

Forecasting methodologies for *Ganoderma* spore concentration using combined statistical approaches and model evaluations

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Abstract High concentration levels of *Ganoderma* spp. spores were observed in Worcester, UK, during 2006–2010. These basidiospores are known to cause sensitization due to the allergen content and their small dimensions. This enables them to penetrate the lower part of the respiratory tract in humans. Establishment of a link between occurring symptoms of sensitization to *Ganoderma* spp. and other basidiospores is challenging due to lack of information regarding spore concentration in the air. Hence, aerobiological monitoring should be conducted, and if possible extended with the construction of forecast models. Daily mean concentration of allergenic *Ganoderma* spp. spores in the atmosphere of Worcester was measured using 7-day volumetric spore sampler through five consecutive years. The relationships between the presence of spores in the air and the weather parameters were examined. Forecast models were constructed for *Ganoderma* spp. spores using advanced statistical techniques, i.e. multivariate regression trees and artificial neural networks. Dew point temperature along with maximum temperature was the most important factor influencing the presence of spores in the air of Worcester. Based on these two major factors and several others of lesser importance, thresholds for certain levels of fungal spore concentration, i.e. low (0–49 s m⁻³), moderate (50–99 s m⁻³), high (100–149 s m⁻³) and very high (150 < n

s m⁻³), could be designated. Despite some deviation in results obtained by artificial neural networks, authors have achieved a forecasting model, which was accurate (correlation between observed and predicted values varied from $r_s=0.57$ to $r_s=0.68$).

Keywords Fungal spore · Prediction model · Urban area · Meteorological parameter · Fungal allergy · Aerobiology

Introduction

Ganoderma spp. is a common bracket fungus infecting both deciduous and coniferous trees, e.g. beech and oak (Gregory and Hirst 1952; Pegler and Young 1973), yew (Pegler and Young 1973), hornbeam (Gibbs and Evans 2000), chestnut (Rose 2004), poplar, maple and ash (McKay 2011). Pegler and Young (1973) identified and described in details six species of *Ganoderma* found in the UK, i.e. *Ganoderma applanatum*, *Ganoderma lucidum*, *Ganoderma adpersum*, *Ganoderma pfifferi*, *Ganoderma resinaceum* and *Ganoderma valesiacum*. A single *G. applanatum* basidiocarp can produce 30 billion spores per day, throughout a period of 6 months (Levetin 1990). Spores have an ovoid shape and they are approximately 8–13 × 4.5–8 µm in size (Pegler and Young 1973). Many clinical studies have already proved that *Ganoderma* spp. (hereafter *Ganoderma*) basidiospores cause allergic responses in people sensitized to this type of spores. The allergenic content and their small dimensions enable them to penetrate the lower part of the respiratory tract (e.g. Gregory and Hirst 1952; Hasnain et al. 1985; Vijay et al. 1991; Horner et al. 1993). People living in locations close to woodlands may be exposed to very high concentration of *Ganoderma* spores (Adams et al. 1968).

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A limited number of clinical studies, investigating sensitization to *Ganoderma* spores and to basidiospores in general, was conducted in the UK (Herxheimer et al. 1966; Herxheimer et al. 1969; Hyde 1972, 1973; Jenkins et al. 1981), as well as in other countries, i.e. New Zealand (Hasnain et al. 1985; Cutten et al. 1988), USA (Lehrer et al. 1986; Sprenger et al. 1988; Horner et al. 1993; Horner et al. 1998), Canada (Tarlo et al. 1979; Vijay et al. 1991) and India (Singh et al. 1995). Moreover, no clinical threshold value has been established for the exposure of *Ganoderma* spores, and a recent review on basidiospores calls for more clinical investigations and exposure studies (Rivera-Mariani and Bolaños-Rosero 2012). It may be difficult for allergists to establish a link between occurring symptoms of sensitization to *Ganoderma* and other basidiospores at their patients because of the lack of information regarding spore abundance in the air. Hence, there is a need to conduct aerobiological monitoring, and if possible to extend this monitoring for fungal spore forecasts.

In aerobiology, there are two ways of data examination, i.e. (1) statistical approach and (2) mechanistic approach. The former has been intensively explored in aerobiological studies since the 1990s. In the majority of published articles, pollen and spore data revealed no normality in distribution (e.g. Toro et al. 1998; del Mar Trigo et al. 2000), what is a common phenomenon observed in environmental studies (e.g. De'ath and Fabricius 2000). However, this causes a tendency of forcing results to approach normality by using different transformation techniques, e.g. square root, logarithmic and Moseholm's (Toro et al. 1998). Often it happens that the transformation of variables does not necessarily bring the expected change. In this situation, a range of forecasting techniques is being limited, and authors decided to apply multivariate regression trees (MRTs) and artificial neural networks (ANNs) for data analyses. The application of multivariate regression trees in environmental studies has been previously described by several authors (De'ath and Fabricius 2000; De'ath 2002; Grinn-Gofroń and Strzelczak 2008; Grinn-Gofroń and Strzelczak 2011; Jedryczka et al. 2015; Kasprzyk et al. 2011).

This article presents a 5-year study of *Ganoderma* spore concentration measured in Worcester between the years 2006 and 2010. Authors collected spore and meteorological data at the University of Worcester with the aid of Hirst type sampler and a weather station adjacent to the spore trap. The relationships between the presence of spores in the air and the weather parameters were examined using several statistical tests. Finally, the forecast models were constructed for the *Ganoderma* spore type using advanced techniques, such as MRTs and ANNs. These models can be employed in spore forecasting for the Worcester area.

Materials and methods

Spore monitoring site and observations

The city of Worcester is a county capital situated in the West Midlands, England. The terrain is relatively uniform in the West Midlands and most of the region is located at 44–200 m height above sea level. Worcestershire forms with Herefordshire the southern part of West Midlands. With Gloucestershire, they play a key role in horticulture and agriculture of England. Apple and pear orchards are characteristic features of the local landscape. Fields of wheat, winter oilseed rape and maize are cultivated in the vicinity of Worcester (DEFRA 2011). Worcester has a high population density approaching 3000 people per km² and a total number of inhabitants was estimated for 100,000 (Rice 2011).

A Hirst type sampler was used for fungal spore sampling between 2006 and 2010 (Hirst 1952). The spore trap was placed on the rooftop of University of Worcester's building (52° 11' N, 2° 14' W) approximately 10 m above the ground level. Microscope slides were prepared according to the British Aerobiology Federation's recommendations (Lacey and Allit 1995). *Ganoderma* spores were identified visually under ×400 magnification. Identification was done at the up to the genus level and along one central longitudinal band in the microscope slide. The daily (00:00 to 23:59) mean concentrations were expressed as number of spores per cubic metre of air [s m⁻³].

Climate and meteorological data

The city of Worcester enjoys relatively warm summers and mild winters, as it is situated in the temperate zone with significant influence of Gulf Stream. This warm ocean current often causes light rain showers (669 mm annual mean) and cloudy skies for more than 255 days each year (Cavan et al. 2004). According to nation-wide mapping done by the UK Met Office (2012), most of West Midlands had during the period 1981–2010 from 5 to 20 days of snow cover as annual mean. The dominating wind direction during the spore season of *Ganoderma* is south west (Sadyś et al. 2014).

The weather station (Weather Link Vantage Pro2, Davis Instruments, Hayward, CA, US) was installed adjacently to the 7-day volumetric spore trap on the rooftop of the university's building (Edward Elgar building, St. John's campus, Worcester). This device provided information of maximum, minimum and mean temperature, dew point temperature, rainfall, relative humidity, wind direction and air pressure. An appropriate transformation of the wind direction parameter was applied in order to perform a statistical evaluation (Sadyś et al. 2015).

Statistical analyses

All statistical analyses were based on the 5 years of *Ganoderma* spore data collected at Worcester. The 90 % method was applied to calculate the main fungal spore seasons following Nilsson and Persson (1981). Onset and termination of seasons were calculated by using 5 % and 95 % of the annual accumulative mean spore concentration, respectively. Each fungal spore season was examined individually in order to investigate annual variations in fungal spore distribution, as well as joined groups of years, i.e. 2, 3, 4 and 5 years in order to indicate the shortest and the most representative sample. Three tests for normality were used to examine the distribution of *Ganoderma* spores, i.e. Kolmogorov-Smirnov, Shapiro-Wilk and Lilliefors. When two out of three tests indicated p value equal to or above 0.05, then the distribution was considered as normal. The overall annual variations in fungal spore occurrence were studied using the ANOVA Kruskal-Wallis test. The data were not normalized because the peak values are often recognized as outliers and then are removed from the data set. The scatter plots were produced to confirm or reject the linear relationship between *Ganoderma* spores and investigated meteorological parameters. Due to the results, Spearman's correlation test was applied to investigate the relationship between *Ganoderma* spore presence in the air and eight meteorological variables.

Associations between spore concentrations and meteorological variables were investigated with the aid of MRTs. The MRT method divides data into two distinctive nodes. These nodes are based on values of dependent variable in relation to independent variable, size of node and the values of the independent variable that characterize it (De'ath and Fabricius 2000). Binary splits of data are continued until the moment when no more homogeneous groups can be distinguished and the size of tree (number of nodes) is reasonably small (De'ath and Fabricius 2000). Relative error indicates the overall fit of the model (defined as a sum of squared Euclidean distances—SSD), which sometimes may be overestimated. Therefore, it was necessary to choose a cross-validated relative error as a predictive principle (CVRE/CV; De'ath 2002). Simplicity of model structure within 1 standard error (1SE) is the main selection criterion for the best predictive tree (Breiman et al. 1984). Multivariate regression tree models were prepared using R software (i386 v. 3.0.1) and *mvpart* (Multivariate Partitioning) package. A model with the lowest Cross-Validated Error (CV Error) was chosen as the most representative regression tree after De'ath and Fabricius (2000) and De'ath (2002), using 2000 multiply cross validations to stabilize the Cross-Validated Error.

ANNs are a group of computational models, which have an ability of learning. They base the accuracy on previous observations and predict time series with a fairly good accuracy (Galán 2003). This kind of modelling is usually applied for

analysis of complex, non-linear relationships between variables (Scheifinger et al. 2013). ANNs have been successfully employed in various disciplines of science (e.g. Burke 1997; Shahin et al. 2002; Lek and Guegan 2000; Khare and Nagendra 2007). In aerobiology (e.g. Li and Flenley 1999), ANNs are mainly used for forecasting the concentration of allergenic and/or pathogenic biological agents, such as fungal spores and pollen grains (e.g. Sánchez-Mesa et al. 2005; Grinn-Gofroń and Strzelczak 2008; Astray et al. 2010; Kasprzyk et al. 2011; Puc 2012; Tomassetti et al. 2013).

In this study, ANNs were designed with the aid of Statistica Statsoft (v. 12) programme. The ANNs were constructed and trained using back propagation by selecting Automatic Problem Solver (Haykin 1994; Fausett 1994). The bootstrap technique that was applied separates the data into three categories, i.e. learning (70 %), testing (15 %) and validating (15 %). Additionally, meteorological observations with 1, 2 and 3 days delay in to relation to *Ganoderma* spore counts were also used. The performance of ANNs was evaluated based on: (1) Pearson's correlation coefficient between observed and predicted spore counts, and (2) the contingency tables prepared accordingly to von Storch and Zwiers (1999).

Results

Ganoderma spore distribution

The characteristics of *Ganoderma* spore seasons (Fig. 1) showed that the daily mean concentration always exceed 7 s m^{-3} . The *Ganoderma* spore season always ended in October, but could start between April (in 2007) and June (in 2006). This caused large differences in duration of both the spore seasons and the Fungal Spore Indexes (i.e. annual sum of mean spore concentration). The difference between annual mean spore concentrations was confirmed by the ANOVA Kruskal-Wallis test at the significance level of $p < 0.05$ (Table 1). The highest daily mean concentrations of spores were found between mid-August and mid-October, and varied between 225 and 376 s m^{-3} .

Spore relationship with meteorological parameters

The *Ganoderma* spore concentrations were not normally distributed. Similar results were obtained for all investigated meteorological parameters, with the exception of air pressure. Hence, Spearman's rank correlation was applied to examine association between spore counts and meteorological variables (Table S1–S4). Lack of linearity between *Ganoderma* and the meteorological variables was confirmed by producing scatter plots presented in Fig. S1.

The strongest statistically significant relationships between spore concentration and most of the meteorological

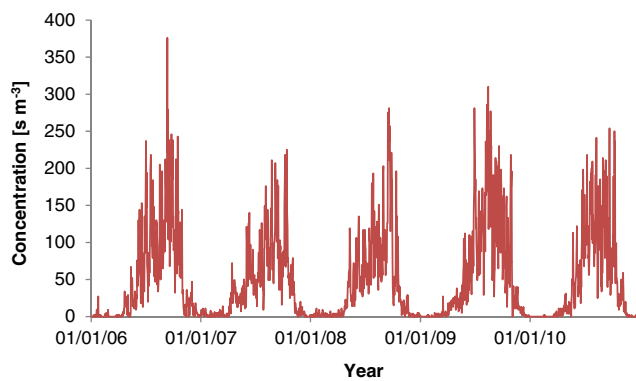


Fig. 1 Distribution of *Ganoderma* spores in the air of Worcester, UK (2006–2010)

parameters were with observations without any time delay (Table S1). The correlation decreased with increased time delay (Table S2–S4), except for a few cases where an increased correlation was obtained for 1-day lag data compared with day-to-day data, and for 3-day lag data compared with 2-day lag data. The main results are the following:

1. Dew point temperature was most strongly associated with *Ganoderma* spore concentration, with the exception of 1-day lag results; the correlation coefficient indicated directly proportional relationship; the highest value was obtained in 2010 reaching $r_s=0.545$; this relationship was declining with a delay of time, with a small twist between the second and the third day backward;
2. Maximum temperature was almost equally associated with the fungal spore presence as dew point temperature; it was a leading factor affecting concentration in 1-day lag data set; the relationship was also found to be directly proportional to *Ganoderma* spore concentration; the highest maximum temperature's association scored $r_s=0.529$ in 2010 and it was gradually declining in time;
3. Minimum temperature revealed similar direction of association with spore counts; on two occasions, it was scored as the second and third most important factor; the highest relationship was found also in 2010 with a r_s value equal to 0.536 in a data set without a time lag;

Table 1 p values from ANOVA Kruskal-Wallis test between years for *Ganoderma* spore concentration in Worcester, UK

Year	2006	2007	2008	2009	2010
2006		0.000*	0.000*	1.000	1.000
2007	0.000*		0.046*	0.000*	0.000*
2008	0.000*	0.046*		0.001*	0.022*
2009	1.000	0.000*	0.001*		1.000
2010	1.000	0.000*	0.022*	1.000	

* $p \leq 0.05$ (significantly different)

4. Mean temperature, derived from maximum and minimum temperatures, followed its roots, and again the maximum correlation coefficient was recorded in 2010 ($r_s=0.534$);
5. Air pressure was ranked as a fifth parameter in all data sets; higher r_s values were found in 1- and 3-day lag data achieving relationships at the r_s level of 0.399 and 0.331, respectively; the direction of this association was directly proportional to *Ganoderma* spore concentration;
6. Wind direction was classified at this position three out of four times; wind direction was mainly inversely correlated with spore counts and all highest coefficients were observed in 2007 within the time perspective $r_s=-0.266$, $r_s=-0.259$, $r_s=0.216$, $r_s=-0.266$, respectively;
7. Rain was inversely correlated with spore counts achieving seven statistically significant correlations for data without a time lag, two correlations for 1- and 2-day lag data, and only one for a 3-day lag data set; the highest relationship was found in 2006 ($r_s=-0.243$; $r_s=-0.242$, $r_s=-0.234$) and in 2007 year ($r_s=-0.266$), respectively;
8. Humidity was the least related meteorological parameter with *Ganoderma* spores; statistically significant relationship was found for a 3-year 2008–2010 ($r_s=0.093$) and 5-year 2006–2010 ($r_s=0.075$) periods of time for a data without a lag in time as well as for a 3-day lag data in 2008, with a r_s value equal to 0.199.

Multivariate regression trees

The MRT analysis showed that dew point temperature was the most important parameter with values between 10.11–12.80 °C and 6.92–8.80 °C (Table 2). On three occasions, dew point temperature was indicated as the only parameter, which had an impact on increased number of *Ganoderma* spores in the air (in 2006, 2010 and 2009–2010). The CV Errors of these models were quite satisfactory as well: 0.912, 0.817 and 0.809, respectively. The second most important parameter was found to be air pressure with a range between 1013 and 1024 hPa (Table 2). These values indicate the presence of high pressure systems above the study area, which brought dry and sunny weather. The maximum temperature between 18.09 °C and 19.61 °C was computed by using decision trees as the third variable affecting *Ganoderma* spore concentration in the air (Table 2). Two times maximum temperature was selected as the major factor (in 2006–2007 and 2006–2008).

The best annual multivariate regression tree model was obtained for 2008 (CV Error 0.544) and the worst for 2006 (CV Error 0.912). The increased number of years of observation did not seem to have an influence on the quality of decision trees; for instance, similar results were found for 2- and 4-year periods of time (2007–2008 and 2007–2010) (Table 2). The number of tree nodes varied, from 2 to 6. However, less

Table 2 Multivariate regression trees for *Ganoderma* spore concentration in Worcester, UK (2006–2010)

Year(s)	Parameter(s)	Value(s)	No of nodes	Error	CV Error	SE Error
1 year of observation						
2006	Dew point temp	12.00 °C	2	0.854	0.912	0.131
2007	Min temp	18.11 °C	5	0.590	0.791	0.108
	Air pressure	1024 hPa				
2008	Dew point temp×2	7.52 °C, 7.155 °C	5	0.354	0.544	0.081
	Air pressure	1021 hPa				
	Max temp, wind direction	18.95 °C, 183.2°				
2009	Dew point temp	8.8 °C	3	0.646	0.724	0.080
	Dew point temp	10.11 °C				
	Air pressure	1013 hPa				
2010	Dew point temp	11.30 °C	2	0.720	0.817	0.096
Two consecutive years of observation						
2006–2007	Max temp	18.09 °C	5	0.690	0.852	0.088
	Dew point temp×2	7.47 °C, 12.00 °C				
	Air pressure	1023 hPa				
2007–2008	Air pressure	1023 hPa	4	0.620	0.727	0.081
	Min temp and	18.16 °C and				
2008–2009	Dew point temp	7.155 °C	3	0.769	0.864	0.075
	Dew point temp	7.595 °C				
	Air pressure	1013 hPa				
2009–2010	Dew point temp	10.11 °C	2	0.768	0.809	0.067
Three consecutive years of observation						
2006–2008	Max temp	19.61 °C	5	0.663	0.807	0.073
	Dew point temp, wind dir	7.485 °C, 222.5°				
	Air pressure	1023 hPa				
2007–2009	Dew point temp	7.515 °C	5	0.681	0.813	0.068
	Max temp	18.16 °C				
	Air pressure	1023 hPa				
2008–2010	Dew point temp	10.27 °C	5	0.665	0.805	0.057
	Dew point temp	10.25 °C				
	Air pressure×2	1021, 1013 hPa				
	Humidity	71.37 %				
Four consecutive years of observation						
2006–2009	Dew point temp	12.00 °C	5	0.732	0.846	0.058
	Dew point temp, mean temp	7.485 °C, 18.8 °C				
	Air pressure	1018 hPa				
2007–2010	Dew point temp	10.25 °C	6	0.668	0.790	0.051
	Air pressure×2	1023, 1013 hPa				
	Dew point temp×2	6.915 °C, 12.8 °C				
Five consecutive years of observation						
2006–2010	Dew point temp	10.23 °C	4	0.785	0.856	0.051
	Air pressure, mean temp	1023 hPa, 19.21 °C				

complicated models were more frequently noticed for 1- and 2-year periods of spore concentration.

A total of 15 models generally provided homogeneous information regarding key meteorological factors together with necessary threshold values, which should be taken into consideration

while forecasting *Ganoderma* spore concentration. To make forecasting feasible using obtained results, authors arranged *Ganoderma* spore records according to the concentration, i.e. low ($0\text{--}49\text{ s m}^{-3}$), moderate ($50\text{--}99\text{ s m}^{-3}$), high ($100\text{--}149\text{ s m}^{-3}$) and very high ($n\geq 150\text{ s m}^{-3}$). This classification

was carried out by analysing the most frequently computed values by multivariate regression trees, as to date no threshold for allergenic responses in sensitized people to *Ganoderma* spores has been established worldwide. Following the described procedure, it received the following: 6 cases for low concentration (10 %), 23 cases for moderate concentration (38 %), 24 cases for high concentration (39 %) and 8 cases for very high concentration (13 %), whereby “case” a single tree node was understood ($n=61$). The weather conditions associated with above-mentioned levels of spores in the air were jointly grouped together and subsequently summarized:

- (a) Low—cold night (dew point temperature below 7 °C), followed by a cool day (maximum temperature equal to or below 17 °C); any rain showers will reduce spore concentration to zero;
- (b) Moderate—slightly warmer, but still a cool night (dew point temperature between 7 and 10 °C) and daily maximum temperature equal to or above 18 °C; lack of light rain showers helps the air to dry up (relative humidity around 71 %);
- (c) High—warm night (dew point temperature around 12 °C) and daily maximum temperature equal to or above 20 °C; lack of light rain showers helps the air to dry up (relative humidity equal to or below 71 %); prevailing winds blowing from south west to north directions may additionally contribute in increased concentration of *Ganoderma* spores in the air;
- (d) Very high—warm night (dew point temperature equal to or above 12 °C) and maximum temperature above 20 °C recorded during the day; lack of any rain showers helps further reduction of moisture in the air (relative humidity below 70 %); prevailing winds blowing from north east to south directions may additionally contribute in increased concentration of *Ganoderma* spores in the air.

Artificial neural network

The best regression ANN model for *Ganoderma* spores was obtained using meteorological data without a time lag. The final Multi Layer Perceptron (MLP) model consisted of 8 input neurons (meteorological parameters), 13 hidden neurons (hidden neurons produced by the model) and 1 output neuron (predicted spore concentration) (Table 3). This model was trained with 135 epochs of back propagation. Table 4 presents results from the sensitivity analysis. They show that mean temperature, followed by minimum and maximum temperature were the most important parameters affecting increased concentration of *Ganoderma* spores in the air of Worcester; all of them obtained a ratio above 3 (Table 4). Remaining parameters also contributed significantly to the final model, as their ratio scored above 1. Correlations between observed and predicted spore counts varied from $r_s=0.573$ to $r_s=0.681$, and this

was the primary criterion for choosing this ANN model compared with other models. Performance of this model has been directly presented in Fig. 2 and described in detail in Table 5.

Spearman's rank association test revealed that the strongest statistically significant relationships were found between *Ganoderma* spore concentration and 1-day lag data for air pressure and wind direction. Hence, an additional “mixed” regression ANN model was constructed. This model was based on the above-mentioned parameters with time lag and the remaining meteorological factors with no time lag. The best results were gained by MLP (8:14:1) network, with 8 input, 14 hidden and 1 output neurons, trained with 70 epochs of back propagation (Table 3). Performance of MLP (8:14:1) network was good, as the correlation coefficients between observed and calculated observations varied from $r_s=0.573$ to $r_s=0.635$ (Table 3). The sensitivity analysis showed that all parameters, regardless of time factor, contributed significantly as their ratio scored above 1 (Table 4). However, the most important parameters were as follows: maximum, minimum and mean temperatures (without a time lag) along with air pressure (1 day of difference in relation to spore counts). The remaining meteorological parameters contributed similarly to the model performance (Table 4). Performance of this model has been directly presented in Fig. 3 and described in detail in Table 5.

Discussion

Ganoderma spores were present in the atmosphere of Worcester, UK, throughout the year. The main fungal spore seasons were used for statistical examination. Spearman's rank correlation indicated a very strong association between the *Ganoderma* spores with dew point temperature and maximum temperature (Table S1–S4). These relationships were confirmed by multivariate regression tree analysis, where presence or absence of spores was determined by a threshold value of 7 °C for dew point temperature and 17 °C for maximum temperature (Table 2). However, the MRT models rated air pressure higher than Spearman's rank test and attributed more importance to its impact on the fungal spore concentration recorded in Worcester. Similar results were obtained by Grinn-Gofroń and Strzelczak (2011), where MRT model revealed dew point temperature as major factor influencing spore load with a border value of 9 °C.

A small difference appears in the second variable indicated by MRT analysis, where mean air temperature was found with a threshold of 15.4 °C in Szczecin (Poland). Classification of spore concentration from low to very high prepared for Worcester reflects accurately the dependence of *Ganoderma* spores from dew point temperature and maximum temperature. Progressive growth of temperature during the night and the following day accelerated a risk of higher spore counts, especially when no precipitation was recorded. Weak, yet

Table 3 The characteristics of MLP (8:13:1) and MLP (8:14:1) neural models based on 5 years of *Ganoderma* spore concentration in Worcester, UK (2006–2010)

	Learn	Test	Validation	Algorithm	Error	Activation function	
						Learn	Validation
MLP 8:13:1 ^a	0.681	0.604	0.573	BFGS 135	SOS	Tangent	Linear
MLP 8:14:1 ^b	0.635	0.573	0.612	BFGS 70	SOS	Tangent	Exponential

BFGS Broyden-Fletcher-Goldfarb-Shanno, *SOS* sum of squares

^a Model based on data without a time lag

^b Model based on data with a time lag

statistically significant relationship with relative humidity was detected by Spearman's rank test, what was also shown by MRT (Table 2). A significant influence of prevailing wind direction was also detected, which was not found by the MRT model produced for *Ganoderma* spores in Szczecin (Grinn-Gofroń and Strzelczak 2011). This result was in an agreement with Spearman's rank correlation (Table S1–S4) and previously published findings, where southern winds were directly connected with woodlands located in South and South-East England, a possible source of *Ganoderma* basidiocarps (Sadyś et al. 2014). Similarly, Jedryczka et al. (2015) found wind direction to be the most influential parameter for *Ganoderma* observations collected at a height of 1 m above ground level. However, the MRT analysis computed mean air temperature as the decisive factor for this type of basidiospores, if the measurements were taken at a height of 18 m above ground level (Jedryczka et al. 2015).

The ANN analysis classified meteorological parameters in a different order than Spearman's rank test and MRT models (Table S1–S4 and Table 4). Dew point temperature went down to the fifth position, whereas the top was occupied by mean and minimum temperatures. Regression-based ANNs could not provide information about threshold values for above-mentioned factors. In a comparison to the ANN model for *Ganoderma* spores computed in Rzeszów (Poland), dew point temperature was also rated as less important than air temperature simultaneously with air pressure (Kasprzyk et al. 2011). The ANN models obtained for Worcester were performing slightly better (Table 3) than the one constructed for Rzeszów because the correlation between observed and predicted results were higher: 0.57–0.68, 0.57–0.64 and 0.50–0.63, respectively

(Kasprzyk et al. 2011). The difference in correlation between these two studies is likely due to the fact that they use a different number of years of observations. Training an ANN with a greater number of observations is likely to result in a more accurate prediction. Another reason that could explain the difference between these three models could be the different number of neurons in a hidden layer, i.e. 13 and 14 (Table 3) and 5, respectively (Kasprzyk et al. 2011). Additionally, the Worcester models were constructed on daily values, whereas the models for Rzeszów used hourly spore concentrations. Grinn-Gofroń and Strzelczak (2011) decided not to publish results from regression neural networks for *Ganoderma* spores in Szczecin (both for original and transformed values), stating that they performed at very low levels. This information could help other researchers to define a level of accepted errors because it will never be possible to create a neural network for any type of spores at level of 100 % accuracy as recently argued by Jedryczka et al. (2015). This type of models is often limited to meteorological observations, although other factors can be also included, e.g. source location as described by Sadyś et al. (2014) affect fungal spore production and their dispersal, before they will be captured by air samplers.

Finally, for forecasting purposes, authors would use MLP (8:13:1) rather than MLP (8:14:1). The aggregation of data points, which reflects the relationship between observed and predicted values, showed less detachment with the MLP (8:13:1). This choice was dictated by two criteria, i.e. the correlation coefficient between observed and predicted values, and the percentage of correctly classified levels of *Ganoderma* spore concentration (i.e. low, moderate, high and very high). These criteria were based on the results presented in the

Table 4 Sensitivity analysis for MLP (8:13:1) and MLP (8:14:1) regression neural models based on 5 years of *Ganoderma* spore concentration in Worcester, UK (2006–2010)

Parameters		Max temp	Min temp	Mean temp	Dew point temp	Rain	Humidity	Wind direction	Air pressure
MLP 8:13:1 ^a	Ratio	3.064	3.457	6.429	1.468	1.051	1.315	1.210	1.475
	Rank	3	2	1	5	8	6	7	4
MLP 8:14:1 ^b	Ratio	1.886	1.517	1.305	1.172	1.003	1.093	1.080	1.205
	Rank	1	2	3	5	8	6	7	4

^a Model based on data without a time lag

^b Model based on data with a time lag

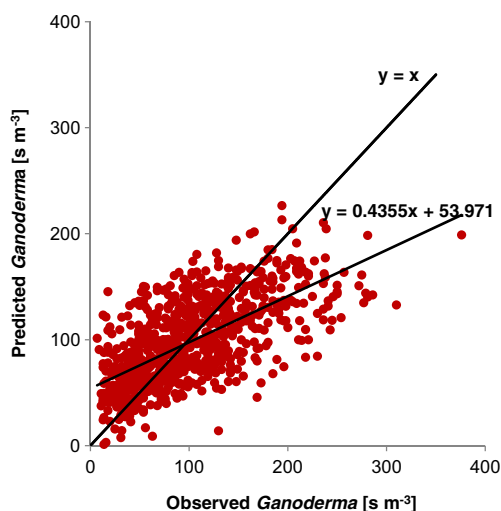


Fig. 2 The correlation between observed and predicted *Ganoderma* spore concentration using the MLP (8:13:1) model for Worcester, UK

contingency Table 5. MLP (8:13:1) predicted low and very high spore concentration scarcely better, i.e. 33 % and 39 % than MLP (8:14:1), 29 % and 32 %, respectively. Moderate spore concentrations by both models reached 60 % of correctly classified values. This was the best result that was obtained among all groups (Table 5). The difference between moderate and high spore concentration levels is the most important difference in practice. In general, it is erroneous to classify a low concentration as very high (Table 5) than a high concentration as moderate or low (Table 5). Larsen and Speckman (2004) stated that multivariate regression trees performed poorly at forecasting new observations. Several authors proved that by plotting a reasonable number of models it was possible to find regular dependences between spore occurrence in the air and meteorological parameters. This could be used to predict *Ganoderma* spore concentration in the future. Although the obtained guideline has had a rather descriptive character than numerical, authors would recommend that MRT analysis was based on at least 2 years of observations in order to obtain fairly representative results.

Table 5 A 4×4 contingency table summarizing the performance of neural models: MLP 8:13:1 (right) and MLP 8:14:1 (left) for prediction of *Ganoderma* spore concentration in the air of Worcester, UK presented in percent ($n=805$)

Observed	Forecast								Total	
	Low	Moderate	High	Very high						
Low	33	29	58	62	9	8	0	1	24	24
Moderate	8	9	60	60	29	28	3	3	35	35
High	0	1	41	42	44	48	15	9	23	23
Very high	1	1	13	18	47	49	39	32	18	18
Total	11	10	46	49	31	32	12	9	100	

Numbers highlighted in *italics* indicate correctly classified spore levels (predicted vs observed) expressed in percentage

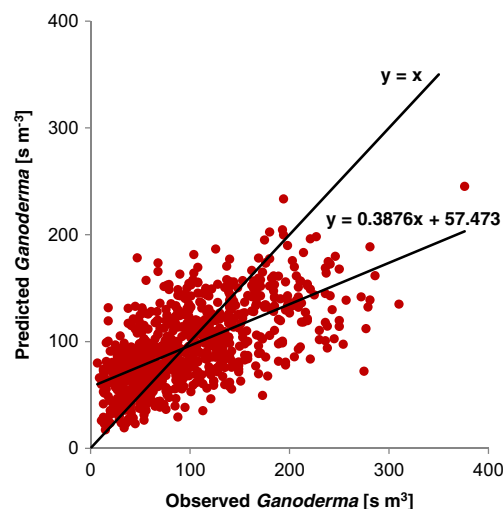


Fig. 3 The correlation between observed and predicted *Ganoderma* spore concentration using the MLP (8:14:1) model for Worcester, UK

Recent research has shown that neural network models can be used to analyse a number of different bioaerosols. In relation to pollen, ragweed (Csépe et al. 2014), plantago (Iglesias-Otero et al. 2015), olive (Oteros et al. 2013), grass (Rodríguez-Rajo et al. 2010) and birch (Puc 2012) pollen have been analysed using neural network models, in relation to fungal spores, *Alternaria* spp. (Grinn-Gofroń and Strzelczak 2013; Tomasetti et al. 2013; Astray et al. 2010), *Cladosporium* spp. (Grinn-Gofroń and Strzelczak 2013) and *Pleospora* spp. (Tomasetti et al. 2013). Most of these studies as well as this study rely on observed meteorological variables and rarely apply forecasted meteorological data (e.g. Tomasetti et al. 2013). It is therefore clear that the application of neural network models is still in its infancy and remains to be tested in a real forecasting environment that rely on grid based and forecasted meteorological data.

Conclusions

In conclusions, dew point temperatures along with the maximum temperature was the most important factor influencing spore presence and their concentration in the atmosphere of the studied area. This was confirmed by multivariate regression trees and the Spearman's rank test. Despite some deviation in results obtained by ANNs, authors have achieved a forecasting model with good accuracy using 5 years of observations. It is therefore recommended to use at least 5-year observations for training ANN models for *Ganoderma*. The comparisons of the ANN model from Worcester with the ANN model from Rzeszów showed that the prediction power could be increased by increasing the number of spore observations or by adding new meteorological variables. Using meteorological parameters with a time delay showed decreased correlation in relation to *Ganoderma* spore concentrations.

Further work

The *Ganoderma* spore study has not been fully explored yet. Authors are planning to expand the analysis for investigation of hourly spore counts using the same statistical methods to test, whether it would be possible to obtain a model with higher precision. In the meantime, authors may try to change some of the setting parameters of neural network estimator, by adding new meteorological parameters or establishing certain threshold values for existing ones, as well as designing models manually rather than using an Automatic Problem Solver. Additionally, authors may combine *Ganoderma* spore observations from two or more monitoring sites to examine, whether it would be possible to receive a spatial model performing well for different biogeographical regions, as the biology of the fungus remains unchanged.

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Supplementary materials to the article

Forecasting methodologies for *Ganoderma* spore concentration using combined statistical approaches and model evaluations

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Table S1 Spearman's rank correlation for *Ganoderma* spore concentration and 0 day lag meteorological parameters for the period 2006-2010

Year	Max temp	Min temp	Mean temp	Dew point temp	Rain	Relative humidity	Wind direction	Air pressure
2006	0.237*	0.220*	0.218*	0.343*	-0.243*	0.135	0.003	-0.038
2007	0.412*	0.415*	0.413*	0.391*	0.089	0.034	-0.266*	0.241*
2008	0.345*	0.346*	0.345*	0.404*	-0.082	0.100	-0.152	0.373*
2009	0.444*	0.446*	0.444*	0.516*	-0.134	0.118	-0.095	0.078
2010	0.529*	0.536*	0.534*	0.545*	-0.143	0.102	-0.045	0.047
2006-07	0.489*	0.173*	0.419*	0.419*	-0.082	0.036	-0.008	0.081
2007-08	0.403*	0.404*	0.404*	0.410*	-0.016	0.056	-0.210*	0.274*
2008-09	0.384*	0.385*	0.384*	0.423*	-0.115*	0.077	-0.147*	0.267*
2009-10	0.477*	0.484*	0.480*	0.506*	-0.140*	0.089	-0.069	0.065
2006-08	0.439*	0.227*	0.395*	0.410*	-0.085	0.055	-0.054	0.178*
2007-09	0.410*	0.411*	0.411*	0.417*	-0.087	0.036	-0.194*	0.225*
2008-10	0.432*	0.433*	0.432*	0.461*	-0.116*	0.093*	-0.094*	0.210*
2006-09	0.402*	0.294*	0.393*	0.415*	-0.118*	0.054	-0.091*	0.162*
2007-10	0.446*	0.445*	0.446*	0.460*	-0.097*	0.070	-0.131*	0.188*
2006-10	0.415*	0.346*	0.418*	0.446*	-0.122*	0.075*	-0.072*	0.143*

Level of statistical significance: $p \leq 0.05$ (*)

Table S2 Spearman's rank correlation for *Ganoderma* spore concentration and 1 day lag meteorological parameters for the period 2006-2010

Year	Max temp	Min temp	Mean temp	Dew point temp	Rain	Relative humidity	Wind direction	Air pressure
2006	0.235*	0.143	0.216*	0.189*	-0.242*	0.011	-0.181*	0.065
2007	0.367*	0.376*	0.375*	0.337*	0.164*	0.024	-0.259*	0.260*
2008	0.306*	0.304*	0.306*	0.306*	0.047	0.035	-0.235*	0.399*
2009	0.404*	0.404*	0.404*	0.369*	0.050	-0.023	-0.193*	0.181*
2010	0.360*	0.361*	0.360*	0.254*	0.009	-0.018	-0.096	0.164
2006-07	0.459*	0.120*	0.397*	0.341*	-0.038	-0.017	-0.070	0.128*
2007-08	0.359*	0.365*	0.365*	0.341*	0.078	0.027	-0.243*	0.296*
2008-09	0.345*	0.345*	0.345*	0.309*	0.046	-0.022	-0.237*	0.331*
2009-10	0.376*	0.379*	0.378*	0.310*	0.029	-0.034	-0.136*	0.174*
2006-08	0.406*	0.178*	0.370*	0.329*	-0.018	0.000	-0.118*	0.220*
2007-09	0.369*	0.374*	0.373*	0.329*	0.039	-0.027	-0.246*	0.270*
2008-10	0.354*	0.353*	0.353*	0.305*	0.038	-0.011	-0.169*	0.289*
2006-09	0.367*	0.247*	0.364*	0.323*	-0.022	-0.021	-0.160*	0.217*
2007-10	0.378*	0.379*	0.380*	0.337*	0.031	-0.004	-0.183*	0.247*
2006-10	0.360*	0.276*	0.365*	0.323*	-0.018	-0.007	-0.139*	0.207*

Level of statistical significance: $p \leq 0.05$ (*)

Table S3 Spearman's rank correlation for *Ganoderma* spore concentration and 2 day lag meteorological parameters for the period 2006-2010

Year	Max temp	Min temp	Mean temp	Dew point temp	Rain	Relative humidity	Wind direction	Air pressure
2006	0.133	0.051	0.098	0.085	-0.234*	0.024	-0.132	0.050
2007	0.282*	0.304*	0.300*	0.268*	0.207*	0.008	-0.216*	0.304*
2008	0.204*	0.202*	0.203*	0.309*	0.069	0.138	-0.074	0.279*
2009	0.289*	0.291*	0.289*	0.314*	0.058	-0.024	-0.193*	0.088
2010	0.245*	0.241*	0.244*	0.224*	0.045	0.032	0.091	0.081
2006-07	0.413*	0.048	0.323*	0.262*	-0.010	-0.029	-0.054	0.142*
2007-08	0.268*	0.281*	0.279*	0.306*	0.107*	0.067	-0.145*	0.265*
2008-09	0.239*	0.240*	0.239*	0.280*	0.068	0.031	-0.156*	0.237*
2009-10	0.262*	0.264*	0.264*	0.263*	0.045	0.001	-0.039	0.085
2006-08	0.348*	0.098*	0.289*	0.277*	0.006	0.024	-0.063	0.194*
2007-09	0.270*	0.282*	0.280*	0.286*	0.066	0.002	-0.181*	0.224*
2008-10	0.245*	0.243*	0.244*	0.270*	0.062	0.045	-0.059	0.199*
2006-09	0.298*	0.161*	0.276*	0.269*	0.000	0.000	-0.115*	0.178*
2007-10	0.276*	0.282*	0.283*	0.291*	0.061	0.031	-0.097*	0.194*
2006-10	0.285*	0.184*	0.272*	0.269*	0.007	0.020	-0.073*	0.162*

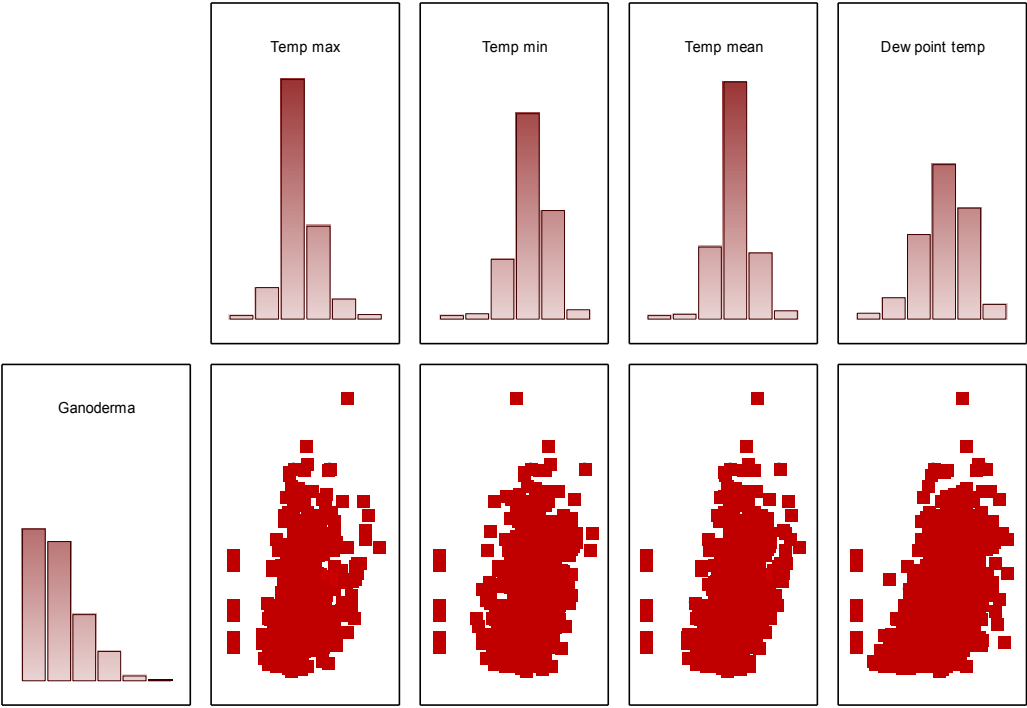
Level of statistical significance: $p \leq 0.05$ (*)

Table S4 Spearman's rank correlation for *Ganoderma* spore concentration and 3 day lag meteorological parameters for the period 2006-2010

Year	Max temp	Min temp	Mean temp	Dew point temp	Rain	Relative humidity	Wind direction	Air pressure
2006	0.126	0.054	0.093	0.014	-0.081	0.002	-0.095	-0.021
2007	0.254*	0.294*	0.286*	0.233*	0.213*	-0.026	-0.266*	0.331*
2008	0.160*	0.159*	0.159*	0.341*	0.006	0.199*	-0.023	0.196*
2009	0.303*	0.305*	0.303*	0.306*	0.049	-0.112	-0.154	0.040
2010	0.125	0.123	0.125	0.054	0.071	-0.024	-0.057	0.081
2006-07	0.409*	0.028	0.316*	0.219*	0.063	-0.051	-0.037	0.127*
2007-08	0.228*	0.253*	0.249*	0.301*	0.093	0.077	-0.150*	0.243*
2008-09	0.214*	0.215*	0.214*	0.287*	0.033	0.030	-0.108	0.176*
2009-10	0.217*	0.219*	0.219*	0.191*	0.047	-0.056	-0.101	0.062
2006-08	0.336*	0.070	0.269*	0.256*	0.047	0.025	-0.036	0.156*
2007-09	0.238*	0.258*	0.254*	0.279*	0.056	-0.005	-0.168*	0.195*
2008-10	0.196*	0.194*	0.196*	0.236*	0.045	0.033	-0.078	0.153*
2006-09	0.287*	0.137*	0.259*	0.250*	0.029	-0.013	-0.085*	0.139*
2007-10	0.230*	0.242*	0.242*	0.262*	0.057	0.018	-0.122*	0.168*
2006-10	0.260*	0.148*	0.241*	0.231*	0.034	0.003	-0.074*	0.126*

Level of statistical significance: $p \leq 0.05$ (*)

Temp max [°C]: Max = 35.10, Min = 0 Temp min [°C]: Max = 24.17, Min = 0
Temp mean [°C]: Max = 26.60, Min = 0 Dew point temp [°C]: Max = 17.65, Min = 0



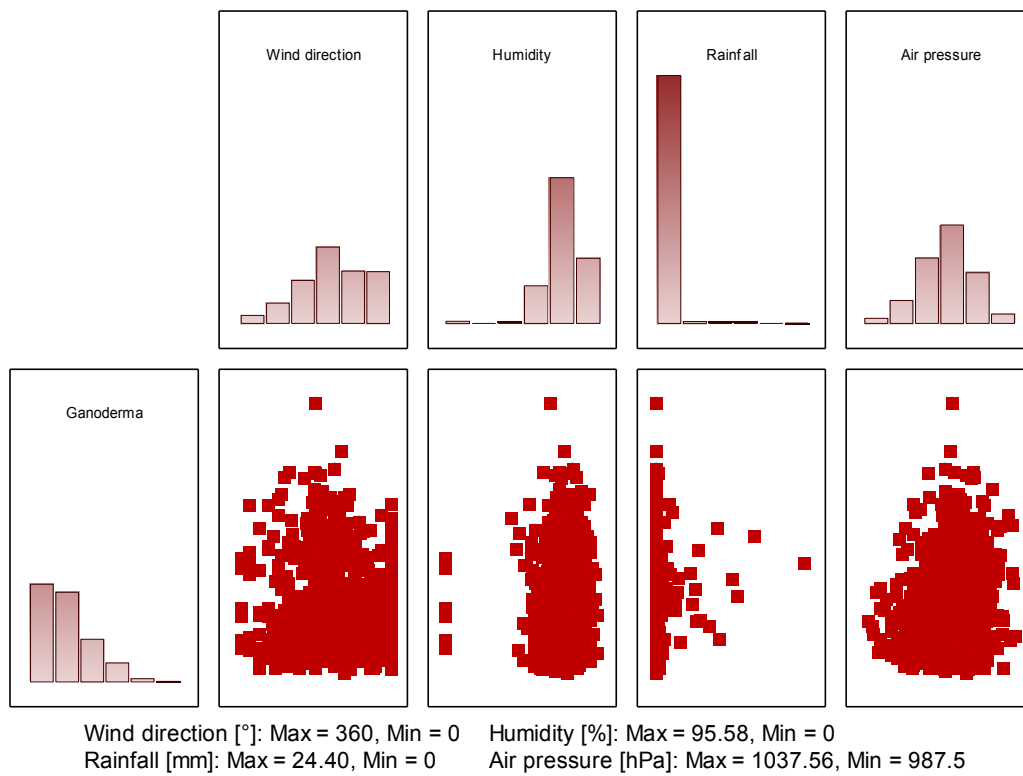


Fig. S1 Matrix scatter plots between *Ganoderma* spores and meteorological parameters measured in Worcester, UK (2006-2010)