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ORIGINAL RESEARCH PAPER

Airborne fungal spores as a potential hazard for vineyard workers – A case report from the Carpathian foothills

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Abstract

Fungal spores often cause allergic diseases in people working outdoors, especially in agriculture. In the case of vineyard workers, the threat of mycotoxins is generally considered, and aerobiological monitoring focuses mainly on pathogenic fungi that cause diseases in grapes. The current requirement is to increase knowledge regarding the risk to vineyard workers coming from highly allergenic fungal spores such as *Alternaria* and *Epicoccum*. Aerobiological monitoring was conducted using the volumetric method. The relationships between meteorological parameters and airborne fungal concentrations were detected using Spearman's correlation and a logistic regression model. It was found that this risk is significant and is particularly strong from July to September, when periods of high concentrations of *Alternaria* and *Epicoccum* spores overlap. High concentrations of spores are favoured by an increase in air temperature, sunshine duration, and low relative humidity. When the weather conditions are favourable for the release and dispersion of spores, their concentration exceeds the threshold to trigger allergic reactions in sufferers. Due to their high concentrations and cross-reactions of their allergens with other aeroallergens, airborne fungal spores may pose a health risk to allergy sufferers working in vineyards. For this reason, aerobiological monitoring is particularly important in agricultural workplace.

Keywords
airborne fungal spores; allergy; occupational biohazard; vineyard workers; meteorological parameters

1. Introduction

Fungal spores are one of the most important components of aeroplankton due to their small size and enormous production by species occurring in each climate zone and habitat condition (Levetin et al., 2016). A majority of studies conducted in various climatic zones prove that *Cladosporium* belongs to the group of the most common spores in the air (Grinn-Gofroń et al., 2019; Katotomichelakis et al., 2015; Olsen et al., 2020; Ščevková & Kováč, 2019). Additionally, *Alternaria*, *Ganoderma*, *Epicoccum*, and *Leptosphaeria* are also frequently detected in the air in temperate climate zones (Bednarz & Pawłowska, 2016; Kasprzyk et al., 2004; Olsen et al., 2019, 2020; Rizzi-Longo et al., 2009; Sadyś et al., 2018; Skjøth et al., 2016).

The length of the spore season, spore concentration, sporulation, transport in the air, and deposition of spores depend on meteorological parameters (mainly temperature and air humidity), climate zones (latitude), geographical location, distance from the sea, the season of the year, the type of vegetation, and finally the availability of nutrients (Amigot Laźaro et al., 2000; Skjøth et al., 2016; Vélez-Pereira et al., 2023). Fungal spores are present in the air almost throughout the entire year. In temperate climates, at the beginning of the growing season of plants (their main source of nutrients), their concentrations systematically increase, reaching their maximum values in summer and early autumn. Weather conditions at this time of year favour sporulation and dispersal of spores (Sadyś et al., 2018; Skjøth et al., 2012).

One of the reasons for frequent research on airborne fungal spores is their allergenic potential. The spectrum of allergic symptoms caused by hypersensitivity reactions includes rhinitis, asthma, and atopic dermatitis (Fukutomi & Taniguchi, 2015; Simon-Nobbe et al., 2007; Twaroch et al., 2015). Knutsen et al. (2012) reported that asthma caused by allergenic fungal spores affect 8.2% of adults and children, roughly amounting to 24.6 million people in the United States.

It has been estimated that, among people with atopy who are sensitive to fungi, about 70% respond to airborne *Alternaria* spores (Knutsen et al., 2012). In their review article, Kustrzeba-Wójcicka et al. (2014) claimed that 16 allergens have been isolated from *Alternaria alternata*. Respiratory allergies induced by *Alternaria* spores, most commonly among children and other sensitive individuals, are well known (Behbod et al., 2015; Knutsen et al., 2012).

Alternaria is also a common pathogen of cereal crops (wheat, maize, millets, and oats) and potatoes, causing serious economic losses. The annual peak concentration of *Alternaria* depends on the local harvest seasons (Skjøth et al., 2012); therefore, it is a serious allergy hazard for agricultural workers. The daily maximum concentration is recorded in the afternoon when the highest temperature and lowest humidity of the day are measured (Recio et al., 2012). Allergic symptoms may appear when the spore concentration in the air exceeds the value of 80 m^{-3} of air (Rapiejko et al., 2004). This value depends on the climate zone and the local concentration of spores in the air; it can be higher, even 100 m^{-3} of air, according to Gravesen (1979). *A. alternata* allergens show cross-reactivity with those of *Aureobasidium pullulans, Aspergillus fumigatus*, and *Cladosporium herbarum*, resulting in antigen sequence identity (Twaroch et al., 2016). Small fragments (less than 1 μm) released from airborne *Alternaria* conidia can enter the lungs and cause respiratory diseases (Lee & Liao, 2014). Furthermore, Kasprzyk (2008) found that *Alternaria* spores occur in the air in temperate climatic zones at the same time as allergenic *Artemisia* pollen, which can increase the threat for allergenic people.

Some species of *Epicoccum* produce highly allergenic spores, which may cause skin reactivity and allergy-mediated respiratory tract diseases (Bisht et al., 2002, 2004; Dixit et al., 1992; Kukreja et al., 2008). The sensitivity to *Epicoccum nigrum*, mainly caused by the allergen Epi p 1, is estimated to range from 5% to 7% (Bisht et al., 2004). In addition, some studies indicate significant cross-reactivity between *E. nigrum* and *A. alternata, Cladosporium herbarum, C. lunata*, and *Penicillium citrinum* antigens (Kukreja et al., 2008; Portnoy et al., 1987). This is a particularly dangerous situation because, in temperate climates, airborne *Epicoccum* spores exhibit similar seasonal patterns as *Alternaria* spores, which is also positively correlated with temperature (Rizzi-Longo et al., 2009). Despite the proven allergenic potential of these spores, the threshold value of the *Epicoccum* spore concentration is still not known and further research on this issue is needed.

Agricultural workers, including vineyard workers, are particularly affected by hazardous agents. They are highly exposed not only to insects, thermal stress, solar radiation, pesticides, organic or inorganic particles, endotoxins, bacteria, and mites, but also to allergenic pollen and fungal spores (Youakim, 2006). Contact with allergenic factors can induce symptoms like rhinitis, asthma, chronic bronchitis, hypersensitivity pneumonitis, and organic dust toxic syndrome (Perotin et al., 2015). *Vitis vinifera* is one of the most widely cultivated fruit crops in the world (Torregrosa et al., 2015), and *Alternaria* and *Epicoccum* fungi have been isolated from grapevines (Del Frari et al., 2019). Fungal spores are often the most prevalent biological particles in the air of a vineyard. Previous studies have attempted to determine seasonal variations and concentrations of selected spores as well as the health risk to workers (Chattopadhyay et al., 2007; Diaz et al., 1997, 1998; Lee & Liao, 2014; Perotin et al., 2015; Youakim, 2006).

The studies mentioned above were carried out in vineyards located in the subtropical climate zone of Southern Europe, where grapevines are cultivated very often (Soltekin & Altındisli, 2022). In Central Europe, particularly Poland, the history of cultivation has over a thousand-year tradition. After the medieval period of prosperity, in the seventh and eighth centuries, there was a regression in winemaking due to the climate cooling. With the climate warming now, it is expected that the cultivation area will expand. In Europe, it is moving towards the north, where an increasing number of people are working in this sector (Martinez-Bracero et al., 2020). However, the degree of aero-allergen risk has not been studied so far in Polish vineyards, and this prompted us to undertake this research. A question was posed whether vineyard workers are exposed to spores of *Alternaria* and *Epicoccum* that endanger their health in the temperate climatic zone, and if so, how strong this threat is. The research was also conducted to verify the hypothesis about the strong influence of weather conditions on fungal spore concentrations and thus on the strength of their impact on the well-being and health of vineyard workers.

2. Material and methods

Aerobiological monitoring was carried out in Rzeszów city located in South-East Poland in the Carpathian Foothills (Figure 1A). Due to the climatic conditions (225 and 230 days of the vegetative period) and topography (predominance of south and south-west slopes) of the Carpathian foothills, the region is the best place in Poland for vineyards. The average temperature in Rzeszów is approximately 9 °C and the mean annual precipitation is approximately 750 mm. The vineyard is located in the south-east part of Rzeszów (Figure 1B) on a south-western slope at a height of 260 to 280 m above mean sea level. In the area, about 2000 grapes were grown of the following varieties: White Johanniter, Solaris, Jutrzenka, Seyval Blanc, Phoenix, Bianca, Marechal Foch, Acolon, Regent, and Hibernal.

Sampling of airborne fungal spores was carried out continuously from May to October 2016 at a height of 2 m from the ground. The volumetric method was applied using a Hirst-type sampler (Lanzoni VPPS 2000). The fungal spores were sucked by the device and attached to the tape covered by silicone oil. Microscopic slides were prepared from the tape and then scanned using a light microscope at 400x magnification. Fungal spores were counted from one horizontal band, which was 48 mm long. Two fungal taxa were investigated: *Alternaria* and *Epicoccum*.

The result was expressed as the daily number of spores m^{-3} of air (Galán et al., 2017). The spore season was determined on the basis of the method of 95% (Caulton & Lacey, 1995). According to the recommendation proposed by Galán et al. (2017), the seasonal spore integral (SSIn) was calculated for the entire monitoring season and for particular months.

To compare the monthly SSIn values between the taxa, λ^2 was calculated. The frequency distributions were not normal, which was confirmed by the Shapiro-Wilk test, and Spearman's rank correlation was applied to check the correlation between the spore concentrations and meteorological parameters.

The influence of weather factors was investigated on the day of the measurements as well as one and two days prior. To test multiple relationships between these parameters, a logistic regression method was used. Based on the results of Spearman's correlation, the following parameters were selected as independent variables: average air temperature (T_{mean}), rainfall (R), solar radiation (irradiation), wind speed (Ws), and air relative humidity (H_{mean}). Meteorological measurements were carried out at the university meteorological station located 1800 m in a straight line from the vineyard. Spore concentration values were classified using a zero-one method based on the threshold value to evoke symptoms due to *Alternaria* (80 spores m^{-3}) and the median value for *Epicoccum*, as no threshold values for this taxon have been established yet. Values equal to or higher than the critical values were defined as the occurrence of an event (appearance of allergy symptoms) and lower values as non-occurrence of the event (no allergy symptoms). The first step before creating an

Figure 1 Location of the study area: (**A**) location of the Rzeszów city in Europe; (**B**) location of the vineyard in the Rzeszów city; (**C**) location of the vineyard against the background of utility grounds.

optimal model was to check the relationship between the spore concentration and each meteorological parameter. A one-way analysis was performed, which verified the possibility of creating an optimal model. The construction of the multivariate logistic regression model was based on the selection of all analysed variables (all effects). The model was created using the Wald stats. The fit of the independent variables to the model was assessed using the Hosmer–Lemeshow test, the likelihood ratio test (chi-square), and the analysis of ROC curves. Discriminant ability was checked by calculating the AUC coefficient. The level of significance was set at $\alpha \leq 0.05$. Statistical analyses were performed using the Statistica v.13 and PQStat v.1.6.8. software.

Table 1 Spores season characteristics of *Alternaria* and *Epicoccum* in vineyard in 2016.

	Start of season [date]	End of season [date]	Length of season [day]	SSIn	Maximum concentration [s m^{-3}]	Day of maximum concentration
Alternaria	18.06	02.10	107	17593	658	18.07
Epicoccum	31.05	27.10	150	5563	283	02.10

SSIn – seasonal spore integral.

Table 2 Spearman's rank correlation coefficients for *Alternaria* and *Epicoccum* spores and meteorological parameters in actual day (n), previous day ($n-1$) and two days earlier ($n-2$).

	Alternaria			*Epicoccum*		
	n	$n-1$	$n-2$	n	$n-1$	$n-2$
T_{min} [°C]	**0.508456***	**0.528470***	**0.494530***	**0.269290***	**0.239897***	0.142439
T_{max} [°C]	**0.457716***	**0.542991***	**0.482239***	**0.162828***	**0.229344***	**0.188871***
T_{mean} [°C]	**0.496168***	**0.563667***	**0.506326***	**0.194111***	**0.232355***	**0.164906***
Humidity [%]	**−0.174933***	**−0.223555***	−0.112409	0.030798	−0.037793	−0.004687
Preasure [hPa]	−0.037643	−0.023474	0.064793	0.112904	**0.156119***	**0.248659***
Wind speed [m/s]	−0.042223	−0.047577	−0.098049	0.014639	0.014635	−0.098863
Rainfall [mm]	−0.116125	**−0.186736***	**−0.188854***	−0.017216	−0.091137	**−0.177469***
Sunshine duration [hour/day]	**0.240328***	**0.312287***	**0.239108***	−0.007579	0.121832	0.092817
Wind speed [m/s]	0.107916	**0.151757***	**0.207394***	0.037486	0.084669	**0.168658***

* Results statistically significant ($p \leq 0.05$).

3. Results

The length of the spore season of *Alternaria* and *Epicoccum* differed significantly. The *Alternaria* spore season began in June, which was three weeks later than the *Epicoccum* season, and ended at the beginning of October, 25 days earlier than the *Epicoccum* season. The season was shorter but more intensive. The seasonal *Epicoccum* SSIn value was lower by more than three times than that of *Alternaria* (Table 1).

The spore concentration of both taxa began to increase during the half of June, but the distribution of their concentration in individual months was completely different, as confirmed by the λ^2 test ($p < 0.000$). The highest *Alternaria* SSIn values were recorded in July and August. For *Epicoccum*, the SSIn values were relatively similar and ranged from 1,137 in August to 1,562 in September, except in May and June when these values were much lower compared to the other months.

The *Alternaria* season was rather continuous, from July to mid-August, and the concentrations were high. An increase in the concentration was also noted in the first half and at the end of September. The highest concentration of *Alternaria* spores, i.e. 658 spores m^{-3} of air, was recorded in July. The *Epicoccum* spore season was comparatively irregular. The highest value was recorded at the beginning of October; it was 283 spores m^{-3}, which was more than two times less than that of *Alternaria* (Table 1, Figure 2).

The concentration of *Alternaria* spores exceeded the allergenic threshold value of 80 spores m^{-3} of air throughout 75 days. The threat to people with allergies appeared at the end of June and occurred almost every day in July and August and in the first half of September. During the entire six-month research period, spanning over 90 days, the concentration of *Epicoccum* spores exceeded the median value. These concentrations were recorded from July to early October (Figure 2).

The results of Spearman's rank correlation indicate that the minimum, maximum, and mean temperatures of actual and days prior to the measurement significantly affected the *Alternaria* and *Epicoccum* spore concentration. The influence of temperature on the increase in the concentration of *Alternaria* spores was greater than the case of *Epicoccum*, as evidenced by the higher values of the correlation coefficients. The

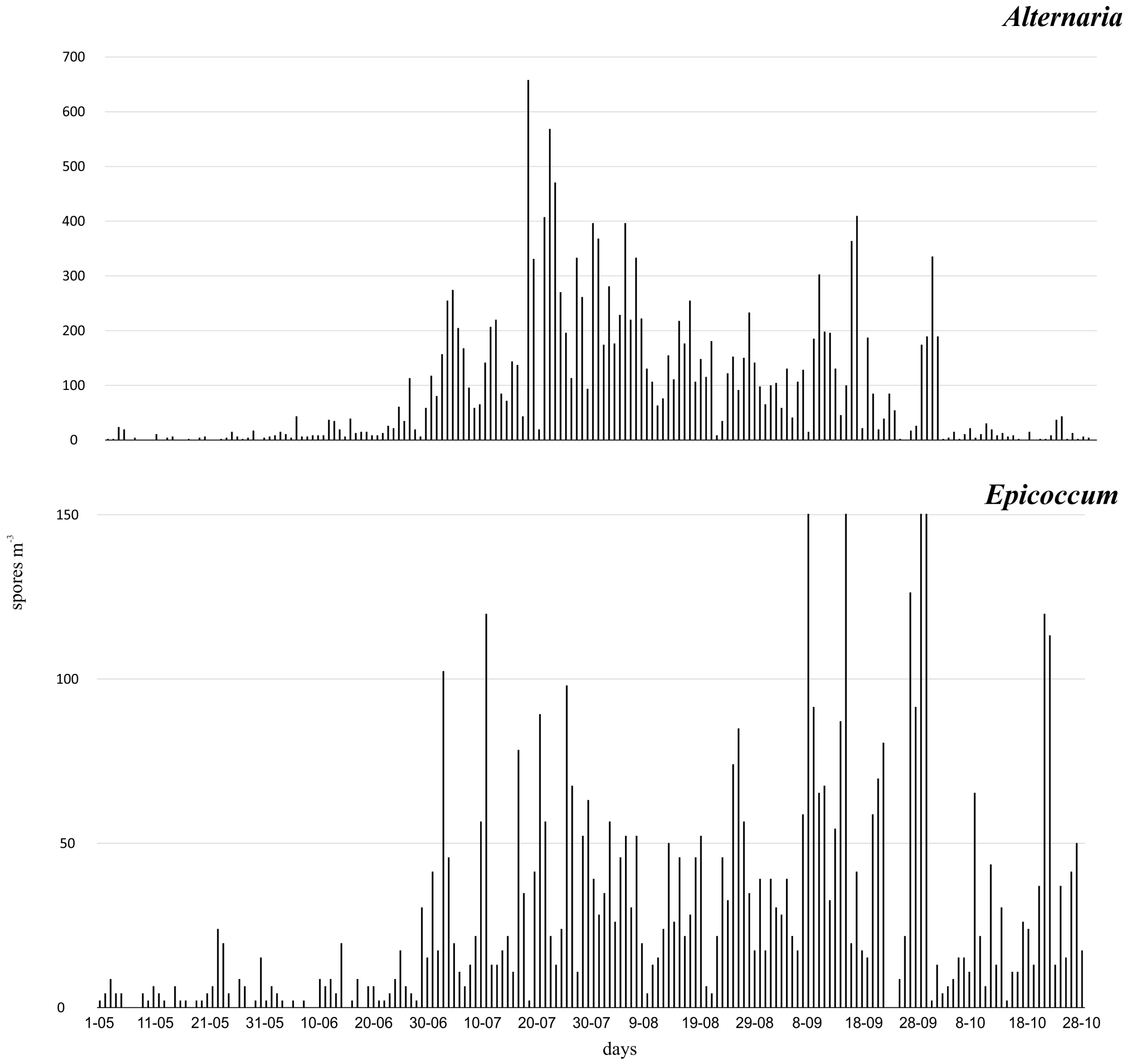

Figure 2 The seasonality of airborne *Alternaria* and *Epicoccum* spores in the vineyard.

increase in the *Alternaria* spore concentration in the air was also shown with the increase in hours of sunshine and the decrease in relative humidity. It seems that the concentrations of *Alternaria* and *Epicoccum* spores in the air decreased when rainfall occurred in days prior to their measurement (Table 2). The "Spores rose" figures show that the highest concentrations of spores of both taxa were noted when the wind was blowing from the west directions (W, SW, NW), where the main part of the vineyard is located, and further from the forest, which is located at a short distance (Figure 1, Figure 3).

The one-way analysis of logistic regression showed that the mean temperature was the only significant parameter favouring the increase in the *Epicoccum* spore concentration ($p = 0.01$), with an increase of nearly 1.08 (Table 3). The one-way analysis of logistic regression also showed that the sunshine duration, relative humidity, and mean temperature of the air could increase the concentration of *Alternaria* spores. However, the increase in prediction that exceeded the value of 1 was found only for the sunshine duration and the mean temperature (Table 3).

To determine factors from the one-way model that had the highest significance in increasing the *Alternaria* spore concentration, the parameters were compared and multivariable analysis was applied. The results showed that the mean temperature of the air was statistically significant and was close to zero ($W = 27.03$). The values of the

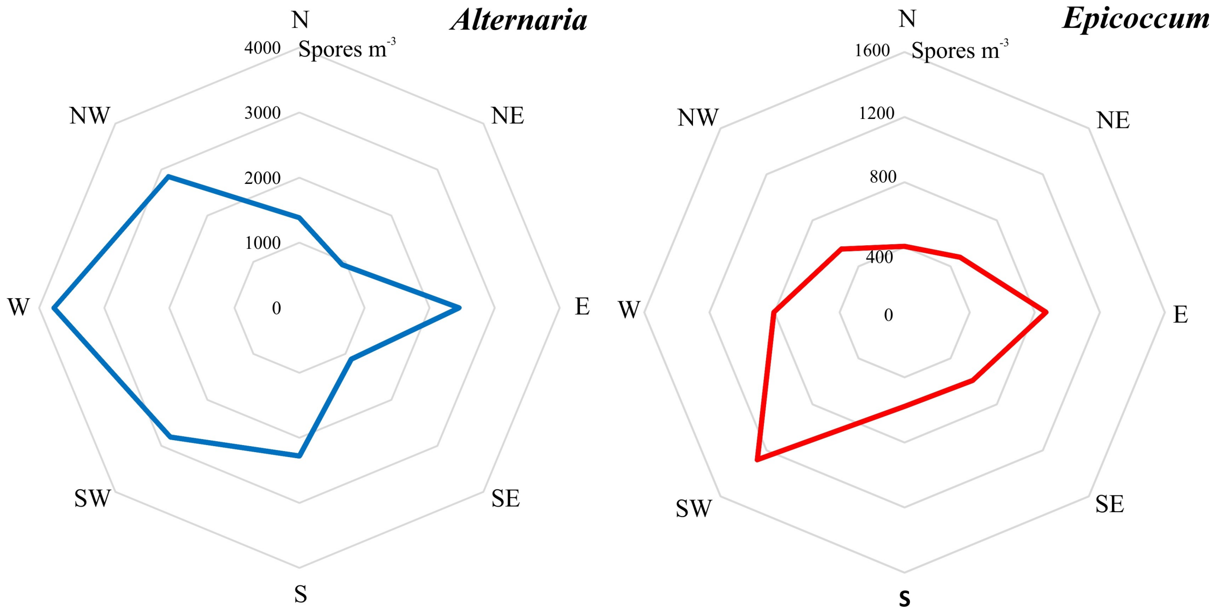

Figure 3 The concentrations of *Alternaria* and *Epicoccum* in the air relative to wind direction.

multivariable logistic regression parameters for relative humidity and sunshine duration were 1.60 and 0.20, respectively. These analyses were statistically insignificant. The odds ratio shows that as the mean temperature rises to a value close to 1.3, the hazard of *Alternaria* spore concentration in the air increases (Table 3, Table 4).

The evaluation of the model based on the analysis of the likelihood ratio (chi-square) test results showed a statistical significance. The area under the ROC curve analysis was above AUC = 0.77, which allowed us to conclude that the model fitted well to the observed data. The result of the Hosmer–Lemeshow test was 9.22 and was statistically non-significant. In the case of the Hosmer–Lemeshow test, a non-significant result indicates similarity between observed frequencies and predicted probabilities (Table S1).

4. Discussion

Fungal spores are a common component of aeroplankton, but the qualitative spectrum and spore concentration in the air vary widely in geographical regions (Bousquet et al., 2007; Martinez-Bracero et al., 2020). In addition, meteorological parameters strongly influence the concentration. The study of the occurrence and season of airborne spores has great practical importance. This study is especially important in occupational medicine because over 80 taxa of fungal spores, including *Alternaria* and *Epicoccum* airborne spores, are known as allergenic and induce type I allergies in sensitive patients (Sánchez et al., 2022; Simon-Nobbe et al., 2007).

Fungal spores are almost always present in the air, which creates dangerous situations for sensitive people working in the agricultural sector, mainly outdoors. Fungal spores, as well as mycelia, pose an additional health risk for humans because they contain mycotoxins of different types (Golec et al., 2004; Góra et al., 2004; Zalewska et al., 2023). The respiratory symptoms among workers also depend on the hours spent working in buildings infected by fungi, around magazines, grain storages, and animal housings (Crook, 1994). In vineyards, a serious risk for hypersensitive workers comes from mycotoxins, components of fungal cell walls, pesticides against powdery mildew (*Erysiphe necator*), downy mildew (*Plasmopara viticola*), and grey mould (*Botrytis cinerea*) pathogens (Youakim, 2006).

Alternaria is considered a cosmopolitan fungus, with a common incidence of its spores in the air in almost all bioclimatic regions. In the air of the vineyard under study, *Alternaria* had a high concentration, although its seasonal occurrence was relatively short. The threshold value of *Alternaria* spores was quickly exceeded at the beginning

Table 3 Results of logistic regression for each parameter separately.

MODEL		Score	SE	−95% CI	+95% CI	Wald test	p	OR	−95% CI	+95% CI
ALTERNARIA	Intercept	−4.19896	0.730694	−5.631098	−2.76683	33.02273	**<0.000001**	0.015011	0.003585	0.062861
	T_{mean}	0.237353	0.042599	0.153861	0.320845	31.04524	**<0.000001**	1.267888	1.166329	1.378292
	Intercept	2.141973	1.280618	−0.367991	4.651938	2.797623	0.094404	8.516228	0.692123	104.7879
	H_{mean}	−0.03339	0.016878	−0.066474	−0.00031	3.914636	**0.047867**	0.967158	0.935688	0.999686
	Intercept	−0.3211	0.160411	−0.635497	−0.0067	4.006904	**0.045314**	0.725352	0.529672	0.993323
	Rainfall	−0.03817	0.038495	−0.113617	0.03728	0.983115	0.321431	0.962551	0.8926	1.037984
	Intercept	−1.1989	0.30691	−1.800432	−0.59737	15.25956	**0.000094**	0.301526	0.165228	0.550259
	Sunshine duration	0.000032	0.00001	0.000012	0.000051	10.14947	**0.001443**	1.000032	1.000012	1.000051
EPICOCCUM	Intercept	−1.11429	0.477572	−2.050315	−0.17827	5.444031	**0.019635**	0.328148	0.128694	0.836718
	T_{mean}	0.07444	0.029375	0.016867	0.132014	6.422002	**0.011272**	1.077281	1.01701	1.141124
	Intercept	0.675896	1.230109	−1.735074	3.086866	0.301906	0.58269	1.965793	0.176387	21.90831
	H_{mean}	−0.00848	0.016092	−0.04002	0.023061	0.277633	0.598257	0.991557	0.96077	1.023329
	Intercept	0.090577	0.157277	−0.21768	0.398834	0.331669	0.564678	1.094806	0.804383	1.490086
	Rainfall	−0.03407	0.033963	−0.100637	0.032496	1.006327	0.315784	0.966504	0.904262	1.03303
	Intercept	−0.1905	0.270923	−0.721497	0.340501	0.494411	0.481966	0.826548	0.486024	1.405652
	Sunshine duration	0.000009	0.000009	−0.000009	0.000027	0.961856	0.32672	1.000009	0.999991	1.000027

SE – standard error; CI – confidence interval; p – error probability; OR - odds ratio – informs about the chance of changing the spores concentration with changes in the values of meteorological parameters.

Table 4 Evaluation of the optimal logistic regression model parameters for *Alternaria* spores.

MODEL	Score	SE	−95% CI	+95% CI	Wald test	p	OR	−95% CI	+95% CI
Intercept	−7.73819	2.89218	−13.406754	−2.06962	7.158592	**0.007461**	0.000436	0.000002	0.126234
T_{mean}	0.258663	0.049746	0.161163	0.356163	27.03662	**<0.000001**	1.295197	1.174876	1.427841
H_{mean}	0.039331	0.031052	−0.021529	0.100192	1.604364	0.205286	1.040115	0.978701	1.105383
Sunshine duration	0.000008	0.000019	−0.000028	0.000045	0.202467	0.652737	1.000008	0.999972	1.000045

SE – standard error; CI – confidence interval; p – error probability; OR – odds ratio.

of May, and the highest concentrations were observed in July and August. It can be concluded that, in the summer, vineyard workers may be exposed to the greatest risk of aeroallergic hazards from *Alternaria*. Studies conducted by other authors also indicate that allergenic *Alternaria* spores are an important component of the air of vineyards. In North-Western Spanish vineyards, the fungal season is irregular and reaches high concentrations from May to the end of August, and the concentrations exceed the risk for sensitive people (Diaz et al., 1998). *Alternaria* spores are also common in Italian vineyards (Magyar et al., 2009). Lee and Liao (2014) pointed out yet another risk of this fungus, which is its production of small particles (<1 μm) that can enter the lungs. *Alternaria* spores can also be dangerous in the production of wine. It was isolated from handmade musts (Fredj et al., 2007).

The study conducted by Katotomichelakis et al. (2015) showed that *Alternaria* is the most prevalent with regard to the sensitization rate, and the allergy symptom score was significantly correlated with its spore concentration. Furthermore, we showed that the mean temperature, sunshine duration, and relative humidity affected the concentration of *Alternaria* in the air. Other authors have also investigated the positive correlation between the air temperature and the *Alternaria* spore concentration (Almeida et al., 2018; Grinn-Gofroń et al., 2015, 2019; Vélez-Pereira et al., 2023). Additionally, Willocquet & Clerjeau (1998) proved that spores can be dispersed at high concentrations on sunny days following a rainy period. Our multivariable model showed that the mean temperature was the most important parameter. The risk of the rise of allergenic *Alternaria* spores increases when the temperature increases to a growth value of 1.26. However, in the one-way model, the odds ratio for solar radiation exceeded the value of 1 and for relative humidity was close to the value of 1. We also proved that solar radiation and relative humidity have an important influence on the appearance of allergy symptoms in vineyard workers expressed as a concentration above the threshold value. Due to climate warming, *Alternaria* spores may become a problem that needs occupational medicine in the winery sector.

Airborne *Epicoccum* spores are not a frequent subject of research. The positive reaction to *Epicoccum* allergens in patients has been described (Bisht et al., 2002; Kukreja et al., 2008; Portnoy et al., 1987), but the threshold value is still not specified and its hazard towards vineyard workers is not understood. In the vineyard "*Winnica Łany*", the rise in the spore concentration was mainly dependent on the growth temperature. Warm and sunny days favoured sporulation and deposition of *Epicoccum* spores. Southeast wind can also transport spores to the vineyard. Grinn-Gofroń et al. (2015) also indicated that the wind velocity and temperature determined the spore season. Furthermore, transportation of spores by the wind in large open agricultural areas can be a threat not only to vineyard workers but also to people living in the neighbourhood (Amigot Laźaro et al., 2000). The *Epicoccum* spore season was very long, lasting around five months. This means that the hazard of exposure to allergenic *Epicoccum* spores began just at the beginning of the agricultural season. However, vineyard workers were exposed to the highest concentration of *Epicoccum* spores in late summer and mainly in autumn. At this time, intensive harvest work is conducted (Olsen et al., 2019). The concentrations of fungal spores increased when some agricultural activity was being undertaken by workers and were positively correlated with some weather conditions. For example, in the harvest period, the concentrations of *Alternaria* and *Epicoccum* spores are very high during sunny days. Therefore, the potential hazard of exposure of workers to fungal aeroallergens can significantly increase.

As in "*Winnica Łany*", Martinez-Bracero et al. (2020) noticed allergenic *Plasmopara, Erysiphe* and *Botrytis* spores in vineyards located in different Spanish bioclimatic regions. The dates of spore occurrence and its intensity depended on the type of climate: temperate and Mediterranean or continental and maritime. The fungi mentioned above are well known as allergenic to 28% of people with atopy (Simon-Nobbe et al., 2007). According to Martinez-Bracero et al. (2020), the highest concentrations of *Botrytis* occurred at the end of May, i.e. before the threatening sensitive concentrations of *Alternaria* and *Epicoccum* spores occurred. Theoretically, the risk period for vineyard workers is very long. At this time, the pollen of herbaceous plants (grasses, mugwort, sorrel, and plantain) is an additional threat, as evidenced by the analysis of pollen calendars (Kasprzyk, 2008).

5. Conclusions

It is of great importance to recognise that agricultural workers have a health risk associated with their occupation. They can develop allergic diseases, including occupational asthma. This can arise from the very high concentration of the most allergenic fungal spores like *Alternaria* as well as their co-occurrence with other aeroallergenic fungal spores and plant pollen in the air. The temperature of the air may be the main factor responsible for an increase in airborne *Alternaria* and *Epicoccum* spores and, as shown many authors, pollen of plants like grass and mugwort. This can be a potential threat for people working in vineyards in a temperate climate. Therefore, research in this area should be deepened. It is important to record allergy symptoms among vineyard workers and compare them with airborne spore concentrations to predict allergy risk periods. Winery industry workers should be updated with information regarding occupational health hazards and about the possibilities of limiting contact with aeroallergens (e.g. using masks). It is crucial to take into account the cross-reaction among fungal allergens and their co-occurrence with other aeroallergens. Research to determine the threshold concentration for *Epicoccum* is also necessary.

6. Supplementary material

The following supplementary material is available for this article:

Table S1. Evaluation of the model for *Alternaria* spores.

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