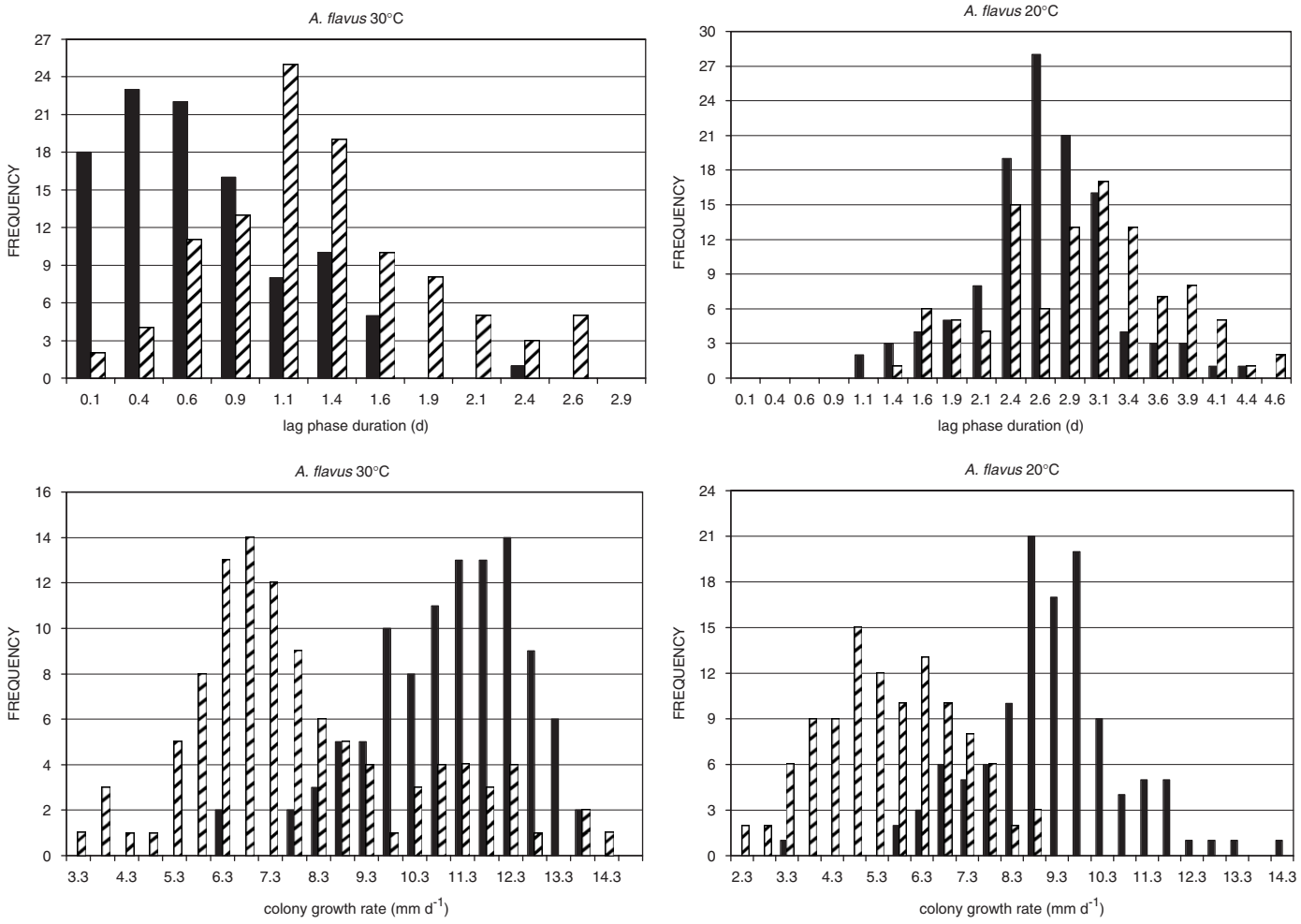

Fig. 2. Influence water activity on the distribution of lag phases and colony growth rates of individual spores of *A. flavus* at a_w 0.88 (black and white columns) and 0.98 (black columns).

estimated lag period for growth, when this parameter is obtained by extrapolation. Estimates of the colony growth rates (g, mm d^{-1}) and lag phase durations (λ, d) of each spore were determined by linear regression of the growth data collected during the linear phase of growth. The colony growth rate was determined as the slope of the curve whereas the lag phase duration was determined by extrapolating the linear regression equation to the time axis. A very significant advantage of the measurement of the colony growth on the petri plates as opposed to the turbidity measurements employed for bacterial cells on the microtitre plates (Francois et al., 2003), was that it was possible to identify and remove most of the petri plates on which more than one spore grew, allowing for the growth data collected to be largely representative of that of single spores. This is not possible with the turbidity measurements where all the growth data from the wells in the last five columns is taken into consideration, some of which contain more than cell.

The normality of the distributions obtained was determined by means of the Kolmogorov–Smirnov test in SPSS® Version 12.0 (SPSS Inc, Chicago, IL, USA). All the distributions were determined to be normal (results not shown). Cumulative distributions of the percentage of individual spores that had completed the lag for growth as a function of time at each condition were then created. A logistic model (Whiting and Call, 1993), also used by Dantigny et al. (2002) to describe the percentage of germinated spores of *Mucor racemosus* as a function of time, was then fitted to the cumulative distributions to describe the percentage of spores that had completed the lag for growth as a function of time:

$$P = P_{\max}/(1 + e^{k(t-t)}), \quad (1)$$

where P was percentage (%) of spores which have completed the lag for growth, $P_{\max}$ (%) the maximum percentage which was set to 100 (as all the spores used to generate the data grew), k (d^{-1}) was the slope term for the rate of increasing single spores that had completed the lag for growth, τ (d) was the time for the inflexion point when half of the spores have completed the lag for growth, and t (d) was the time. The logistic function was fitted to the cumulative distributions using the nonlinear regression function of SPSS® Version 12. A modified Gompertz

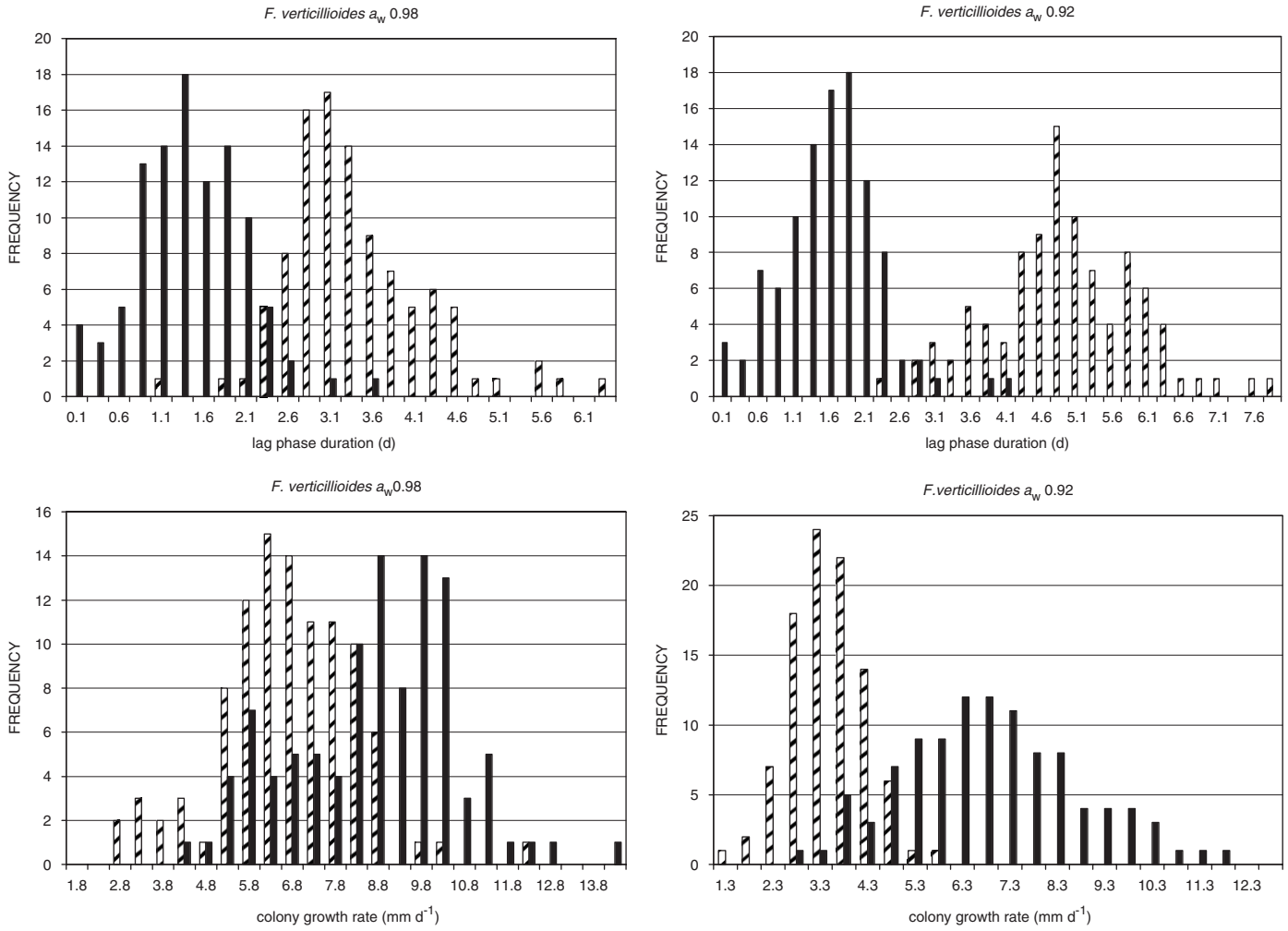

Fig. 3. Influence of temperature on the distribution of the lag phases and colony growth rates of individual spores of *F. verticillioides* at 20 (black and white columns) and 30 °C (black columns).

function has also been used to model such distributions (Marin et al., 1996; Pardo et al., 2005, 2006), but according to Dantigny et al. (2005), the logistic function appears to perform better than the Gompertz equation.

3. Results and discussion

3.1. Protocol evaluation

Tables 1 and 2 show the results of the evaluation of the protocol. As observed for bacterial cells (Francois et al., 2003) the dilution series do not necessarily follow the exact pattern expected theoretically, with some cells actually having no spores followed by cells with one or more spores. Although the mean number of spores per column were not identical to the theoretical mean values, they followed the expected trend and were not significantly different to those of theoretical values according to a *t*-test ($P<0.05$). As observed for bacterial cells by Francois et al. (2003), single spores were mostly found in the last five columns, with 78% and 74% of the wells which contained spores having single spores of *A. flavus* and *F. verticillioides*, respectively. This rose significantly to 93% and 89% when only the last four columns were considered for *A. flavus* and *F. verticillioides*, respectively. Similar values of 80% and 87.5% were obtained for *L. lactis* cells in the last five and four columns, respectively by Francois et al. (2003). A high yield of individual spores was obtained, 77 for *A. flavus* and 69 for *F. verticillioides* from 48 $\frac{1}{2}$ dilution series. This yield is much higher than that obtained by Francois et al. (2003) for *L. lactis* of 75 from 72 trials. Microscopic images of the fungal spores observed during the enumeration on the counting chamber showed very little clumping of the spores, which may very well play an important role in the higher yields noted in this study. On the basis of these results, the protocol could therefore be accepted as a simple high yielding means of isolating single fungal spores and was subsequently applied as such for the study to determine the growth kinetics of single fungal spores and the effect of a_w and temperature on the trends observed.

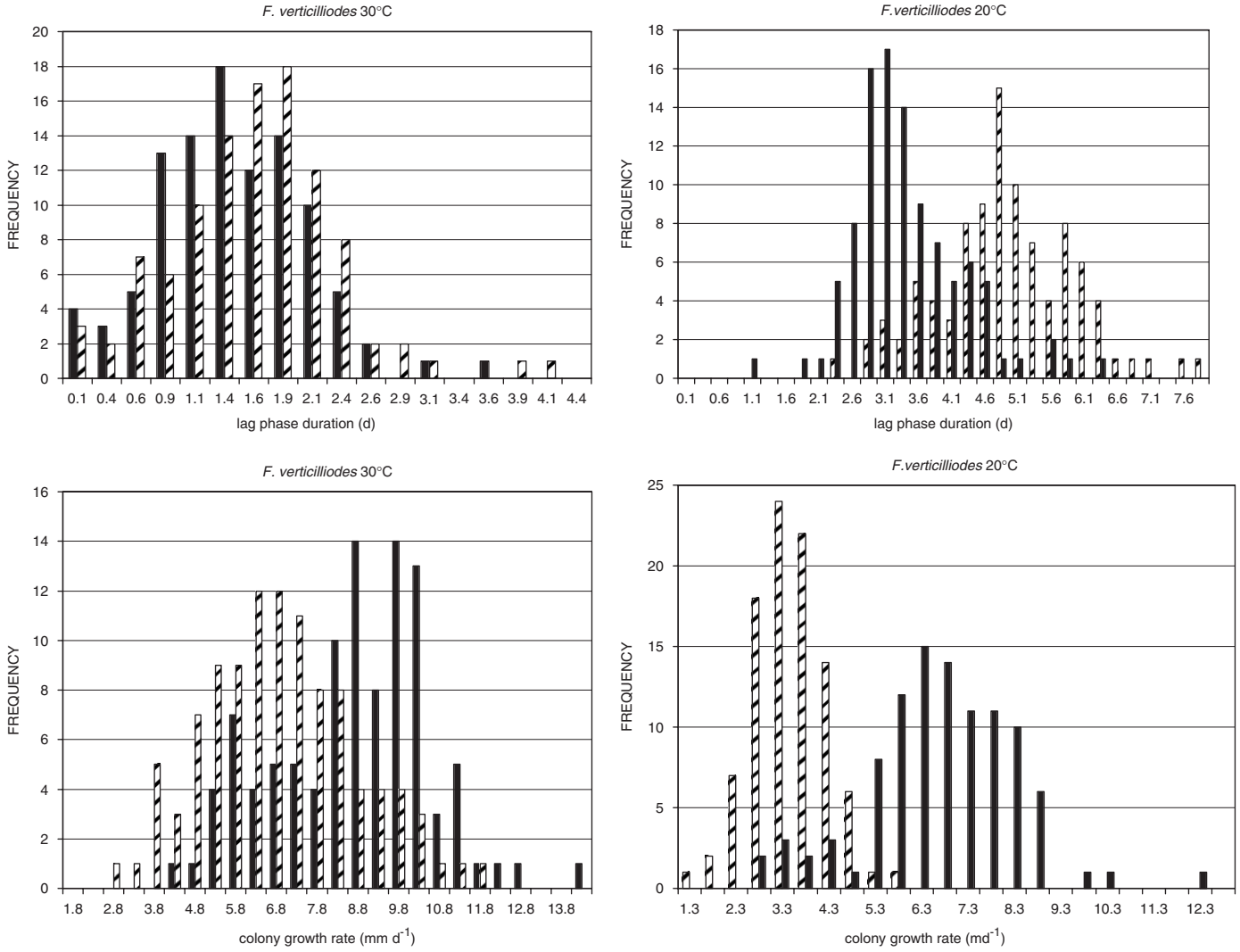

Fig. 4. Influence of temperature on the distribution of the lag phases and colony growth rates of individual spores of *F. verticillioides* at a_w 0.88 (black and white columns) and 0.98 (black columns).

3.2. Effect of water activity and temperature on the growth kinetics of single spores of A. flavus and F. verticillioides on corn meal

Table 3 shows the mean colony growth rates (g, mm d^{-1}) and lag phase durations (λ, d) of the two isolates investigated at different conditions. Also shown are the standard deviations of the estimated growth parameters. Generally high determination coefficients (r^2) were obtained for the linear regression of growth data of at least 0.92; these are however lower than those normally obtained for fungal growth, where r^2 is usually $\geqslant 0.99$ (Dantigny et al., 2002). This could have been due to the uneven surface of the corn meal. The mean colony growth rate decreased whereas the mean lag phase increased with a decrease in a_w and/or temperature. The same trends have also been observed when high inoculation levels have been used (Le Bars et al., 1994; Cahagnier et al., 1995; Marin et al., 1995, 1998, 1999; Samapundo et al., 2005a, b).

Important to note was that the yield of germinating single spores did not differ at any of the conditions within the investigated range and also did not differ from the yield that had being obtained on PDA during validation of the protocol. This indicated that ability of the individual spores to germinate on the corn meal was not significantly affected by the a_w and temperature range investigated in this study. This could change if more growth limiting conditions are investigated as Pitt and Miscamble (1995) reported that the a_w minima for germination of *A. flavus* and related species changed with temperature from 0.82, 0.81 to 0.80 at 25, 30 and 37 °C, respectively. Marin et al. (1998) also reported similar minima a_w for germination between 0.80 and 0.85 for *Aspergillus* species including *A. flavus*.

The effect of temperature and a_w on the distribution of lag phases and colony growth rates of the individual spores of *A. flavus* is illustrated by the histograms in Figs. 1 and 2, respectively. Those showing the effect of temperature and

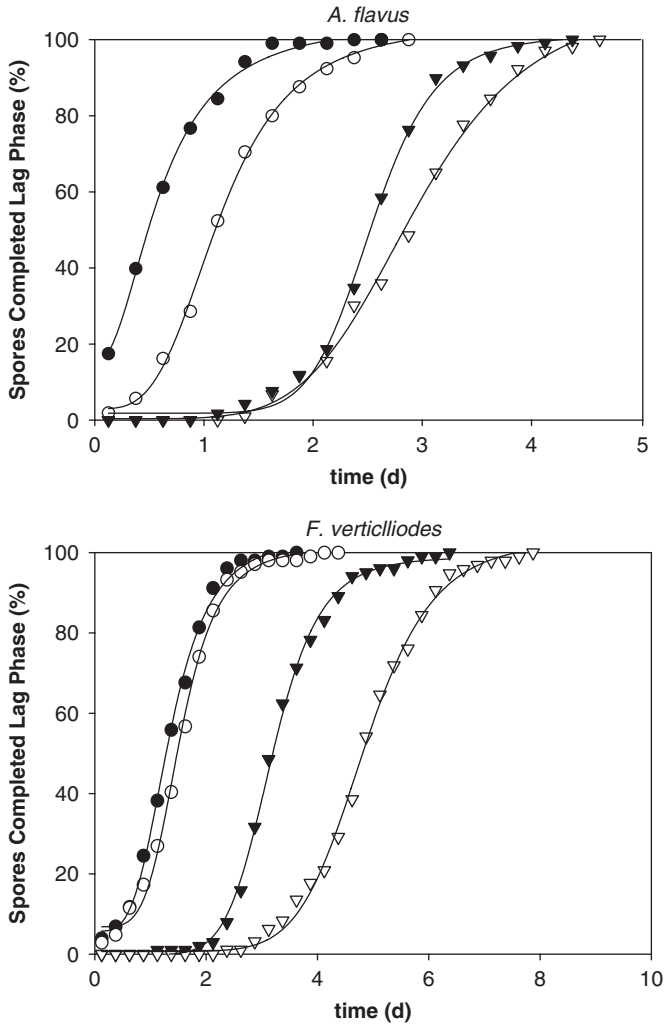

Fig. 5. Influence of water activity on the rate of germination of individual lag phases and colony growth rate of *A. flavus* at 30 °C a_w 0.98 (●), 30 °C a_w 0.88 (○), 20 °C a_w 0.98 (▼) and 20 °C a_w 0.88 (▽) and *F. verticillioides* at 30 °C a_w 0.98 (●), 30 °C a_w 0.92 (○), 20 °C a_w 0.98 (▼) and 20 °C a_w 0.92 (▽).

a_w on the individual spores of *F. verticillioides* are shown in Figs. 3 and 4, respectively. It can clearly be seen that both temperature and a_w had a very large impact on the distribution of the lag phases and the colony growth rates of the individual spores of both isolates. Most notable is the shift to longer lag phases and shorter colony growth rates when a_w and/or temperature is decreased, alone or in combination. The range (spread of the histograms) of the lag phases and colony growth rates of the individual spores was also observed to be generally larger at more growth compromising conditions than those observed at optimal growth conditions, indicating greater variability when growth is stressed. An example of this being for *A. flavus* at 30 °C where at a_w 0.98 the individual spore colony growth rates ranged from 6.16 to 13.76 mm d^{-1} and the lag phases from 0 to 2.45 d, whereas at a_w 0.88 the individual colony growth rates ranged from 3.45 to 14.36 mm d^{-1} and the lag phases from 0 to 2.69 d. At the most growth-limiting condition investigated for *A. flavus* of a_w 0.88 and 20 °C, the ranges had changed to individual colony growth rates between 2.38 and 8.97 and lag phases between 1.32 and 4.63 d.

The effect of temperature appears to be more pronounced on the distribution of the lag phase durations for growth than it is on the colony growth rates of the individual spores. This can be deduced from the greater degree of overlapping that occurs between the histograms of the colony growth rates compared to those for the lag phases of the individual spores. This may be explained by considering that the temperature range investigated may simply be larger in terms of stress imposed on growth compared to the a_w ranges evaluated. Generally at a high a_w value, the distributions of both growth parameters at 20 and 30 °C show a greater degree of overlapping than those at a lower less optimal a_w, indicating that an interaction between a_w and temperature occurs on the distribution of the individual spore lag phases and colony growth rates.

Table 4
Estimated parameters of the logistic function used to describe the cumulative distribution of the lag phases of *A. flavus* and *F. verticillioides* as a function of time on corn meal

Temperature (°C)	a_w 0.98				a_w 0.88			
	k (d^{-1})[a]		τ (d)[b]		k (d^{-1})		τ (d)	
A. flavus								
30	3.36	[2.99–3.73]*	0.69	[0.60–0.78]	3.12	[2.77–3.48]	1.14	[1.10–1.18]
20	3.33	[3.12–3.54]	2.65	[2.55–2.76]	2.28	[2.13–2.43]	2.85	[2.82–2.88]
F. verticillioides								
	a_w 0.98				a_w 0.92			
30	2.72	[2.59–2.84]	1.31	[1.29–1.33]	2.65	[2.53–2.78]	1.50	[1.48–1.51]
20	2.27	[2.05–2.48]	3.24	[3.20–3.29]	1.71	[1.64–1.77]	4.84	[4.81–4.87]

*95% confidence interval.
[a]k-rate of completion of lag phase period.
[b]τ-time for half of spores to complete the lag phase.

3.3. Effect of water activity and temperature on the cumulative distribution of the lag phase durations of single spores of A. flavus and F. verticillioides on corn meal

The cumulative distributions of the lag phases of the individual spores of both isolates are shown in Fig. 5. The parameters of the logistic function fitted to these cumulative distributions are shown in Table 4. Very high determination coefficients (r^2) of $\geqslant 0.995$ were obtained for the fit of the logistic function to the cumulative distributions of both isolates. The effect of both a_w and temperature on the range of single spore lag phases (discussed before in Section 3.2) can also be clearly seen in Fig. 5. As observed and discussed before, the spread of the plots became wider the more growth limiting the conditions became. For both *A. flavus* and *F. verticillioides*, at the highest a_w value evaluated of 0.98, the slopes of the curves depicting the rate of completion of the lag phase period (k, d^{-1}) at 20 and 30 °C are not much different for both isolates. However a comparison of the 95% confidence limits shows that a significant difference occurs for *F. verticillioides*, with a slower rate of completion at 20 °C. At the lower a_w values evaluated, the differences between the slope of the curves (and hence the value of k) at 20 and 30 °C becomes more significant for both isolates as seen in both Fig. 5 and Table 4. This indicates that the rate at which individual spores of both isolates complete the lag period decreases with an increase in the level of stress imposed and further highlights the interaction of the effects of a_w and temperature at the individual spore level. In agreement, Sautour et al. (2001) also found that a significant positive interaction occurred between a_w and temperature on the rate of germination of *P. chrysogenum* spores on an artificial growth medium. Interaction between the effects of a_w and temperature on the germination and germination rates of *Penicillium verrucosum* and *Aspergillus ochraceus* on malt extrat agar and green coffee-based medium, respectively, was also reported by Pardo et al. (2005, 2006).

The influence of both a_w and temperature on τ (d), the time for half of the spores to complete the lag phase, is even more apparent than that on k. This observation has also been reported in studies of *M. racemosus* growth on PDA (Dantigny et al., 2002). Even more striking is that effect of temperature on τ (at the a_w values investigated) is much larger than the effect of a_w at the temperature investigated. An example of this being the increase of τ from 0.69 to 2.65 d for *A. flavus* at a a_w value of 0.98 and from 1.50 to 4.84 d for *F. verticillioides* at a_w value of 0.92 as temperature is lowered from 30 to 20 °C. This was also observed in the histograms depicting the distribution of single spore colony growth rates and lag phase durations and was explained by assuming that the temperature range investigated may simply be larger in terms of stress imposed compared to the a_w ranges evaluated. It can be concluded from the trend observed for the two parameters that an interaction between a_w and temperature also occurs on the length of time that the individual spore takes to pass through the lag period. As the end of the lag phase is characterized by formation of a visible growing colony which results in a product being considered spoiled, the interaction observed clearly has an important bearing on the shelf-life of food products spoiled by moulds.

4. Conclusions

The protocol of Francois et al. (2003), originally developed for the isolation and growth of single bacterial cells, was modified to suit the specificities of fungal growth. The protocol developed fulfilled the criteria required of a simple, reliable and high yielding method. This method was applied to study the growth kinetics of single spores of *A. flavus* and *F. verticillioides* on corn meal and the effects of a_w and temperature on the distribution of the colony growth rates and lag phase durations. The effect of the same environmental factors on the rate at which the singles spores pass through the lag period was also discussed.

Both environmental factors significantly influenced the distributions of both growth parameters and the rate at which single spores from both isolates completed the lag period. Interaction was observed between the effects of these two environmental factors on all the growth parameters. In general the spread of the colony growth rates and lag phases for growth of the single spores of both isolates generally became wider the more limiting the conditions for growth became. The rate at which the single spores completed the lag phases (k, d^{-1}) generally reduced with a decrease in temperature and/or a_w. These results showed that within the limits investigated, the yield of the spores was unaffected and that growth still occurs at the lowest inoculum level possible yield. In view of these and previously reported results and the continuing worldwide research on methods for the protection of stored cereal grains from fungal spoilage and the prevention of mycotoxin production, it is also important that the influence of these potential techniques be investigated at the individual spore level in addition to the high levels usually studied.

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