BIOLOGICAL RESEARCH

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THE INFLUENCE OF AGRICULTURAL LAND ON THE LEVEL OF AIRBORNE ALTERNARIA SPORES

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Aim *of the research was to investigate the impact of agricultural activity on the concentration of Alternaria spores.*

Materials and methods. *The study was carried out at the Department of Medical Biology, Parasitology and Genetics of the ZSMPhU. Samples were collected using a 7-day volumetric sampler of the Hirst type, using the volumetric method. Samples were identified under a light microscope, and spore identification and counting were limited to genus levels. The relationship between seasonal Alternaria spore levels and harvest rates was analysed using Pearson's correlation method. The effect of meteorological conditions and agricultural activity on the daily concentration of Alternaria was analysed using stepwise correlation based on logarithmically transformed daily average spore counts. Classical leave-one-out cross-validation (LOOCV) was used to estimate the mean square error (MSE), associated with this model and Bayesian information criterion (BIC) was used to assess its accuracy.*

Results. *Seasonal characteristics of Alternaria spores and agricultural activity in Zaporizhzhia and Dnipro regions were analysed. The connection of some seasonal and daily indicators with harvesting rates and meteorological conditions was determined. Two models with 5 and 9 parameters were found that best explain the dynamics of Alternaria spores.*

Conclusions. *The most significant parameters positively correlated with Alternaria spore levels were temperature, pressure, westerly wind and wheat yield; relative humidity was negatively correlated.*

Keywords: *aeromonitoring, airborne fungal spores, Alternaria, agricultural activity and spore level, crops, LOOCV, BIC*

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1. Introduction

Long-term aerobiological monitoring in Europe and worldwide has shown that fungi of the genus Alternaria are widespread. According to reports from two air monitoring stations in Vinnytsia [1] and Zaporizhzhya [2], the fungus Alternaria is predominant in the air of Ukraine.

At concentration of 100 spores per m3, Alternaria fungal spores are aeroallergenic and may cause allergy symptoms [3]. According to some authors, Alternaria spores cause to allergic rhinitis, rhinosinusitis, and asthma [4]. Additionally, high concentration of Alternaria spores during summer thunderstorms is associated with an increase in the number of hospitalisations of people with asthma [5]. Alternaria fungi are known to inoculate various field crops and negatively affect the quantity and quality of the crop, indicating their role in agriculture. Cereal crops including barley, corn, wheat [6], rapeseed [7], and potatoes [8] are susceptible to infection by Alternaria fungi. From the above, it can be seen that fungi belonging to the Alternaria genus are of great clinical and economic value, thus generating interest among scientists worldwide.

The concentration of fungal spores can be affected by the species composition of the nearby forests, plants, and agricultural land. Apangu et al. [9] conducted a study on the effect of grain harvesting on the peak concentration of Alternaria using remote sensing and an atmospheric transport and dispersion model, integrated with Sentinel-2 satellite imagery. The study revealed a connection between the intensive harvesting of agricultural land and a considerable rise in the concentration of Alternaria spores in the atmosphere of Northern and Central Europe. The main sources of peak concentration of Alternaria spores were cereal crops, as noted by the authors. According to the authors, remote sources may have also contributed to the peak concentration of Alternaria spores. Pastures, hayfields and green urban areas also contributed to peak concentration of Alternaria spores, yet to a relatively lesser extent, as the authors note. The concentration of Alternaria in two localities, León and Villadolid in Spain, was investigated by Fernández et al. [10]. The results indicate significant differences in spatial patterns between the two nearby regions. The high amount of spores, detected in Valladolid, can be attributed to the dominant winds blowing from cereal-covered areas during the harvesting season. Similar research was carried out by Grinn-Gofroń et al. [11] in Turkey. According to their observations, pastures and agricultural lands, particularly those situated near the trap, are the primary potential sources of fungal spores.

Therefore, the composition of airborne fungal spores and the timing of their sporulation season are determined by local factors, especially the vegetation and land use in a particular area or nearby regions. This is because spores can be transported over distances. Therefore, **the aim of the research** was to investigate the impact of agricultural activity on the concentration of Alternaria spores.

2. Materials and methods

The results of investigation are based on aerobiological monitoring, performed in the Aerobiology Laboratory at Zaporizhzhia State Medical and Pharmaceutical University from 2016 to 2020for eight months (from March 1 to October 31). The sampling was carried out by the volumetric method, using a 7-day volumetric sampler of the Hirst type. The trap was set on the roof of the educational building No. 3 of Zaporizhzhia State Medical and Pharmaceutical University at a height of 30 metres above the ground level and calibrated to collect air samples at a rate of 10 l/min. The sampler drum was changed weekly. The adhesive tape was cut into 7 strips, each of which represented a specific day of the week. Before the exposure, the slides were coated with a glycerin-fuchsin mixture. The samples were examined under a light microscope at 400× magnification. The spore identification and counting were limited to genus levels. The final number of spores was expressed as the number of spores per cubic meter.

The meteorological data were obtained from the weather station, located at Zaporizhzhia airport. The weather station measurements included the following parameters: average daily temperature, pressure, relative humidity, wind speed, wind direction, weather phenomena, cumulative rainfall (mm), cloud classification (cumulus, cumulonimbus or stratocumulus, stratus, nimbostratus, altocumulus or altostratus, cirrus or cirrocumulus or cirrostratus). A number of other indicators, such as temperature, dew point, horizontal visibility, minimum and maximum temperature, etc. were tested separately and only those indicators with the lowest test error were included in the analysis. The VIF (variance inflation factor) was used to assess positive multicollinearity. For our data, all factors are low or moderate.

The influence of meteorological factors on daily Alternaria concentrations was analysed using stepwise correlation on log-transformed daily mean spore data. Classical leave-one-out cross-validation (LOOCV) and Bayesian information criterion (BIC) were used to estimate the mean squared error (MSE), associated with a given model to assess its accuracy. The connection between seasonal indicators of Alternaria and annual meteorological conditions was analysed using the Pearson correlation method. Seasonality was analysed using the indicators: total number of spores, beginning, end and duration of the season, average day, peak value, number of days with more than 100 spores/m^3.

Zaporizhzhia city borders on Dnipro region to the north and northwest, so the indicators of both regions were used for the analysis. Data on agricultural activity in the two regions were obtained from open sources, including weekly data from the Department of Agricultural Development (https://www.zoda.gov.ua/) and the State Statistics Service of Ukraine (https://ukrstat.gov.ua/).

The five most common crops were analysed:

– Winter: barley, wheat, rapeseed

– Spring: sunflower and maize.

All of them are a potential environment for the formation of Alternaria fungi. The productivity of land (yield), start and end of harvest, average and peak day are highlighted. Daily data reflect the total area, harvested in thousands of hectares.

3. Research results

The main characteristics of seasonality are presentedinTable 1 and include:

– Total number of spores (ALTE_sum,) calculated as the sum of daily spore concentration during the observation period;

– start (ALTE_ssn_start), duration (ALTE_ssn_len), end (ALTE_ssn_end) of the season, determined using the 90 % method (Nilsson and Persson 1981) [12];

– day with the maximum value (ALTE_peak_date) and peak value (ALTE_peak_val,) in spores/m3;

– average day – 50th percentile (ALTE_ssn_mean);

– number of days exceeding 100 spores/m3 (ALTE_above100)

Table 1

Seasonal characteristics of airborne Alternaria during 2016–2020

Year	ALTE_sum, spor/m^3	ALTE_peak_val, spor/m^3	ALTE_above100	ALTE_peak_date	ALTE_ssn_start	ALTE_ssn_mean	ALTE_ssn_end	ALTE_ssn_len
2016	23880	1356	75	2016-07-04	2016-05-28	2016-07-16	2016-10-01	126
2017	15542	1918	43	2017-07-06	2017-06-05	2017-07-30	2017-10-01	118
2018	14174	415	49	2018-07-27	2018-05-27	2018-07-31	2018-10-15	141
2019	24127	964	91	2019-07-05	2019-05-26	2019-07-27	2019-10-02	129
2020	14727	359	51	2020-06-14	2020-05-29	2020-07-29	2020-10-24	148

The highest total number of Alternaria spores per season was recorded in 2016 and 2019 (23880 and 24127 spores/m3, respectively). It should be noted, that in these years the peak values of the daily concentration of Alternaria were recorded on the same day and only one day later in 2017. The dates of other seasonality indicators were also often similar. The peak values and the average day were always in July. A similar seasonal behaviour of Alternaria spores was observed in the studies of Grinn-Gofroń et al. [11], where peak concentration occurred in summer (July-August), and in the studies of Sánchez Reyes et al. [13], where monthly and daily peaks were also recorded in summer.

Zaporizhzhia city borders on Dnipro region in the north and northwest. According to Fig. 1, high levels of Alternaria spores were detected in Zaporizhzhia during periods of prevailing winds from Dnipro region. Consequently, the agricultural activity in both Zaporizhzhia and its neighbouring region was investigated.

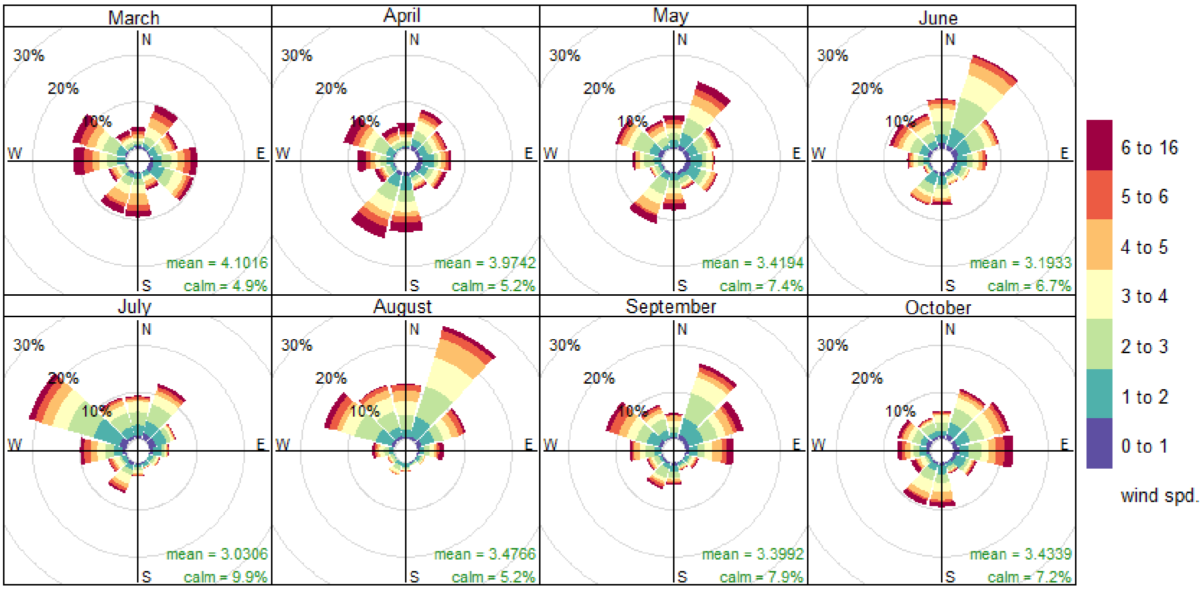

Fig. 1. Polar charts that show the direction and strength of the wind

Zaporizhzhia and Dnipro have the total area of 27183 km² and 31923 km², respectively. Farmland covers 62 % of the total area with 17079 km² and 19808 km² farmland in Zaporizhzhia and Dnipro, respectively. The distribution of crops in Zaporizhzhia and Dnipro regions appears to be somewhat similar, with the exception of maize, which is predominantly grown in Dnipro, as shown in Fig. 2.

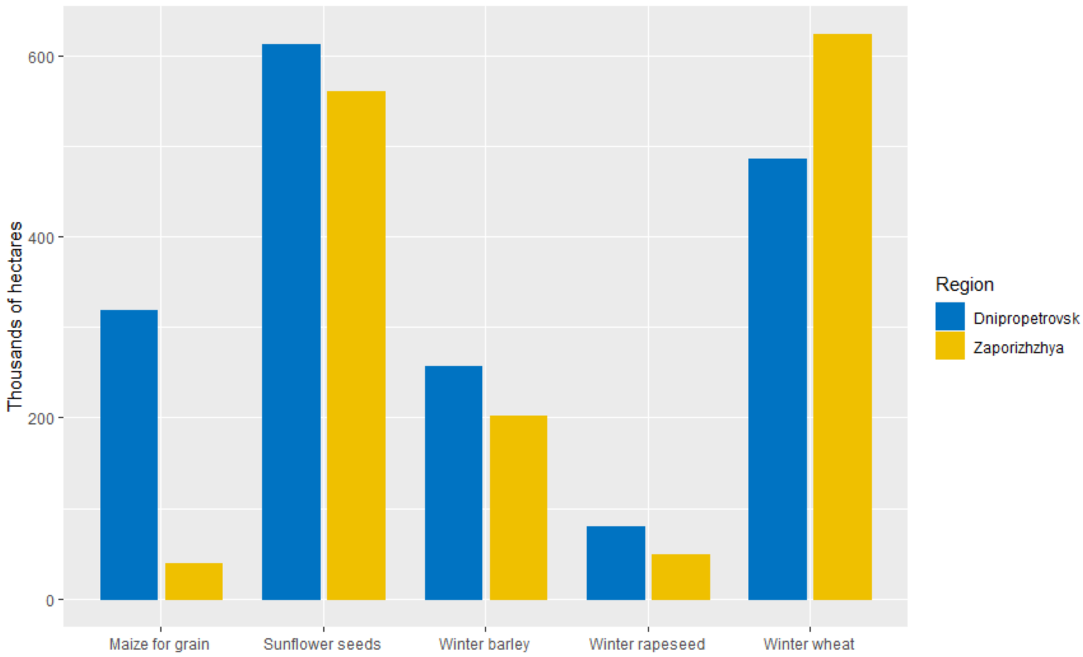

Fig. 2. Total harvested area in Zaporizhzhia and Dnipro region

Comparative trends in yield can be observed in Fig. 3. The year 2018 had the lowest yield, whereas 2019 had the highest yield. In those years, the lowest and highest number of spores per season were recorded, respectively.

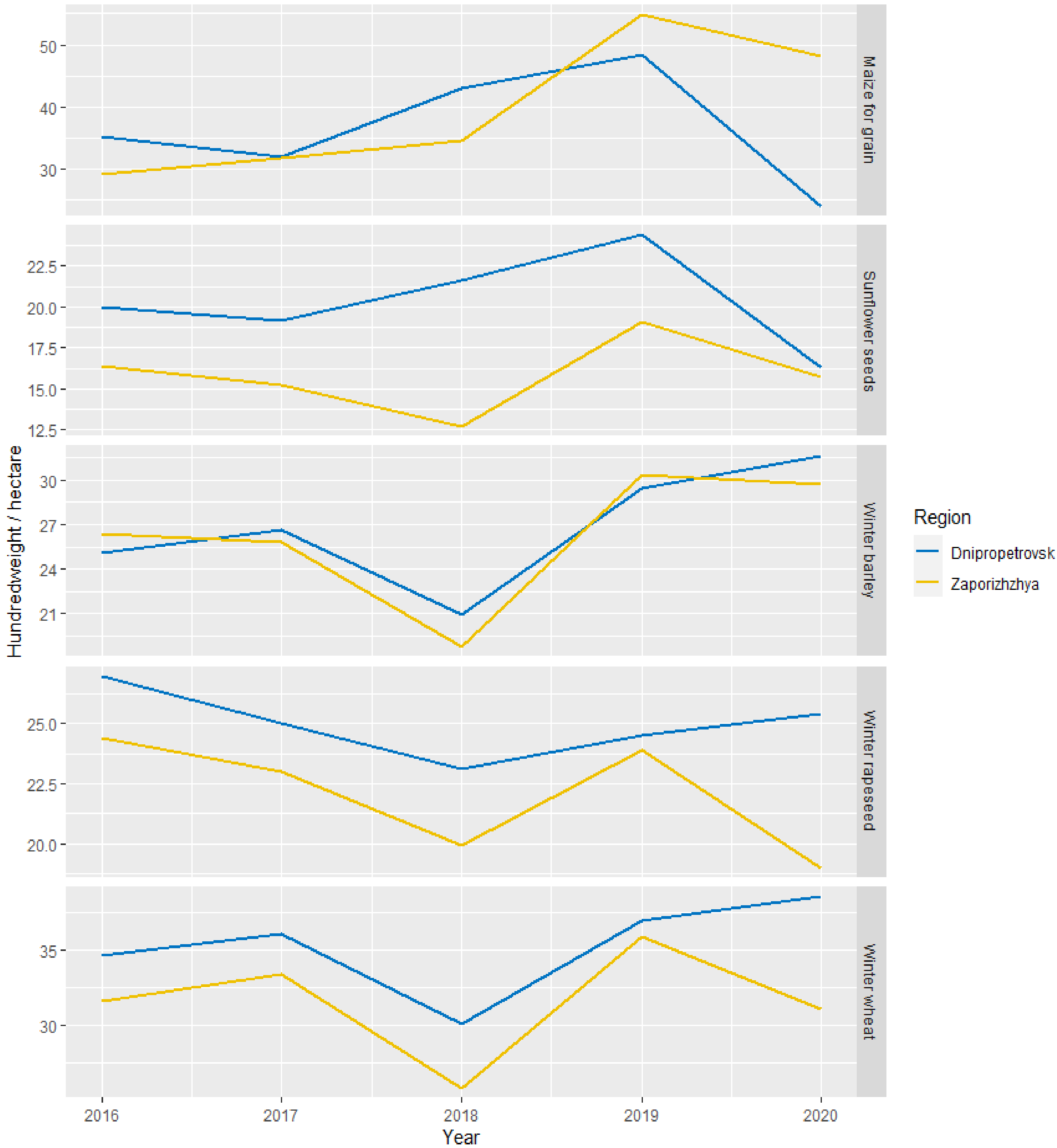

Fig. 3. Annual yields in Zaporizhzhia and Dnipro regions

Fig. 4 depicts the harvest data for the Zaporizhzhia and Dnipro regions and the log-transformed daily mean Alternaria data. It is evident from the figure, that high concentration of Alternaria overlap with the harvest period, suggesting that agricultural practices potentially influence spore levels. Table 2 shows a correlation between seasonal parameters of Alternaria and annual agricultural activity.

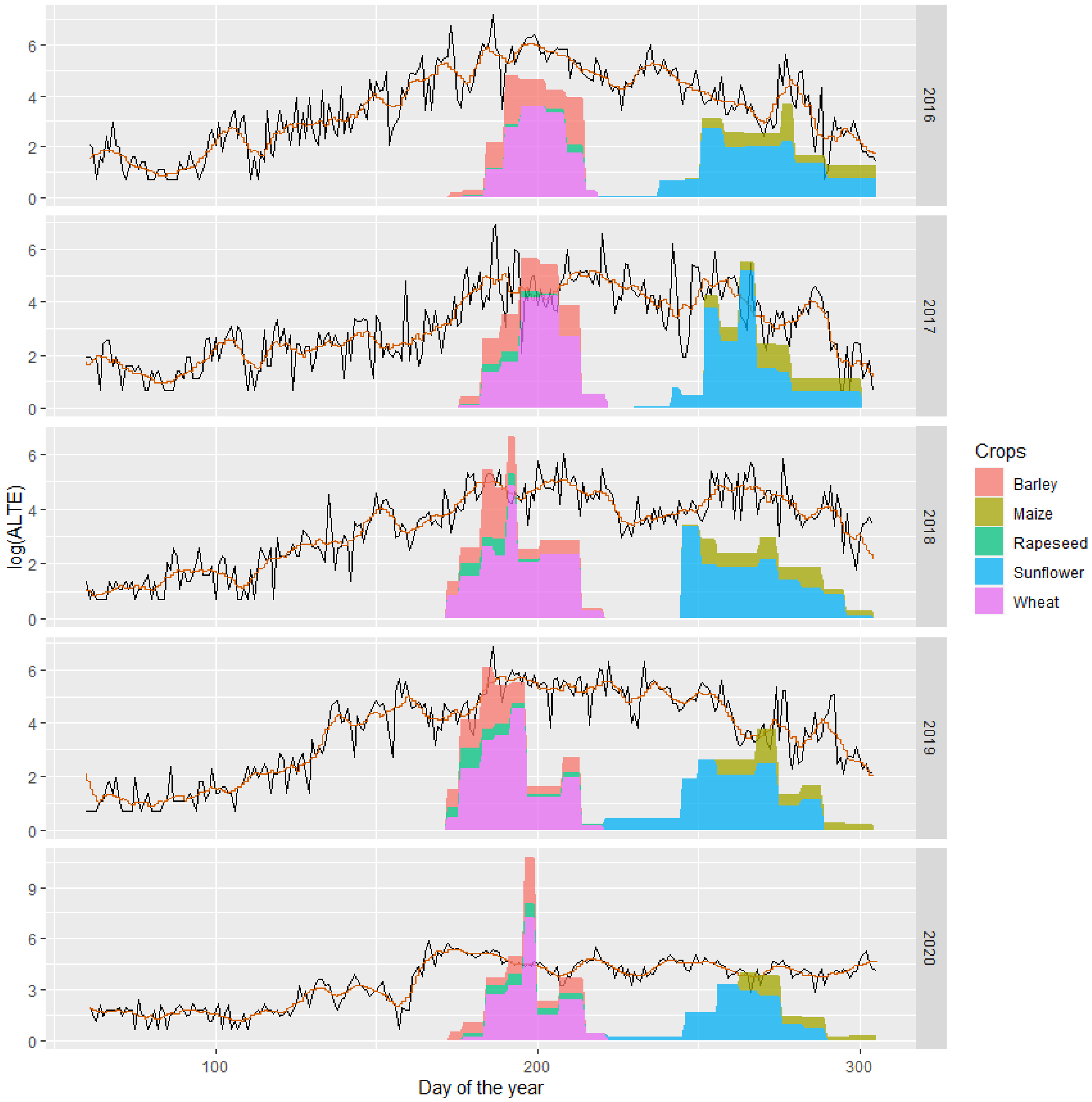

Fig. 4. Logarithmic daily average Alternaria data, moving average of order 15 and harvest data in Zaporizhzhia and Dnipro region

Table 2

Pearson's correlation coefficient between seasonal parameters of Alternaria and yield indicators

Seasonality parameters	Yield indicators	Correlation coefficient
ALTE_ssn_start	wheat_start_zp	0.8360566
ALTE_ssn_start	barley_start_zp	0.8549672
ALTE_ssn_start	rapeseed_start_zp	0.9235181
ALTE_sum	yield_sunflower_zp	0.8016962
ALTE_sum	yield_rapeseed_zp	0.8324114
ALTE_above100	rapeseed_start_zp	–0.7129644
ALTE_above100	yield_sunflower_zp	0.8325328
ALTE_above100	sunflower_start_zp	–0.8585362

However, five years of data are insufficient to obtain statistically significant results. The multiple linear regression model, obtained by regressing Alteranaria onto the full set of parameters, is shown in Table 3.

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Table 3

The multiple linear regression model with full set of predictors

Coefficients:		Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)		–25.516192	9.801464	–2.940	0.00340**
Temperature		0.107833	0.012184	8.850	<2e–16 ***
Pressure		0.042085	0.012861	3.272	0.00112 **
Humidity		–0.072771	0.014860	–4.897	1.23e–06 ***
Wind direction	E	0.097283	0.057821	1.682	0.09297
	N	0.015102	0.040564	0.372	0.70980
	N.E	–0.035865	0.044503	–0.806	0.42061
	N.W	0.012554	0.043395	0.289	0.77245
	S	0.042505	0.058512	0.726	0.46784
	S.E	–0.276256	0.112563	–2.454	0.01438 *
	S.W	–0.027472	0.078284	–0.351	0.72576
	W	0.105820	0.043983	2.406	0.01641 *
Types of clouds	Cumulus	–0.008282	0.005329	–1.554	0.12062
	Cumnb_Strcum	–0.003720	0.003093	–1.203	0.22952
	Stratus	0.002068	0.012689	0.163	0.87057
	Nimbostrat	–0.021707	0.146342	–0.148	0.88213
	Alto	–0.001269	0.002641	–0.480	0.63110
	Cirro	–0.005146	0.003324	–1.548	0.12209
Rain_shower		–0.021136	0.023430	–0.902	0.36733
Fog		–0.013984	0.029653	–0.472	0.63737
Thunder storm		0.00350	0.028878	0.121	0.90336
Rain		–0.091416	0.078379	–1.166	0.24392
Precipitation		0.010828	0.040268	0.269	0.78809
Wheat_delt		0.009120	0.004165	2.190	0.02890 *
Maize_delt		–0.008611	0.014915	–0.577	0.56390
Barley_delt		0.027250	0.010137	2.688	0.00737 **
Rapeseed_delt		–0.038832	0.024212	–1.604	0.10925
Sunflower_delt		0.007365	0.003835	1.921	0.05522

*Note: *0.05≥p>0.01; **0.01≥p>0.001; ***p≤0.001*

The F-statistic 8.3 ($p<0.05$) suggests that at least one of the factors presented must be related to daily spore concentration. The LOOCV MSE of the model is 0.73, R-squared 0.26. Stepwise regression data (an automatic procedure for selecting significant variables) was obtained using the R software environment. To compare the models, we use the Bayesian Information Criterion (BIC), which provides an approximate estimate of the model's test error (Fig. 5).

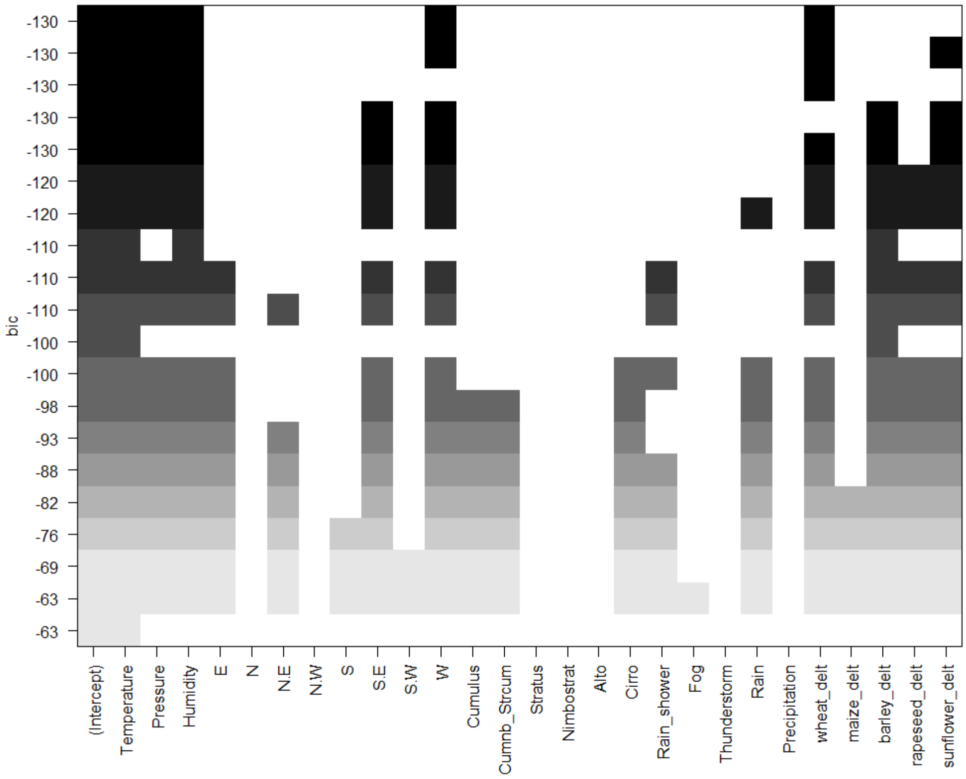

Fig. 5. Stepwise regression results

It can be seen, that the indicators of agricultural activity (on the right) are often important factors. Fig. 6 shows how the error estimate changes for models with different numbers of variables. BIC is used on the left and LOOCVMSE on the right. The minimum error is achieved with 5 and 9 parameters – these models are presented in Tables 4 and 5.

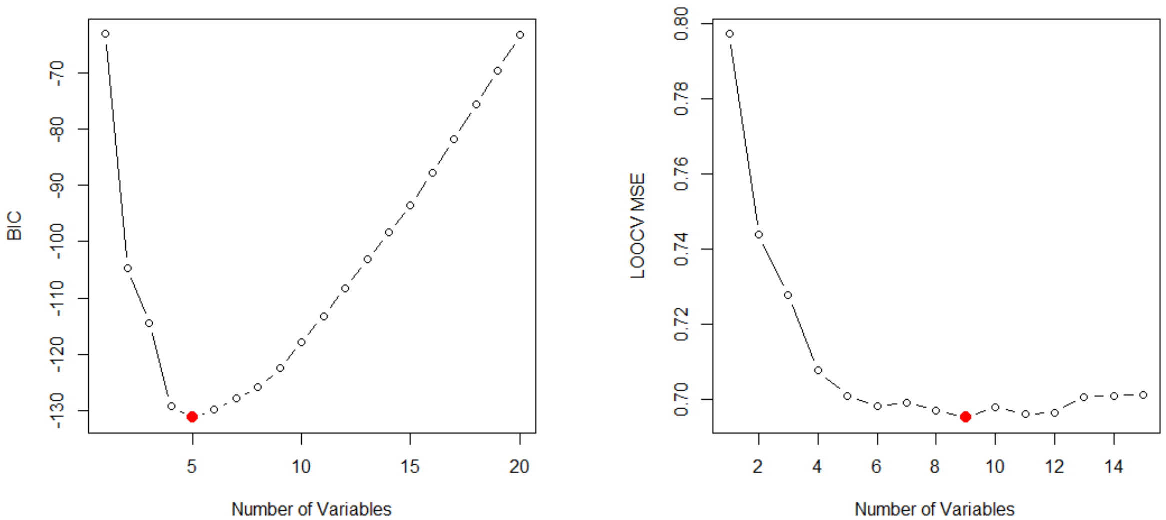

Fig. 6. MSE changes with the number of parameters

Table 4

The best linear model with 5 parameters, selected by stepwise regression

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	–40.337206	7.670206	–5.259	1.96e–07 ***
Temperature	0.108207	0.010099	10.715	< 2e–16 ***
Pressure	0.057063	0.010109	5.645	2.46e–08 ***
Humidity	–0.059932	0.010411	–5.757	1.31e–08 ***
W	0.106142	0.036219	2.931	0.0035 **
wheat_delt	0.014337	0.002247	6.380	3.34e–10 ***

*Note: *0.05≥p>0.01; **0.01≥p>0.001; ***p≤0.001*

Table 5

The best linear model with 9 parameters, selected by stepwise regression

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	–35.667067	7.697010	–4.634	4.33e–06 ***
Temperature	0.114710	0.010760	10.661	<2e–16 ***
Pressure	0.050674	0.010149	4.993	7.63e–07 ***
Humidity	–0.064687	0.010661	–6.068	2.19e–09 ***
S.E	–0.229842	0.104966	–2.190	0.02890 *
W	0.092356	0.036422	2.536	0.01145 *
Wheat_delt	0.009761	0.004009	2.435	0.01518 *
Barley_delt	0.026074	0.009873	2.641	0.00846 **
Rapeseed_delt	–0.041511	0.023501	–1.766	0.07780
Sunflower_delt	0.007512	0.003040	2.472	0.01371 *

*Note: *0.05≥p>0.01; **0.01≥p>0.001; ***p≤0.001*

We can see that the 5 parameter model is not very different from the 9 parameter model. The second model includes four yield indicators, most of which are positively correlated with spore concentration, indicating a weak connection.

Similar patterns are reported in the literature by scientists from around the world. According to the investigation, conducted by Sánchez Reyes et al. [13], most of the Alternaria spores were collected during the summer harvest, and a positive correlation with temperature and a negative correlation with relative humidity were observed. Moreover, wind direction has a favourable impact on Alternaria spore concentration. The research, carried out by Skjøth et al. [14], validates the conjecture that the primary source of Alternaria spores in Denmark is agricultural areas. The study's results demonstrate that a considerable amount of fungal spores were formed during harvesting, despite the application of fungicides in the fields. Mitakakis et al. [15] suggested the influence of precipitation, temperature, and wind direction on spore concentration, along with the impact of crop maturation. Treated crops release spores into the air, which can be carried to other locations by winds. The concentration of fungal spores is reported to be greater during crop ripening and harvesting compared to when the crop is already ripe, as the authors' observations. The importance of local spore sources, as well as wind speed and direction, is emphasised in the research by Grinn-Gofrońetal. [11]. The research was carried out in some cities across Turkey, with varying climates and agricultural practices. In specific cities with low wind speeds, local spore sources were found to have a significant influence, whereas in other cities, low spore concentrations were linked to wind direction from areas with little vegetation, dominated by rocks and bare soil. The authors identified pastures, crop land, and coniferous forests as the primary potential sources of fungal spore sin the study area, based on a combination of wind conditions and land use data.

Limitations of the study. The limitation of the study is the presence of only one trap in the city, as well as the location of the weather station at a quite distance from it.

Prospects for further research are to apply more sophisticated nonlinear methods to obtain a better model.

4. Conclusions

According to the results of this study, the impact of agricultural activity on the level of fungal infection of the airborne Alternaria spores was analysed, considering meteorological conditions. Peak spore concentrations and high rates were observed in July, just as the harvest was taking place. The results of the stepwise regression using BIC and LOOCV MSE as model selection criteria revealed a positive relationship with relative humidity, southeast wind and rapeseed yield. And a positive relationship with temperature, pressure, westerly wind, wheat, barley and sunflower yields.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results, presented in this article.

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Data availability

Data will be made available on reasonable request.

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