

Hourly predictive artificial neural network and multivariate regression tree models of *Alternaria* and *Cladosporium* spore concentrations in Szczecin (Poland)

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Abstract A study was made of the link between time of day, weather variables and the hourly content of certain fungal spores in the atmosphere of the city of Szczecin, Poland, in 2004–2007. Sampling was carried out with a Lanzoni 7-day-recording spore trap. The spores analysed belonged to the taxa *Alternaria* and *Cladosporium*. These spores were selected both for their allergenic capacity and for their high level presence in the atmosphere, particularly during summer. Spearman correlation coefficients between spore concentrations, meteorological parameters and time of day showed different indices depending on the taxon being analysed. Relative humidity (RH), air temperature, air pressure and clouds most strongly and significantly influenced the concentration of *Alternaria* spores. *Cladosporium* spores correlated less strongly and significantly than *Alternaria*. Multivariate regression tree analysis revealed that, at air pressures lower than 1,011 hPa the concentration of *Alternaria* spores was low. Under higher air pressure spore concentrations were higher, particularly when RH was lower than 36.5%. In the case of *Cladosporium*, under higher air pressure (>1,008 hPa), the spores analysed were more abundant, particularly after 0330 hours. In artificial neural networks, RH, air pressure and air temperature were the most important variables in the model for *Alternaria* spore

concentration. For *Cladosporium*, clouds, time of day, air pressure, wind speed and dew point temperature were highly significant factors influencing spore concentration. The maximum abundance of *Cladosporium* spores in air fell between 1200 and 1700 hours.

Keywords *Alternaria* · *Cladosporium* · Weather variables · Hourly pattern · Szczecin (Poland) · Artificial neural networks · Multivariate regression trees

Introduction

Airborne fungal spores are a major biopollutant, and their seasonal distribution in the atmosphere has important health implications for allergic disorders (Burge 1989; Lacey and Dutkiewicz 1994; Verhoeff and Burge 1997). Given that a number of fungal genera are allergenic (e.g. *Alternaria* and *Cladosporium*), characterization of fungal spore seasons would allow physicians and allergy sufferers to more accurately establish preventative strategies (Mitakakis and Guest 2001).

The first records of airborne fungal spores in Poland come from Kraków and Rabka (Weiss 1962). Studies of the concentration of fungal spores performed to date in Poland refer both to outdoor (Gaweł et al. 1996) and indoor (Mędreła-Kuder 1991) analyses.

Since the sporulation process and spore dispersion of fungi are heavily dependent on local climatic conditions, many authors have correlated meteorological parameters with the presence or prevalence of airborne spores in the atmospheres of climatically different locations (Hasnain 1993; Hjelmroos 1993; Munuera Giner and Carrión García 1995; Herrero et al. 1996). In this paper, we use three different statistical methods: Spearman's correlation coeffi-

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cient, artificial neural networks (ANN) and multivariate regression trees (MRT) to analyse the effect of certain variables—air temperature, dew point temperature, relative humidity (RH), air pressure, wind direction, wind speed, mist, rain and clouds—on the atmospheric levels of hourly concentrations of *Alternaria* and *Cladosporium* spores in Szczecin, Poland.

Materials and methods

The results of this investigation are based on aerobiological monitoring performed in Szczecin in 2004–2007. Hourly summed counts of airborne fungal spores were performed using a 7-day recording Lanzoni volumetric trap. The trap was installed on a rooftop in the Szczecin city district of Śródmieście, at a height of 21 m above ground level. Hourly records of airborne fungal spores were obtained by means of counting all spores in one longitudinal transect at $\times 400$ magnification using a light microscope (MK 348) with phase contrast optics. After counting the spores in each sampling area, a correction factor specific for the microscope used was applied. Thus, the final counts of fungal spores were expressed as hourly numbers of spores per cubic metre of air. Meteorological data covering 4 years of studies were provided by an Automatic Weather Station (Vaisala MAWS101, Helsinki, Finland). The meteorological station was located in the immediate neighbourhood of the Lanzoni trap. The spore data were analysed to determine the start, end and duration of the season using the 90% method. The start of the season was defined as the date on which 5% of the seasonal cumulative spore count was trapped, and the end of the season as the date on which 95% of the seasonal cumulative spore count was reached.

For this study, we chose days in all four seasons (a total of 53) with conditions in which the concentration of spores of these types typically exceeded threshold values, causing allergic symptoms among sensitive people, i.e. days on which the level of spores exceeded 100 and 3,000 spores m^{-3} for *Alternaria* and *Cladosporium*, respectively; days with maximum daily temperature above 20°C, minimum daily temperature above 10°C, dew point temperature above 13°C, rainfall below 10 mm/ m^2 ; and days without wind. These weather criteria were chosen based on our previous analysis (Grinn-Gofroń and Strzelaczak 2008a, b). Allergy symptoms and threshold concentrations were described and defined by Rapiejko et al. (2004). They analysed the clinical picture of upper respiratory tract allergic disease and conjunctival symptoms that appear in response to (mainly) these two aeroallergens. The frequency and severity of allergic rhinitis and conjunctivitis in a group of 660 subjects with hypersensitivity to *Alternaria* and *Cladosporium* allergens were studied. Aerobiological measurements, patients symptom

score cards, and the results of physical examination were analysed. Subjects with hypersensitivity to *Alternaria* and *Cladosporium* allergens experienced symptoms during exposure to concentrations of approximately 100 and 3,000 spores m^{-3} , respectively.

The hourly pattern of these spore types, and the external conditions leading to their high concentration in air, were investigated. For days meeting the selected meteorological values, we created predictive models of diurnal rhythms of both analysed taxa.

Data analysis

The obtained data set contained spore concentrations, time of day and meteorological variables: air temperature, dew point temperature, RH, air pressure, wind direction (0–360°), wind speed, lack (0) or presence (1) of mist, lack (0) or presence (1) of rain and clouds (1 = clear, 2 = mostly clear, 3 = mostly cloudy). Since our intention was to predict hourly patterns of *Alternaria* and *Cladosporium* spore concentrations, it was necessary to use “hour” as an input variable. Another reason to include time of day in the analysis was that it was weakly correlated with meteorological parameters—correlating only with temperature and dew point temperature (Spearman’s correlation coefficients 0.3–0.5, results not shown). Thus, we assumed that “hour” might represent some other unknown factor influencing spore concentrations that was not measured or taken into account in the analyses.

Due to non-linearity, non-normality and the presence of categorical variables (mist, rain and clouds), the following methods were applied in data analysis: Spearman’s rank correlation, ANN and MRT models. Meteorological parameters were used as input variables while *Alternaria* and *Cladosporium* spore concentration were output variables.

The MRT technique was chosen because it analyses community data, but makes no assumptions about the form of relationships between airborne mycoflora and meteorological factors. Moreover, this method is applicable for complex ecological data with imbalanced, non-linear relationships and high-order interactions. The clusters, and their dependence on environmental variables, are presented graphically as a tree. The general idea of MRT is to form clusters of sites by repeated splitting of the data along axes of the explanatory environmental variables. Each split is chosen to minimise the dissimilarity (sum of squared Euclidian distances, SSD) of the taxa and sites within the clusters (Breiman et al. 1984; De’ath and Fabricus 2000; De’ath 2002). The overall fit of the tree is specified as relative error (error, defined as SSD in the clusters divided by SSD of the undivided data) and the predictive accuracy is assessed by cross-validated relative error (CVRE; Breiman et al. 1984; De’ath and Fabricus 2000). In this

Table 1 Spearman's rank correlation coefficients between *Alternaria* and *Cladosporium* spore concentrations, meteorological variables and time of the day

Variable	<i>Alternaria</i>	<i>Cladosporium</i>
"Hour"	−0.248 *	0.110
Air temperature	0.581 ***	0.290 *
Dew point temperature	0.247 *	0.091
Relative humidity	−0.657 ***	−0.314 **
Air pressure	0.563 ***	0.359 **
Wind direction	0.189	0.062
Wind speed	0.290 *	0.121
Mist	−0.050	−0.189
Rain	−0.172	−0.189
Clouds	−0.524 ***	0.088

* $P < 0.05$. ** $P < 0.01$, *** $P < 0.001$

study, the tree finally selected was the most complex model within one standard error (1 SE) from the best predictive tree (Breiman et al. 1984), using 2000 multiple cross validations to stabilize CVRE. Analyses were carried out in R 2.1.1 (R Development Core Team 2004) using the mvpart (Multivariate Partitioning) package (De'ath 2002).

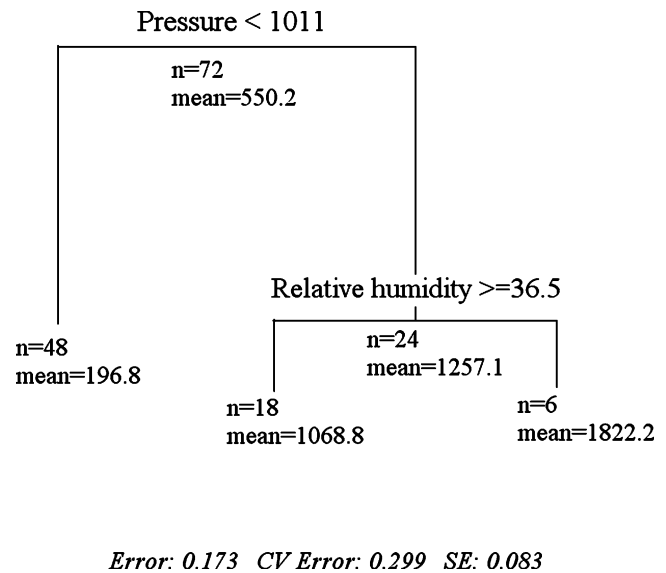
MRT models were complemented by ANN. In this study, multi layer perceptrons (MLP) were applied, which perform a stochastic approximation of multivariate functions mathematically (Osowski 1996; Carling 1992; Lek and Guegan 1999). Calculations were performed using StatSoft software Statistica 6.1, with implementation of the neural network module (Lula 2000; Tadeusiewicz 1993, 2001).

Consecutive neural networks were designed and trained using back propagation (Fausett 1994; Haykin 1994) and conjugate gradient algorithms (Bishop 1995) using Automatic Problem Solver. Cases were divided using the bootstrap method into three subsets:

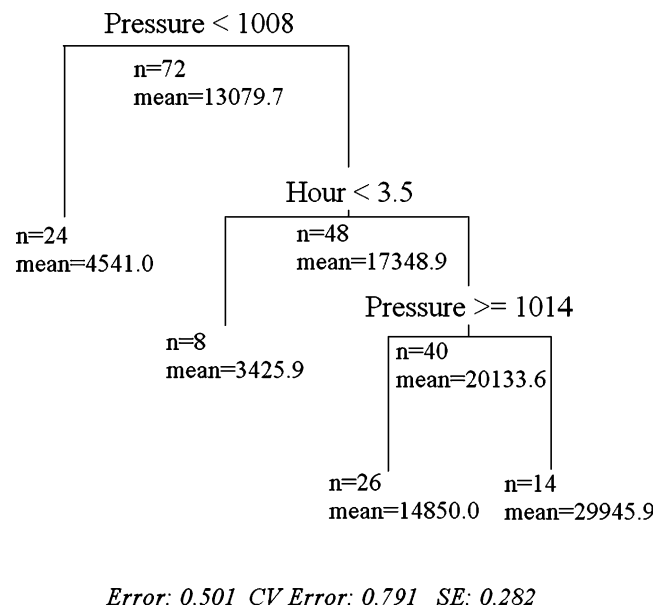
- training (Tr)—used for training a neural network (67% of the cases);
- verification (Ve)—used for verifying performance of a network during training (33% of the cases);
- testing (Te)—used for assessing predictability and accuracy of a neural model on data not presented during training and validation (cases remained after creating a training subset during bootstrap).

The choice criteria of the best neural networks were: value of SD ratio (ratio between error standard deviation and standard deviation of experimental data) and correlation (Pearson's correlation coefficient between experimental and calculated data).

Special emphasis was placed on sensitivity analysis and response plots. Sensitivity analysis creates ranking of input

**Fig. 1** Multivariate regression tree (MRT) for *Alternaria* spores. *n* Number of cases, *mean* average spore concentration

variables and is based on calculations of the error when a given input variable is removed from the model. The ratio of the error for the complete model to that with the ignored variable allows ordering of variables according to their importance. The response plot is the model response (output) as a function of a single selected input variable, assuming constant values of other variables, or, in other words, a one-dimensional section through the response surface in the N-dimensional space of input variables.

**Fig. 2** MRT for *Cladosporium* spores. *n* Number of cases, *mean* average spore concentration

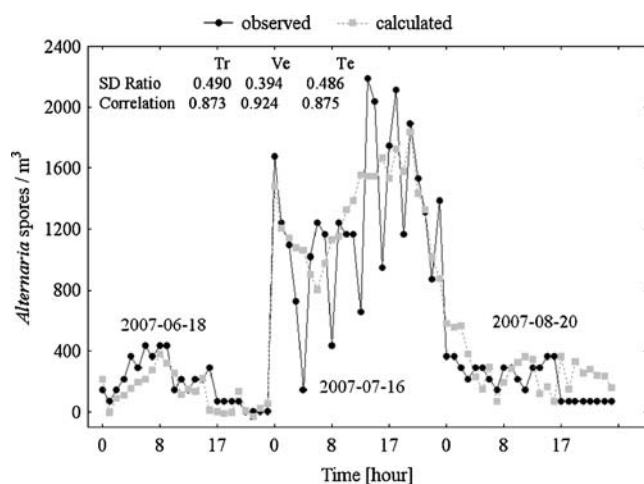


Fig. 3 Comparison of observed *Alternaria* spore concentrations and those calculated from a multi layer perceptrons (MLP) 10:10-12-1:1 neural network

Results

Analysis of Spearman's rank correlations (Table 1) showed that RH, air temperature, air pressure and clouds influenced the concentration of *Alternaria* spores most strongly and significantly. The relationships were inversely proportional in the case of RH and clouds, and directly proportional for the rest of the above mentioned factors. Weaker, negative correlations were observed with "hour" and clouds. *Cladosporium* spores correlated less strongly and significantly with meteorological factors than *Alternaria*. Negative relationships of a moderate level were noted for RH, while relationships for air pressure and air temperature were directly proportional.

MRT analysis revealed that, for air pressure lower than 1,011 hPa, the concentration of *Alternaria* spores was much lower (left branch of the tree, average 196.8 m^{-3}) than values equal or higher than the threshold (right branch, average $1,257.1 \text{ m}^{-3}$). Under higher air pressure, RH turned out to be an important parameter. Higher average *Alternaria* spore concentrations ($1,822.2 \text{ m}^{-3}$) occurred at humidity lower than 36.5%. Below that threshold, average spore concentrations amounted to $1,068.8 \text{ m}^{-3}$ (Fig. 1). MRT analysis did not reveal the time of day at which the abundance of *Alternaria* spores reached a maximum.

Somewhat similar results were obtained in case of MRT analysis of *Cladosporium*. Again, under higher air pressure ($>1,008 \text{ hPa}$, right branch of the tree) spores were more abundant ($17,348.9 \text{ m}^{-3}$) compared to values at pressures lower than the threshold ($4,541.0 \text{ m}^{-3}$). Under higher air pressure, higher concentrations of *Cladosporium* spores occurred after 0330 hours ($20,133.6 \text{ m}^{-3}$). During the rest of the day, the highest number of airborne spores was observed under air pressures ranging from 1,008 to 1,014 hPa ($29,945.9 \text{ m}^{-3}$; Fig. 2).

Of the ANN regression models between *Alternaria* spore concentration and meteorological variables as well as time of the day tested, best performance was achieved with network MLP 10:10-12-1:1, trained with 100 epochs of back propagation, and 8 epochs of conjugate gradient algorithm. The corresponding statistics were satisfactory (SD ratio between 0.394 and 0.490, correlation between 0.873 and 0.924). Direct comparison of observed and calculated values (Fig. 3) confirmed the good predictability of the model obtained, as well as the ability to recreate most of the variations.

Sensitivity analysis (Table 2) indicated RH, air pressure and air temperature to be the most important variables in the model. The remaining variables also contributed to the high network performance (ratio above 1) but their impact was less distinct.

Response plots (Fig. 4) indicated almost linear dependencies. Assuming constant values of the remaining factors, the *Alternaria* spore concentration increased with air pressure and temperature but decreased with RH. The ANN model did not reveal when the abundance of spores was maximal. The conclusions were consistent with the results of MRT analysis.

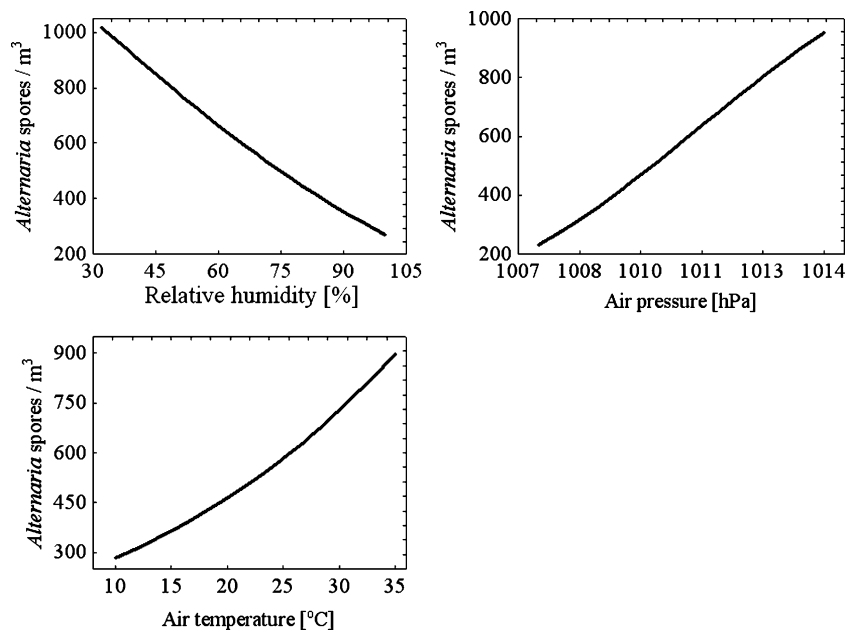
The best regression model of relationships between *Cladosporium* spore concentration, "time of day" and meteorological variables was obtained for network MLP 10:10-10-1:1, trained with 100 epochs of back propagation and 20 epochs of conjugate gradient algorithm. The model had good performance (SD ratio between 0.247 and 0.499, correlation between 0.879 and 0.970). Direct comparison of observed and calculated values (Fig. 5) revealed quite good accuracy of prediction.

Sensitivity analysis (Table 3) revealed that clouds, time of day, air pressure, wind speed and dew point temperature

Table 2 Sensitivity analysis for a multi layer perceptron (MLP) 10:10-12-1:1 neural network. Regression model for *Alternaria* spore concentration

	"Hour"	Air temperature	Dew point temperature	Relative humidity	Air pressure
Ratio	1.004	1.153	1.055	1.222	1.192
Rank	10	3	5	1	2
	Wind direction	Wind speed	Mist	Rain	Clouds
Ratio	1.021	1.063	1.010	1.028	1.016
Rank	7	4	9	6	8

Fig. 4 Response plots for the most important input variables in the MLP 10:10-12-1:1 neural network. Regression model for *Alternaria* spore concentration



were highly significant factors influencing concentration of *Cladosporium* spores. The remaining variables, except mist, also contributed to the network performance (ratio above 1).

Response plots (Fig. 6) indicated nonlinear dependencies. Assuming constant values of the remaining factors, the *Cladosporium* spore concentration turned out to increase with clouds, wind speed and air pressure but to decrease with dew point temperature. Maximum abundance of *Cladosporium* in air was present between 1200 and 1700 hours. The conclusions confirm and complement the results of Spearman's rank analysis and MRT.

Discussion

On days during which the potential meteorological conditions meant that high spore concentrations could be expected, both spore types included in this study exhibited distinct diurnal rhythms in the air. In a study in the suburbs of Mexico City, Calderon et al. (1997) noted *Cladosporium* concentration peaks at 0200–0400 hours, again at approximately 1400 hours, and at 20:00–22:00 hours. Peak concentration of *Alternaria* occurred between 1200 and 2000 hours. In our study, we found maximum abundance of *Cladosporium* in air between 1200 and 1700 hours and abundant occurrence after 0330 hours. *Alternaria* did not show any diurnal pattern. In MRT analysis, time was not important, and there was only a weak correlation with time in ANN. Fernandez et al. (1998) recorded the same time—midday to 1400 hours—interval for highest *Alternaria* and *Cladosporium* spore distribution in León (Spain). *Alternaria* spore concentration showed a clear maximum at 1300 hours, whereas the

majority of *Cladosporium* spores were collected between midday and 1400 hours. The clear hourly variation in the *Alternaria* spore count has also been noted in Murcia in Spain (Munuera Giner et al. 2001). Concentrations were the lowest in the early morning (about 0800 hours), increased rapidly until ca. 1400 hours followed by a slight rise to maximum at about 1900 hours, and then decreased steadily until the early morning minimum. In Derby in the United Kingdom, Corden and Millington (2001) noted the hourly spore diurnal periodicity for *Alternaria* spore concentration. The peak time for spore capture was from 1400 to 2200 hours, and more than half of the daily *Alternaria* spore catch was between 1800 and 2400 hours.

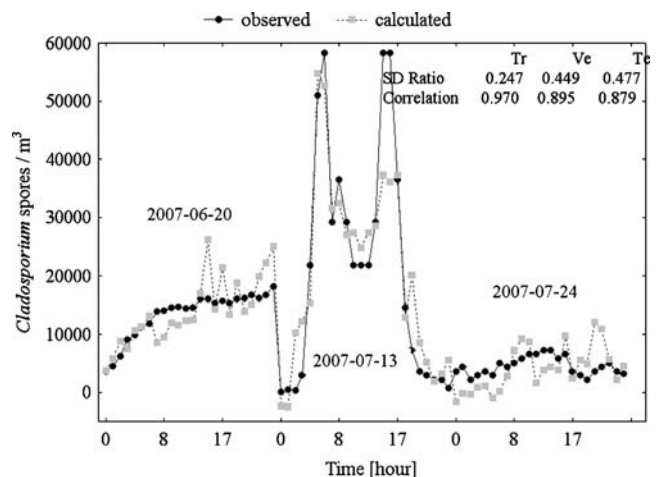


Fig. 5 Comparison of observed *Cladosporium* spore concentrations and those calculated from the MLP 10:10-10-1:1 neural network

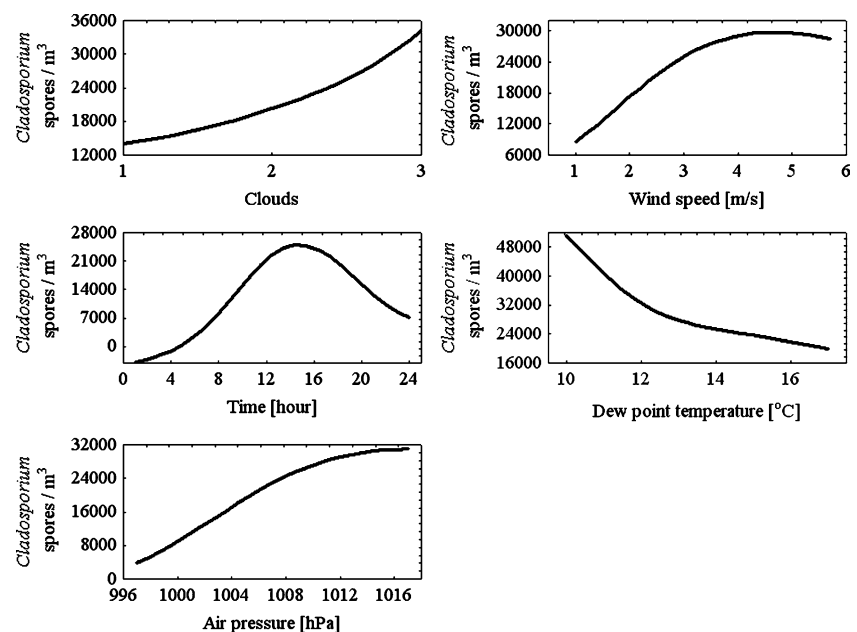
Table 3 Sensitivity analysis for MLP 10:10-10-1:1 neural network. Regression model for *Cladosporium* spore concentration

	"Hour"	Air temperature	Dew point temperature	Relative humidity	Air pressure
Ratio	2.867	1.241	1.630	1.277	1.781
Rank	2	8	5	7	3
	Wind direction	Wind speed	Mist	Rain	Clouds
Ratio	1.415	1.723	0.980	1.050	3.044
Rank	6	4	10	9	1

The reason for the different patterns at different locations is differences in weather parameters. High concentrations generally occur at times of day when the temperature and wind speed are sufficient to break conidial chains, with the largest concentrations being measured in the afternoon during the dry season (Calderon et al. 1997). Lin and Li (2000) investigated the relationships between fungal spore concentrations and meteorological conditions. Fungal concentrations were found to be highly dependent on weather conditions, with maximum concentrations at temperatures of 25–30°C, RH 60–70%, and wind speeds less than 1 m/s. They also noted that fungal concentration decreased with increasing temperature in the summer, but increased with increasing temperature in the winter. In our ANN model, *Alternaria* spore concentration turned out to increase with temperature and air pressure but to decrease with RH. Clouds, "hour", air pressure, wind speed and dew point temperature were highly significant factors influencing the concentration of *Cladosporium* spores. Assuming constant values of the remaining factors, the *Cladosporium* spore concentration was seen to increase with clouds, wind speed and air pressure, but to decrease with dew point temperature.

In South-East Spain, temperature was correlated consistently with *Alternaria* spore concentration. Linear stepwise regression analysis showed that wind and RH, together with minimum temperature, were the meteorological factors that best explained variations in airborne spore concentrations, but only in 13%, thus the model was not satisfactory (Munuera Giner et al. 2001). In both England (Corden and Millington 2001) and Spain (Fernandez et al. 1998), a positive correlation was found between *Alternaria* spore concentrations and maximum temperature, and a slight negative correlation with precipitation.

In many other locations, temperature was the most important and significant factor correlated with *Cladosporium* spore concentration (Hasnain 1993; Herrero et al. 1996; Hollins et al. 2004; Kurkela 1997; Munuera Giner and Carrión García 1995; Sabariego et al. 2000; Troutt and Levetin 2001). Troutt and Levetin (2001) noted a significant negative correlation between dew point of temperature and *Cladosporium* spore concentration. Similar to our results, the *Cladosporium* spore count decreased with dew point of temperature. In Finland, in addition to the positive correlation of *Cladosporium* spore concentration with precipitation and negative correlation with RH (Kurkela

Fig. 6 Response plots for the most important input variables in the MLP 10:10-10-1:1 neural network. Regression model for *Cladosporium* spore concentration

1997), a positive correlation with wind speed was also found (similar to our studies). Contradictory results regarding wind speed were noted in United States by Lyon et al. (1984). Other results also show correlations with different factors; Hjelmroos 1993 (Stockholm, Sweden; precipitation); Katial et al. 1997 (Denver, CO, United States; RH, precipitation); Sabariego et al. 2000 (Granada, Spain; hours of sunshine).

Mathematical analyses revealed different significant correlations between *Alternaria* and *Cladosporium* spore counts. For the former taxon, the most important variables were RH, air pressure and temperature, and for the latter, clouds, “hour”, air pressure, wind speed and dew point of temperature. The results indicate a possible dependence on the concentrations of both taxa on the atmosphere, ongoing climatic factors, and the time of day. From the theoretical patterns obtained by ANN and MRT analysis of these factors, all of them could be regarded as forecasting variables.

Our study has shown that advanced mathematical and statistical methods such as MRT and ANN are useful in predicting the hourly content of fungal spores in air. The good performance of the models obtained indicates that they can be used successfully in practical applications.

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