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M.V. BOGACH[1*], A.P. PALIY[2], D.M. BOHACH[1], L.V. KOVALENKO[2],
N.V. SELISHCHEVA[1], L.O. GANOVA[3], B.T. STEGNIY[2], O.V. PAVLICHENKO[4], D.V. VOVK[2]

[1] Odesa Research Station of the National Scientific Center «Institute of Experimental and Clinical Veterinary Medicine», 2 Svobody Ave., Odesa, 65037, Ukraine
[2] National Scientific Center «Institute of Experimental and Clinical Veterinary Medicine», 83 Pushkinska Str., Kharkiv, 61023, Ukraine
[3] Zabolotny Institute of Microbiology and Virology, NAS of Ukraine, 154 Akademika Zabolotnogo Str., Kyiv, 03143, Ukraine
[4] State Biotechnological University, 44 Alchevskyh Str., Kharkiv, 61002, Ukraine
[*] Author for correspondence; e-mail: bogach_nv@ukr.net

INFLUENCE OF WEATHER CONDITIONS ON CONTAMINATION OF GRAIN FODDER BY MICROMYCETES IN THE NORTHWESTERN BLACK SEA REGION OF UKRAINE

The amount of precipitation and its seasonal distribution affect the contamination of grain fodder with micromycetes. The use of grain from different crops as animal feed and in the food industry raises concerns about its quality and adherence to sanitary and medical standards. The ***aim*** *of this study was to investigate the degree of micromycetes contamination in grain fodder (wheat, corn, barley) in the northwestern Black Sea region, taking into account weather conditions during 2017—2022.* ***Methods.*** *After harvesting, grain samples were taken and inoculated into Petri dishes containing potato dextrose agar to establish the total number of fungi and determine their species composition. The number of fungal spores in 1 g of feed was calculated using the method of serial dilutions. Micromycetes species were identified using classical methods based on cultural and morphological characteristics. Moisture conditions during the period of active vegetation, defined as having average daily temperatures above 10* °C, were assessed using the hydrothermal coefficient (HTC) of the *Selyaninov method. The HTC was not calculated for months without active air temperatures above 10* °C. ***Results.*** *In the northwestern Black Sea region of Ukraine, the moderate and dry period has increased from four to eight months, with an HTC of 0.4—0.8. The period of sufficient moisture (2018, 2021) with an HTC of 1.0—1.5 lasted five months. The amount of precipitation and its seasonal distribution in the northwestern Black Sea region af-*

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fects the contamination of grain fodder with micromycetes. During the years of severe drought (2017, 2022), wheat grain was primarily contaminated with Aspergillus fungi, while corn and barley were contaminated with Penicillium. In the years of moderate drought (2019, 2020), grain feed was most contaminated with Penicillium fungi, and during the period of sufficient moisture (2018, 2021), it was contaminated with Penicillium and Fusarium fungi. ***Conclusions.*** *The mycobiota of grain fodder in the northwestern Black Sea region of Ukraine is dependent on natural and climatic conditions. Filamentous fungi were the most commonly found in corn grain. During a period of severe drought, the grain was contaminated with fungi from the genera Penicillium (81%) and Aspergillus (78%). The other four fungal genera found were Fusarium (42%), Alternaria (31%), Mucor (16%), and Rhizopus (5%). In years with adequate moisture, the occurrence of Penicillium and Aspergillus fungi decreased by 17% and 14%, respectively, while the incidence of Fusarium fungi increased by 9%. During the period of sufficient moisture, wheat grain samples were primarily affected by fungi of the genera Penicillium (52%) and Aspergillus (45%). Fungi of the genera Rhizopus, Mucor, and Alternaria were isolated at a frequency ranging from 5% to 10%. In severe drought, barley and corn samples were primarily affected by fungi of the genera Penicillium (51%) and Aspergillus (42%). During the period of sufficient moisture, the frequency of isolation for Alternaria, Mucor, and Rhizopus fungi was 9%, 8%, and 4%, respectively.*

Keywords: *hydrothermal coefficient, wheat, corn, barley, Aspergillus, Fusarium, Penicillium.*

Micromycetes and their secondary metabolites, known as mycotoxins, are among the many environmental factors that can negatively impact the safety of feed raw materials and fodder. Their danger lies in their latent impact on animal health. The presence of microscopic fungi in feed can lead to a decrease in consumption due to a deterioration of organoleptic qualities, as well as a decrease in the absorption of nutrients and a disruption of metabolic processes in the body. Mycotoxigenic fungi can cause economic damage to the producer and spoil the crop. Additionally, they pose a serious threat to human and animal health due to contamination of commercial products with carcinogenic and neurotoxic secondary metabolites (Chalivendra et al., 2020; Tian et al., 2022). The state of animal health, biological integrity, and safety of livestock products significantly depends on the sanitary quality of feed, which also determines the degree of contamination with pathogenic microorganisms and toxic substances of both natural and human-made origin (Kolchyk et al., 2022; Stanley & Bajagai, 2022; Tadele et al., 2023).

During the growing season, harvesting, transportation, and storage, feeds can be affected by mold saprophytes, which can cause them to have a darker color and unpleasant odor. The development of microscopic fungi not only changes the physical properties of the feed but also leads to the decomposition of 281 organic compounds, resulting in the formation of toxic compounds that can cause poisoning in farm animals (Santos Pereira, 2019; Keriene et al., 2020; Adelusi et al., 2022).

Cereal crops are often affected by environmental stressors such as water and mineral availability, as well as bacterial diseases and microscopic fungi. In particular, wheat is susceptible to various diseases that can significantly reduce yields. Fusarium head blight is a major threat to wheat caused mainly by several members of the genus *Fusarium*. The severity of disease epidemics and the accumulation of related trichothecene mycotoxins in wheat grains are highly dependent on meteorological factors (Vujanovic et al., 2012; Vaughan et al., 2016).

Various factors, including geographical location, climate, relationships between microorganisms, and environmental conditions, play a crucial role in determining the diversity and distribution of different ecosystem representatives (Bellgard et al., 2011; Bogach et al., 2020). Additionally, microscopic fungi can cause plant diseases, with weather conditions being the primary contributing factor (Anderson et al., 2004; Ospanova et al., 2021).

Aspergillus flavus is an opportunistic pathogen that infects cereals and oilseeds. It is most commonly found in the southern region of Ukraine (Bogach et al., 2023). This fungus produces mycotoxins that can contaminate food and feed, and it has been reported in many countries (Munkvold et al., 2019). *Fusarium verticillioides* (formerly known as *F. moniliforme*) is the predominant contaminant of raw materials (Munkvold, 2003). The fungi *A. flavus* and *F. verticillioides* are responsible for ear rot in maize (*Zea mays* L.), a globally significant crop for both food and feed production. These fungi can also contaminate grain (Guo et al., 2017).

Aspergillus and *Fusarium* ear rot are more frequently observed in warm and dry years, or warm and humid conditions during harvesting, and due to damage caused by insect pests during storage (Santiago et al., 2015; Dong et al., 2018).

Stepanenko (2007) reported long dry and hot periods in the southern regions of Eastern Europe and the Mediterranean from 2005. The impact of global climate warming on economic sectors, including agriculture, which depends on climatic conditions, was significant (Mendelsohn, 2009; Gornall et al., 2010; Paliy et al., 2021).

The use of grain from various crops as animal feed and in the food industry raises questions about its quality and compliance with sanitary and medical conditions. From the moment it is in the field, during harvesting, transportation, and storage, grain is affected by various fungal pathogens, among which molds, as well as *Fusarium* and *Alternaria* pathogens, are the most common. All of them belong to the group of toxin-producing pathogens and pose a threat not only to grain storage but also to the food industry and livestock. Therefore, it is important and timely to investigate the causes of grain contamination in different crops and the possibility of preventing the spread of infection. The region where the research was conducted not only produces grain but also stores it for further transportation, making the research results even more relevant.

The study **aims** to investigate the degree of contamination of grain feed (wheat, corn, barley) with micromycetes, taking into account weather conditions in 2017—2022 in the north-western Black Sea region of Ukraine.

Materials and Methods. Toxin-forming fungi in grain crops were monitored from 2017 to 2022 in farms of various ownership forms in the Odesa Region of Ukraine. The monitoring was conducted at the Laboratory of Epizootology, Parasitology, Animal Disease Monitoring and Providing of the Odesa Research Station of the NSC «IECVM» and the Department of Toxicology, Safety and Quality of Agricultural Products of the NSC «IECVM». A total of 450 grain samples consisting of wheat, corn, and barley, were examined during the specified period. Each year, 25 samples of each grain crop were collected, with each sample weighing at least 2 kg. The samples were packed in linen bags after harvesting and stored until they were analyzed for mycological purposes in the laboratory.

All grain samples were tested by direct inoculation. To isolate the mycobiota, one hundred grains of wheat, corn, and barley were selected from each sample and disinfected in a 2% solution of active chlorine for one minute at room temperature. They were then rinsed twice for one minute with sterile distilled water, and the surface was dried before direct inoculation. Inoculation was performed in Petri dishes (90 mm diameter, 10 grains per plate) containing potato dextrose agar (CONDA, Pronadisa, Madrid, Spain) and incubated at 25 °C for seven days.

The pure cultures of micromecetes were identified according to cultural and morphological characteristics using modern guides for different genera of fungi (Domsch et al., 2007; Pitt & Hocking, 2009).

Data on precipitation and average air temperature were obtained from the Bolhrad Meteorological Station (Bolhrad, Odessa Region). The hydrothermal coefficient (HTC) according to the method of Selianinov (1937) was used to assess the humidity conditions of the period with average daily temperatures above 10 °C, i.e.

the period of active vegetation. Since there are no active air temperatures above 10 °C in some months of the year, the HTC was not calculated.

The HTC indicator was calculated by dividing the amount of precipitation (ΣR) in mm for the period with temperatures above 10 °C by the sum of active temperatures (Σt > 10) for the same period, reduced by a factor of 10:

$$HTC = \frac{\Sigma R}{0.1 \cdot \Sigma t_{act>10}} \text{ or } HTC = \frac{\Sigma R \cdot 10}{\Sigma t_{act>10}}$$

if HTC < 0.4 — very severe drought,
HTC from 0.4 to 0.5 — severe drought,
HTC from 0.6 to 0.7 — moderate drought,
HTC from 0.8 to 0.9 — mild drought,
HTC from 1.0 to 1.5 — quite humid,
HTC > 1.5 — excessively humid.

All experiments were performed in triplicate. The results were processed by methods of variation statistics using Microsoft Excel for Windows 2010. To compare mean values, Student's *t*-test was used (Van Emden, 2019).

Results. According to the agroclimatic zones of Ukraine, the territory of the southern districts of the Odesa Region (Bessarabia) belongs to the Southern Steppe with a very dry and hot climate (Adamenko, 2014). Atmospheric precipitation is one of the most unstable elements of the climate and, at the same time, its amount and seasonal distribution in the south of Ukraine affects the contamination of grain feed with micromycetes.

The HTC indicator was used to assess the humidity conditions of the period with average daily temperatures above 10 °C. According to the Bolhrad Meteorological Station, in 2017 and 2022, the HTC for seven months ranged from 0.4 to 0.8, indicating severe to moderate drought. The data on hydrometeorological conditions indicating severe drought are shown in the example of 2022 (Table 1).

In 2019 and 2020, the average drought was recorded for five months, and for three months, the HTC was in the range of 1.0—1.1.

In the northwestern region of the Black Sea, there was sufficient humidity in 2018 and 2021. The HTC was 1.0—1.5 for five months, indicating sufficient humidity, and only three months of average drought were recorded. The data on hydrometeorological conditions indicating sufficient humidity in 2021 are shown in Table 2.

Table 1. **Characteristics of hydrometeorological conditions in the northwestern Black Sea region in 2022 (severe drought)**

Month	Precipitation, mm	Average air temperature, ˚C	Sum of active temperatures ($t_{act>10}$), ˚C	HTC
January	30.5	1.1	0.0	—
February	44.9	5.6	0.0	—
March	26.4	10.9	338	0.8
April	31.1	13.3	399	0.5
May	28.4	18.5	574	0.5
June	26.1	23.1	693	0.4
July	29.5	24.9	772	0.4
August	27.7	25.5	791	0.4
September	29.1	19.7	591	0.5
October	30.0	13.8	428	0.7
November	42.2	9.6	0.0	—
December	45.5	3.7	0.0	—

Table 2. **Characteristics of hydrometeorological conditions in the northwestern Black Sea region in 2021 (sufficiently humid)**

Month	Precipitation, mm	Average air temperature, ˚C	Sum of active temperatures ($t_{act>10}$), ˚C	HTC
January	20.1	0.9	0.0	—
February	33.0	2.4	0.0	—
March	42.4	11.6	360	1.2
April	51.5	13.5	405	1.3
May	68.1	20.4	632	1.1
June	46.3	22.9	687	0.7
July	49.9	26.7	828	0.6
August	48.7	25.5	791	0.6
September	52.4	21.3	639	0.8
October	58.8	13.6	422	1.4
November	46.2	10.2	306	1.5
December	53.2	2.9	0.0	—

Thus, in terms of weather conditions, the northwestern Black Sea region is characterized by severe to moderate drought, and the climate is sufficiently humid only once every four to five years (Fig. 1).

According to our research, natural and climatic conditions affect the level of contamination and the species affiliation of micromycetes in grain feed.

The highest contamination of wheat grains was determined for micromycetes of the genera *Penicillium* (from 39% to 54%), *Aspergillus* (from 38% to 52%) and *Fusarium* (from 28% to 37%), and for other fungi of the genera *Mucor, Rhizopus,* and *Alternari,* it ranged from 4% to 20% of the examined samples (Table 3).

According to the HTC, 2017 and 2022 were characterized by severe drought, while 2018 and 2021 — by sufficient humidity. In the years of severe drought, the highest number of wheat grains infected with fungi of the genus *Aspergillus* was recorded, which is 5% more than in the years with sufficient humidity (Fig. 2). Infection of wheat with *Penicillium* and *Fusarium* fungi was 16% and 7% higher, respectively, in wet years than in dry years.

The infection of corn grain by filamentous fungi was significantly higher than that of wheat and barley (Table 4).

Fungi of the genus *Aspergillus* were isolated from 60—81% of the corn samples. Almost similar figures were obtained for fungi of the genus *Penicillium* (from 66% to 82%). In comparison

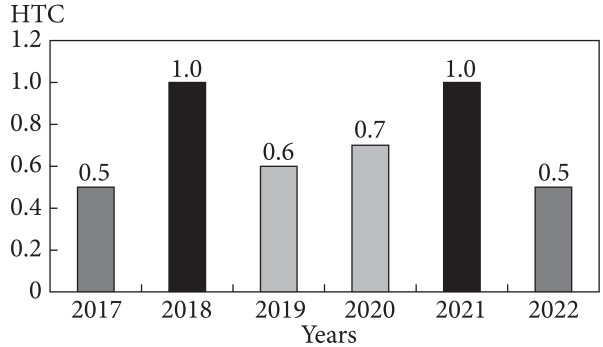

Fig. 1. Parameters of hydrothermal coefficients from 2017 to 2022 in the northwestern Black Sea region

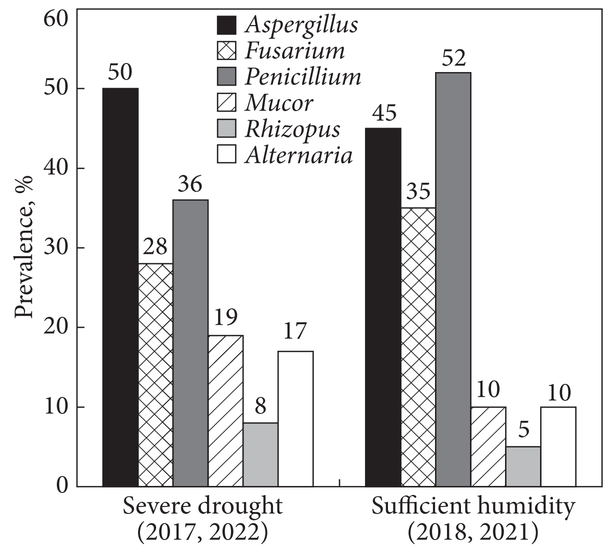

Fig. 2. The average rate of wheat grain infection by fungi of different genera in years of severe drought and sufficient humidity

Table 3. **Taxonomic structure of wheat grain mycobiota studied in 2017—2022**

Genus of fungi	Years of research / Percentage of samples affected by fungi					
	2017	2018	2019	2020	2021	2022
Aspergillus	48	45	40	38	46	52
Fusarium	29	34	30	32	37	28
Penicillium	39	54	49	45	50	34
Mucor	18	12	10	12	9	20
Rhizopus	7	6	4	5	5	9
Alternaria	19	11	9	8	10	16

Table 4. **Taxonomic structure of the corn grain mycobiota studied in 2017—2022**

Genus of fungi	Years of research / Percentage of samples affected by fungi					
	2017	2018	2019	2020	2021	2022
Aspergillus	75	60	68	70	62	81
Fusarium	43	50	51	49	52	42
Penicillium	80	68	70	72	66	82
Mucor	17	24	19	20	21	15
Rhizopus	6	10	7	7	8	5
Alternaria	32	22	26	28	24	31

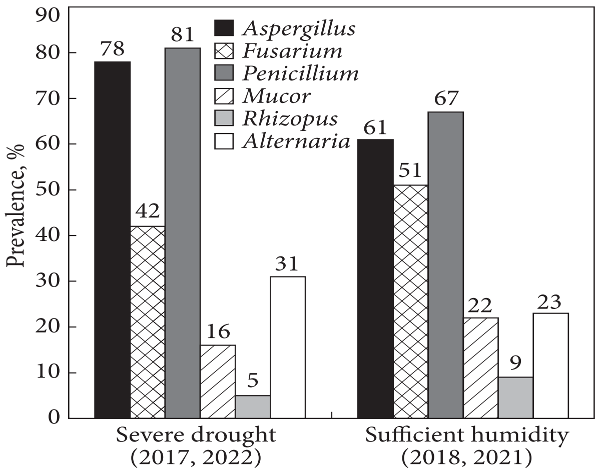

Fig. 3. Average infection of corn grain by fungi of different genera in years of severe drought and sufficient humidity

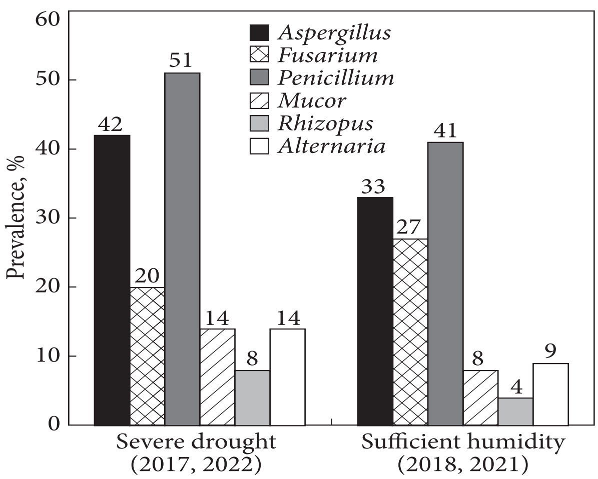

Fig. 4. The average rate of barley grain infection by fungi of different genera in years of severe drought and sufficient humidity

Table 5. **Taxonomic structure of barley grain mycobiota studied in 2017—2022**

Genus of fungi	Years of research / Percentage of samples affected by fungi					
	2017	2018	2019	2020	2021	2022
Aspergillus	42	34	36	38	32	43
Fusarium	21	28	22	24	26	20
Penicillium	52	42	45	46	40	50
Mucor	13	8	10	11	9	15
Rhizopus	8	4	4	4	4	8
Alternaria	15	10	12	11	9	13

with wheat grain, the infection rates of corn grain with fungi of the genus *Fusarium* were 1.5 times higher, and with fungi of the genus *Alternaria* — 2.3 times higher.

Comparing the mycobiota structure of corn grains in years of severe drought and adequate humidity, we found that fungi of the genera *Penicillium* (81%) and *Aspergillus* (78%) were most prevalent in 2017 and 2022. In 2018 and 2021, the rates of grain infection by these fungi were 14% and 17% lower, respectively. It should be noted that the infection of corn grain by *Fusarium* fungi was 9% higher in years with sufficient humidity than in years with severe drought (Fig. 3).

In the study of barley grain samples, it was found that among all mycobiota, filamentous fungi of the genus *Penicillium* were the most common (from 42% to 52%) (Table 5).

Barley grain was slightly less contaminated with micromycetes compared to corn and wheat, and fungi of the genera *Penicillium* (from 42% to 52%), *Aspergillus* (from 32% to 43%), and *Fusarium* (from 20% to 28%) were found in the samples, while fungi of the genera *Mucor, Rhizopus, and Alternaria* were revealed in a smaller number of samples (from 4% to 15%).

The average indicators of micromycetes contamination of barley grains in years of severe drought and adequate humidity were slightly different (Fig. 4).

In dry years, the average rate of infection of barley grains by *Penicillium* was 51%, while in wet years it was 41%. Almost similar results were obtained for *Aspergillus*, with 42% and 33%, respectively. The infection of barley grains by *Fusarium* fungi was also 7% higher in years with sufficient humidity, while the infection by *Mucor, Rhizopus*, and *Alternaria* fungi was 6%, 4%, and 5% higher in years with severe drought than in years with sufficient humidity.

Taking into account the data of mycological monitoring of grain feed in the Odesa region, it should be noted that the main isolates were filamentous fungi of the genus *Penicillium* represented by *P. lanosum* (33%) and *Penicillium* spp. (64%), which were most often identified in wheat, corn, and barley.

The toxinogenic genus *Aspergillus* was represented by *Aspergillus flavus* (14.3%), *A. niger* (28.6%), *A. sydowi* (7.1%), *A. amstelodami* (7.1%), *A. proliferans* (7.1%), *A. oryzae* (21.5%), and *A. candidius* (14.3%).

The genus *Fusarium* was represented by the species *F. oxysporum* (66.7%), and *F. sporotrichiella* (33.3%), which were most frequently isolated and identified in corn and wheat grains.

Discussion. The main fungi that cause frequent and problematic contamination of food and feed with mycotoxins belong to the genera *Aspergillus, Fusarium,* and *Penicillium* (Bucheli et al., 2008; Marin et al., 2013; Leslie et al., 2021). While the mycobiota of the genera *Aspergillus* and *Penicillium* often develops on stored food and feed, fungi of the genus *Fusarium* typically infect growing crops such as wheat, barley, and corn in the field and multiply within the plants (Bennett & Klich, 2003).

Unfortunately, approximately 25% of the world's crops are contaminated with mycotoxins annually, resulting in significant agricultural and industrial losses totaling billions of dollars (Marin et al., 2013).

Contamination with mycotoxins can occur before harvest, when the plant is growing, or after harvest during food processing, packaging, distribution, and storage (Bhatnagar et al., 2006; Pereira et al., 2014). In general, all crops and cereals that are improperly stored under high temperature and high humidity conditions for a long time can be susceptible to micromycetes growth and mycotoxin contamination (Bennett & Klich, 2003). Corn is considered to be the crop the most susceptible to mycotoxin contamination, while rice is the least susceptible (Dong et al., 2018; Chulze, 2010).

The World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the European Food Safety Authority (EFSA) are addressing the global problem of mycotoxin contamination in food and feed by adopting strict regulatory requirements for the main classes of mycotoxins (Bennett & Klich, 2003).

Environmental conditions related to storage can be controlled to affect the presence of mycotoxins in food or feed. However, other factors such as climate, fungal strain specificity, strain variability, and instability of toxigenic properties are more difficult to control. *Fusarium* is commonly found in corn, wheat, barley, sorghum, and rye. In the United States and Canada, corn and wheat are more commonly contaminated with *Fusarium*, whereas in European countries, *Fusarium* contamination is mainly found in wheat, rye, and oats (Hussein & Brasel, 2001).

Fungi of the genera *Aspergillus, Penicillium, Fusarium*, and *Alternaria* were found in both stored and freshly harvested samples in Tunisia. *Alternaria* (70.83%), *Eurotium* (62.50%), *Aspergillus* (54.17%), and *Penicillium* (41.67%) were the most frequent fungi isolated from wheat grain. *Penicillium* (75%), *Aspergillus* (70%), *Eurotium* (65%), and *Alternaria* (65%) were the most frequently isolated fungi from barley grain. In corn grain, fungi of the genera *Aspergillus* (76.19%), *Eurotium* (42.86%), and *Penicillium* (38.09%) predominated (Jedidi et al., 2018).

The study of wheat from Algeria identified the following fungal species: *A. terreus* (50%), *A. niger* (28%), *A. fumigatus* (40%), *A. versicolor* (18%), and *Penicillium* spp. (21.7%) (Riba, 2008).

In the southern province of Buenos Aires (Argentina), wheat (in the form of bran) and corn (in the form of dry grain or fermented feed) were studied, which are the main ingredients of feed used on local cattle and pig farms. The most commonly isolated fungi from corn grains were *Penicillium* (70%), *Fusarium* (47%), and *Aspergillus* (34%). *Penicillium* (42%), *Fusarium* (27%), and *Alternaria* (25%) were most often isolated from wheat grain (Roigé et al., 2009).

Fungi of the genus *Aspergillus* often colonize corn grains when the weather is hot and dry in late summer, eventually causing mold or cob rot. The fungi were isolated from three highly susceptible wheat genotypes in the North China Plain. The 21 isolates represented 11 fungal species. The most common species were from the genera *Alternaria* (isolation frequency 56.7%), *Bipolaris* (16.1%), and *Fusarium* (6.0%). The other eight species were *Curvularia, Aspergillus, Cladosporium, Exserohilum, Epicoccum, Nigrospora, Penicillium,* and *Ulocladium*; their frequency of isolation ranged from 0.8% to 4.8% (Xu et al., 2018).

Fusarium species cause two distinct diseases of corn cobs: *Fusarium* head blight or pink cob rot (main causative agent is *F. verticillioides*, but *F. subglutinans* and *F. proliferatum* are also important) and *Gibberella* head blight or red cob rot (causative agent is *Gibberella zeae* — teleomorph of *F. graminearum*), both of which can lead to mycotoxin contamination of corn grain (Munkvold, 2003).

The most frequently isolated fungi from corn in Iran, Brazil, and China were *Fusarium* spp. (17.3%, 17.9%, and 37.1%), *Aspergillus* spp. (9.3%, 17.4%, and 19.7%), *Penicillium* spp. (5.8%, 15.2%, and 17.6%), *Rhizopus* spp. (2.4%, 3.2%, and 3.5%), *Mucor* spp. (1.1%, 1.6%, and 1.3%), *Cladosporium* spp. (1.6, 1.9, and 1.9%), and *Alternaria* spp. (1.1%, 1.6%, and 1.3%), respectively (Khosravi et al., 2007). However, in Nigeria, the incidence of *Aspergillus* spp. and *Penicillium* spp. was 74% and 24%, respectively (Ekpakpale et al., 2021).

A total of 220 wheat samples were collected from four farms in the southwestern region of Uruguay. The main fungi isolated were *Fusarium* (43%) and *Aspergillus* (36%). The most common species were *F. graminearum* sensu lato and *Aspergillus* section *Flavi* (Del Palacio et al., 2016).

In 53 whole-grain samples collected in South Brazil, *Fusarium verticillioides* (34%) and *F. graminearum* (30.2%) prevailed among *Fusarium* species. For *Aspergillus* species, 37.7% of *A. flavus* was identified. As for *Penicillium* species, the most common species was *P. digitatum* (49%) (Savi et al., 2014).

A dry and cool spring may result in low levels of wheat grain contamination with microscopic fungi. Perkowski et al. observed similar dependences in the analysis of oat samples (Stuper-Szablewska & Perkowski, 2014; Perkowski et al., 2008).

In eastern Ukrainian farms, wheat grain was found to be contaminated with micromycetes from various genera, including *Fusarium, Aspergillus, Penicillium* (ranging from 30% to 58%), as well as *Rhizopus, Mucor*, and *Alternaria* (ranging from 6% to 17%). Barley grain was found to be less contaminated with micromycetes compared to corn and wheat. The samples contained fungi of the genera *Fusarium, Aspergillus*, and *Penicillium* (from 21% to 44%), as well as the genera *Rhizopus, Mucor*, and *Alternaria* in a smaller number of samples (from 2% to 11%) (Kutsan et al., 2021).

Based on mycological monitoring of 87 samples of feed and feed raw materials used for cattle feeding from the first half of 2018 to the first half of 2019, 73.6% were of poor quality. This indicates a consistent trend over the past four years, as the previous data from the authors have shown**.** In 2014 and 2015, the degree of contamination with fungi was 51—52%, which increased to 79% in 2016 and 88% in 2017 (Yaroshenko, 2016).

Environmental conditions, particularly air temperature and humidity, play a crucial role in the growth and reproduction of micromycetes (Mykhalska et al., 2019). Fungal growth thrives at an optimal temperature range of 18—25 °C, while toxin formation requires lower temperatures ranging from 4 °C to 12 °C, and humidity levels from 40% to 50%. Alterations in environmental parameters can affect not only the quantity but also the type of mycotoxin produced (Ogórek et al., 2020).

The presence of toxigenic fungi in a substrate does not necessarily indicate the natural occurrence of mycotoxins in the field. However, it does indicate a potential risk of contamination. Therefore, it is important to minimize the impact of fungi and mycotoxins on humans and animals

(Rodionova et al., 2021). Improper grain storage facilities can cause infection with fungal pathogens and the accumulation of mycotoxins during the post-harvest period of grain. Proper handling of grain in the post-harvest phase is crucial for its preservation over an extended time. It should also be noted that the yield of grain crops and their further preservation are directly affected by the soil (Demyanyuk et al., 2020).

The studies suggest the necessity of conducting yearly monitoring of crop grains during their technological periods to detect possible contamination with fungal pathogens and their toxins.

Conclusions. In the northwestern Black Sea region, the mycobiota of grain fodder is dependent on natural and climatic conditions. Filamentous fungi were found most commonly in corn grain. During a period of severe drought, the grain was contaminated with fungi from the *Penicillium* (81%) and *Aspergillus* (78%) genera.

The other four fungal genera were *Fusarium* (42%), *Alternaria* (31%), *Mucor* (16%), and *Rhizopus* (5%). In years with adequate humidity, infection by *Penicillium* and *Aspergillus* decreased by 17% and 14%, respectively, while infection by *Fusarium* increased by 9%. On the contrary, wheat grain samples were most affected by fungi of the genus *Penicillium* (52%) and *Aspergillus* (45%) during the period of sufficient humidity. The frequency of isolation of fungi of the genera *Rhizopus, Mucor,* and *Alternaria* ranged from 5% to 10%. Barley and corn samples in severe drought were most affected by fungi of the genera *Penicillium* (51%) and *Aspergillus* (42%). The frequency of isolation of *Alternaria, Mucor,* and *Rhizopus* fungi during the period of sufficient humidity was 9%, 8%, and 4%, respectively.

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REFERENCES

Adamenko, T. I. (2014). *Agroclimatic zoning of the territory of Ukraine taking into account climate change*. Ria Blitz. https://www.gwp.org/globalassets/global/gwp-cee_files/idmp-cee/idmp-agroclimatic.pdf [In Ukrainian].

Adelusi, O. A., Gbashi, S., Adebiyi, J. A., Makhuvele, R., Aasa, A. O., Oladeji, O. M., Khoza, M., Okoth, S., & Njobeh, P. B. (2022). Seasonal diversity and occurrence of filamentous fungi in smallholder dairy cattle feeds and feedstuffs in South Africa. *Journal of Fungi*, *8*(11), 1192.

Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R., & Daszak, P. (2004). Emerging infectious diseases of plants: Pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology & Evolution*, *19*(10), 535—544.

Bellgard, S., & Williams, S. (2011). Response of mycorrhizal diversity to current climatic changes. *Diversity*, *3*(1), 8—90.

Bennett, J. W., & Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews*, *16*(3), 497—516.

Bhatnagar, D., Cary, J. W., Ehrlich, K., Yu, J., & Cleveland, T. E. (2006). Understanding the genetics of regulation of aflatoxin production and *Aspergillus flavus* development. *Mycopathologia*, *162*(3), 155—166.

Bogach, M. V., Paliy, A. P., Perots'ka, L. V., Pyvovarova, I. V., Stoyanova, V. Y., & Palii, A. P. (2020). The influence of hydro-meteorological conditions on the spread of chicken cestodiasis. *Regulatory Mechanisms in Biosystems*, *11*(3), 414—418.

Bogach, M., Selischeva, N., & Bogach, D. (2023). Spread of mold fungi and their contamination of feed in Southern Ukraine. *Bulletin of Agricultural Science*, *101*(9), 30—36. [In Ukrainian].

Bucheli, T. D., Wettstein, F. E., Hartmann, N., Erbs, M., Vogelgsang, S., Forrer, H.-R., & Schwarzenbach, R. P. (2008). *Fusarium* mycotoxins: Overlooked aquatic micropollutants? *Journal of Agricultural and Food Chemistry*, *56*(3), 1029—1034.

Chalivendra, S., Huang, F., Busman, M., Williams, W. P., & Ham, J. H. (2020). Low aflatoxin levels in *Aspergillus flavus*-resistant maize are correlated with increased corn earworm damage and enhanced seed fumonisin. *Frontiers in Plant Science*, *11*, 565323.

Chulze, S. N. (2010). Strategies to reduce mycotoxin levels in maize during storage: A review. *Food Additives & Contaminants: Part A*, *27*(5), 651—657.

Del Palacio, A., Bettucci, L., & Pan, D. (2016). *Fusarium* and *Aspergillus* mycotoxins contaminating wheat silage for dairy cattle feeding in Uruguay. *Brazilian Journal of Microbiology*, *47*(4), 1000—1005.

Demyanyuk, O. S., Symochko, L. Y., & Mostoviak, I. I. (2020). Soil microbial diversity and activity in different climatic zones of Ukraine. *Regulatory Mechanisms in Biosystems, 11*(2), 338—343.

Domsch, K. H., Gams, W., & Anderson, T.-H. (2007) *Compendium of soil fungi* (2nd ed., Vols. 1—2). IHW Verlag.

Dong, C., Wu, Y., Gao, J., Zhou, Z., Mu, C., Ma, P., Chen, J., & Wu, J. (2018). Field inoculation and classification of maize ear rot caused by *Fusarium verticillioides*. *Bio-Protocol*, *8*(23), e3099.

Ekpakpale, D. O., Kraak, B., Meijer, M., Ayeni, K. I., Houbraken, J., & Ezekiel, C. N. (2021). Fungal diversity and aflatoxins in maize and rice grains and cassava-based flour (pupuru) from Ondo State, Nigeria. *Journal of Fungi*, *7*(8), 635.

Gornall, J., Betts, R., Burke, E., Clark, R., Camp, J., Willett, K., & Wiltshire, A. (2010). Implications of climate change for agricultural productivity in the early twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1554), 2973—2989.

Guo, B., Ji, X., Ni, X., Fountain, J. C., Li, H., Abbas, H. K., Lee, R. D., & Scully, B. T. (2017). Evaluation of maize inbred lines for resistance to pre-harvest aflatoxin and fumonisin contamination in the field. *The Crop Journal*, *5*(3), 259—264.

Hussein, H., & Brasel, J. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*, *167*(2), 101—134.

Jedidi, I., Soldevilla, C., Lahouar, A., Marín, P., González-Jaén, M. T., & Said, S. (2018). Mycoflora isolation and molecular characterization of *Aspergillus* and *Fusarium* species in Tunisian cereals. *Saudi Journal of Biological Sciences*, *25*(5), 868—874.

Keriene, I., Mankeviciene, A., & Blazyte, J. (2020). The effect of antifungal extracts on the contamination of grain with microfungi. *Food Science & Nutrition*, *8*(3), 1375—1382.

Khosravi, A. R., Mansouri, M., Bahonar, A. R., & Shokri, H. (2007). Mycoflora of maize harvested from Iran and imported maize. *Pakistan Journal of Biological Sciences*, *10*(24), 4432—4437.

Kolchyk, O., Illarionova, T., Buzun, A., Paliy, A., & Palii, A. (2022). Influence of probiotic microorganisms on microbial biofilms in feeds. *Scientific Horizons*, *25*(1), 41—50.

Kutsan, O. T., Nychyk, C. A., Zakharova, O. M., & Tarasov, O. A. (2021). Mycotoxicological risks by grain feeds. *Veterinary Biotechnology, 38*, 131—144. [In Ukrainian].

Leslie, J. F., Moretti, A., Mesterházy, Á., Ameye, M., Audenaert, K., Singh, P. K., Richard-Forget, F., Chulze, S. N., Ponte, E. M. D., Chala, A., Battilani, P., & Logrieco, A. F. (2021). Key global actions for mycotoxin management in wheat and other small grains. *Toxins*, *13*(10), 725.

Marin, S., Ramos, A. J., Cano-Sancho, G., & Sanchis, V. (2013). Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology*, *60*, 218—237.

Mendelsohn, R. (2009). The impact of climate change on agriculture in developing countries. *Journal of Natural Resources Policy Research*, *1*(1), 5—19.

Munkvold, G. P. (2003). Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. *European Journal of Plant Pathology*, *109*(7), 705—713.

Munkvold, G. P., Arias, S., Taschl, I., & Gruber-Dorninger, C. (2019). Mycotoxins in corn: Occurrence, impacts, and management. In S. O. Serna-Saldivar (Ed.), *Corn* (3rd ed., pp. 235—287). Elsevier.

Mykhalska, L. M., Zozulia, O. L., Hrytsev, O. A., Sanin, O. Y., & Schwartau, V. V. (2019). Distribution of species of *Fusarium* and *Alternaria* genera on cereals in Ukraine. *Biosystems Diversity*, *27*(2), 186—191.

Ogórek, R., Kurczaba, K., Łobas, Z., Żołubak, E., & Jakubska-Busse, A. (2020). Species diversity of micromycetes associated with *Epipactis helleborine* and *Epipactis purpurata* (Orchidaceae, Neottieae) in Southwestern Poland. *Diversity*, *12*(5), 182.

Ospanova, A., Anuarova, L., Shapalov, S., Gabdulkhayeva, B., Kabieva, S., Baidalinova, B., & Maui, A. (2021). Fungal pathogens found in tissues of herbaceous plants growing in the Yereymentau District, Akmola Region. *Saudi Journal of Biological Sciences*, *28*(1), 55—63.

Paliy, A. P., Mashkey, A. N., Faly, L. I., Kysterna, O. S., Rebenko, H. I., & Palii, A. P. (2021). Ecology of zoophilic flies in livestock biocenoses of Ukraine. *Biosystems Diversity*, *29*(3), 258—263.

Pereira, V. L., Fernandes, J. O., & Cunha, S. C. (2014). Mycotoxins in cereals and related foodstuffs: A review on occurrence and recent methods of analysis. *Trends in Food Science & Technology*, *36*(2), 96—136.

Perkowski, J., Buśko, M., Stuper, K., Kostecki, M., Matysiak, A., & Szwajkowska-Michałek, L. (2008). Concentration of ergosterol in small-grained naturally contaminated and inoculated cereals. *Biologia*, *63*(4), 542—547.

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and Food Spoilage*. Springer.

Riba, A. (2008). Mycoflora and ochratoxin A producing strains of *Aspergillus* in Algerian wheat. *International Journal of Food Microbiology*, *122*(1—2), 85—92.

Rodionova, K., Khimych, M., & Paliy, A. (2021). Veterinary and sanitary assessment and disinfection of refrigerator chambers of meat processing enterprises. *Potravinarstvo Slovak Journal of Food Sciences*, *15*, 616—626.

Roigé, M. B., Aranguren, S. M., Riccio, M. B., Pereyra, S., Soraci, A. L., & Tapia, M. O. (2009). Mycobiota and mycotoxins in fermented feed, wheat grains and corn grains in Southeastern Buenos Aires Province, Argentina. *Revista Iberoamericana de Micología*, *26*(4), 233—237.

Santiago, R., Cao, A., & Butrón, A. (2015). Genetic factors involved in fumonisin accumulation in maize kernels and their implications in maize agronomic management and breeding. *Toxins*, *7*(8), 3267—3296.

Santos Pereira, C., C. Cunha, S., & Fernandes, J. O. (2019). Prevalent mycotoxins in animal feed: Occurrence and analytical methods. *Toxins*, *11*(5), 290.

Savi, G. D., Piacentini, K. C., Tibola, C. S., & Scussel, V. M. (2014). Mycoflora and deoxynivalenol in whole wheat grains (*Triticum aestivum* L.) from Southern Brazil. *Food Additives & Contaminants: Part B*, *7*(3), 232—237.

Stanley, D., & Bajagai, Y. S. (2022). Feed safety and the development of poultry intestinal microbiota. *Animals*, *12*(20), 2890.

Stepanenko, S. N. (2007). Climate change. What awaits us in the future. *Bulletin of the Hydrometeorological Center of the Black and Azov Seas, 2*, 8. [In Ukrainian].

Stuper-Szablewska, K., & Perkowski, J. (2014). Contamination of wheat grain with microscopic fungi and their metabolites in Poland in 2006—2009. *Annals of Agricultural and Environmental Medicine*, *21*(3), 504—509.

Tadele, F., Demissie, B., Amsalu, A., Demelash, H., Mengist, Z., Ambelu, A., & Yenew, C. (2023). Aflatoxin contamination of animal feeds and its predictors among dairy farms in Northwest Ethiopia: One Health approach implications. *Frontiers in Veterinary Science*, *10*, 1123573.

Tian, M., Feng, Y., He, X., Zhang, D., Wang, W., & Liu, D. (2022). Mycotoxins in livestock feed in China—Current status and future challenges. *Toxicon*, *214*, 112—120.

Van Emden, H. F. (2019). *Statistics for terrified biologists* (2nd ed.). John Wiley & Sons.

Vaughan, M., Backhouse, D., & Ponte, E. M. D. (2016). Climate change impacts on the ecology of *Fusarium graminearum* species complex and susceptibility of wheat to *Fusarium* head blight: A review. *World Mycotoxin Journal*, *9*(5), 685—700.

Vujanovic, V., Mavragani, D., & Hamel, C. (2012). Fungal communities associated with durum wheat production system: A characterization by growth stage, plant organ and preceding crop. *Crop Protection*, *37*, 26—34.

Xu, K.-G., Jiang, Y.-M., Li, Y.-K., Xu, Q.-Q., Niu, J.-S., Zhu, X.-X., & Li, Q.-Y. (2018). Identification and pathogenicity of fungal pathogens causing black point in wheat on the North China Plain. *Indian Journal of Microbiology*, *58*(2), 159—164.

Yaroshenko, M. O. (2016). Mold saprophytes — biotic contaminants of feed as a possible source of fungal infections poultry. *Veterinary Medicine, 102*, 235–240. [In Ukrainian].

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М.В. Богач[1], А.П. Палій[2], Д.М. Богач[1], Л.В. Коваленко[2], Н.В. Селіщева[1], Л.О. Ганова [3], Б.Т. Стегній[2], О.В. Павліченко[4], Д.В. Вовк[2]

[1] Одеська дослідна станція Національного наукового центру «Інститут експериментальної і клінічної ветеринарної медицини», пр. Свободи, 2, Одеса, 65037, Україна

[2] Національний науковий центр «Інститут експериментальної і клінічної ветеринарної медицини», вул. Пушкінська, 83, Харків, 61023, Україна

[3] Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, вул. Академіка Заболотного, 154, Київ, 03143, Україна

[4] Державний біотехнологічний університет, вул. Алчевських, 44, Харків, 61002, Україна

ВПЛИВ ПОГОДНИХ УМОВ НА КОНТАМІНОВАНІСТЬ МІКРОМІЦЕТАМИ ЗЕРНОВИХ КОРМІВ У ПІВНІЧНО-ЗАХІДНОМУ ПРИЧОРНОМОР'Ї УКРАЇНИ

Кількість атмосферних опадів та їх сезонне розподілення впливає на контамінацію зернових кормів цвілевими грибами. Використання зерна різних сільськогосподарських культур як кормів для тварин та в харчовій промисловості ставить питання щодо його якості і відповідності санітарно-медичним умовам. **Метою** роботи було дослідити ступінь контамінації мікроміцетами зернових кормів (пшениця, кукурудза, ячмінь) із урахуванням погодних умов у 2017—2022 роках в північно-західному Причорномор'ї. **Методи.** Проби зерна відбирали після збору врожаю. Для встановлення загальної кількості грибів та визначення їх видового складу досліджуваний матеріал засівали в чашки Петрі, що містили середовище картопляного агару з декстрозою. Методом серійних розведень розраховували кількість діаспор грибів в 1 г корму. Видову ідентифікацію мікроміцетів проводили класичними методами за культурально-морфологічними ознаками. Показник ГТК за методом Селянінова використовували для оцінки умов зволоження в період із середньодобовими температурами понад 10 °C, тобто в період активної вегетації. Оскільки в окремих місяцях року відсутні активні температури повітря понад 10 °C, тому ГТК не розраховували. **Результати.** У північно-західному Причорномор'ї України помірний і сухий період збільшився з чотирьох до восьми місяців, впродовж яких гідротермічний коефіцієнт становив 0,4—0,8. Період достатньої вологи (2018, 2021 рр.) за значень гідротермічного коефіцієнту 1,0—1,5 тривав п'ять місяців. Кількість атмосферних опадів та їх сезонне розподілення в умовах північно-західного Причорномор'я впливає на контамінацію зернових кормів цвілевими грибами. У роки сильної посуха (2017, 2022 рр.) зерно пшениці було контаміновано найбільше грибами роду *Aspergillus*, а кукурудзи і ячменю — *Penicillium*. У середню посуху (2019, 2020 рр.) найвища контамінованість зернових кормів була грибами роду *Penicillium*, а в період достатньої вологи (2018, 2021 рр.) — грибами роду *Penicillium* та *Fusarium.* **Висновки.** У північно-західному Причорномор'ї України мікобіота зернових кормів залежить від природно-кліматичних умов. Цвілевими грибами найбільше було уражено зерно кукурудзи. У період сильної посухи зерно було контаміновано грибами роду *Penicillium* (81%) і *Aspergillus* (78 %). Іншими чотирма родами грибів були *Fusarium* (42 %), *Alternaria* (31 %), *Mucor* (16 %) та *Rhizopus* (5 %). У роки з достатньою вологою ураження грибами роду *Penicillium* і *Aspergillus* було на 17 % і 14 % менше, а грибами роду *Fusarium* — на 9 % більше. Проби зерна пшениці, навпаки, найбільше були уражені грибами роду *Penicillium* (52 %) і *Aspergillus* (45 %) в період достатньої вологи. Частота виділення грибів роду *Rhizopus, Mucor* та *Alternaria* коливалася від 5 до 10%. Зразки ячменю, як і кукурудзи, у сильну посуху були уражені найбільше грибами роду *Penicillium* (51 %) і *Aspergillus* (42 %). Частота виділення грибів *Alternaria*, *Mucor* та *Rhizopus* у період достатньої вологи склала відповідно 9, 8 і 4 %.

Ключові слова: *гідротермічний коефіцієнт, пшениця, кукурудза, ячмінь, Aspergillus, Fusarium, Penicillium.*