

Aerobiological monitoring of *Aspergillus*/*Penicillium* spores during the potato storage

Isabel Iglesias Fernández · Maria Carmen Seijo Coello ·
Maria Fernández González · Olga Escuredo Pérez

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Abstract Aerobiological studies are widely used to determine the fungal spectrum in the air. These studies have revealed that *Aspergillus*/*Penicillium* spores are the most abundant spores in both outdoor and indoor environments. In this study, we have presented the variations in the concentration of these spores in an indoor environment (a potato store). Aerobiological sampling was conducted during five storage period (from 2002 to 2008 year) using a Hirst-type spore trap. The maximum spore concentrations were counted during the second fortnight of January and in the months of February and March, with values higher than 6,000 spores/m³ per day. A correlation analysis between the *Aspergillus*/*Penicillium* spores and the main environmental parameters was performed; significant coefficients were obtained for spores present in the store previous days and mean temperature of the same day and previous days ($P < 0.001$). Moreover, a regression model was established and predicted 53% variability of the data included in the analysis. The best obtained model took into account the *Aspergillus*/*Penicillium* spore type levels of 1 previous day and the mean temperature in the preceding 2 days.

Keywords Aerobiology · *Aspergillus*/*Penicillium* spores · Indoor environment · Potato · Environmental parameters

1 Introduction

Many aerobiological studies have confirmed that *Aspergillus* and *Penicillium* spores are the most abundant species found in indoor ambient (Garret et al. 1998; Ren et al. 1999; Aira et al. 2002; Gorny and Dutkiewicz 2002; Archana and Aarti 2004; Escuredo et al. 2007; Cho et al. 2008; Sen and Asan 2009). The small size and the ease dispersion of their propagules favour the presence of high concentrations of fungal spores in both indoor and outdoor environments (Aira et al. 2002). Evaluation of airborne fungal contaminants has gained importance because of the health hazards caused by the spores or microbial metabolites (Sen and Asan 2009). Some studies have related the environmental exposure to biological agents in grain processing plants, waste materials treatment and other products of industrial culture to the effects on worker's health (Solans et al. 2007). Workers in potato stores may be exposed to high ambient concentrations of fungi during receipt, handling and shipment of these products. The fungal spores behave as pathogens causing respiratory diseases such as allergy and asthma (Espinosa et al. 1992; García et al. 2001; Erbek et al. 2008).

I. Iglesias Fernández (✉) · M. C. Seijo Coello ·
M. Fernández González · O. Escuredo Pérez
Department of Plant Biology and Soil Sciences,
University of Vigo, 'As Lagoas', 32004 Ourense, Spain
e-mail: oescuredo@uvigo.es

Potato is the fourth most important crop in the world; as a result, large quantities of this product are stored for long periods. The potatoes stores are a good site for mould growth since some species of fungi live on damp substrates and can grow on food, walls and wood. *Fusarium* or *Alternaria* may also propagate inside the store due to their pathogenicity in the potatoes cultures (Alonso et al. 2008). However, other fungi such as *Aspergillus* and *Penicillium* are the most abundant fungi indoor (Docampo et al. 2010). Previous studies have evaluated the association between exposure to dust and high concentrations of airborne microorganisms in potato processing industries and have confirmed the potential health risks for the workers in these industries (Dutkiewicz et al. 2002; Hollander et al. 1994; Zock et al. 1995).

The study was performed in a store located in 'Xinzo de Limia' (Ourense, NW Spain), an area where potatoes are intensively cultivated. Due to its geographical location and altitude, the weather in this region shows a combination of oceanic and Mediterranean characteristics, with an inclination towards continental weather. These characteristics favour the growth of this crop, which is the main source of income for the people in this region (Escuredo et al. 2011). Every year, tons of 'Kennebec' potatoes, the only variety protected by the Protected Geographical Indication (PGI), are stored after harvesting.

Prediction of aeroallergens from pollen grains and fungal spores has been a scientific goal of many studies (Grinn-Gofron and Strzelczak 2008). The majority of these studies focused on predicting the airborne fungal spores circulating in the air (Grinn-Gofron and Strzelczak 2008; Iglesias et al. 2010; Escuredo et al. 2011). Since weather is one of the most important factors influencing the development of fungus, these models were based on the weather.

The aim of the present work was to analyse the behaviour of *Aspergillus*/*Penicillium* spore type during the potatoes storage and to study the relationship between the fungal spore levels and the main environmental factors.

2 Materials and methods

The study was carried out in a potato store located in 'Xinzo de Limia' (Ourense, NW Spain) during five storage period (2002/2003–2007/2008); each study

period began from October 15th to April 15th. The store had a total area of 1,900 m², and the potatoes were stored in 500-kg wooden boxes and placed in towers of five units on the ground. This allowed the free circulation of air through the boxes and a comfortable work for the operators.

The storage conditions were assessed by an automatic recording device (HOBO ProSeries, HO8-032-08) located in the same place, which provided temperature and humidity data at hourly intervals.

A Hirst-type volumetric spore trap (Lanzoni VPPS 2000) (Hirst 1952) was used for the sampling. This equipment was localised in a high area of the store above the height of the tuber boxes, and the aspiration rate was adjusted to 5 L of air per minute. The collector included a drum, which contained a Melinex tape coated with 2% silicone solution on which the spores are deposited; the drum was replaced on a weekly basis. The tapes were processed according to the methodology recommended by the Spanish Aerobiology Network (REA) (Domínguez et al. 1991; Galán et al. 2007). The identification and quantification of these spores were realised with a Nikon Optiphot II microscope. Four horizontal bars were made in each slide. This method has an average error of less than 20%, which varies depending on the size of the sample and the spores selected for the study. This procedure provides acceptable values that allow proper identification of the spores collected and facilitates analysis of their relationship with different meteorological parameters (Comtois et al. 1999).

The spores of *Aspergillus* and *Penicillium* were identified using various handbooks (Ellis 1971, 1976; Dennis 1978; Nilsson 1983) and reference preparations. Microscopic examination revealed that the morphology of these 2 fungal spores is similar, and hence, they were grouped together as *Aspergillus*/*Penicillium* spore type. The spore concentrations were expressed as the number of spores per cubic metre of filtered air and collected in 24 h.

In order to determine the relation between the *Aspergillus*/*Penicillium* spore concentrations and the principal environmental factors influencing the indoor air of the store, a linear correlation analysis was performed. Since the data were not normally distributed, nonparametric statistical analysis was performed by using the Spearman rank correlation analysis. The main environmental factors analysed were mean temperature and mean humidity. Environmental

conditions may affect spore production directly or indirectly through their effect on the substrates colonised by the fungus. For that reason, this study also determined the correlation between spore counts for a given day and the main environmental parameter values for the previous 1–3 days. The spore concentrations were considered as dependent variables, and temperature and humidity were considered as independent variables. Significance was calculated for $P < 0.001$. A linear regression analysis was performed for the spore data and the environmental factors to obtain a model for predicting *Aspergillus/**Penicillium* spore concentrations. All statistical analyses were performed using SPSS 17.0 statistical software package.

3 Results

The aerobiological study showed that *Aspergillus/**Penicillium* spores were the most abundant in the environment. These spores comprised 80% of the total spores in all the study periods, except 2005/2006, when the proportion of these spores was 50% (Fig. 1). Other important spore types found in the air of the store were *Cladosporium*, *Fusarium*, *Alternaria* and *Helminthosporium*, but these types were found in very low concentrations. *Cladosporium* was found to be the second most abundant taxa (2–12%), except in 2005/2006 (45%).

*Aspergillus/**Penicillium* spores were present throughout the study period, but the concentrations of these spores differed across years (Fig. 2). The highest levels were detected in 2007/2008, with a total content of 474,539 spores, which represented a daily

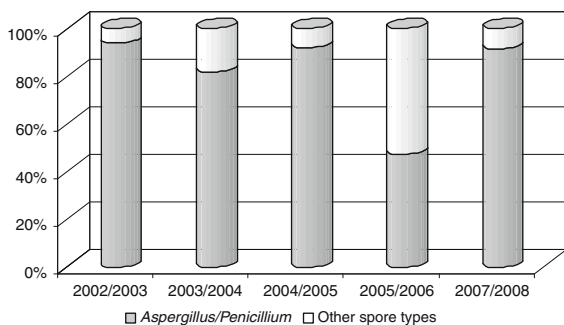


Fig. 1 *Aspergillus/**Penicillium* spore percentages regarding other spore type in the different storage periods

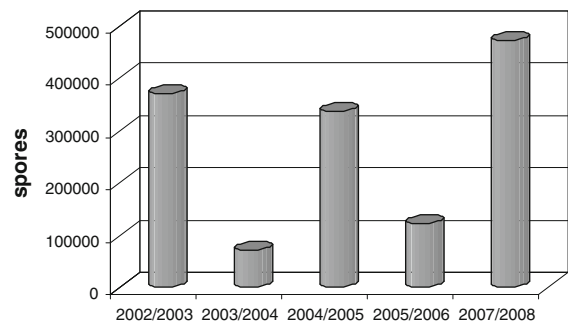
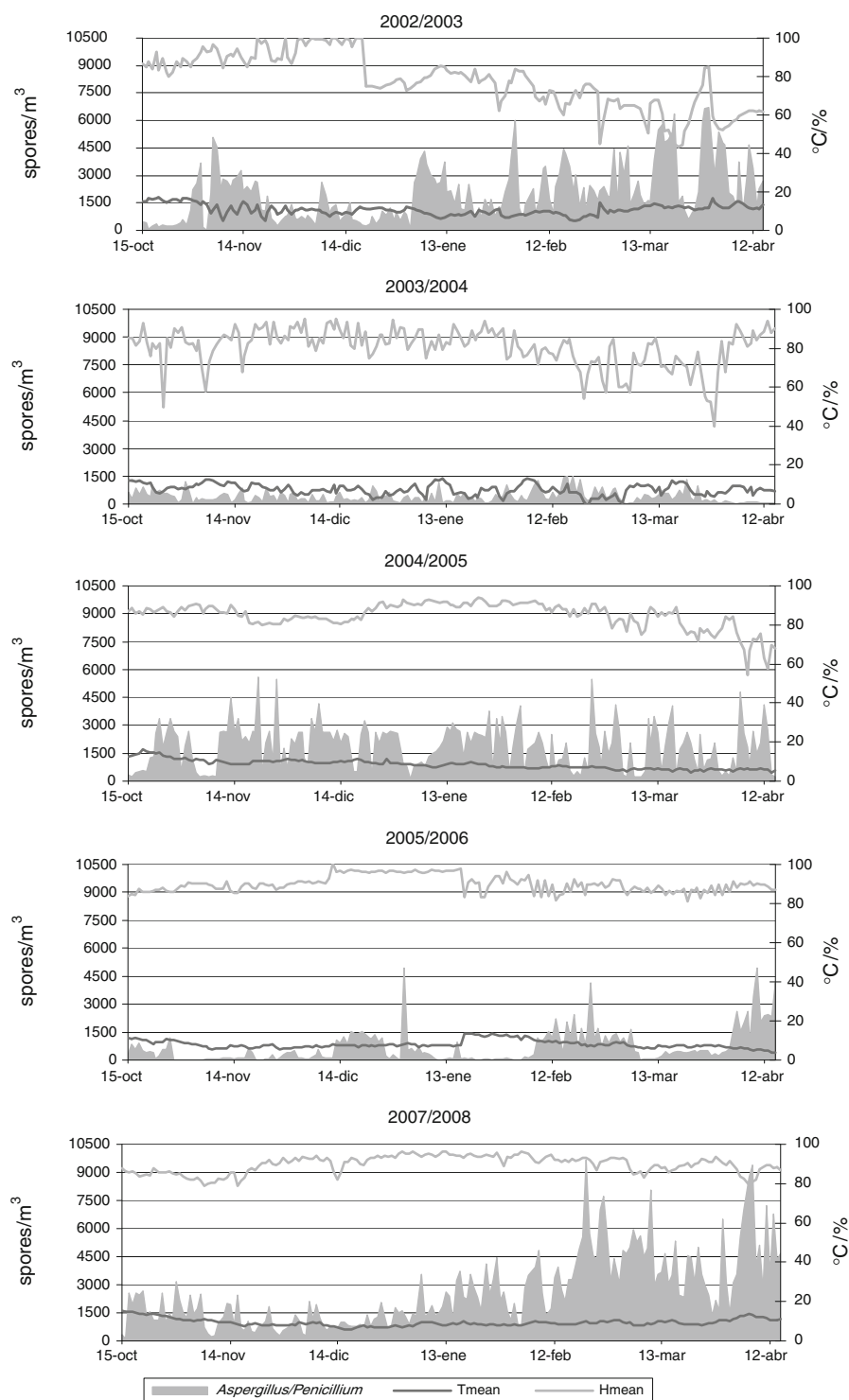


Fig. 2 Total *Aspergillus/**Penicillium* spores in each storage period

mean concentration of 2,593 spores/m³. The number of *Aspergillus/**Penicillium* spores counted in 2002/2003 and 2004/2005 were similar, with a total spore concentrations of 334,566 and 254,913, respectively. However, the levels in the others 2 years were much lower, with 157,918 spores (2005/2006) and 70,184 spores (2003/2004). The daily mean *Aspergillus/**Penicillium* spore concentrations and the main environmental factors recorded during the study period are shown in Fig. 3. The maximum daily mean concentration was detected in the late storage period (February, March and April). The maximum daily peak levels were recorded in 2007/2008 (9693 spores/m³ on 21 February) and in 2002/2003 (6,690 spores/m³ on 30 March). The daily peak levels in 2004/2005 (maximum concentration of 5,606 spores/m³ on 23 February) and in 2005/2006 (4932 spores/m³ on 10 April) were slightly lower. The lowest daily peak was recorded in 2003/2004 with a maximum value of only 1,511 spores/m³ on 18 February.

The relationships between *Aspergillus/**Penicillium* spores and mean temperature and mean humidity during five storage period were studied (Table 1). In general, higher mean temperatures were in the years 2002/2003 and 2007/2008. During the last year of the study period (2007/2008), the daily humidity was quite regular, and highest humidity values were recorded with a total mean of 88.8%. The lowest humidity values were recorded in the year 2002/2003, with a total mean daily value of 76.9%. The concentration of *Aspergillus/**Penicillium* spores was the highest in these 2 years (2002/2003 and 2007/2008). In contrast, in 2003/2004, we recorded the lowest spore levels and total mean temperature (7.7°C) and a high total mean humidity (80.6%). The maximum

Fig. 3 Evolution of *Aspergillus/Penicillium* spores during the storage periods studied



concentrations in the last period of storage coincided with high daily mean temperature and low daily mean humidity.

Correlation between spore counts for a given day and the main environmental parameters values for the previous 3 days was observed using the data for all the

Table 1 Monthly mean temperature (°C) and mean humidity (%) of the store and their total mean of each storage period

	2002/2003		2003/2004		2004/2005		2005/2006		2007/2008	
	T	H	T	H	T	H	T	H	T	H
October	15.8	86.2	9.1	82.3	13.3	87.2	9.9	86.5	13.4	85.6
November	10.6	92.2	10.6	78.8	10.3	88.4	6.8	89.6	10.4	81.6
December	10.1	89.9	6.4	88.4	9.7	83.1	7.0	92.3	8.6	92.1
January	8.8	78.5	7.0	84.8	8.2	91.3	9.9	93.6	8.5	94.4
February	8.4	70.8	6.3	75.6	7.2	88.2	9.2	88.8	9.1	92.5
March	11.8	62.5	7.2	68.8	5.9	80.6	7.2	86.8	9.2	89.1
April	12.7	58.3	7.4	85.6	5.9	71.1	5.5	89.2	11.8	86.2
Total	11.2	76.9	7.7	80.6	8.6	84.3	7.9	89.6	10.1	88.8

T mean temperature, *H* mean humidity

study periods. The results showed significant correlation coefficients with *Aspergillus/Penicillium* spores of previous days ($P < 0.001$); highest coefficients were with *Aspergillus/Penicillium* spores of 1 day before (Table 2). The mean temperature on the same day and 3 previous days also showed significant statistical correlation ($P < 0.001$). However, there was no significant correlation between the mean humidity and the levels of *Aspergillus/Penicillium* spores. On the basis of these results, the predictive capacity of the variables was evaluated through a linear regression analysis. The best model included *Aspergillus/Penicillium* spore levels 1 day before and the mean temperature 2 before days (Table 3). R^2

Table 2 Correlation coefficients and significance levels between the number of *Aspergillus/Penicillium* spores and the main environmental parameters, applying Spearman's test

<i>Aspergillus/Penicillium</i> spores	
<i>Aspergillus/Penicillium</i> -1	0.780*
<i>Aspergillus/Penicillium</i> -2	0.715*
<i>Aspergillus/Penicillium</i> -3	0.668*
Mean temperature	0.196*
Mean temperature-1	0.210*
Mean temperature-2	0.230*
Mean temperature-3	0.246*
Mean humidity	-0.035
Mean humidity-1	-0.029
Mean humidity-2	-0.016
Mean humidity-3	-0.005

Values of the three previous days were also considered

* $P < 0.001$

coefficient value (0.532) explained the 53% of variability in *Aspergillus/Penicillium* spore levels in the environment of the potato store.

4 Discussion

Aspergillus/Penicillium spores were the main fungal spores of the airborne mycoflora of the potato store studied, with a mean concentration of 1,505 spores/m³ per day. These spores and *Cladosporium* spores have been found as the most abundant fungi in many indoor environments, such as coffee processing plants (Alonso et al. 2008), household waste treatment plants, (Solans et al. 2007), residential houses (Archana and Aarti 2004; Sen and Asan 2009), caves (Docampo et al. 2010, 2011) and potato processing plants (Dutkiewicz 1997).

Indoor air contamination is an important area of concern, especially considering the fact that many people spend about 80–90% of their time indoors, either at home or at work (Sen and Asan 2009). In the case of *Aspergillus* and *Penicillium*, the conidia are potentially pathogenic risk factors in the workers (Dutkiewicz et al. 2002). These fungi are predominant in indoor environments and colonise a wide variety of substrates (Fairs et al. 2010), such as potato, which is a good substrate for their growth. Moreover, the high respiratory activity of this product creates suitable conditions for the development and subsequent dispersion of spores.

The variation in the sporulation and dispersion of spores are related to the environmental factors. Many studies have shown that the concentration of fungal

Table 3 Linear regression analysis for *Aspergillus/**Penicillium* spores during studied period

Model summary					
<i>R</i>	<i>R</i> ²	<i>R</i> ² adjusted	Est. error	<i>F</i>	<i>P</i>
0.73	0.53	0.53	1,058.55	515.33	0.000
Coefficients					
	<i>B</i>	Est. Err. B	Beta	<i>t</i>	<i>P</i>
Intercept	42.76	122.88		0.35	0.730
<i>Aspergillus/</i> <i>Penicillium</i> -1	0.71	0.02	0.71	30.67	0.000
Mean temperature-2	45.08	13.60	0.08	3.31	0.001

spores in indoor and outdoor air is strongly related to meteorological factors (Burch and Levetin 2002; Sakiyan and Inceoglu 2003; Sen and Asan 2009; Iglesias et al. 2010; Escuredo et al. 2011). The two storage periods studied with higher number of spores counted (2002/2003 and 2007/2008) coincided with the higher mean temperature of all the period, and this relation has been seen in the main peaks recorded. Therefore, the increase the *Aspergillus/**Penicillium* spores was due principally to an increase in mean temperature. This behaviour was detected in other indoor spaces (Ren et al. 2001; Padmanabhan and Themath 2004). Besides higher spore levels coincided with higher activity levels in the store, such as those observed during moving of boxes or packing of potatoes. These movements generate particles in the form of dust suspended in the air. Some authors (Lehtonen et al. 1993; Docampo et al. 2010) have reported that variations in spore levels indoors are mainly caused by human activities such as cleaning, object movement or by transport of spores on clothes. As already mentioned, the mean temperature of previous days had the highest correlation coefficients influencing positively in the dispersion of these spores in the studied environment. Thus, the influence of these factors on the concentrations of fungal spores suspended in the air seems to be additive and not independent. Many of these factors are interrelated, and it is often difficult to determine the most important factor (De-Wei and Bryce 1995).

Therefore, there is an increasing interest in the development of statistical models for determining the airborne fungal spore levels. These models will allow employees to take preventative action against occupational hazards in the industry. The better model obtained for the prediction of *Aspergillus/**Penicillium*

spores considered the mean temperature of 2 previous days.

5 Conclusions

High amounts of *Aspergillus/**Penicillium* spores were recorded throughout the study period. Thus, *Aspergillus* and *Penicillium* may cause respiratory diseases in the employees; improvement in the cleaning techniques of surfaces would reduce the levels of biological agents in the environment. Regression analysis showed a good relationship between *Aspergillus/**Penicillium* spore concentrations and the mean temperature of 2 before days. Further, control of the environmental factors inside the store may prevent the development of these spore types.

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