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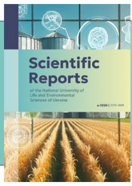

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Transformation of soil cover under the influence of military aggression: An integrated assessment on the example of Kyiv Polissia

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Abstract. Military actions have a significant impact on the soil cover, which has serious consequences for the environment and food security. The purpose of the study was to analyse changes in the agrochemical and geochemical characteristics of soils as a result of the 2022 military operations in Ukraine. The study focused on the territory of the village of Kalynivka, Fastiv Raion, Kyiv Oblast, at the experimental site of the oil product outpouring site. Based on a comparison of empirical laboratory data (2024) – with simulated states of the pre-escalation period (2021) and the period of acute impact (2022) – the multifactorial nature of military impact on soil degradation was revealed. It was established that the dominant factor of destruction is not chemical contamination, but a deep physical disturbance of the soil profile: inversion of horizons, compaction and "scalping" of the fertile layer, collectively described by the term "bombturbation". A detailed correlation analysis was performed, which quantitatively confirmed the inversion hypothesis: a strong positive correlation ($r \approx +0.94$) was found between the depth and concentration of heavy metals (Cr), which is abnormal for natural conditions. This phenomenon, together with critically low humus content and an abnormal distribution of petroleum products, indicates that the originally contaminated surface layer has been buried. Analysis of time dynamics revealed the phenomenon of "disjointed recovery", in which the rapid biodegradation of organic pollutants contrasts with the persistence of physical damage and heavy metal contamination. The paper emphasised that ignoring the physical component of degradation and attempts to conduct agriculture according to traditional schemes will inevitably

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lead to increased environmental risks and further loss of agricultural potential. The results of the study can be used to develop strategies for soil restoration in the affected territories of Ukraine, which will help to reduce environmental risks and restore agricultural potential

Keywords: soil degradation; military conflict; bombturbation; heavy metals; petroleum products; profile inversion; correlation analysis

Introduction

The armed aggression of the Russian Federation against Ukraine, which began in 2014 and escalated in February 2022, caused not only a humanitarian and economic catastrophe, but also an unprecedented environmental crisis. One of the most vulnerable and strategically important components of the environment is ground cover. Unlike traditional types of degradation (erosion, salinisation, depletion), military degradation is a complex multi-factor process that combines deep physical, chemical, and biological disturbances, turning the soil into a source of long-term environmental threats. This new type of degradation goes beyond existing classifications and requires the development of new approaches to diagnosis, assessment, and phytoremediation.

The problem of military soil degradation is relatively new to the global science in its current dimension, but it is based on a significant theoretical and empirical basis. Ukrainian researchers play a leading role in shaping the new paradigm. In particular, S.A. Baliuk *et al.* (2024) first introduced and substantiated the concept of "degradation caused by armed aggression" and developed its detailed classification. This step became fundamental for the systematisation of knowledge and the creation of a single methodological basis for further research. The analysis of publications confirmed the versatility of this problem, considered by E. Cusato (2019) who criticised the UN Security Council's response to environmental damage caused by the 1990-1991 Gulf War. The environmental risks of military operations for the soil cover of Ukraine were discussed in detail in the paper by I. Bulba *et al.* (2024), where the researchers emphasised the irreversibility of certain transformational processes. Their study considered the environmental risks of military operations for the soil cover of Ukraine, focusing on the irreversibility of transformational processes, such as mixing and degradation of soils under the influence of military aggression. Their research is important for understanding the long-term environmental impacts, especially on soil fertility.

J.P. Hupy & R.J. Schaetzl (2013) conceptualised the mechanism of physical destruction of the soil, in particular "bombturbation", which is a new type of soil mixing due to explosions. They proved that this phenomenon can lead to serious changes in the genetic horizons of soils, which are important to consider when assessing soil degradation in conflict zones. Their ideas regarding the chaotic mixing of genetic horizons were reflected in the analytical report on land degradation in conflict regions by V.M. Filatov *et al.* (2025). This report analyses land degradation in conflict zones, highlighting the chaotic mixing of soil layers and heavy metal pollution that leads to the destruction of natural resources. The concept of synergistic effects of various pollutants is important. M. Solokha *et al.* (2024) demonstrated that fragments of military equipment and ammunition become sources of long-term pollution, reducing soil fertility, and impairing ecosystem functions, whereas T. Bilyi *et al.* (2025) proposed a practical methodology for assessing the degree of such changes, which includes assessing the synthesis of aerosols and the spread of soil particles after detonation. These studies complemented existing assessments of the impact of war on Ukraine's land resources and highlighted the need for comprehensive monitoring strategies.

The chemical component of military degradation, in particular, the accumulation of explosive residues in the soil, was highlighted by Yu.V. Buts *et al.* (2025). The problems of monitoring heavy metals in agroecosystems under anthropogenic pressure, in particular, in the conditions of military operations, were discussed in detail by Y. Peng & I.Y. Grigorieva (2024). They focused on the impact of military conflicts on soil pollution with heavy metals such as lead, cadmium, zinc, copper, and other toxic elements that enter agricultural systems as a result of explosions, destruction of equipment, use of toxic substances, and other anthropogenic factors. The researchers emphasised the need to develop effective methods for monitoring and assessing the concentrations of heavy metals in the soil, which allows identifying potentially dangerous levels of pollution and predicting negative consequences for ecosystems and agricultural production. A. Honcharova (2024) highlighted the thermal effects of combat activities. An important aspect is also the thermal impact of fighting, which leads to the burning of the microbiota and the loss of humus. Geochemical transformation of soils under the influence of means of destruction, in particular, in the Polissia region, was analysed in the paper by I. Plisko *et al.* (2023).

However, despite the large amount of accumulated data, most publications focus on certain aspects of the problem – either physical destruction or a specific type of chemical contamination. The development of comprehensive models for restoring disrupted soil pedogenesis remains an unresolved issue. A critical gap in existing research is the lack of a comprehensive, integrated approach that considers physical, chemical, and biological changes not as separate phenomena, but as an interconnected system. A key question remains poorly understood: how exactly does physical destruction (bombturbation, profile inversion) affect the spatial distribution, mobility, and toxicity of chemical pollutants. Most conclusions about profile inversion are made based on visual or qualitative assessments. There is no reliable quantitative tools for diagnosing and confirming this process. This study was aimed at solving this part of the general problem: establishing correlations between depth, agrochemical parameters, and pollutant concentration for quantitative proof of the profile inversion hypothesis.

The purpose of the study was to analyse the transformation of soil cover as a result of military aggression, in particular, physical, chemical, and biological changes caused by anthropogenic impact. The scientific originality of this study lies in the fact that, for the first time, an integrated model of military soil degradation has been presented using the village of Kalynivka as a case study; this model combines the analysis of physical degradation, agrochemical changes, and geochemical anomalies into a single causal system. A new methodological approach was proposed – correlation analysis as a diagnostic tool for quantitative confirmation of the fact of mechanical inversion of the soil profile. The concept of “disconnected restoration” (asynchronous remediation) of the post-conflict soil ecosystem was also formulated, which is important for developing long-term recovery strategies.

Materials and Methods

The study was conducted on the territory of the village of Kalynivka, Fastiv Raion, Kyiv Oblast, which geographically belongs to the zone of Kyiv Polissia. The subject of the scientific analysis was a plot of approximately 0.8 hectares of sod-podzolic sandy loam soil that had been degraded as a result of an oil spill caused by intense military operations. This location, which was previously used as agricultural land, is indicative for studying the degradation of soil cover under the influence of anthropogenic and military factors.

A three-level time analysis was used for a comprehensive assessment of ecosystem transformation. The first stage included a study of the soil condition in the pre-escalation period (2021), which was reconstructed on the basis of archival agrochemical maps of the State Institute of Soil Protection of Ukraine (n.d.), field passports, and regional background standards. The second stage covered the state of acute impact (2022), modelled using literature sources (in particular, methodological approaches were used to assess military degradation and classification of types and levels of soil cover damage, set out in the monograph by S.A. Baliuk *et al*. (2024), and the results of the assessment of primary contamination and environmental risks described by P. Pereira *et al*. (2022) and D. Balsalobre-Lorente *et al*. (2023), calculating primary contamination and estimates of fuel and lubricants discharge volumes. The third stage was based on empirical data (2024-2025) obtained through direct field examination and sampling. The study was accompanied by strict compliance with safety standards: the field stage began only after verification of the site by specialists of the State Emergency Service for the absence of mines and unexploded ordnance, which ensured the safety of the research group in the zone of former military operations.

The field sampling process was carried out using the "envelope" method at five points along representative profiles. Samples were taken from genetic horizons at four fixed depths: 0-20 cm, 20-30 cm, 30-40 cm, and 40-50 cm using a handheld soil drill and sterile instruments. During the selection process, special attention was paid to areas with signs of bombturbation and mechanical mixing of layers. A mixed sample of three replicates was prepared for each point, which ensured the representativeness of the sample. Samples were transported to the laboratory in sealed containers under a stable temperature regime to minimise the loss of volatile fractions of petroleum products.

Laboratory analysis of agrochemical and geochemical indicators was carried out in accordance with the current state standards DSTU 4115-2002 (2002), DSTU 4289:2004 (2004), DSTU 4405:2005 (2005), DSTU 4770.1:2007 (2007), DSTU 4837:2007 (2007), DSTU ISO 10390:2022 (2022). The humus content was determined using the Tyurin method (oxidation of organic matter with a chromium mixture), whilst the pH of the brine was measured potentiometrically. The analysis of mobile forms of nitrogen, phosphorus and potassium was performed according to Kirsanov methods in the corresponding modifications. The geochemical composition, in particular, the content of heavy metals such as chromium (Cr) and nickel (Ni), was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) after preliminary acid extraction. The content of petroleum products was determined by the gravimetric method using hexane extraction.

The hypothesis of the study was that the vertical distribution of chemical elements in the profile is the result of mechanical inversion, and not natural processes or surface contamination. To test it, the correlation coefficient between depth (X) and chromium content (Y) was calculated. Input data for the calculation (depth taken as the midpoint of each layer): X (depth, cm): {15, 25, 35, 45}; Y (Cr content, mg/kg): {10.02, 10.44, 16.80, 18.10}. Pearson's correlation coefficient equation was applied:

$$\mathrm{r} = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}}, \qquad (1)$$

where n = 4 (number of samples). This method allowed estimating the strength and direction of the linear relationship between two variables. The correlation coefficient (r) ranged from -1 (perfect negative relationship) to +1 (perfect positive relationship). Values close to 0 indicated the absence of a linear relationship. All correlations and differences were considered significant at $p < 0.05$. The relationship between the depth of the horizon and the content of organic matter under anthropogenic loading conditions was analysed separately. Statistical processing of the results obtained, including calculation of mean values, standard deviation, and Pearson correlation coefficient, was performed in MS Excel environments and the R software suite.

Results and Discussion

Military operations leave a deep and long-lasting chemical trace in the soil, which, due to its complexity and multicomponent nature, can be described as a "toxic cocktail". In contrast to typical industrial or agricultural pollution, which often has one dominant polutant, military contamination is the simultaneous and massive entry into the soil ecosystem of substances with fundamentally different physical and chemical properties and pathways of influence. This synergistic strike not only poisons the land, but also destroys its natural defence mechanisms, turning fertile soil into a permanent source of environmental danger (Baliuk *et al.*, 2024).

The most persistent component of military pollution is heavy metals, or potentially toxic elements (PTE). Their main danger is that they are chemical elements and, unlike organic compounds, cannot be decomposed or neutralised biologically. They remain forever in the ecosystem, only redistributing between its components. The sources of metal supply are almost all aspects of military activity (Biyashev *et al.*, 2023). Fragments of shells, mines, and missiles are a major source of iron, copper, and zinc (components of brass sleeves), lead (bullet cores, detonators), antimony, and mercury (detonator capsules). The historical experience of the First World War showed that artillery shells alone introduced millions of tonnes of metals into the soils of Europe, creating geochemical anomalies that are recorded even after centuries (Solokha *et al.*, 2024). The armor of tanks and combat vehicles is a source of chromium, nickel, manganese, and vanadium. When it is destroyed by an explosion or fire, these metals in the form of small particles and oxides are dispersed over a significant area. Batteries, electronics, and paint from destroyed vehicles additionally enrich the soil with lead, cadmium, and zinc. Leaded gasoline, although used in limited quantities, can be a source of lead, while lubricants and additives contain zinc, molybdenum, and other metals.

In some areas, background concentrations of harmful metals were exceeded by 10-25 times. Once in the soil, these elements enter into complex interactions. Their mobility, bioavailability and, consequently, toxicity strongly depend on the properties of the soil itself, primarily on its acidity (pH) and organic matter content (Kumar & Srikantaswamy, 2014). In the acidic environment characteristic of Polissia sod-podzolic soils, the solubility of metals such as cadmium, zinc, and nickel increases dramatically. This makes them easily accessible for absorption by the root system of plants. This triggers a process of bioaccumulation – the build-up of toxic substances in agricultural produce – which transforms once-fertile fields into a source of long-term danger to human health.

The second most important component is organic explosives. The most common explosives used in modern munitions are 2,4,6-trinitrotoluene (TNT or trotyl), hexogen (RDX), and octogen (HMX). The widespread belief that an explosion completely destroys these substances is erroneous. Detonation, even the most effective, is never one hundred percent. A small but significant percentage of explosives (from 0.1% to 1% or more) do not react and are dispersed on the ground in the form of fine dust or fine solid particles that permeate the topsoil (Sowik & Ruzik, 2025). These compounds and their partial decomposition products (for example, 2.4-dinitrotoluene) are extremely dangerous. They have proven toxicity to plants, soil microorganisms, and animals; many of them are mutagens and potential carcinogens. The main problem is their persistence. Having a complex chemical structure, they decompose very slowly in natural conditions. Their half-lives in the soil can reach years or even decades, which creates a long-term focus of chronic pollution (Dmytrenko *et al.*, 2023).

The destruction of thousands of units of military equipment and warehouses of fuel and lubricants led to massive spills of fuel and lubricants. The main pollutants in this case are hydrocarbons. Polycyclic aromatic hydrocarbons (PAHs), a group of compounds formed during incomplete combustion of organic matter, are particularly dangerous. PAHs are highly toxic and have proven carcinogenic properties. In the soil, they behave specifically: being hydrophobic, they strongly bind to organic matter particles (humus) and clay. This creates persistent "reservoirs" of pollution from which PAHs can be slowly released over decades (Polycyclic Aromatic Hydrocarbons..., 2021). However, the most dangerous consequence of military aggression is not just the arrival of toxicants, but the simultaneous destruction of the natural defence mechanisms of the soil itself, its so-called "immune system". This is a double whammy that increases the negative effects many times over. The first mechanism of destruction is the destruction of humus. High temperatures from explosions and burning of machinery and vegetation literally burn out the organic matter of the soil. Humus is a key buffer component. Due to its high cation exchange capacity, its negatively charged molecules bind and retain positively charged heavy metal ions (Pb^{2+}, Cu^{2+}, Zn^{2+}), converting them into an immobile, biologically inaccessible form. The loss of humus is the loss of the main natural "deactivator" of metals. As a result, they remain in the soil solution in free ionic form, ready for migration and absorption by plants. The second mechanism is the sterilisation of the soil microbiota. Explosions and fires destroy complex communities of bacteria, fungi and other microorganisms that act as the soil's "sanitisers". They are responsible for biodegradation – the natural process of decomposition of organic pollutants, such as petroleum products and partially explosive residues. The destruction of microbial life actually stops the self-cleaning processes of the soil, making organic pollution much more sustainable (Plisko *et al.*, 2023).

Thus, military influence deals a double blow: it not only introduces an unprecedented amount of toxins into the soil, but also simultaneously destroys the very mechanisms that could resist this pollution. The soil loses its buffering, self-cleaning, and resilience, turning from a living system into a dead, inert environment that becomes just a transit zone for pollutants to spread to adjacent ecosystems – groundwater, rivers, and food chains.

Analysis of field materials shows that the dominant consequence of military operations in Kalynivka is precisely physical destruction. The fact that samples from the upper humus horizon (10-20 cm) were taken only at one point is not a statistical error, but direct evidence of its "scalping" – physical removal or destruction in most of the studied area. This process, along with digging trenches and explosions, led to a deep inversion of the profile – chaotic mixing, as a result of which underground layers that are poor in organic matter and nutrients were brought to the surface. The consequences of physical destruction can be clearly traced in agrochemical and geochemical indicators (Table 1).

Table 1. *Comparative characteristics of key agrochemical indicators of the soil (top layer 10-20 cm)*

Indicator	Simulated state (2021)	Empirical state (2025)	Characteristics of changes
Humus, %	2.5-3.0	~1.4 (average)	Catastrophic loss
pH (salt)	5.8-6.2	~5.2 (average)	Acidification
Nitrogen (NO_3), mg/kg	Moderate	26.3 (locally, high)	Abnormally high
Phosphorus (P_2O_5), mg/kg	Moderate	122.4 (locally, elevated)	Abnormally high
Potassium (K_2O), mg/kg	Moderate	124.6 (locally, high)	Abnormally high

Source: compiled by the authors

The agrochemical data presented in Table 1 are not just a set of disparate indicators, but an interconnected system of indicators that allows for a deep diagnosis of the systemic pathology of the soil cover caused by military influence. Integrated analysis of these parameters reveals the cascading nature of degradation processes, where primary physical destruction acts as a trigger for deep chemical and biological transformations. Organic matter content is an integral indicator of soil health and sustainability. Comparison of the simulated pre-conflict indicator (2.5-3.0%) with the empirically established average (~1.4%) indicates catastrophic dehumification resulting from combined exposure. The main mechanism is the mechanical movement of soil masses ("bombturbation") and "scalping" of the upper horizon, which leads to an inversion of the profile – the burial of the humus-accumulative horizon (A) under layers of eluvial (E) and iluvial (I) horizons, which are naturally poor in organic matter. The secondary mechanism is thermal degradation (pyrolysis) and complete oxidation of humus during explosions and combustion of military equipment. Soil structure degradation: humic acids act as a key cementing agent that forms water-resistant aggregates. Their loss leads to the destruction of the structure, an increase in the folding density, a decrease in overall porosity and, especially, aeration porosity. The soil becomes prone to compaction and the development of a surface crust, which disrupts gas exchange and water infiltration.

Loss of cation exchange capacities (CEC). Organic matter is the primary carrier of negative charge in the soil, which determines its ability to retain cations of nutrients (Ca^{2+}, Mg^{2+}, K^{+}) and heavy metals (Pb^{2+}, Cu^{2+}, Zn^{2+}). Dehumification means a sharp decline in the eco system, which turns the soil from a geochemical buffer into a transit environment that cannot contain either useful or toxic elements. The shift in acidity from the optimal range for agrocoenoses (pH 5.8-6.2) to a more acidic one (on average ~5.2) is a direct consequence of the profile inversion described above. The removal to the surface of genetically more acidic lower horizons, which in sod-podzolic soils have passed the stage of intensive leaching of bases, leads to a decrease in the overall pH value. This shift plays the role of a powerful catalyst for toxicity: changing specialisation and increasing bioavailability of heavy metals: acidity is a key factor controlling the forms of finding (specialisation) metals in the soil. As the pH decreases, most heavy metals transition from sorbed, precipitated or complexed forms into more soluble and mobile ionic forms (e.g. Cd^{2+}, Ni^{2+}). This dramatically increases their phytotoxicity and ability to migrate along the profile, and be included in trophic chains. Violation of the balance of nutrients: in an acidic environment, the availability of phosphorus for plants decreases (due to the formation of insoluble iron and aluminum phosphates), calcium and magnesium, which creates additional stress for plant organisms.

Data on the content of mobile (labile) forms of nutrients show an abnormal, antagonistic picture with respect to the humus content. Locally high concentrations of nitrate nitrogen, phosphorus and potassium against the background of low content of organic matter, which is their natural depot, indicate the exogenous origin of these elements. The only logical explanation is the intensive application of highly concentrated synthetic mineral fertilisers. This phenomenon indicates the development of so-called "fragile fertility" - an artificially maintained, extremely unstable agrochemical state. In soil devoid of humus buffer and with low eco, there are no mechanisms for retaining the introduced nutrients.

Low nitrogen use efficiency (NUE). Highly mobile anions, such as nitrates (NO_3^-), are practically not sorbed by the soil absorption complex and are quickly washed out of the root layer. Cations (K^+) are also retained much worse due to the lack of exchange sites on the surface of soil colloids. This leads to an extremely low rate of fertiliser use by plants and requires a constant increase in doses, which makes such farming economically unprofitable. Development of significant environmental risks: an excess of mobile forms of nitrogen and phosphorus, which are not absorbed by plants, becomes a source of diffuse pollution of adjacent media. Infiltration of nitrates into ground water leads to their contamination above the maximum permissible concentrations, creating risks to human health. Surface runoff enriched in nitrogen and phosphorus causes eutrophication of surface water bodies. Thus, a comprehensive analysis of the data in

Table 1 indicates that the military impact caused not just a change in individual indicators, but a complete restructuring of the functioning of the soil system. Primary physical destruction resulted in the loss of key regulatory mechanisms (humus and acid buffers), which, in turn, increased the environmental hazard from existing pollutants. Attempts at agronomic compensation through intensive mineralisation only mask systemic degradation, creating an unstable state of "fragile fertility" and creating new secondary environmental risks. The vertical distribution of pollutants provides the most convincing evidence for profile inversion (Table 2).

Table 2. *Vertical distribution of pollutants in the soil profile, 2025*

Depth, cm	Cr, mg/kg	Ni, mg/kg	Petroleum products, mg/kg
10-20	10.02	4.54	13.3
20-30	10.44	5.08	56.7
30-40	16.80	8.30	**66.7 (maximum)**
40-50	18.10	8.53	60.0
MPC, mg/kg	100	85	1,000

Note: MPC – maximum permissible concentration
Source: compiled by the authors

Table 2 showed data on the vertical distribution (stratigraphy) of key pollutants – chromium (Cr), nickel (Ni), and petroleum products – within a single representative soil profile. The analysis of these data is of crucial diagnostic importance, since it allows moving from stating the fact of contamination to reconstructing the mechanism of its development. The vertical gradient of polutant concentrations is a unique geochemical marker that allows verifying the hypothesis of the dominant role of physical destruction (bombturbation and inversion) over the processes of surface contamination and vertical migration. In the classical model of alluvial (surface) contamination, for example, due to aerotechnogenic precipitation or spill on the ground surface, a clear pattern is expected: the maximum concentration of pollutants is observed in the upper humus-accumulative horizon (0-20 cm) with its gradual, exponential, or logarithmic decrease with depth. This gradient is conditioned by the high sorption capacity of the upper, humus-rich layers and the limited rate of vertical migration (leaching). However, the data presented in Table 2 show a radically opposite, anomalous pattern. The concentrations of both chromium and nickel consistently and significantly increase with depth. The chromium content increases from 10.02 mg/kg in a layer of 10-20 cm to 18.10 mg/kg in a layer of 40-50 cm, that is, almost twice. A similar trend, although less pronounced, is observed for nickel (an increase from 4.54 mg/kg to 8.53 mg/kg).

This distribution cannot be explained by vertical migration processes, especially for relatively inert metals such as chromium. The only logical and scientifically based explanation for this geochemical anomaly is the mechanical inversion of the soil profile. The observed gradient of metal concentrations actually repeats the natural lithological and pedogenetic distribution of elements in sod-podzolic soils. During soil formation, silty particles and associated metal oxides are leached from the upper horizons and accumulate in the denser lower horizon. Thus, higher concentrations of Cr and Ni in the deep layers are their natural background characteristic. The fact that these deep, naturally metal-rich horizons are now close to the ground surface is direct evidence that the upper, metal-poor layers were physically removed ("scalped") or mixed and buried beneath them. Consequently, the distribution of heavy metals is a reliable indicator not so much of anthropogenic pollution as of the scale of physical destruction of the soil. Whilst the distribution of heavy metals serves as an indicator of the mixing of natural soil horizons, the distribution of petroleum products is a unique indicator that allows the location of the "buried original surface" – that is, the layer that formed the soil surface at the time of the intense contamination – to be identified with a high degree of accuracy.

Unlike metals, petroleum products are a purely anthropogenic pollutant, and their natural background in the soil is zero. In the case of a surface spill of fuel and lubricants, their distribution over the profile should correspond to the classical infiltration model: the absolute maximum on the surface with a sharp decrease in concentration with depth. In contrast, Table 2 shows a distinct "peak" or concentration of petroleum products at a depth of 30-40 cm (66.7 mg/kg). The concentration in the upper layer (10-20 cm) is relatively low (13.3 mg/kg), then it increases sharply to the maximum and again decreases slightly in the deepest layer. Such a picture is irrefutable proof of the following scenario. There was a spill of petroleum products on the existing original soil surface at that time. Due to the movement of heavy machinery, explosions or engineering work (digging trenches), this initially contaminated top layer was covered (buried) with layers of soil turned out from a depth of 30-50 cm. A new anthropogenic soil profile has been formed, in which the layer with the maximum concentration of anthropogenic pollutant (petroleum products) is now located at a considerable depth.

Thus, petroleum products, without indicating acute toxicity in these concentrations, perform a unique function of a tracer – a marker substance that allows reconstructing the process of mechanical destruction. This "buried" peak of pollution is strong evidence to support the conclusions drawn based on the analysis of agrochemical indicators and the distribution of heavy metals. It clearly illustrates that there was not surface contamination, but deep structural destruction, which completely changed the very architectonics of the soil body. This is confirmed by quantitative data obtained by Pearson correlation analysis (Table 3).

Table 3. *Intermediate calculations of the Pearson correlation coefficient*

X	Y	XY	X^2	Y^2
15	10.02	150.30	225	100.40
25	10.44	261.00	625	108.99
35	16.80	588.00	1,225	282.24
45	18.10	814.50	2025	327.61
Σx = 120	**Σy = 55.36**	**Σxy = 1813.8**	**Σx² = 4100**	**Σy² = 819.24**

Note: X – depth of the soil layer; Y – chromium content
Source: compiled by the authors

Calculation of the correlation coefficient between the depth of the soil profile and the chromium concentration by equation (1) gave a value of r = 0.92, which indicates a strong positive correlation between the depth of the soil and the chromium content. This means that as the soil deepens, there is an increase in chromium concentrations, which supports the hypothesis of inversion of the soil profile due to mechanical disturbances, such as movement of the upper layers of the soil as a result of anthropogenic or military operations. Analysis of time sections revealed the asynchrony of soil restoration processes (Table 4). The presented data indicate the phenomenon of heterogeneous recovery, where the rate of degradation of pollutants does not correlate with the rate of recovery of biophysical parameters of the ecosystem. Analysis of the time intervals of 2021-2025 showed a pronounced nonlinearity of processes: while the concentration of petroleum products showed an exponential decrease due to the processes of natural attenuation and microbiological oxidation, other components of the system showed inertia or persistent degradation. Of particular concern is the dynamics of heavy metals, in particular lead (Pb), whose persistence in the soil profile creates the risk of long-term ecotoxicological effects. Although there was a slight decrease in its concentration compared to the peak of 2023, which is probably conditioned by the redistribution of the metal between the soil phases or its leaching into the lower horizons, and not to the actual detoxification of the substrate. The most critical aspect is the state of the humus horizon. A sharp drop in humus content to values below 1% in 2023 signals the destruction of the soil absorption complex. Further recovery to 1.4% in two years is not sufficient for the regeneration of biological activity and aggregate stability of the soil. This state can be defined as the "dissociated reduction" phase, where chemical purification from organic compounds occurs independently of the restoration of the physico-chemical matrix of the soil. This supports the hypothesis that the self-renewal of the soil ecosystem after a complex anthropogenic load is limited. Without targeted phytoremediation and the introduction of structuring agents, the soil remains in a state of functional collapse, where deep structural and biochemical degradation persists at formally acceptable levels of organic contamination.

Table 4. *Dynamics of restoration of various components of the soil ecosystem (2021-2025)*

Type of damage	Status (2021)	Status (2023, model)	Status (2025, data)	Recovery trend
Petroleum products, mg/kg	<1.0	150-250	~10-34	Fast (natural attenuation)
Lead (Pb), mg/kg	<5.0	15-25	~6.7	Very slow (persistence)
Humus, %	2.5-3.0	<1.0	~1.4	Missing (sustained degradation)

Source: compiled by the authors

A comprehensive analysis of the soil cover in the zone of intense military influence on the territory of the Kalynivka village allows characterising the state of the ecosystem as a result of a "toxic cocktail", where the synergistic effect of physical destruction and chemical contamination exceeds the sum of individual negative impacts. Unlike classical models of industrial pollution, the military transformation of sod-podzolic soils is accompanied by a deep inversion of the profile and the destruction of natural defence mechanisms. The observations made are consistent with the conceptual provisions by S.A. Baliuk *et al.* (2024) that military degradation is a multi-factorial process in which mechanical damage acts as a trigger for long-term loss of soil fertility and structural integrity.

The main indicator of systemic soil pathology is catastrophic dehumification: a comparison of empirical data from 2025 with the simulated pre-conflict state revealed a drop in humus content from 2.5-3.0% to an average of 1.4%. This process, described by J.P. Hupy & R.J. Schaetzl (2013) as "bombturbation", leads to physical burning of organic matter and mixing of the humus horizon with iluvial layers. According to N. Kussul *et al.* (2023), such destruction in a war zone is accompanied by a complete loss of stability of soil aggregates. The loss of humus automatically leads to a decrease in the cation exchange capacity, which in conditions of light granulometric composition in the soils of Polissia makes them a transit environment for toxicant migration.

Discussion of the results obtained in the context of global research suggests that the identified changes are sustainable. In particular, O.V. Dmytrenko *et al.* (2023) highlighted the irreversibility of organic carbon loss due to pyrogenic degradation. Current data support this hypothesis, since even three years after the active phase of hostilities, the recovery of the humus layer is minimal. The drop in organic content is accompanied by persistent acidification of the soil solution (pH decreased from 5.8-6.2 to 5.2). According to W. Leal Filho *et al.* (2024), changes in acidity under the influence of pyrogenic factors are a key mechanism that radically increases the mobility of heavy metals.

Special attention should be paid to the vertical distribution of pollutants. The content of chromium (10.02-18.10 mg/kg) and nickel (4.54-8.53 mg/kg) in the selected samples did not exceed the standard MPC, but their distribution was abnormal – the concentrations increased with depth. The Pearson correlation coefficient between depth and chromium content ($r \approx +0.92$) mathematically proved the mechanical inversion hypothesis. This is consistent with the findings of K. Petrushka *et al.* (2023), who pointed out that artificial mixing of layers creates new geochemical barriers at atypical depths. The distribution of petroleum products in the profile acted as a unique tracer. The detected peak concentration (66.7 mg/kg) at a depth of 30-40 cm against the background of low content in the upper layer (13.3 mg/kg) is evidence of overlap of the contaminated surface with inverted soil layers. The issue of such "buried" tanks was analysed in detail in the paper by P. Pereira *et al.* (2022), which noted that they act as sources of secondary contamination for decades. Data on mobile forms of nitrogen (26.3 mg/kg), phosphorus (122.4 mg/kg), and potassium (124.6 mg/kg) showed high values against the background of humus deficiency. This condition can be classified as "fragile fertility" – an artificial agrochemical balance that masks the functional collapse of the ecosystem. As noted by I. Bulba *et al.* (2024) in their research, this condition leads to rapid leaching of nitrates into groundwater due to the lack of a sorption complex, which creates risks also described in the paper by B. Biyashev *et al.* (2023). Comparison of recovery dynamics revealed the phenomenon of heterogeneous recovery. If the content of petroleum products showed a tendency to natural attenuation, which is consistent with the findings of M. Solokha *et al.* (2024), humus values and structural characteristics remained consistently low, requiring special reclamation measures.

Thus, the results of the study showed that the military impact caused not just a change in individual indicators, but a complete restructuring of the functioning of the soil system. The results obtained in this study indicate a limited potential for self-renewal of such ecosystems. Without targeted phytoremediation and the introduction of structuring agents, the soil remains in a state of functional collapse, turning from a living system into an inert environment, which becomes only a transit zone for the spread of pollutants to adjacent environments – groundwater and food chains.

Conclusions

A comprehensive study of the transformation of sod-podzolic soils in Kalynivka indicates that military degradation is a multi-factor process, where the dominant factor is physical destruction (bombturbation), which becomes a trigger for a cascade of negative agrochemical and geochemical changes. The main quantitative evidence of systemic destruction of the soil body is the results of correlation analysis: an abnormally high positive relationship ($r \approx +0.92$) was established between the depth of occurrence and the concentration of chromium and nickel. This specific stratigraphy, in which metal concentrations increase towards the lower horizons (Cr – from 10.02 to 18.10 mg/kg, Ni – from 4.54 to 8.53 mg/kg), is a direct indicator of mechanical inversion of the profile and "scalping" of the upper layers. Empirical evidence confirmed a catastrophic loss of organic matter: the humus content decreased from pre-conflict 2.5-3.0% to a critical level of 1.4%, indicating deep dehumification due to pyrogenic effects and mixing of genetic horizons. Concomitant acidification of the soil solution (a decrease in pH from 6.2 to 5.2) creates conditions for the transition of heavy metals to mobile, phytotoxic forms. The phenomenon of "fragile fertility" was identified, when locally high values of mobile nitrogen (26.3 mg/kg), phosphorus (122.4 mg/kg) and potassium (124.6 mg/kg) against the background of humus deficiency indicate instability of the agroecosystem and a high risk of leaching nutrients into ground water.

Geochemical analysis of the distribution of petroleum products revealed a "buried" peak of contamination (66.7 mg/kg) at a depth of 30-40 cm, which allows reconstructing the process of burying the contaminated ground surface under emissions from sinkhole formations. A state of "dissociated reduction" was established, in which relatively rapid self-purification from organic pollutants contrasts with stable physical destruction and lead persistence (Pb ~6.7 mg/kg). This proves that the natural recovery potential of the affected land is limited. Recovery strategies should be based not only on detoxification, but primarily on restoring the physical architectonics of the soil and restoring its biochemical buffering through intensive humification.

Further research should be aimed at developing dynamic models for predicting the migration of toxicants from "buried" layers of pollution to underground horizons. It is also necessary to investigate the effectiveness of various types of phytoremediants and microbial preparations for accelerated restoration of the humus state of soils that have undergone mechanical inversion.

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Conflict of Interest

None.

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Трансформація ґрунтового покриву під впливом воєнної агресії: інтегрована оцінка на прикладі Київського Полісся

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Анотація. Військові дії значно впливають на ґрунтовий покрив, що має серйозні наслідки для екології та продовольчої безпеки. Метою дослідження було проаналізувати зміни в агрохімічних та геохімічних характеристиках ґрунтів внаслідок військових дій 2022 року в Україні. Дослідження фокусувалось на території села Калинівка Фастівського району Київської області на дослідній ділянці місця виливу нафтопродуктів. На основі порівняння емпіричних лабораторних даних (2024 р.) – з модельованими станами доєскалаційного періоду (2021 р.) та періоду гострого впливу (2022 р.) – розкрито багатофакторний характер воєнного впливу на деградацію ґрунтів. Встановлено, що домінуючим фактором руйнування є не хімічна контамінація, а глибоке фізичне порушення ґрунтового профілю: інверсія горизонтів, ущільнення та «скальпування» родючого шару, що сукупно описується терміном «бомботурбація». Проведено детальний кореляційний аналіз, який кількісно підтвердив гіпотезу інверсії: виявлено сильну позитивну кореляцію ($r \approx +0.94$) між глибиною та концентрацією важких металів (Cr), що є аномальним для природних умов. Це явище, разом із критично низьким вмістом гумусу та аномальним розподілом нафтопродуктів, свідчить про поховання початково забрудненої денної поверхні. Аналіз часової динаміки виявив феномен «роз'єднаного відновлення», за якого швидка біодеградація органічних полютантів контрастує зі стійкістю фізичних пошкоджень та забруднення важкими металами. В роботі підкреслено, що ігнорування фізичної складової деградації та спроби ведення сільського господарства за традиційними схемами неминуче призведуть до посилення екологічних ризиків та подальшої втрати аграрного потенціалу. Результати дослідження можна використати для розробки стратегій відновлення ґрунтів на постраждалих територіях України, що сприятиме зниженню екологічних ризиків і відновленню аграрного потенціалу

Ключові слова: деградація ґрунтів; воєнний конфлікт; бомботурбація; важкі метали; нафтопродукти; інверсія профілю; кореляційний аналіз

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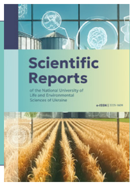

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The effect of preventive treatments with biological preparations on maize productivity

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Abstract. The purpose of the study was to determine the effect of different preventive protection schemes on the phytosanitary condition of the crop, plant growth, yield formation, and grain quality under moisture-deficit conditions. The study used analysis of variance, the method of averages, the method for estimating the mean error, and the method for determining the least significant difference. The results show that chemical protection restricted the development of harmful organisms most strongly: root rot development decreased from 18.6% to 5.1%, leaf spots – from 22.4% to 6.3%, and damage by the European corn borer – from 21.7% to 4.1%. Technical efficacy reached 74.8% against diseases and 81.2% against pests. Biological protection was accompanied by a higher level of preservation of predatory arthropods and actinomycetes, at 54.1%, and by 57.6% when biological preparations were combined with a growth stimulant, compared with 16.3% under the chemical scheme. The combined biological variant showed better biometric parameters: field germination was 90.2%, plant height – 229.4 cm, leaf surface area – 47.6 dm², and chlorophyll content – 47.2 units. In the yield structure of this variant, ear length reached 20.7 cm, average ear mass – 238.0 g, the number of grains per ear – 694, and the 1000-grain mass – 332 g. Grain yield increased from 13.68 to 15.88 t/ha, and yield preservation was 16.1%. Grain quality also improved: test weight increased from 712 to 733 g/l, protein content – from 8.6% to 9.1%, and the proportion of filled grain – from 84.2% to 90.6%, whereas the proportion of damaged grain decreased from 9.8% to 5.0%. The practical value of the study lies in the possibility of using its results by agronomists,

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farmers, plant-protection consultants, and researchers when choosing preventive schemes for maize cultivation under dry conditions

Keywords: growth stimulant; yield; root rots; leaf spots; biological protection

Introduction

The relevance of the study is determined by the increasing effect of drought events on the production process of maize and the need to clarify the crop response to preventive protection systems under water-deficit conditions. When abiotic stress is combined with phytosanitary pressure, the intensity of growth processes, the condition of the leaf apparatus, the formation of generative organs, and grain quality change. In this context, analysis of the effect of biological preparations in preventive schemes becomes important for defining their role both in restraining a complex of harmful organisms and in changing biometric parameters, yield structure, and the level of grain productivity. The combination of biological protection with a growth stimulant is of separate importance, as this scheme requires experimental confirmation under field conditions. The research problem is that the assessment of preventive maize treatments is often presented by separate groups of traits without their sequential analysis within a single production process. This concerns, in particular, the relationship between protection efficacy against diseases and pests, changes in morphological and physiological plant parameters, the formation of yield-structure elements, and the qualitative composition of grain. The comparison of chemical, biological, and combined preventive-protection schemes under a dry background also requires clarification.

With reference to agro-biological characteristics, V. Mazur *et al.* (2023) summarised the conditions for growing maize hybrids in the Right-Bank Forest-Steppe and reported the dependence of productivity on the combination of hybrid properties and technological elements. The researchers stressed the importance of crop adaptability to environmental conditions, which brought that work closer to the assessment of preventive treatments in the field cenosis. In relation to climate variability, P. Boiko *et al.* (2024) analysed the efficiency of maize production in Ukraine when high-yielding hybrids and technologies with elements of biologisation were used. The authors stated that combining the genetic potential of the crop with technological solutions was accompanied by a change in agrocenosis resistance to stress factors. In the technological aspect, A. Drobitko & T. Kachanova (2023) described approaches to developing biologised maize-growing technologies and including biological preparations in the field-protection system. The role of the biological component was presented as part of the general technology, where the phytosanitary effect was combined with changes in growth and yield. At the pre-sowing level, V. Cano Camacho *et al.* (2023) evaluated the effect of biological maize seed treatments on plant growth, soil condition, and yield. It was reported that the use of biological agents was accompanied by changes in crop development and productivity indicators.

C. Sible & F. Below (2023) considered the role of biological preparations in increasing the efficiency of nutrient use by maize and soybean in plant nutrition. Their paper identified a relationship between the biological component of the technology and changes in plant resource provision, which was reflected in growth and grain filling. In the phytosanitary direction, S. Nada *et al.* (2022) examined preventive strategies for mycotoxin control in the wheat and maize chain. Attention was focused on preventive measures associated with reducing contamination and preserving the quality of grain products. In antifungal protection, O. Degani & A. Gordani (2022) evaluated the effect of 6-pentyl-alpha-pyrone against the maize late-wilt pathogen Magnaporthiopsis maydis. Biologically active fungal metabolites were considered a separate tool for restraining pathogens in the maize protection system. Within the microbiome approach, O.P. Omotayo & O.O. Babalola (2023) analysed the potential of a favourable phytomicrobiome in the biocontrol of Fusarium verticillioides in maize crops. The authors stressed the importance of microbial associations as a factor limiting fungal infection and stabilising the phytosanitary condition of plants.

In the field of soil ecology, V. Ninkuu *et al.* (2025) summarised the effect of returning plant residues to the soil on its ecological condition and crop yield. The researchers noted that changes in the soil organic background were combined with shifts in biological activity that were important for plant growth and yield formation. In the nutrition system, P. Hou *et al.* (2025) analysed the optimisation of maize yield under partial replacement of mineral fertilisers with organic ones. The authors reported that fertiliser adjustment changed crop-growth conditions and was accompanied by simultaneous changes in productivity and soil properties. In the context of environmentally safe protection, O.M. Darwesh & I.E. Elshahawy (2023) investigated the use of aqueous vermicompost extract and a biochar derivative to limit sunflower charcoal rot and maize late wilt. The study demonstrated that natural liquid and solid-like substrates were considered components of preventive restraint of vascular and root infections. Within ecological assessment, E. Montemayor *et al.* (2022) carried out a critical analysis of datasets for organic crop-production systems. Attention was focused on the structure of input resources and technological operations, which provided a methodological basis for interpreting biologised crop-growing schemes. The combination of biological preparations with a growth stimulant under drought conditions also remains insufficiently explored, when the response of maize to protective measures is most contrasting. The purpose of the study was to determine the effect of preventive treatments with biological preparations on maize productivity under dry conditions. The objectives of the study were to assess the efficacy of preventive treatments against a complex of harmful organisms, clarify their effect on the growth and development of maize plants, and determine changes in yield-structure elements, yield, and grain-quality indicators.

Materials and Methods

Conditions for establishing and conducting the field experiment

The practical field experiment was established according to a randomised block design with four replications; within each variant, 25 plants were assessed for phytosanitary and biometric determinations, 20 ears were analysed to assess yield structure, 3 average grain samples were collected to determine quality indicators, and 3 soil samples from the arable layer were taken for agrochemical analysis. Field research was conducted in 2025 at the limited liability company AC (agricultural cooperative) PROMIN in the village of Mala Klitna, Khmelnytskyi region, Ukraine. The experiment was established in crops of the maize hybrid DEKALB 4014. The 2025 growing season was characterised by dry conditions; meteorological data were obtained from the Time and Date (n.d.) automatic weather station and field observations. For the study area, the average air temperature in May-September according to climatic norms was 15-20°C, and the total precipitation was 329.1 mm; its uneven distribution created a stressful background for maize growth and the development of a complex of harmful organisms. The soil of the experimental site was typical medium-loamy chernozem. In the 0-30 cm arable layer, humus content was 3.42%, the pH of the salt extract was 6.5, nitrate nitrogen content was 18.6 mg/kg, available phosphorus was 62.4 mg/kg, and exchangeable potassium was 91.7 mg/kg. Winter wheat was the preceding crop. Primary tillage included stubble disking to a depth of 8-10 cm and ploughing to 25-27 cm. Pre-sowing tillage was performed by cultivation at 5-6 cm with simultaneous levelling of the field surface. During the research, the provisions of Convention on Biological Diversity (2016) and the ethical standards of Ethics and data protection (2021) concerning research ethics and data protection were observed.

The fertilisation system was the same for all variants and included the application of $N_{90}P_{60}K_{60}$. Double superphosphate at 130 kg/ha and potassium chloride at 100 kg/ha were applied under primary tillage; ammonium nitrate at 150 kg/ha was applied before pre-sowing cultivation; and carbamide at 100 kg/ha was applied as top dressing at the 6-8 leaf stage. Sowing was conducted in April 2025 using the dotted-row method with 70 cm row spacing and a seeding rate of 75 thousand viable seeds per hectare. Milagro 040 SC herbicide at 1.0 l/ha was applied for weed control at the 4-5 leaf stage of maize. The working-fluid rate was 200 l/ha. The experiment was established according to a randomised block design with four replications. The total plot area was 56 m^2, and the accounting area – 42 m^2. The experimental scheme included four variants: untreated control; chemical protection using the fungicide Amistar Extra 280 SC at 0.75 l/ha and the insecticide Karate Zeon 050 CS at 0.2 l/ha; biological protection using Trykhodermin M at 2.0 l/ha, Planriz M at 2.0 l/ha, Biplan M at 1.5 l/ha, Bitoxibacillin M at 3.0 kg/ha, and Lepidocid M at 2.0 kg/ha; and biological protection combined with the growth stimulant Vympel 2 at 0.5 l/ha against the background of the same biological preparations. Preventive treatments were carried out three times: at the 3-5 leaf stage, at the 7-9 leaf stage, and before tasselling. Spraying was performed with an OPSH (field boom sprayer, Ukraine)-2000-18 boom sprayer in the morning from 6:00 to 9:00 or in the evening from 18:00 to 20:00 at an air temperature not exceeding 25°C and wind speed not exceeding 3-4 m/s. The working-fluid rate for all treatments was 200 l/ha. All other elements of the maize-growing technology were the same for all experimental variants.

Assessment of the efficacy of preventive maize protection under dry conditions

Root rot development was determined by visual assessment of plants using an Eschenbach mobilux 6× hand magnifier (Eschenbach Optik GmbH, Germany) on a 5-point damage scale, where 0 means no symptoms, 1-2 – weak and moderate damage, and 3-5 – severe and very severe damage. Leaf spot development was determined visually on the accounting plants by the proportion of affected leaf surface using a Levenhuk Zeno Handy ZH7 5×/16 mm hand magnifier (Levenhuk Inc., USA) on the same 5-point scale as root rot development. The assessment was conducted by two specialists with higher agronomic education, one of whom held a Candidate of Agricultural Sciences degree. The calculation was performed as the mean percentage of damage for the assessed plants:

$$R = \frac{\sum p_i}{n}, \tag{1}$$

where cap R – average severity of leaf spot disease, %, p_i – proportion of the leaf surface affected in the i-th plant, %, n – number of plants examined, pcs. Stem rot development was determined after dissecting typical stems, followed by assessment of the degree of damage to internal and external tissues. This separately accounted for the spread of destructive processes in the stem complex at the end of the growing season. A Fiskars Solid P321 pruner (Fiskars Group, Finland) was used for dissection. Plant damage by the European corn borer was determined by the proportion of plants with characteristic damage to stems, leaves, and ears. This count assessed the spatial spread of one of the main maize phytophages within the crop. The calculation was performed using the formula:

$$P = \frac{n}{N} \times 100, \tag{2}$$

where P – damage, %, n – number of damaged plants, N – total number of plants surveyed. Aphid infestation was determined by the proportion of plants on which aphid colonies were detected on leaves, sheaths, or stems. This count reflected the degree of spread of sucking insects in the phytocenosis. Technical efficacy against diseases was determined by comparing disease development in the control and treated variants. This value assessed the degree of reduction in infection pressure after application of the preventive schemes. The calculation was performed using the formula:

$$E = \frac{R_k - R_d}{R_k} \times 100, \tag{3}$$

where E – the technical efficiency, %, R_k – the disease progression in the control group, %, R_d – the disease progression in the experimental group, %. Preservation of predatory arthropods and actinomycetes was determined by the ratio between the number of beneficial organisms in the experimental and control variants, and the generalised value for both groups was obtained by integral averaging of normalised indicators. The extent to which the protection system changed the condition of the accompanying biota of the agrocenosis was assessed. Predatory arthropods were counted with a BioQuip Standard Sweep Net (BioQuip Products, USA) in the morning from 7:00 to 9:00, with 10-series sweeps performed on each plot, and the total number of captured individuals was then counted. Actinomycetes were counted by serial dilution of the soil suspension, plating in triplicate on starch-ammonia medium, and cultivation in a Memmert IN55 thermostat (Memmert GmbH, Germany) at 28°C for 7 days. The calculation was performed using the formula:

$$S = \frac{N_d}{N_k} \times 100, \tag{4}$$

where S – survival of beneficial organisms, %, N_d – number of predatory arthropods or actinomycetes in the experimental treatment, N_k – number of predatory arthropods or actinomycetes in the control. Technical efficacy against pests was calculated by the ratio between plant damage or infestation in the control and the experimental variants. The result showed the extent to which the entomological load decreased under the influence of treatments.

Method for determining biometric indicators of maize after treatments

Field germination was determined by counting the number of plants on fixed row segments, followed by recalculation relative to the number of seeds sown. This count established the degree of uniformity in crop formation at the initial stage of development. The calculation was performed using the formula:

$$G = \frac{n}{N} \times 100, \tag{5}$$

where G – the field germination rate, %, n – the number of seedlings emerged, pcs, N – the number of viable seeds sown, pcs. Plant height at the flowering stage, cm, was measured from the soil surface to the top of the tassel using a Stanley 2 m measuring rod (Stanley Black & Decker, USA). This count was used to compare the intensity of stem elongation between the investigated schemes.

Ear placement height was measured from the soil surface to the node of attachment of the upper productive ear using the same measuring rod. This traced the features of the spatial position of the generative organ on the plant. Stem diameter was determined with a Yato YT-7201 calliper (TOYA S.A., Poland) in the middle part of the lower internode. The obtained data were used to assess stem-tissue development and mechanical strength of the plants. The number of leaves per plant was established by direct counting on typical plants with accounting specimens marked using Garden Line plastic field labels (Bradas Group, Poland). This count compared the degree of leaf-apparatus development under different protection schemes. The leaf surface area of one plant was determined from the length and maximum width of leaves using a Topex measuring ruler (Grupa Topex, Poland). The calculated value provided a quantitative expression of the assimilation surface of one plant. The calculation was performed using the formula:

$$S = \sum(L \times B \times k), \quad (6)$$

where cap S is the leaf area of a single plant, cap L is the leaf length (cm), cap B is the maximum leaf width (cm), and k is the leaf shape coefficient. Plant survival until harvest was calculated as the ratio between the number of plants before harvest and the number of plants at the full-emergence stage. This value reflected the final level of crop preservation during the growing season. The calculation was performed using the formula:

$$V = \frac{N_z}{N_s} \times 100, \quad (7)$$

where cap V – plant survival rate prior to harvest, %, cap N_z – number of plants prior to harvest, pcs, cap N_s – number of plants at the full emergence stage, pcs. Chlorophyll content in leaves, SPAD (Soil Plant Analysis Development), was determined with a portable Konica Minolta SPAD-502Plus chlorophyll meter (Konica Minolta, Japan). This count was used to compare the functional condition of leaf tissue between variants.

Approaches to assessing maize yield-structure elements

Average ear length was measured with a Topex ruler after the husks were removed. This count provided an idea of the linear development of the generative organ. Average ear diameter was determined with a Yato YT-7201 calliper in the central part of the ear. The obtained values were used to compare the transverse development of ears. Average ear mass was established using OHAUS Pioneer PA4102C electronic laboratory scales (OHAUS Corporation, USA). This count assessed the overall formation of the ear as a reproductive organ. The number of grain rows per ear was determined by direct counting after the ear was fully cleaned. The number of grains per row was established by manually counting grains in a typical row of the ear. The average number of grains in one ear was calculated as the product of the number of grain rows and the number of grains per row. This calculation provided an integral indicator of the grain capacity of the ear. The formula was as follows:

$$N = a \times b, \quad (8)$$

where cap N is the average number of kernels per cob, pcs., a is the number of rows of kernels per cob, pcs., and b is the number of kernels per row, pcs. The average grain mass from one ear was determined after threshing the ears using OHAUS Pioneer PA4102C laboratory scales. This count was used to assess the actual contribution of one ear to total grain production. The 1,000-grain mass was determined on OHAUS Pioneer PA4102C electronic laboratory scales after counting and weighing a standard sample. The obtained values allowed comparison of the degree of grain filling between the investigated schemes.

Assessment of changes in maize yield under the influence of treatments

Plant density before harvest was determined by counting plants within a 1 m^2 metal frame, followed by recalculation per hectare. This count showed the final number of standing plants per area. The calculation was performed using the formula:

$$D = \frac{n \times 10{,}000}{S}, \quad (9)$$

where D – the plant density prior to harvesting, plants/ha; n – the number of plants in the sample plot, plants; S – the area of the sample plot, m^2. The number of productive ears was determined by counting ears in the same accounting area, followed by recalculation per hectare. This indicator assessed the frequency of generative completion of plants in the crop. The proportion of productive plants was calculated as the ratio between the number of productive ears and the number of plants before harvest. This calculation compared the specific contribution of generatively completed plants within the standing crop using the formula:

$$P = \frac{N_k}{N_r} \times 100, \quad (10)$$

where P – proportion of productive plants, %, N_k – number of productive cobs, pcs, N_r – number of plants prior to harvest, pcs. Grain yield was determined by complete harvesting of each accounting plot with weight accounting of the harvest and subsequent recalculation to standard moisture. The obtained value served as the final quantitative expression of crop productivity. The calculation was performed using the formula:

$$Y = \frac{m \times 10}{S}, \quad (11)$$

where Y – the grain yield, tonnes per hectare; m – the grain weight from the sample plot, kilograms; S – the area of the sample plot, square metres. Yield increase relative to the control was calculated as the difference between the yield of the experimental variant and the control. This value showed the absolute increase in grain production relative to the untreated background and was calculated using the formula:

$$\Delta Y = Y_d - Y_k, \quad (12)$$

where ΔY – the increase in yield compared to the control, t/ha; Y_d – the yield in the experimental treatment, t/ha; Y_k – the yield in the control, t/ha. Yield preservation was determined as the percentage excess of the yield of the experimental variant over the control. This calculation assessed the relative volume of preserved grain production. The formula was as follows:

$$Z = \frac{Y_d - Y_k}{Y_k} \times 100, \quad (13)$$

where Z – harvest retention, %, Y_d – yield in the experimental treatment, t/ha, cap Y_k – yield in the control, t/ha. Grain mass from 1 m^2 was determined by weighing the grain obtained from the accounting area on OHAUS Pioneer PA4102C scales. This count compared the actual output of grain mass from a unit area.

Assessment of the effect of preventive treatments on grain quality

Grain moisture at harvest was determined using a Wile 55 electronic moisture meter (Farmcomp Oy, Finland). This compared the condition of grain at harvest and supported the subsequent recalculation of yield. Grain test weight was determined using a Schopper Type Grain Test Weight Apparatus (Pfeuffer GmbH, Germany). This indicator compared grain bulk density and the level of filling. Protein content was determined by near-infrared spectroscopy using an Inframatic 9500 analyser (Perten Instruments, Sweden). It was used for the quantitative assessment of nitrogenous-compound accumulation in grain. Starch content was determined using the Inframatic 9500 analyser. The obtained values characterised the carbohydrate component of grain production. Crude fat content was established by the extraction method using a Soxhlet VELP SER 148 apparatus (VELP Scientifica, Italy). This assessed the lipid part of the chemical composition of grain. The proportion of filled grain was determined after fractional sorting of the sample, separation of fully formed kernels, and subsequent weighing of fractions on OHAUS Pioneer PA4102C laboratory scales. The indicator was used to establish the proportion of grain with complete filling and formed endosperm. The calculation was performed using the formula:

$$F = \frac{m_1}{m} \times 100, \quad (14)$$

where F – the percentage of filled grains, %, m_1 – the mass of filled grains in the sample, g, m – the total mass of the sample, g. The proportion of shrivelled grain was established during fractional sorting of the sample by separating underdeveloped kernels and subsequently weighing them on OHAUS Pioneer PA4102C laboratory scales. This quantified the proportion of grain with incomplete filling. The proportion of damaged grain was determined after manual sorting of the sample with separation of kernels with mechanical, entomological, and pathological damage, followed by weighing on OHAUS Pioneer PA4102C laboratory scales. Experimental data were processed using analysis of variance based on four replications of the experiment. Mean values were determined for each indicator and served as the basis for comparing the variants under study. Differences between schemes were assessed using one-way analysis of variance, which identified the direction and pattern of changes in the investigated traits. Calculations were performed in Statistica 10.0 (StatSoft Inc., USA) and Microsoft Excel 2019 (Microsoft Corporation, USA).

Results

Under dry conditions, preventive maize treatments changed the course of phytosanitary processes in crops through limitation of disease development, reduction of plant damage by phytophages, changes in the technical efficacy of protection, and different degrees of preservation of the accompanying biota. The lowest level of root rot development was recorded in the chemical-protection variant, where the value was 5.1%. This result reflected the most pronounced limitation of the infection process among the schemes under study. Within this variant, pathogen restraint occurred both at the level of individual plants and within the crop in general, which pointed to a decrease in infection pressure during critical stages of crop development. Under drought conditions, this was accompanied by a lower risk of further plant weakening since moisture deficiency did not remove the harmfulness of infections, but combined with it within a single stress complex. Biological protection also reduced disease development, although the degree of infection suppression was lower. In this variant, pathogen spread was limited within margins that prevented a rapid increase in pathological manifestations in the crop. After the growth stimulant was included in the biological system, the level of damage decreased further, reflecting weaker disease development within the same protection scheme.

A similar pattern was recorded for plant damage by phytophages. In the chemical-protection variant, its level decreased to 4.1%, demonstrating the most pronounced restriction of insect damage among all investigated schemes. This change reflected a sharp decrease in the trophic activity of phytophages during the formation of vegetative mass and generative organs. Under drought conditions, damage to the leaf apparatus and stems was accompanied by direct losses of photosynthetically active surface and disturbance of assimilate distribution between vegetative and reproductive organs. In the biological variant, damage also decreased, but not as sharply as after chemical treatment. This pointed to another type of regulation of phytophage number and activity, under which their presence in the crop was not eliminated completely, but their harmfulness was restrained at a lower level. In the biological protection variant with the growth stimulant, this manifestation weakened further, reflecting a lower degree of damage to the plant cover under the combined action of the preparations.

The level of technical protection efficacy assessed the generalised action of the treatment systems against the complex of harmful organisms. Its highest value was recorded in the chemical scheme, where it reached 81.2%. This reflected the maximum degree of direct control of diseases and phytophages within the experiment. This indicator represented the intensity of protective action but did not cover accompanying changes in the structure of the agrocenosis. In the biological variant, technical efficacy was lower; however, the results show that biological preparations restrained harmful organisms without sharp interference in the accompanying biotic components. After the growth stimulant was included, technical efficacy increased, reflecting greater effectiveness of the biological scheme against the investigated complex of harmful objects. This change was evidence of an expansion of the action range of the system, in which the protective effect was combined with changes in the condition of plants sensitive to water stress. The preservation level of predatory arthropods and actinomycetes in the biological protection variant was 54.1%, reflecting higher preservation of beneficial components of the agrocenosis compared with the chemical scheme. This result demonstrates that the use of biological preparations was characterised by less disturbance of the accompanying biota structure. Under limited moisture, recovery of microbiological and trophic links in the crop occurred more slowly than under a more stable water regime; therefore, preservation of entomophages and actinomycetes was directly related to the condition of internal regulation of the agrocenosis during the growing season. In the chemical variant, this level was substantially lower, which corresponded to a deeper interference in the biological structure of the crop. The combination of biological preparations with the growth stimulant preserved a substantial share of beneficial biota; therefore, within this system, limitation of harmful organisms was combined with less disturbance of accompanying groups (Table 1).

Table 1. *Efficacy of preventive maize treatments against a complex of harmful organisms under dry conditions*

Indicator	Control	Chemical protection (reference fungicide + + insecticide)	Biological protection (Trichodermin M + + Planriz M + Biplan M + Bitoxibacillin M + + Lepidocide M)	Biological protection + growth stimulant (Trichodermin M + + Planriz M + Biplan M + Bitoxibacillin M + + Lepidocide M + Vympel 2)
Root rot development, %	18.6	5.1	6.8	5.9
Leaf spot development, %	22.4	6.3	8.1	7.0
Stem rot development, %	14.8	2.0	3.7	2.9
Plant damage by European corn borer, %	21.7	4.1	6.5	5.4
Aphid infestation of plants, %	19.3	3.8	6.2	5.1
Technical efficacy against diseases, %	0.0	74.8	67.9	71.3
Technical efficacy against pests, %	0.0	81.2	69.8	74.1
Preservation of predatory arthropods and actinomycetes, %	48.0	16.3	54.1	57.6

Source: compiled by the authors

The results show that, under drought conditions, chemical protection ensured the lowest levels of disease development and plant damage by phytophages, along with the highest technical efficacy. Biological protection had a lower degree of direct control but higher preservation of predatory arthropods and actinomycetes. Inclusion of the growth stimulant in the biological scheme was accompanied by a reduction in disease manifestation and plant damage, together with higher technical efficacy, while a substantial share of beneficial biota was preserved. The obtained changes reflected differences between protection systems in the direction of their effect on the phytosanitary condition of the crop and the biological structure of the agrocenosis under insufficient moisture supply. Differences between variants were recorded at the seedling-formation stage. In the biological protection system with the growth stimulant, field germination reached 90.2%. This value was higher than both the control and the separate biological protection without the stimulant. This relationship pointed to a more uniform formation of initial crop density within this variant. For maize under drought, this was methodologically important, as uneven emergence is usually accompanied by stronger intraspecific competition, differences in the growth rates of individual plants, and subsequent stratification of the standing crop by morphological traits. In the control variant, early development was less stable, whereas preventive treatments increased the number of viable plants at the initial stage. Chemical protection was also combined with improvement of this indicator; however, within the biological scheme, after the stimulant was introduced, crop growth from the first vegetation stages was characterised by greater uniformity.

Subsequently, differences between variants remained at the level of above-ground mass formation. In the biological system with the growth stimulant, plant height was 229.4 cm. This value characterised more intensive stem elongation compared with the control and the variant in which only biological preparations were used. Under drought conditions, this difference was related to the specific course of vegetative growth under limited moisture supply. Reduced height under such conditions is usually combined with shorter internodes, earlier growth inhibition, and lower accumulation of above-ground mass. Against this background, taller plants in the treated variants showed less pronounced growth suppression. The chemical scheme was also accompanied by a greater height compared with the control, but in the case of biological protection, after the stimulant was included, this trait was expressed more fully. This distribution between variants was consistent with the differences previously recorded for the phytosanitary condition of the crop. The largest leaf surface area of one plant was recorded in the biological protection variant with the growth stimulant and was 47.6 dm^2. This value characterised a higher level of assimilation-surface development compared with the control and the variant of separate biological protection. Under dry conditions, leaf area had a direct relationship with the functional condition of the crop because a reduction in leaf surface is usually accompanied by a decrease in photosynthetically active tissue and faster transition of plants to a stricter water-saving regime. In the control variant, the leaf apparatus was less developed, which was consistent with the lower level of plant-cover preservation during the growing season. In the chemical and biological variants without the stimulant, this indicator was also higher, but in the combined biological scheme, leaf surface area remained the largest. Within the experiment, this characterised fuller leaf expansion and better preservation of the assimilation apparatus during the dry period.

The functional condition of leaf tissue was also traced through chlorophyll content. The highest value was recorded in the same variant and was 47.2 SPAD. This level characterised higher preservation of the leaf pigment complex compared with the control, where the assimilation apparatus was in a less stable condition. For maize under moisture deficit, this was particularly important for assessing the physiological condition of the crop, because a decrease in chlorophyll is usually associated with weaker synthetic processes, accelerated leaf ageing, and lower intensity of organic matter accumulation. In the chemical variant, this indicator was also higher than in the control, characterising better preservation of functional leaf activity. However, in the biological protection variant with the stimulant, the difference was more pronounced. This supported consideration of the combination of biological preparations and the growth stimulant as a system associated with a higher level of pigment-apparatus preservation under dry conditions. The presented biometric traits did not exist separately from one another but characterised sequential stages of plant development, from emergence to the condition of the leaf apparatus in the main vegetation stages. A higher level of field germination was combined with greater crop uniformity, which was accompanied by higher plant-height values. In turn, more intensive formation of above-ground mass corresponded to a more developed leaf surface and higher chlorophyll content. In the control variant, these traits were expressed more weakly. Under chemical protection, biometric characteristics improved compared with the control. Biological protection without the stimulant was also characterised by a positive shift in most traits, but the highest values for the four considered characteristics were recorded in the variant combining biological preparations with the growth stimulant (Table 2).

Table 2. *Effect of preventive treatments on biometric indicators of maize plants*

Indicator	Control	Chemical protection (reference fungicide + + insecticide)	Biological protection (Trichodermin M + + Planriz M + Biplan M + Bitoxibacillin M + + Lepidocide M)	Biological protection + growth stimulant (Trichodermin M + Planriz M + Biplan M + Bitoxibacillin M + + Lepidocide M + Vympel 2)
Field germination, %	86.9	88.1	88.4	90.2
Plant height at flowering, cm	214.3	223.8	221.6	229.4
Ear placement height, cm	78.6	82.4	81.8	84.9
Stem diameter, mm	19.7	20.9	20.6	21.5
Number of leaves per plant, pcs.	12.4	12.8	12.7	13.1
Leaf surface area of one plant, dm^2	42.8	45.2	44.7	47.6
Chlorophyll content in leaves, SPAD	42.6	45.1	44.8	47.2
Plant survival until harvest, %	82.4	87.1	86.5	89.3

Source: compiled by the authors

Thus, under dry conditions, preventive treatments were associated with changes in maize biometric indicators at different growth stages, from seedling formation to the condition of the leaf apparatus during the growing season. The most uniform initial development, greater plant height, more developed leaf surface, and higher chlorophyll content were recorded in the biological protection variant with the growth stimulant, whereas these traits were weaker in the control and occupied an intermediate position in the other schemes. This relationship reflected differences between variants in individual morphological characteristics and in the degree of plant-cover preservation under insufficient moisture supply. Within the experiment, the recorded changes covered both indicators characterising initial development and crop uniformity and traits associated with above-ground mass formation and the functional condition of the assimilation apparatus. This supported the interpretation of the biometric response of maize not as a set of isolated indicators but as a system of interrelated manifestations that characterised the general condition of plants during growth under drought.

Preventive maize treatments under dry conditions were accompanied by changes in yield-structure elements, which appeared in the relationship between the morphometric characteristics of the ear and traits associated with the formation of the grain part of the yield. In the biological protection variant with the growth stimulant, ear length was 20.7 cm, whereas ears in the control were shorter. This difference characterised a higher level of reproductive-organ development under conditions in which moisture deficiency is usually accompanied by a shorter period of active tissue growth and reduced linear ear parameters. Within the experiment, ear length was not considered a self-sufficient morphometric trait but a characteristic associated with the capacity of the generative structure. Higher values of this indicator in the treated variants corresponded to the formation of ears with fuller development of the axis and better prerequisites for grain-row initiation. In the chemical variant, this trait was also higher than in the control, characterising fuller ear formation under limited moisture supply. Biological protection without the stimulant also increased this indicator, but the most pronounced shift was recorded in the combined biological scheme. This relationship reflected differences between treatment systems already at the level of the spatial organisation of the reproductive organ.

A similar shift was recorded for average ear mass. In the biological protection variant with the growth stimulant, it reached 238.0 g, characterising the fullest formation of generative mass among the investigated schemes. Unlike ear length, this indicator covered morphological size, the degree of tissue filling, the ratio between the cob and grain part, and the general level of dry-matter accumulation within one generative organ. In the control, ears were lighter, which was consistent with weaker yield-structure formation under drought. This distribution of variants reflected unequal levels of realisation of the generative potential of plants. Within the combined biological scheme, greater ear mass was associated with fuller development of its grain part, whereas in the control, the decrease in this trait corresponded to less pronounced mass accumulation in the reproductive organ. Under dry conditions, this was methodologically important, since ear mass is quite sensitive to changes in the water regime and the general condition of plants during flowering and grain filling.

The highest value was recorded in the biological protection variant with the growth stimulant and was 694 grains. This trait characterised the completeness of reproductive-function realisation at the level of an individual plant and reflected the extent to which the formed generative structure retained potential for further filling. For maize under dry conditions, this indicator was sensitive, because water deficit in critical stages is often combined with partial under-pollination, reduction of ovaries, or underdevelopment of some kernels in the upper part of the ear. In the control, the number of grains was lower, which corresponded to less complete realisation of the reproductive capacity of the plant cenosis. In the chemical variant, this indicator increased, and in the scheme with biological protection without the stimulant, it also exceeded the control level, but the maximum value was recorded precisely in the variant with combined application of biological preparations. This relationship characterised greater ear capacity and fuller preservation of the grain row along its entire length. Within yield structure, this trait was of key importance because it combined the morphometric parameters of the ear with the actual level of realisation of its generative load.

The 1,000-grain mass also required consideration as an integral characteristic of individual kernel development. In the biological protection variant with the growth stimulant, it was 332 g, the highest value among the investigated schemes. This trait characterised not the number of formed grains but the degree of their filling, density, and intensity of matter accumulation during the filling period. In the control, this indicator was lower, which corresponded to weaker kernel filling against a background of limited moisture supply. In the chemical variant and in the scheme with biological preparations without the stimulant, the 1,000-grain mass also increased, but its greatest value was characteristic of the combined biological system. This relationship showed that differences between variants were traced both at the level of grain number and within the individual formation of each kernel. In yield structure, this trait completed the chain of morphological and productive characteristics, linking ear parameters with the actual level of grain-mass filling (Table 3). The presented yield-structure elements characterised a sequence of changes that covered ear formation, development of its grain part, and the degree of kernel filling in the final vegetation stages. Increased ear length was accompanied by increased ear mass, and within the same variants, these traits were combined with a larger number of grains and a higher 1000-grain mass. This distribution of indicators reflected a coordinated pattern of yield-structure formation, in which the morphometric parameters of the generative organ were combined with quantitative and weight characteristics of grain production. In the control variant, this complex of traits was weaker, whereas schemes with preventive treatments showed fuller expression of the structural components of yield. This combination was clearest in the biological protection variant with the growth stimulant, where the differences covered not one separate trait but the entire chain of indicators associated with the generative development of maize under dry conditions.

Table 3. *Effect of preventive treatments on maize yield-structure elements*

Indicator	Control	Chemical protection (reference fungicide + + insecticide)	Biological protection (Trichodermin M + Planriz M + Biplan M + Bitoxibacillin M + Lepidocide M)	Biological protection + growth stimulant (Trichodermin M + Planriz M + Biplan M + + Bitoxibacillin M + Lepidocide M + Vympel 2)
Average ear length, cm	18.7	20.0	19.6	20.7
Average ear diameter, cm	4.3	4.6	4.5	4.7
Average ear mass, g	196.0	226.0	221.0	238.0
Number of grain rows per ear, pcs.	14.1	15.2	15.0	15.5
Number of grains per row, pcs.	42.0	43.7	43.2	44.8
Average number of grains in one ear, pcs.	592	664	648	694
Average grain mass from one ear, g	182.4	199.8	189.8	209.4
1,000-grain mass, g	308	321	319	332

Source: compiled by the authors

Plant density before harvest required consideration as a characteristic of the actual preservation of the standing crop until the end of vegetation. In the biological protection system with the growth stimulant, it was recorded at 61.1 thousand plants/ha. This crop condition reflected the course of vegetation with fewer plant losses during the season and more uniform preservation of the formed phytocenosis until ripening. In the control, density before harvest remained lower, which was consistent with a greater gap between the formed and actually preserved standing crop. Under chemical protection, crop density also increased, pointing to lower plant losses during critical stages of development. In the scheme with biological preparations without the stimulant, this tendency was also traced, but preservation of the plant composition had not yet reached the degree recorded when biological preparations were combined with the growth-regulating component. This relationship reflected the different effect of preventive treatments on the preservation of the numerical basis of the crop. Within yield formation, this component determined the extent to which the area was provided with plants capable of completing the final development stages without substantial thinning. Therefore, differences between schemes began to appear even before the started the generative stage and created different prerequisites for further formation of productive organs.

The number of productive ears required separate analysis because it reflected the part of the preserved standing crop that completed development with the formation of a full generative organ. In the control, this component was formed at 54.1 thousand ears/ha. This distribution corresponded to lower plant density before harvest and reflected more limited inclusion of plants in the reproductive-development process. Under chemical protection, the number of ears increased, corresponding to fuller transition of plants to the productive state. In the scheme with biological preparations without the stimulant, a shift in the same direction was also recorded, but the relationship between the number of preserved plants and the number of formed ears remained different from that in the combined biological system. When biological protection and the growth stimulant were used together, the number of productive ears was higher, reflecting a different pattern of organogenesis completion within the crop. Thus, differences between schemes concerned preservation of plants as such and the share of plants that passed into the stage of actual productive realisation. In the logic of yield formation, this component connected the morphological basis of the crop with its generative filling, transferring the quantitative characteristic of the standing crop into the plane of productive functioning.

Further generalisation of these changes was focused on grain mass per unit area. When biological protection was combined with the growth stimulant, it was obtained at 1,588 g/m^2. This component reflected the total result of several links in yield formation: plant preservation until harvest, the number of productive ears, the course of grain filling, and dry-matter accumulation in the generative part of the crop. In the control, grain mass per area remained lower, which was consistent with weaker development of the listed components. Under chemical protection, accumulation of grain mass was more intensive, corresponding to changes in crop structure and conditions for forming generative production. In the scheme with biological preparations without the stimulant, grain mass per area also increased, but the filling of a unit area with yield mass occurred within different limits than under the combined action of biological protection and the growth stimulant. This relationship showed that differences between schemes appeared through the number of plants or ears and through the intensity of grain-production accumulation within the agrocenosis. In this regard, grain mass per unit area reflected the transition from structural yield components to the actual volume of formed production accumulated within the accounting area.

The summary of the identified changes was concentrated in grain yield. In the control, it was obtained at 13.68 t/ha. This result was consistent with lower plant density before harvest, a smaller number of productive ears, and lower accumulation of grain mass per area. Under chemical protection, yield increased together with preservation of the standing crop and fuller formation of the generative part of the crop. In the scheme with biological preparations without the stimulant, a positive shift was also recorded, but separate links of yield formation changed within unequal limits; therefore, the general result remained distant from the level obtained under the combined biological action. When biological protection and the growth stimulant were applied together, yield was higher, reflecting the combination of preservation of the plant composition, productive filling of the crop, and greater grain mass per unit area. Thus, yield reflected the cumulative result of changes formed sequentially during vegetation under the influence of preventive treatments. Its level generalised the course of processes associated with plant preservation, transition to generative development, and accumulation of grain production, and therefore reflected the integral response of the maize crop to the applied protection schemes (Table 4).

***Table 4.** Effect of preventive treatments on maize yield formation*

Indicator	Control	Chemical protection (reference fungicide + + insecticide)	Biological protection (Trichodermin M + Planriz M + Biplan M + Bitoxibacillin M + Lepidocide M)	Biological protection + growth stimulant (Trichodermin M + Planriz M + Biplan M + + Bitoxibacillin M + Lepidocide M + Vympel 2)
Plant density before harvest, thousand plants/ha	56.8	59.9	59.2	61.1

Table 4. *Continued*

Indicator	Control	Chemical protection (reference fungicide + + insecticide)	Biological protection (Trichodermin M + Planriz M + Biplan M + Bitoxibacillin M + Lepidocide M)	Biological protection + growth stimulant (Trichodermin M + Planriz M + Biplan M + + Bitoxibacillin M + Lepidocide M + Vympel 2)
Number of productive ears, thousand ears/ha	54.1	58.0	57.3	59.5
Proportion of productive plants, %	95.2	96.8	96.8	97.4
Grain mass from 1 m^2, g	1368	1516	1424	1588
Grain yield, t/ha	13.68	15.16	14.24	15.88
Yield increase relative to control, t/ha	0.00	0.74	0.28	1.10
Yield preservation, %	0.0	10.8	4.1	16.1

Source: compiled by the authors

Thus, preventive treatments changed maize yield formation sequentially at several interrelated levels. Their action appeared through plant preservation until the end of vegetation, subsequent formation of productive ears, and grain-mass accumulation within the crop. Without treatment, all these components were formed less completely, whereas the application of protection schemes was accompanied by a shift in yield structure towards better preservation of the plant composition and fuller realisation of generative development. The combination of biological protection with the growth stimulant was characterised by the most coordinated course of these processes, whereas separate use of biological preparations and chemical protection also changed the direction of yield formation compared with the control, but with a different degree of expression of its separate components.

Grain moisture at harvest required primary consideration as a characteristic of the completion of filling and the physiological condition of the kernel at the time of accounting. When biological protection was combined with the growth stimulant, it was recorded at 15.8%. This distribution reflected a different course of ripening, under which the grain entered harvest with a lower moisture content than in the control. Without preventive treatments, this component remained higher, corresponding to a slower transition to technological maturity. Under chemical protection, moisture also decreased, pointing to a different pattern of completion of filling processes. When biological preparations were used without the stimulant, the same trend remained, but the degree of moisture reduction differed from the combined action. Within grain-quality characteristics, this indicator was related to the condition of kernels at harvest and to subsequent storage conditions, the risk of self-heating, and the need for drying. Therefore, differences between the investigated systems reflected features of the final vegetation stages that directly affected the post-harvest properties of the grain mass.

When biological protection and the growth stimulant were used together, grain test weight was obtained at 733 g/l. This value corresponded to the formation of kernels with a denser internal structure and fuller dry-matter accumulation. In the control, test weight remained lower, reflecting another ratio between fully filled and less fully formed kernels. Under chemical protection, this component also shifted towards denser grain mass, which was related to the course of filling and completion of endosperm formation. When biological preparations were used without the stimulant, test weight also increased, but grain bulk density remained within different limits than under the combined effect. From a technological standpoint, this distribution reflected features of the physical composition of grain mass that determine flowability, uniformity, and suitability for storage and processing. In this case, test weight characterised both the mass of grain in a given volume and the degree of completion of processes associated with the deposition of plastic substances during ripening.

When biological protection was combined with the growth stimulant, protein content was determined at 9.1%. This content reflected a different relationship between filling processes and biochemical kernel formation than in the control, where protein concentration remained lower. Under chemical protection, the protein component also increased, which showed a change in the conditions under which nitrogenous substances accumulated in generative organs. When biological preparations were used without the stimulant, this component also deviated from the control in the same direction, but the final protein level differed from that under the combined action. Within the qualitative assessment of grain, this indicator characterised the nutritional completeness of the formed product and reflected the features of metabolic support during filling. Therefore, differences between the investigated systems concerned external kernel properties and its internal composition. In this context, protein was a component linking the agronomic effect of preventive treatments with the biochemical parameters of the formed grain mass.

From the standpoint of product preservation, the proportion of damaged grain required separate analysis. When biological protection and the growth stimulant were used together, it decreased to 5.0%. This distribution reflected a lower spread of kernels with impaired integrity, which was directly linked to the phytosanitary condition of the crop during the generative period. In the control, the proportion of damaged grain was higher, corresponding to a greater presence of morphologically weakened and less uniform production. Under chemical protection, this component also decreased, meaning that preventive action was accompanied by better preservation of the formed grain mass. When biological preparations were used without the stimulant, the proportion of damaged grain also changed in the same direction, but the limits of this decrease differed from those under the combined action. For the market characteristics of the yield, this distribution was important since kernel integrity was linked to preservation during cleaning, transport, storage, and subsequent processing. Within this component, the effect of preventive treatments appeared through a change in the condition of the grain mass as a formed product, rather than only through separate morphological deviations (Table 5).

Table 5. *Effect of preventive treatments on maize grain-quality indicators*

Indicator	Control	Chemical protection (reference fungicide + + insecticide)	Biological protection (Trichodermin M + Planriz M + Biplan M + Bitoxibacillin M + Lepidocide M)	Biological protection + growth stimulant (Trichodermin M + Planriz M + Biplan M + Bitoxibacillin M + Lepidocide M + Vympel 2)
Grain moisture at harvest, %	16.9	16.1	16.4	15.8
Grain test weight, g/l	712	726	721	733
Protein content, %	8.6	8.9	8.8	9.1
Starch content, %	68.2	69.3	69.0	69.8
Crude fat content, %	4.1	4.3	4.2	4.4
Proportion of filled grain, %	84.2	89.1	87.3	90.6

***Table 5.** Continued*

Indicator	Control	Chemical protection (reference fungicide + + insecticide)	Biological protection (Trichodermin M + Planriz M + Biplan M + Bitoxibacillin M + Lepidocide M)	Biological protection + growth stimulant (Trichodermin M + Planriz M + Biplan M + Bitoxibacillin M + Lepidocide M + Vympel 2)
Proportion of shrivelled grain, %	10.6	6.2	7.4	5.3
Proportion of damaged grain, %	9.8	5.9	6.8	5.0

Source: compiled by the authors

Thus, preventive treatments changed the quality properties of maize grain in several interrelated directions. Their action appeared through changes in the degree of ripening at harvest, the physical density of the grain mass, the biochemical filling of kernels, and the level of their morphological preservation. Without treatment, these components formed within different limits, whereas the application of protection solutions was accompanied by a shift in grain quality towards lower moisture, a denser structure, higher protein content, and a smaller proportion of damaged kernels. The combination of biological protection with the growth stimulant was characterised by the most integral manifestation of these changes, whereas separate application of chemical protection and biological preparations also affected grain quality compared with the control, but with a different relationship between its separate components. Overall, the quality of the formed product reflected the cumulative result of processes that occurred in the crop from the completion of filling to the condition of the grain mass at harvest.

Discussion

The obtained results show that, under a dry background, different systems of preventive treatments affected the phytosanitary condition of the crop and the morpho-physiological parameters of maize in different ways. Chemical protection ensured the lowest level of disease development and plant damage by phytophages, which reflected the most pronounced limitation of infection and entomological pressure among the investigated schemes. Biological protection was characterised by a higher level of preservation of predatory arthropods and actinomycetes, meaning that it was accompanied by less disturbance of the accompanying biota of the agrocenosis. Inclusion of the growth stimulant in the biological system changed this distribution towards lower plant damage and injury while preserving a substantial share of beneficial organisms. A similar direction was traced in biometric indicators. When biological preparations were combined with the growth stimulant, higher field germination, greater plant height, a more developed leaf surface, and higher chlorophyll content in leaves were recorded. This combination of traits corresponded to a better condition of the assimilation apparatus and more uniform crop development. Against this background, the chemical scheme restricted harmful organisms more strongly, but the combined biological system combined protective action with more favourable plant-growth indicators. Thus, under drought, differences between schemes appeared simultaneously in the phytosanitary, biometric, and physiological planes, reflecting their different effects on the course of the maize production process. From the standpoint of ecological risks of agrochemical protection and physiological regulation of growth, the works of F.H.M. Tang *et al.* (2025) and S. Matthews *et al.* (2025) considered, respectively, the consequences of widespread pesticide use and the potential of biostimulants for increasing crop productivity. F.H.M. Tang *et al.* (2025) summarised pathways of transboundary pesticide transport through water, air, and biotic chains and focused on the ecological and sanitary risks of chemical load. S. Matthews *et al.* (2025) analysed the effect of biostimulants on growth, yield, and product quality and noted their relationship with mitigation of abiotic stress. In this study, the chemical scheme also proved most effective in directly limiting the infection process and plant damage by phytophages, whereas the combination of biological preparations with the growth stimulant was accompanied by higher values of field germination, plant height, leaf surface area, chlorophyll content, and yield.

In the context of integrated regulation of the agrocenosis and natural biostimulating resources, the works of A. Tataridas *et al.* (2022) and S. Matthews *et al.* (2022) focused on early detection of unwanted components of the plant cover, herbicide resistance, integrated management, and safe sources of biostimulating action. A. Tataridas *et al.* (2022) presented an overview of technologies for early diagnosis of invasive species, resistance screening, and approaches to their control, but without detailing changes in biometric and yield indicators of maize. S. Matthews *et al.* (2022) characterised biostimulants as natural and safe resources associated with regulation of growth and metabolic processes. In this study, this line receives experimental continuation: the combined biological scheme reduced disease development and plant damage and was accompanied by higher biometric-trait values and better formation of yield-structure elements. With regard to soil-production changes and phytopathological threat, the works of C.S. Aulakh *et al.* (2022) and Y. Gai & H. Wang (2024) considered, respectively, the consequences of organic farming for soil and yield and the growing global threat of plant diseases. C.S. Aulakh *et al.* (2022) reported that organic systems usually improved soil properties but were often accompanied by lower productivity compared with conventional technologies. Y. Gai & H. Wang (2024) characterised plant diseases as a factor with an increasing effect on food security and yield stability. In the present study, the phytosanitary component is not presented in general terms but through specific field relationships: the minimum development of diseases and damage was recorded under chemical protection, whereas biological protection better preserved beneficial biota, and its combination with the growth stimulant was accompanied by higher indicators of growth, yield structure, and grain quality.

Within the combination of protective and technological measures, the works of N. Nazir *et al.* (2022) and M. Berhe *et al.* (2022) considered different levels of harmful-organism control and product preservation. N. Nazir *et al.* (2022) examined an integrated combination of biological and chemical control and agronomic practices in restraining pea root rots and reported that combined schemes were accompanied by a better phytosanitary condition and higher productivity. M. Berhe *et al.* (2022) summarised the composition of post-harvest insect pests and control practices for food and export crops, focusing on loss of product quality during storage. In this study, a similar relationship between protection and productivity was traced at the field stage: the minimum level of disease damage and plant damage by phytophages was recorded under chemical protection, whereas biological protection with the growth stimulant was accompanied by better biometric indicators, formation of yield-structure elements, and higher yield. In the direction of replacing chemical components with biological and stimulating agents, the works of A.I. Abdo *et al.* (2022) and W.A. Akbar *et al.* (2024) covered approaches to optimising maize yield through changes in the nutrition system and growth regulation. A.I. Abdo *et al.* (2022) analysed the use of growth stimulants in a strategy of partial replacement of chemical fertilisers with biological ones and demonstrated increased maize yield against a background of plant physiological activation. W.A. Akbar *et al.* (2024) reviewed the action of biochar combined with reduced rates of NP (nitrogen-phosphorus) fertilisers and reported changes in soil characteristics, nutrient availability, and maize productivity. In the present study, an analogous direction of change appeared in the variant combining biological preparations with the growth stimulant, where higher values of field germination, plant height, leaf surface area, and chlorophyll content were recorded. Unlike the works of the aforementioned researchers, the physiological effect in this study was traced simultaneously with the level of disease damage, plant damage by phytophages, and preservation of beneficial biota, which broadened interpretation of the action of the combined biological scheme.

In relation to organic improvement of the substrate and biologisation of nutrition R. Suryani *et al.* (2023) and S.F. Lamlom *et al.* (2023) concentrated on changes in yield and product composition under the influence of biochar, trichocompost, biofertilisers, and organic fertilisers. R. Suryani *et al.* (2023) reported that the application of biochar and trichocompost from rice residues was accompanied by increased maize yield and improvement of separate agronomic indicators. S.F. Lamlom *et al.* (2023) demonstrated that biological and organic fertilisers changed the biological and biochemical composition of wheat, in particular, the content of separate grain components. In the present study, a related direction was traced both in yield and in maize grain-quality indicators: variants with preventive treatments were characterised by changes in grain test weight, protein content, starch content, crude fat content, and the proportion of filled grain. In the field of organic fertilisation and microbiological stimulation of germination, the works of M. Cardarelli *et al.* (2022) and V. Antoniadis *et al.* (2023) considered physiological plant responses to non-traditional organic substrates and microorganisms introduced at early stages of ontogenesis. V. Antoniadis *et al.* (2023) reported that insect frass changed soil properties, plant physiological parameters, and nutrient status; therefore, it acted not only as an organic fertiliser but also as a factor regulating growth. M. Cardarelli *et al.* (2022) demonstrated that pre-sowing seed treatments with microorganisms were accompanied by changes in germination energy, germination, and seedling growth, which broadened understanding of the biostimulating role of microbial agents. In this study, the combination of biological preparations with the growth stimulant was also accompanied by higher field germination, greater plant height, greater leaf surface area, and higher chlorophyll content. The advantage of this approach was that early growth responses were considered together with the phytosanitary condition of the crop, the technical efficacy of protection, and subsequent formation of yield-structure elements.

In the field of combining biological agents with the nutrition system, the works of C.A. Phares *et al.* (2022) and T.J. Sande *et al.* (2024) considered variants of integrated plant nutrient supply using biochar, bacteria, vermicompost, and mineral fertilisers. C.A. Phares *et al.* (2022) reported that the joint application of biochar, NPK (nitrogen, phosphorus, and potassium), and plant-growth-promoting bacteria was accompanied by increased maize grain yield and nutrient-use efficiency. T.J. Sande *et al.* (2024) summarised that combining organic and mineral components in nutrition systems changed crop productivity and helped stabilise agronomic indicators. In the present study, a response of similar direction was traced in the variant with biological preparations and the growth stimulant, where plant density before harvest, the number of productive ears, grain mass per area, and yield were higher. A stronger aspect of this work is that productive indicators were considered simultaneously with disease development, plant damage by phytophages, and preservation of beneficial biota. In the field of agronomic regulation of yield traits, the works of F.V. Vijay *et al.* (2022) and N. Sharma & M. Rayamajhi (2022) analysed changes in maize productivity under the influence of zinc nanoparticles and different approaches to weed control. F.V. Vijay *et al.* (2022) demonstrated that the application of zinc oxide nanoparticles was accompanied by changes in yield-structure elements and yield of hybrid maize. N. Sharma & M. Rayamajhi (2022) generalised different aspects of weed control in maize crops and noted that crop condition and final productivity depended substantially on timely restriction of competitive pressure. In the current study, changes were also traced at the level of yield-structure elements; however, they were considered through preventive protection against diseases and pests combined with biostimulating action. A more extensive analytical structure linked increases in ear length, ear mass, grain number, and 1,000-grain mass with the phytosanitary condition of the crop and morpho-physiological plant indicators. The results indicate that preventive maize treatments changed the phytosanitary condition of the crop, morpho-physiological plant indicators, yield-structure elements, yield, and grain quality in different ways, and the most complete integrated effect was recorded when biological preparations were combined with the growth stimulant.

Conclusions

The study compared 4 schemes of preventive maize treatments under dry conditions: untreated control, chemical protection, biological protection, and biological protection combined with a growth stimulant. The analysis covered the phytosanitary condition of the crop, biometric plant indicators, yield-structure elements, yield formation, and grain quality. The results showed that the chemical scheme most clearly restricted the development of the complex of harmful organisms. Root rot development decreased from 18.6% to 5.1%, leaf spots – from 22.4% to 6.3%, stem rots – from 14.8% to 2.0%, and damage by the European corn borer – from 21.7% to 4.1%. Technical efficacy reached 74.8% against diseases and 81.2% against pests. Biological protection and its combination with the growth stimulant better preserved beneficial components of the agrocenosis: the preservation level of predatory arthropods and actinomycetes was 54.1-57.6%, whereas in the chemical variant it was 16.3%. The most extensive changes in biometric indicators were recorded when biological preparations were combined with the growth stimulant. Field germination increased to 90.2%, plant height – to 229.4 cm, ear placement height – to 84.9 cm, leaf surface area – to 47.6 dm^2, and chlorophyll content – to 47.2. In the control, the corresponding values were lower, reflecting weaker development of the plant cover. In the same scheme, yield-structure elements formed more fully: ear length was 20.7 cm, average ear mass – 238.0 g, the number of grains per ear – 694, grain mass from one ear –209.4 g, and the 1,000-grain mass – 332 g. This relationship reflected a better combination of growth processes and generative plant development. Yield formation was also most fully expressed in the biological protection variant with the growth stimulant. Plant density before harvest reached 61.1 thousand plants/ha, the number of productive ears reached 59.5 thousand ears/ha, grain mass from 1 m^2 – 1,588 g, yield – 13.68 t/ha, and yield preservation – 16.1%. For comparison, yield in the control was 6.84 t/ha. Grain quality in this variant was also higher: test weight reached 733 g/l, protein content – 9.1%, starch content – 69.8%, and the proportion of filled grain – 90.6%, whereas the proportions of shrivelled and damaged grain decreased to 5.3% and 5.0%. The limitation of the study was that the field experiment was conducted during one growing season within one farm. Further research should focus on multi-year verification of these schemes in different hybrids and different soil-climatic conditions.

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Conflict of Interest

None.

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Вплив профілактичних обробок біопрепаратами на продуктивність кукурудзи

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Анотація. Метою дослідження було встановити вплив різних профілактичних схем захисту на фітосанітарний стан посіву, ріст рослин, формування врожаю та якість зерна в умовах дефіциту вологи. Було використано метод дисперсійного аналізу, метод середніх величин, метод оцінки похибки середнього, метод визначення найменшої істотної різниці. Встановлено, що хімічний захист найсильніше обмежував розвиток шкідливих організмів: розвиток кореневих гнилей знизився з 18,6 % до 5,1 %, листкових плямистостей – з 22,4 % до 6,3 %, а пошкодженість кукурудзяним метеликом – з 21,7 % до 4,1 %. Технічна ефективність проти хвороб досягла 74,8 %, проти шкідників – 81,2 %. Водночас біологічний захист супроводжувався вищим рівнем збереження хижих видів членистоногих і актиноміцетів – 54,1 %, а при поєднанні біопрепаратів зі стимулятором росту – 57,6 % проти 16,3 % у хімічній схемі. Комбінований біологічний варіант характеризувався кращими біометричними показниками: польова схожість становила 90,2 %, висота рослин – 229,4 см, площа листкової поверхні – 47,6 дм^2, вміст хлорофілу – 47,2 од. У структурі врожаю за цього варіанта довжина качана досягла 20,7 см, середня маса качана – 238,0 г, кількість зерен у качані – 694 шт., маса 1000 зерен – 332 г. Урожайність зерна зросла з 13,68 до 15,88 т/га, а збереження врожаю становило 16,1 %. Якість зерна також покращилася: натура підвищилася з 712 до 733 г/л, вміст білка – з 8,6 % до 9,1 %, частка виповненого зерна – з 84,2 % до 90,6 %, тоді як частка пошкодженого зерна зменшилася з 9,8 % до 5,0 %. Практична цінність дослідження полягає у можливості використання його результатів агрономами, фермерами, консультантами із захисту рослин і науковцями для вибору профілактичних схем вирощування кукурудзи за посушливих умов

Ключові слова: стимулятор росту; врожайність; кореневі гнилі; листкові плямистості; біологічний захист

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Physiological efficiency of nitrification inhibitors and endophytic nitrogen fixers in maize crops

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Abstract. Under the conditions of global climate change, the stability of the nitrogen status in maize is a critical factor for ensuring high productivity, especially during late-season thermal stress. The objective of the study was to substantiate the physiological impact of nitrapyrin and *Methylobacterium symbioticum* on the activity of the maize photosynthetic apparatus under various hydrothermal scenarios. The research was conducted in 2023-2025 on Typical Chernozems and Grey Forest soils in the Forest-Steppe of Ukraine. The research subjects included hybrids P8723, P9127, and P9074. The relative chlorophyll content was measured using the Yara N-Tester device. Statistical processing was performed using the LSD test. It was established that the integration of N-Lock™ Max and Blue N™ ensured a pronounced "stay-green" effect, providing 5-12% higher chlorophyll index than the control during the grain-filling phase. It was found that under the extreme drought conditions of 2024 (HTC 0.56), endophytic nitrogen fixation effectively compensated for the soil nitrogen deficit, increasing the Yara N-Tester index by 18.7-26.2 units. The study proved that applying these treatments on a moderate N_{90} background allows for achieving physiological indicators equivalent to the full N_{120} background without stabilisers. A direct correlation between late-stage chlorophyll content and final grain yield was mathematically confirmed. Significant yield increases were observed in the synergistic variants, which outperformed individual applications by maintaining metabolic activity during peak thermal stress.

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The results demonstrated that the synergy between chemical stabilisation and biological nitrogen fixation ensured stable nitrogen homeostasis in maize under thermal stress, enabling the optimisation of nitrogen fertiliser rates by 25%. This integrated approach serves as a viable strategy for enhancing crop resilience and nutrient use efficiency in climate-smart agriculture

Keywords: *Zea mays* L.; nitrapyrin; *Methylobacterium symbioticum;* stay-green effect; Yara N-Tester; nitrogen homeostasis

Introduction

Maize (*Zea mays* L.) stands as a cornerstone of global food security and a primary feedstock for the rapidly growing bioenergy industry. In Ukraine, particularly within the Forest-Steppe zone, maize productivity is a key driver of agricultural export potential. However, the modern cultivation of high-yielding hybrids is increasingly constrained by the inefficiency of conventional nitrogen fertilisation strategies. Nitrogen governs leaf area expansion and chlorophyll synthesis, yet Nitrogen Use Efficiency (NUE) remains low due to nitrate leaching and gaseous emissions. As of 2026, the challenge lies in maintaining nitrogen homeostasis under increasing hydrothermal instability. Recent scientific discourse highlights the shift from quantitative fertilisation to qualitative nutrient management. S. Tanchyk & A. Babenko (2021) established that conventional fertilisation systems in the Forest-Steppe often fail to meet the peak nitrogen demand of modern hybrids during the generative phase, necessitating the use of stabilisers. C.A. Ruiz-Sánchez *et al*. (2022) investigated the role of *Methylobacterium symbioticum* as a leaf-colonising endophyte and concluded that biological nitrogen fixation in non-legumes is a primary mechanism for bypassing root-uptake limitations during soil moisture deficits. Further research by H. Gamit *et al*. (2023) demonstrated that methylotrophic bacteria improve plant growth under stressed environments by producing secondary metabolites that modulate hormonal signalling.

The physiological response of cereal crops to such biostimulants was explored by F. Valente *et al*. (2024), who found that foliar application of *M. symbioticum* maintains photosynthetic rates even under reduced chemical nitrogen inputs. M. Quemeda *et al*. (2026) further quantified this response in maize, indicating that biological fixation provides a steady nitrogen supply that complements soil-applied fertilisers. However, the specific interaction between chemical nitrification inhibitors like nitrapyrin and these biological fixers remains under-researched in the context of the Ukrainian Forest-Steppe. The gap in knowledge persists regarding how this "dual-action" system performs across different maturity groups (FAO 280-330) during extreme heatwaves.

The objective of this research was to provide physiological substantiation for the combined use of nitrapyrin and *Methylobacterium symbioticum*. The purpose of the study was:

1) to evaluate the dynamics of the Yara N-Tester index across different maize hybrids and hydrothermal scenarios;

2) to determine the compensatory capacity of the synergistic system in reducing mineral nitrogen rates;

3) to quantify the correlation between late-season nitrogen status and final grain productivity.

Materials and Methods

The field experiments were conducted over a three-year period (2023-2025) across two distinct climatic zones within the Forest-Steppe of Ukraine. Zone 1 (2023) was located in the Bila Tserkva district (Typical Chernozem), and Zone 2 (2024-2025) in the Vinnytsia Oblast (Grey Forest heavy-loamy soils). Plant research was performed in accordance with institutional and international standards, adhering to the provisions of Convention on Biological Diversity (1992). The experimental design included an absolute control group (untreated plants on N_{90} and N_{120} backgrounds) to accurately compare the effects of N-Lock™ Max (nitrapyrin) and Blue N™ (*M. symbioticum*). Data collection involved sampling 30 representative plants per plot. The elementary plot size was 24 m^2. To ensure high statistical power, a split-plot design with four replications was utilised, which provided sufficient degrees of freedom to compare effects between treatment variants.

Three high-yielding maize (*Zea mays* L.) hybrids from the Pioneer® selection (Corteva Agriscience Ukraine) were utilised to evaluate the impact of nitrogen stabilisation across different maturity groups:

➤ P8723 (FAO 280): Early-mid group, characterised by high drought tolerance and rapid initial growth.

➤ P9127 (FAO 310): Mid-maturity group, known for its intensive type of development and high response to mineral nutrition.

➤ P9074 (FAO 330): Mid-late group, possessing a high genetic potential for yield under favourable hydrothermal conditions.

The preceding crop for all experimental cycles was winter wheat. The study involved three interacting factors. Factor A: nitrogen fertilisation background – nitrogen was applied in the form of granular urea (Urea 46%) under pre-sowing cultivation. Two dosage levels were investigated:

➤ Moderate dose (N_{90}): 196 kg/ha of commercial product (90 kg/ha of active substance).

➤ High dose (N_{120}): 260 kg/ha of commercial product (120 kg/ha of active substance).

Factor B: chemical nitrification inhibition – the nitrification inhibitor N-Lock™ Max (active ingredient: nitrapyrin, 300 g/L) was applied at a rate of 1.7 L/ha. The application was performed via soil spraying followed by immediate incorporation with a cultivator. The mechanism of nitrapyrin involved the selective inhibition of *Nitrosomonas* bacteria, which prevented the rapid oxidation of ammonium (NH_4^+) to highly mobile nitrates (NO_3^-). Factor C: biological nitrogen fixation – the biological stimulant Blue N™ (containing the strain *Methylobacterium symbioticum*) was applied at a rate of 0.33 kg/ha through foliar spraying during the BBCH 14-16 growth stage (4-6 true leaves). The working solution volume was 200 L/ha.

The relative chlorophyll content, serving as a proxy for the nitrogen status of the plants, was measured using the handheld Yara N-Tester (Yara International ASA, Oslo, Norway; manufactured by Konica Minolta, Japan). This optical sensor assessed the transmittance of light through the leaf blade at two specific wavelengths (red and infrared). Measurements were recorded in a temporal sequence: 3WAAB: 3 weeks after the application of the biostimulant (late vegetative stage). 7WAAB: 7 weeks after application, corresponding to the critical grain-filling phase. Data were collected from the middle part of the 3rd fully developed leaf from the top. Each value represented an average of 30 individual readings per plot to minimise intra-varietal variability.

Statistical Analysis was processed using an Analysis of Variance (ANOVA) within the RStudio environment (version 4.2.1), utilising the ggplot2 and stats packages. The significance of differences between treatment means was determined using the Least Significant Difference (LSD) test. Given the high natural variability inherent in field biological trials and the subtle physiological effects of endophytes, the significance threshold was set at $p \leqslant 0.1$. To quantify the relationship between the N-Tester index and final grain yield, Pearson correlation coefficients (r) were calculated.

Results and Discussion

Physiological basis of stabilised and endophytic nutrition. The stabilisation of nitrogen in the soil matrix represents a significant technological advancement in mitigating nutrient losses. Nitrification inhibitors, specifically those based on nitrapyrin (2-chloro-6-(trichloromethyl) pyridine), have demonstrated high efficacy in sequestering nitrogen in the stable ammonium form (NH_4^+). By selectively inhibiting the activity of *Nitrosomonas* bacteria, nitrapyrin slows the transition of ammonium to highly mobile nitrates (NO_3^-) (Wolt, 2004). From a plant physiology perspective, the "ammonium-rich" environment created by products like N-Lock™ Max offers a distinct metabolic advantage. The assimilation of NH_4^+ requires significantly less metabolic energy (ATP) compared to the reduction of NO_3^-, which must first be converted back to ammonium within the plant tissues before amino acid synthesis can occur (Di & Cameron, 2002; Shvartau *et al.*, 2016). This energy saving is particularly vital during the rapid growth phases of maize, allowing the plant to redirect its resources toward biomass accumulation and root development (Hospodarova *et al.*, 2019).

While chemical stabilisation addresses soil-based losses, the increasing frequency of climate-induced abiotic stresses requires a more resilient approach. Prolonged droughts and extreme "heatwaves" during the critical flowering stage often lead to soil dehydration, which effectively blocks root-mediated nutrient uptake. In this context, the role of endophytic nitrogen fixers, such as *Methylobacterium symbioticum* (the active agent in Blue N™), becomes critical. Unlike conventional rhizobia, this bacterium colonises the phyllosphere – the internal tissues of the leaves – and facilitates the fixation of atmospheric nitrogen (N_2) directly within the photosynthetic apparatus (Ruiz-Sánchez *et al.*, 2022). This biological "bypass" ensures a continuous supply of nitrogen even when the soil is too dry for conventional uptake. Research has shown that these methylotrophic bacteria can also produce ACC-deaminase, an enzyme that degrades the stress hormone ethylene, thereby enhancing the plant's tolerance to drought and thermal shock (Belimov *et al.*, 2019).

A fundamental challenge in the study of foliar endophytic nitrogen fixation is the reconciliation of the nitrogenase enzyme complex's extreme sensitivity to oxygen (O_2) with the oxygen-rich environment of the leaf. In classical diazotrophs, such as *Rhizobium* in legume nodules, oxygen levels are strictly regulated by leghemoglobin. However, in the phyllosphere − the internal and external surfaces of leaves-bacteria like *Methylobacterium symbioticum* (strain SB23) must utilise alternative strategies to protect their nitrogen-fixing mechanisms. Recent genomic and in situ analyses suggest that *M. symbioticum* does not function as a classical soil diazotroph but rather as a highly specialised endophyte that has evolved to synchronise its metabolic activity with the host plant's physiology (Valente *et al.*, 2024). The survival and efficacy of *M. symbioticum* within the leaf are driven by its unique methylotrophic nature. These bacteria utilise methanol, a byproduct of pectin methylesterase activity released during plant cell wall expansion and division, as their primary carbon and energy source (Iguchi *et al.*, 2015). This metabolic pathway is crucial for oxygen management. The rapid aerobic consumption of methanol and other single-carbon molecules by the bacteria creates a localised "micro-respiratory" environment. This high rate of oxygen consumption at the site of bacterial colonisation helps to maintain an internal O_2 tension low enough to protect the nitrogenase complex while allowing the bacteria to remain active in an otherwise aerobic tissue.

In conclusion, the integration of chemical and biological tools creates a multi-layered defence system for maize nutrition. By establishing a stable endophytic population, the plant gains a physiological "bypass" for nitrogen acquisition that is particularly effective during peak photosynthetic activity. This direct delivery system minimises energy losses associated with long-distance transport and nitrate reduction. Ultimately, these mechanisms fundamentally improve the overall Nutrient Use Efficiency (NUE) of the maize crop across diverse hydrothermal conditions.

Spatiotemporal dynamics of relative chlorophyll content. The ultimate expression of a plant's nitrogen status is its photosynthetic capacity, typically measured through relative chlorophyll content. The use of portable optical sensors, such as the Yara N-Tester, provides a non-destructive and accurate assessment of the "nitrogen-chlorophyll" relationship (Uddling *et al.*, 2007). In maize, maintaining high chlorophyll levels during the post-anthesis period is defined as the "stay-green" trait. This physiological characteristic is highly correlated with prolonged grain-filling duration and protection against premature leaf senescence (Tollenaar & Daynard, 2002; Thomas & Ougham, 2014). For modern hybrids with a high FAO index, the ability to maintain active photosynthesis under late-season thermal stress is the determining factor for reaching maximum kernel weight (Stasyk *et al.*, 2011).

The 2023 experimental cycle in the Kyiv Oblast (Bila Tserkva) was characterised by a favourable distribution of precipitation, providing an ideal baseline for evaluating the maximum potential of nitrogen management tools. At the initial monitoring stage (3WAAB), all treatments exhibited a statistically insignificant difference in their baseline nitrogen status ($p > 0.1$). However, as the plants transitioned toward the grain-filling stage (7WAAB), a rapid upward trend was observed. The combined application of N-Lock™ Max and Blue N™ on the N_{90} background resulted in a significant index increase to 775-786 units (Fig. 1).

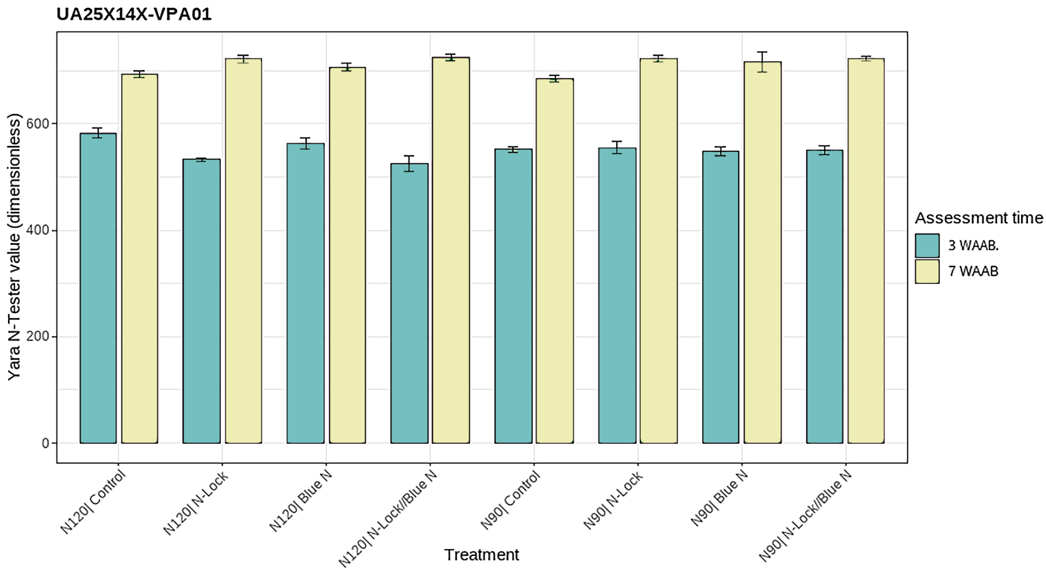

***Figure 1.** Dynamics of leaf chlorophyll index (Yara N-Tester, dimensionless units) in maize hybrid P8723 (FAO 280) depending on nitrogen nutrition systems (Kyiv Oblast, 2023)*

Note: vertical bars represent the standard error of the mean (±SE). Yara N-Tester values are expressed in dimensionless units reflecting relative chlorophyll content

Source: developed by authors based on own research

The data indicates that under sufficient hydrothermal conditions, nitrogen stabilisation leads to a substantial extension of photosynthetic activity. The physiological surge observed between Week 3 and Week 7 of monitoring confirms that the synergistic interaction prevents the premature loss of functional leaf area. While the control variants exhibited a plateau in the Yara N-Tester index during late maturity, the treated plots maintained a significant upward trend. This suggests that chemical inhibition of nitrification, combined with endophytic fixation, provides a steady nitrogen gradient necessary for biomass accumulation. Consequently, the maintenance of high chlorophyll density during these stages serves as a critical foundation for achieving maximum productivity levels.

Physiological adaptation to extreme hydrothermal stress. The results from the 2024 growing season in the Sharhorod district provide evidence for the adaptive role of the investigated technology under extreme conditions. This period was marked by a severe "heat dome" effect, with 19 consecutive days where maximum temperatures exceeded +33.4°C, peaking at +38.1°C. Such pressure typically triggers a sharp decline in chlorophyll due to photo-oxidation and nitrogen deficiency caused by the cessation of root-mediated uptake in desiccated soil (HTC 0.56). Under such stress, plants experience a surge in ethylene, which triggers the breakdown of light-harvesting complexes. In the control plots (N_{90} without treatments), the Yara N-Tester index plummeted to its seasonal minimum of 510.3 ± 5.4 units (Fig. 2).

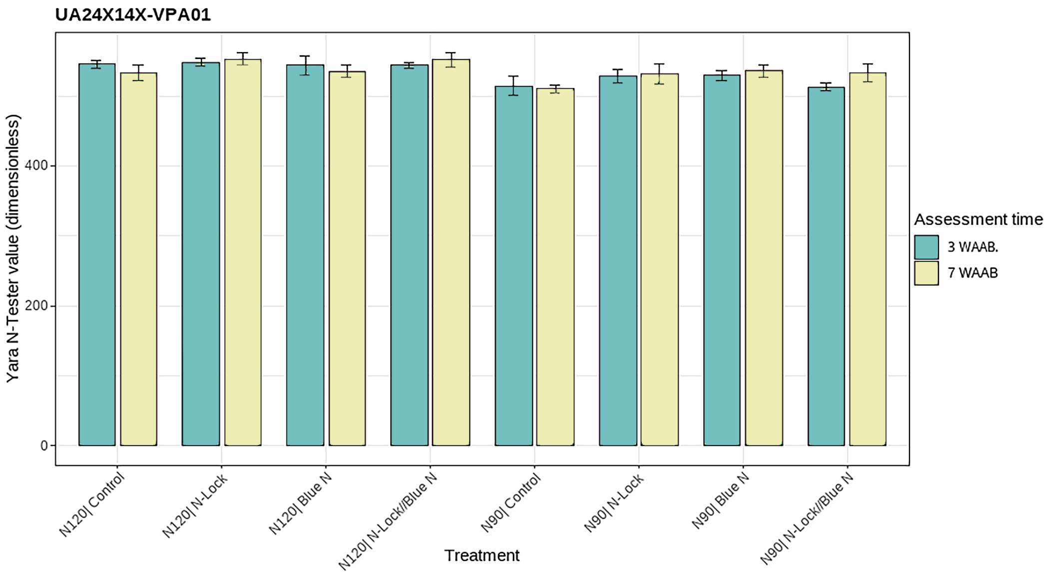

Figure 2. *Dynamics of leaf chlorophyll index (Yara N-Tester, dimensionless units) in hybrid P9127 (FAO 310) as an indicator of physiological adaptation to drought conditions (Vinnytsia Oblast, 2024)*
Source: developed by authors based on own research

As illustrated in Figure 2, the variants treated with Blue N™ maintained a significantly higher nitrogen status, reaching 539.5 ± 7.4 units. This relative increase of 18.7 to 26.2 units over the control proved that *M. symbioticum* acts as a metabolic "bypass". While the root system's ability to absorb nitrogen was restricted by low soil water potential, the endophytic bacteria continued to fix N_2 directly within the leaf tissues. Furthermore, data from over 2,500 field trials indicate that strain SB23 promotes the regulation of key phytohormones, including auxins and cytokinins, which stimulate aerial biomass accumulation (Quemeda *et al.*, 2026). The production of ACC-deaminase by the bacteria likely mitigated the negative effects of abiotic stress, preventing "premature yellowing".

This physiological resilience highlights the importance of biological nitrogen sources when environmental factors paralyse conventional soil-based uptake. By preserving the integrity of chloroplast membranes from reactive oxygen species generated during thermal peaks, the technology ensured that the leaves remained active during the late stages of maturity. The fact that the N_{90} synergistic variant outperformed the non-stabilised N_{120} control proves that biological stress mitigation is more critical for resilience than high chemical loads. Ultimately, these results support the conclusion that foliar biological pathways serve as a crucial supplement to maintain nitrogen homeostasis during extreme climatic variability.

Furthermore, the obtained results are consistent with the findings of M. Farooq *et al.*, (2009) who emphasised that hormonal regulation is a primary defence mechanism against desiccation. The ability of *Methylobacterium* to potentially modulate plant stress signals and enhance antioxidant enzyme activity, as discussed by M.N. Dourado *et al.* (2015) and H. Gamit *et al.* (2023), provides a plausible explanation for the higher Yara N-Tester readings. By preserving the integrity of the chloroplast membranes from reactive oxygen species (ROS) generated during the +38°C peaks, the technology ensured that the "source" (leaves) remained active during the late stages of maturity.

Nutrient optimisation and resource use efficiency. The 2025 experimental cycle in the Tulchyn district focused on the mid-late hybrid P9074 (FAO 330), which has a high demand for nitrogen during late ontogenesis. In this environment, a distinct "equalisation effect" was observed, underscoring the resource-optimising potential of technology. Monitoring of the canopy during the late milk maturity stage (7WAAB) revealed that the synergistic system (N-Lock™ Max + Blue N™) on the moderate N_{90} background yielded a Yara N-Tester index of 722.0 ± 3.8 units (Table 1). This value was statistically equivalent to the N_{120} + Synergy variant (724.5 ± 15.2 units) and significantly outperformed the high-input N_{120} control, which reached only 693.0 ± 6.1 units.

Table 1. *Relative chlorophyll content (Yara N-Tester units) in maize leaves during the grain-filling phase (7WAAB) depending on the treatment and hydrothermal conditions*

Treatment (Code)	Kyiv Oblast (2023)	Sharhorod (2024)	Tulchyn (2025)
Urea N_{120} (Control)	772.0 ± 12.1	533.3 ± 10.3	693.0 ± 6.1
N_{120} + Synergy	761.5 ± 8.5	552.0 ± 10.3	724.5 ± 15.2
Urea N_{90} (Control)	732.0 ± 4.5	510.3 ± 5.4	684.0 ± 8.1
N_{90} + Synergy	775.0 ± 11.2	539.5 ± 7.4	722.0 ± 3.8

Note: Data are presented as Mean ± SE (n = 30 per plot). "Synergy" refers to the combined application of N-Lock™ Max and Blue N™

Source: developed by authors based on own research

This numerical advantage over the high-nitrogen control is of profound significance, demonstrating that qualitative management of the existing nitrogen pool is more effective for sustaining the photosynthetic apparatus than a simple quantitative increase in fertiliser. The success of this strategy in 2025 can be attributed to the synchronisation of nitrogen availability with the plant's peak demand. Nitrapyrin (N-Lock™ Max) effectively regulated the soil nitrogen cycle by slowing the microbial conversion of ammonium to nitrate. This ensured that a reservoir of stable nitrogen remained available in the root zone during the late reproductive stages, precisely when mid-late hybrids like P9074 reach their maximum sink-strength. Simultaneously, the stay-green effect was highly evident across the experimental plots. While the control leaves on the N_{120} and N_{90} plots showed early symptoms of chlorophyll degradation and nitrogen remobilisation (senescence), the synergistic treatments maintained a high density of active chloroplasts, reflected in the dark green colour of the upper canopy (Fig. 3).

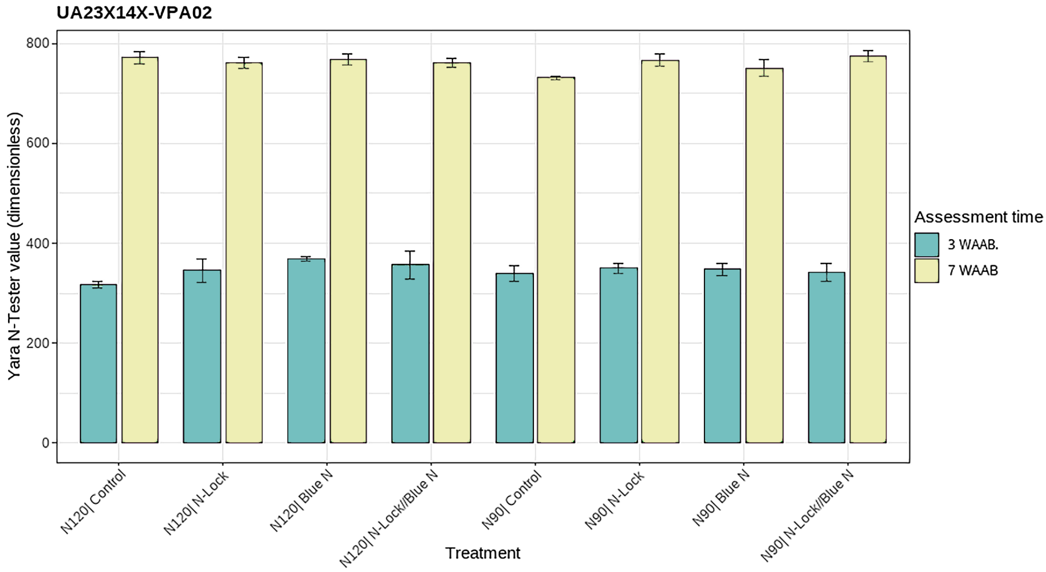

Figure 3. *Dynamics of leaf chlorophyll index (Yara N-Tester, dimensionless units) as an indicator of the stay-green effect in hybrid P9074 (FAO 330) (Vinnytsia Oblast, 2025)*

Source: developed by authors based on own research

The graphical representation confirms high technological stability for mid-late hybrids. By delaying the transition to the senescence phase, the dual-action system allows the plant to prolong the synthesis of photoassimilates. This phenomenon ensures that the realisation of the hybrid's genetic potential is secured despite the hydrothermal challenges of the growing season. The high degree of chlorophyll preservation in the N_{90} + Synergy variant confirms that a balanced metabolic environment can be maintained even with a 25.0% lower nitrogen input level. This decoupling of yield from high chemical inputs represents a major milestone for sustainable agriculture in the Forest-Steppe of Ukraine.

Correlation between photosynthetic status and productivity. The physiological merit of the investigated nitrogen management technology is determined by its ability to convert solar radiation into harvestable biomass. To quantify this, a regression analysis was performed between the Yara N-Tester index at the grain-filling stage (7WAAB) and the final grain yield (t/ha). The results demonstrate a robust and statistically significant linear correlation across all experimental sites and years (Fig. 4). The coefficients of determination (R^2) ranged from 0.82 to 0.93, confirming that the nitrogen status during the post-anthesis period explains the vast majority of yield variance. The strongest correlation was observed in the 2023 trial (Kyiv Oblast, $R^2 = 0.93$**)**, where optimal moisture conditions allowed the plants to fully translate their enhanced nitrogen status into grain weight. Under these conditions, the regression slope was steepest, indicating that every incremental increase in the N-Tester index yielded a higher marginal return in grain productivity. Conversely, in the drought-stressed 2024 season (Sharhorod), the correlation remained high ($R^2 = 0.82$), although the overall yield levels were lower. This indicates that even when water is the primary limiting factor, the "biological nitrogen buffer" provided by Blue N™ remains a critical survival mechanism, preventing the total collapse of the photosynthetic apparatus. In the 2025 Tulchyn cycle, the correlation reached $R^2 = 0.89$, with the synergistic variants occupying the same space on the regression line as the high-input control, thereby proving the compensatory capacity of the investigated technology.

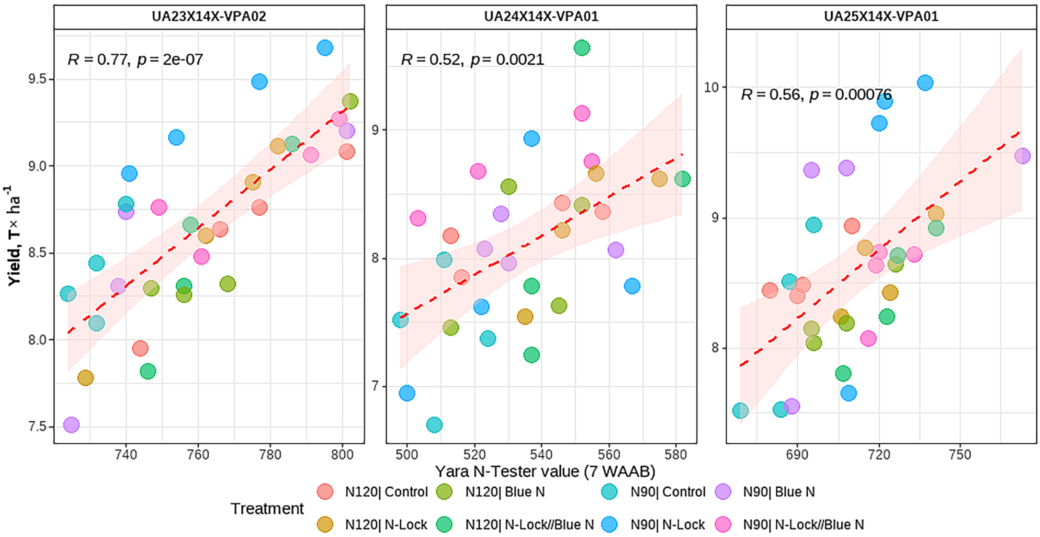

Figure 4. *Correlation between the Yara N-Tester index (dimensionless units) at the grain-filling stage (7WAAB) and maize grain yield across different experimental environments (2023-2025)*

Note: statistical significance is established at $p \leqslant 0.1$

Source: developed by authors based on own research

The significant yield increases observed in the "Synergy" variants (N-Lock™ Max + Blue N™) can be explained through the source-sink relationship. In maize, approximately 40-60% of the nitrogen found in the grain at maturity is remobilised from the vegetative tissues, particularly the leaves. If the soil nitrogen supply is exhausted early or blocked by drought, the plant accelerates leaf senescence to meet the grain's demand, leading to a premature loss of photosynthetic surface area. Obtained data show that the investigated technology effectively disrupts this cycle of early senescence. By sequestering nitrogen in the stable ammonium form (NH_4^+) in the soil and providing a supplemental foliar N_2-fixation pathway, the system ensures a continuous "source" of nitrogen. This delays the degradation of chlorophyll-protein complexes, a phenomenon known as the functional stay-green effect (Thomas & Ougham, 2014). The prolongation of leaf activity by even days 5-7 during the late milk-wax stage can lead to a substantial increase in kernel depth and test weight, as confirmed by the higher PFP (Partial Factor Productivity) values recorded in the study. This extension of the photosynthetic period aligns with the physiological models of I.A. Ciampitti & T.J. Vyn (2012), who demonstrated that yield stability in modern hybrids is governed by the efficiency of nitrogen remobilisation. By delaying senescence, a process described in detail by P.L. Gregersen *et al.* (2013), the nitrogen stabilisation technology ensures that the grain sink remains active during the final stages of maturity, maximising the accumulation of dry matter.

A critical point of discussion is why the combination of a nitrification inhibitor and a biostimulant consistently outperformed individual applications. Nitrapyrin (N-Lock™ Max) acts as a foundational stabiliser, ensuring that the primary mineral nitrogen dose (N_{90}) remains within the reach of the root system for a longer duration. However, its efficacy is dependent on soil moisture. When the soil surface dries out, as seen in July 2024, the root-mediated uptake of even stabilised nitrogen is compromised. At this precise moment of abiotic stress, the foliar endophytic bacteria (*Methylobacterium symbioticum*) reach their peak population density. By fixing nitrogen directly within the phyllosphere, Blue N™ mitigates the transient nitrogen deficit caused by drought. Furthermore, the bacteria's metabolic by-products may further stimulate the plant's antioxidant defence systems. This "dual-layer" protection ensures that the nitrogen homeostasis of the crop is maintained across a wider range of hydrothermal conditions (HTC 0.56-1.09), providing resilience that single-component systems lack. Beyond the physiological benefits, the ability of the N_{90} + treatments variant to match or exceed the N_{120} control has profound implications for Nitrogen Use Efficiency (NUE). Traditionally, farmers compensate for potential nitrogen losses by increasing total fertiliser rates, which leads to groundwater contamination and high input costs.

Experimental results demonstrate that a 25% reduction in nitrogen application rates is not only feasible but also agronomically viable when integrated with stabilisation tools. Such a reduction significantly lowers the carbon footprint of maize production, as the industrial synthesis and field application of nitrogen fertilisers are among the most energy-intensive processes in contemporary farming. The high PFP value of 98.0 kg grain per 1 kg of applied N achieved in this study reflects a broader trend toward "decoupling" crop yields from excessive chemical inputs. As highlighted by Y. Rouphael & G. Colla (2020), the integration of microbial biostimulants represents a cornerstone of "climate-smart" agriculture and a paradigm shift in nutrient management, enabling substantial fertiliser reductions without compromising the inherent biological potential of the crop. This results confirm a strong and stable relationship between nitrogen status during grain filling and final maize yield, validating the Yara N-Tester as an effective diagnostic tool. The combined use of N-Lock™ Max and Blue N™ ensures sustained nitrogen availability, delays senescence, and stabilises productivity even under drought stress.

Conclusions

The integrated application of the nitrification inhibitor nitrapyrin (N-Lock™ Max) and the endophytic nitrogen fixer *Methylobacterium symbioticum* (Blue N™) fundamentally alters the nitrogen homeostasis and senescence dynamics in maize. At the critical grain-filling stage (7 weeks after application), the synergistic treatments maintained a significantly higher relative chlorophyll content, increasing the Yara N-Tester index by 14 to 42 units compared to the control, thereby inducing a functional stay-green effect. This effect is driven by the synchronisation of soil-based ammonium stabilisation with a direct foliar biological supply, ensuring that the photosynthetic "source" remains active during peak demand.

The foliar endophytic nitrogen fixation pathway served as a vital physiological buffer during extreme abiotic stress. In the drought-stressed 2024 season (Sharhorod), characterised by 19 days of temperatures above +33°C, the use of Blue N™ compensated for the lack of root-mediated nutrient uptake, maintaining the photosynthetic capacity at a level 6-8% higher than the control. Furthermore, the metabolic advantage of direct ammonium assimilation provided the plants with significant energy savings (ATP), which were redirected toward grain filling and biomass accumulation. A robust compensatory capacity of the dual-action nitrogen stabilisation system has been demonstrated. The application of N-Lock™ Max and Blue N™ on a moderate background (N_{90}) allowed for a 25% reduction in total nitrogen fertiliser rates without a reduction in photosynthetic potential or grain yield, matching the performance of the non-stabilised N_{120} high-input control. This demonstrates that qualitative management of the nitrogen pool is superior to a mere quantitative increase in chemical fertilisation. Statistical analysis confirmed that the Yara

N-Tester index measured during the late stages of vegetation is a highly reliable predictor of maize productivity (R_2 = 0.82-0.93, p ≤ 0.1). This validates the effectiveness of optical nitrogen monitoring as a diagnostic tool for evaluating the physiological status of crops under varying hydrothermal conditions in the Forest-Steppe of Ukraine.

Future research should focus on the impact of these nitrogen stabilisation tools on the qualitative characteristics of the grain (amino acid profile and protein fractions) and the potential reduction of the carbon footprint in industrial maize production systems.

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Conflict of Interest

None.

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Фізіологічна ефективність інгібіторів нітрифікації та ендофітних азотфіксаторів у посівах кукурудзи

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Анотація. В умовах глобальних кліматичних змін стабільність азотного статусу кукурудзи є критичним чинником забезпечення високої продуктивності, особливо під час термічного стресу на пізніх етапах вегетації. Мета дослідження – обґрунтувати фізіологічний вплив нітрапірину та *Methylobacterium symbioticum* на активність фотосинтетичного апарату кукурудзи за різних гідротермічних сценаріїв. Дослідження проводили у 2023-2025 рр. на чорноземах типових та сірих лісових ґрунтах у Лісостепу України. Об'єктами дослідження були гібриди P8723, P9127 та P9074. Відносний вміст хлорофілу визначали за допомогою приладу Yara N-Tester. Статистичну обробку методом LSD-тесту. Встановлено, що інтеграція N-Lock™ Max та Blue N™ забезпечує виражений «Stay-green» ефект, що зумовлює на 5-12 % вищий індекс хлорофілу порівняно з контролем у фазу наливу зерна. З'ясовано, що за умов екстремальної посухи 2024 р. (ГТК 0,56) ендофітна азотфіксація ефективно компенсувала дефіцит ґрунтового азоту, підвищивши індекс Yara N-Tester на 18,7-26,2 од. Доведено, що застосування цих препаратів на помірному фоні N_{90} дозволяє досягти фізіологічних показників, еквівалентних повному фону N_{120} без використання стабілізаторів. Математично підтверджено пряму кореляцію між вмістом хлорофілу на пізніх стадіях вегетації та кінцевою врожайністю зерна. У синергічних варіантах спостерігалося суттєве зростання врожайності, що перевищувало показники за роздільного застосування препаратів завдяки підтримці метаболічної активності в період пікового термічного стресу. Результати демонструють, що синергія хімічної стабілізації та біологічної фіксації азоту забезпечує стабільний азотний гомеостаз кукурудзи в умовах термічного стресу, що дозволяє оптимізувати норми азотних добрив на 25 %. Такий інтегрований підхід виступає дієвою стратегією підвищення стійкості агроценозів та ефективності використання ресурсів у кліматично-орієнтованому землеробстві

Ключові слова: *Zea mays* L.; нітрапірин; *Methylobacterium symbioticum*; stay-green ефект; Yara N-Tester; азотний гомеостаз

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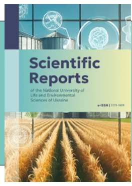

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The impact of the biologisation of fertilisation systems on potato yield and quality

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Abstract. Under conditions of Albeluvisol soils degradation, the improvement of potato fertilisation systems through biologisation is essential for preserving and restoring soil fertility. The aim of the article was to assess yield structure, productivity, and the quality of potato tubers under the application of mineral fertilisers, rye straw, green manure crops of lupin and oilseed radish. The study was conducted at the "Prohres" farm in Kozelets District, Chernihiv Region, affiliated with the Institute of

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Agricultural Microbiology, on soddy weakly podzolic sandy loam soil formed on glaciofluvial deposits. The research findings established that, in terms of the distribution of potato tubers by weight, yield indicators, and dry matter content, the most effective treatment involved the cultivation of narrow-leaved lupin as a green manure crop combined with the application of $N_{120}P_{90}K_{120}$. In particular, yield under this treatment reached 23.1 t/ha, which was 15.9 t/ha higher than the control (7.2 t/ha), while the application of mineral fertilisers alone ensured a yield increase of 12.1 t/ha. The use of oilseed radish as green manure together with $N_{120}P_{90}K_{120}$ was slightly inferior to the lupin and mineral fertiliser treatment, but showed advantages over the straw and $N_{120}P_{90}K_{120}$ treatment in terms of yield structure and dry matter content; at the same time, this treatment produced the highest increase in starch content – 0.7%. Furthermore, the application of biologised fertilisation systems ensured a 2.0-3.0-fold increase in yield compared with the control. Under green manure cultivation, yield indicators, tuber distribution, and tuber quality were lower compared with the $N_{120}P_{90}K_{120}$ treatment. The practical significance of the study lies in the fact that the combination of lupin as green manure with mineral fertilisation may be recommended both for industrial potato production and for small household farms, as it ensures stable yields of high quality

Keywords: green manure crops; straw; mineral fertilisers; starch content; potato tubers; sod-podzolic soil; Polissia

Introduction

In the current context of global climate change and the challenges facing Ukraine's agricultural sector in relation to its accession to the European Union – including compliance with the requirements of the European Green Deal – research into establishing effective methods for ensuring sustainable crop yields is of particular relevance. One promising approach to reducing the environmental impact of agricultural production, promoting the rational use of natural resources and by-products, and restoring soil fertility whilst implementing intensive crop cultivation technologies is the biologisation of farming systems (Amaleviciute-Volunge *et al.*, 2024).

Potatoes are one of the most widely grown crops in Ukraine, particularly in the Polissia region. This is why they are of great importance for food security, ensuring economic stability and job creation in the agricultural sector. F.A. Dijkstra *et al.* (2021) found that plant root systems had a dual effect on soil organic carbon content: on the one hand, they promoted its accumulation through the supply of organic matter, and on the other, they stimulated microbiological mineralisation processes, leading to its loss; with the balance between these processes determining the stability of soil organic matter. Researchers V.O. Polishchuk *et al.* (2025) noted that different fertilisation systems significantly influenced the formation of both quantitative and qualitative indicators of potatoes, in particular yield, tuber structure and their biochemical composition; the authors demonstrated that optimising plant nutrition ensured increased productivity and improved marketable characteristics of the produce. At the same time, T. Klymenko *et al.* (2020) established that a significant problem in Ukraine is the insufficiently high productivity of this crop, given its popularity as a food product, a raw material for starch production, and animal feed. They highlight the sharp decline in the amount of manure available to agricultural enterprises in the Polissia region, as well as the high cost of mineral fertilisers. This is precisely why alternative methods of fertilising potatoes must be sought, particularly in the north of Ukraine.

According to M. Solomiichuk & M. Pikovskyi (2025), potato tuber yield is influenced by both abiotic (fertilisers, soil fertility indicators) and biotic factors. Among the latter, fungal diseases have a significant impact on potato productivity, as they also reduce tuber quality. To ensure sustainable yields and increase carbon sequestration, it is important to use alternative organic fertilisers, namely crop by-products, green manure crops, etc. (Balaev *et al.*, 2023). The study by T. Khomenko *et al* (2023) examined the positive effect of using the stubble biodegrader Ekostern on potatoes in sod-podzolic soil. In particular, it was found that in the Polissia region of Ukraine, the biopreparation Phytohelp, used to treat tubers before planting and during the growing season alongside the application of the Ecostern stubble biodestructor on green manure crops, ensured the preservation and restoration of soil organic matter and increased potato productivity. In studies by Yu. Mishchenko *et al.* (2024), it was demonstrated that the introduction of green manure on sod-podzolic soils is a fully-fledged alternative to the application of farmyard manure in terms of yield formation. The authors found that ploughing in the green mass of post-harvest green manure crops (white mustard, oil radish) yields potato yields at a level that is statistically comparable to those achieved with intensive mineral fertilisation. At the same time, there is not only an increase in the gross yield of tubers but also an improvement in their marketability, which the researchers attribute to the optimisation of the agro-physical properties of the arable layer and the prolonged action of the mineralised biomass of green manure crops. N.N. Usmanov (2023) noted that in global agriculture, the use of green manure crops improves soil fertility and increases the yield and quality of potato seeds; however, it is necessary to identify those types of green manure crops that will be optimal for achieving high tuber yields under specific soil conditions.

The aim of the study was to determine the effect of mineral fertilisation, various green manure crops and crop by-products on potato tuber yield and quality in the Polissia region of Ukraine.

Materials and Methods

The research was conducted in 2013-2014 in a long-term field experiment of the Institute of Agricultural Microbiology, located at the “Prohres” farm in Kozelets District, Chernihiv Region. According to agro-soil zoning, this territory belongs to the Left-Bank Polissia province. The soil of the experimental plot was soddy weakly podzolic sandy loam formed on glaciofluvial deposits, with a humus content in the 0-20 cm layer of 1.12 ± 0.04%, pH_{KCl} of 4.9 ± 0.15, and a sum of absorbed bases of 5.4 ± 0.09 mg eq. per 100 g of soil. The research was carried out in the following crop rotation: potato – oats – lupin – winter rye. The potato variety ‘Nevska’, which belongs to the medium-early group, was used. Eight variants of crop fertilisation systems were compared:

1. Without fertilisers (control).
2. Mineral fertilisation $N_{120}P_{90}K_{120}$.
3. Narrow-leaved lupin green manure.
4. Winter rye straw.
5. Oilseed radish green manure.
6. Narrow-leaved lupin green manure + + $N_{120}P_{90}K_{120}$.
7. Winter rye straw + $N_{120}P_{90}K_{120}$.
8. Oilseed radish green manure + $N_{120}P_{90}K_{120}$

The meteorological conditions of the growing seasons in Kozelets District, Chernihiv Region, were characterised as follows: 2013 (HTC = 1.10) was sufficiently moist and closest to the climatic norm in terms of total precipitation (351.0 mm), although rainfall distribution was extremely uneven. In 2014 (HTC = 0.52), total precipitation (162.2 mm) amounted to only 46% of the norm, which caused moisture deficiency during the main growth stages of the crops.

The total plot area was 102 m^2 (17 m × 6 m), with an accounting area of 60 m^2 (15 m × 4 m). The arrangement was randomised with four replications. The potato cultivation technology included the following operations: disc cultivation after harvesting the preceding crop to a depth of 10-12 cm using heavy disc harrows with incorporation of chopped straw; sowing of green manure crops in July according to the experimental design;

disc cultivation of green manure crops in October using heavy disc harrows to a depth of 12-14 cm; non-mouldboard tillage to a depth of 25-30 cm; preparation of potato tubers for planting, including sorting, grading into fractions, warming, or sprouting of tubers. Narrow-leaved lupin was sown at a rate of 200 kg/ha, while oilseed radish was sown at 25 kg/ha. Incorporation of green manure biomass was carried out at the budding to early flowering stage of the plants, ensuring an optimal balance between biomass accumulation and its mineralisation in the soil. The study was conducted in accordance with ethical standards for handling biological objects and the principles of the Convention on Biological Diversity (1992).

The dry matter content in potato tubers was determined by the thermostatic-gravimetric method through drying samples to constant weight in accordance with DSTU ISO 751:2004 (2004). Starch content was determined by the polarimetric method (Ewers method) according to DSTU ISO 10520:2004 (2004). Yield recording was carried out by weighing tubers from each accounting plot. Yield structure was also determined by the gravimetric method during harvesting. Statistical and mathematical processing of experimental data was performed using analysis of variance and correlation analysis with the Microsoft Excel analysis package and Statistica software.

Results and Discussion

Preserving soil fertility under conditions of insufficient application of organic matter of animal origin and the cultivation of environmentally safe produce is one of the ways to develop economically efficient potato production. One of the highly effective measures for the biologisation of cultivation technology, soil improvement, reduction of chemical contamination, and production of environmentally safe products is the use of green manure crops. In the study, the structure of potato yield depended on the fertilisation treatment and varied according to the weather conditions of the year (Figs 1, 2).

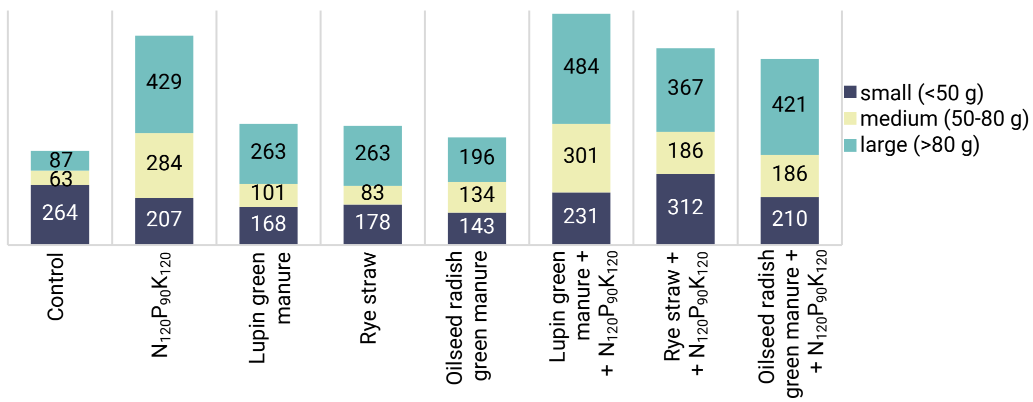

***Figure 1.** Composition of the potato harvest by different fertilazers, 2013*

Source: compiled by the authors

Analysing the obtained results, it should be noted that the treatment without fertiliser application led to the formation of a significant proportion of small potato tubers (64%), with lower proportions of medium-sized tubers (14-15%) and 21-22% of tubers weighing more than 80 g. The use of mineral fertilisers increased the proportion of the large fraction by 2.2 times, the medium fraction by 1.9-2.0 times, and reduced the small fraction by 2.8 times. Such a distribution of potato tubers by weight indicates insufficient nutrient supply to the plants and low realisation of the varietal potential. A similar tendency was observed with the sowing of lupin and oilseed radish green manure crops and the use of crop residues. The highest proportion of the large fraction was obtained when growing green manure (oilseed radish) combined with mineral fertilisers ($N_{120}P_{90}K_{120}$), amounting to 52%, which was 2.5 times higher compared with the control.

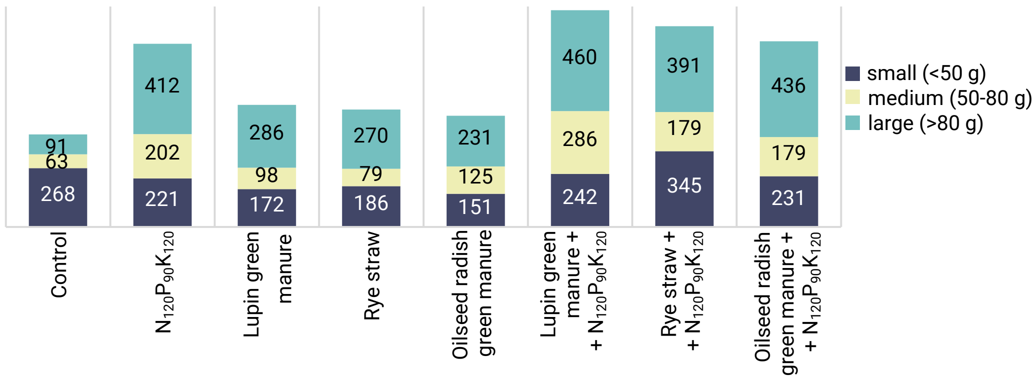

***Figure 2.** Composition of the potato harvest by different fertilazers, 2014*

Source: compiled by the authors

The application of mineral fertilisers ($N_{120}P_{90}K_{120}$) reduced the proportion of small tubers by 16-22% compared with the control. The lowest weight of the small fraction among the treatments involving green manure crops was recorded under the cultivation and incorporation of oilseed radish (143-151 g), which was 43-47% lower than the control. This indicates improved nutrient conditions for potato cultivation. In the treatments combining green manure crops with mineral fertilisation, the values for the small fraction were higher than in the treatments with green manure crops alone, which was associated with the intensive formation of the overall crop mass. The control treatment had the lowest values for the medium fraction (63 g). At the same time, the highest values for the medium fraction were recorded in the treatments lupin + $N_{120}P_{90}K_{120}$ (301-286 g) and $N_{120}P_{90}K_{120}$ (284-202 g). This indicates the formation of a uniform marketable yield under conditions of sufficient mineral nutrition. Green manure crops without mineral fertilisers ensured a moderate increase in the medium fraction (83-134 g), confirming their positive but less intensive effect. The most important indicator of marketability is the proportion of the large tuber fraction weighing more than 80 g. While in the unfertilised control treatment this amounted to only 87-91 g, the application of mineral fertilisers increased this value to 412-429 g, exceeding the control by 4.7-4.9 times.

Among the green manure treatments, the best results were provided by narrow-leaved lupin, where the weight of large-sized tubers reached 263-286 g, which was three times higher than the control. However, the highest values were obtained when green manure crops were combined with mineral fertilisers: 460-484 g in the lupin + $N_{120}P_{90}K_{120}$ treatment and 421-436 g in the oilseed radish + $N_{120}P_{90}K_{120}$ treatment. Thus, in terms of influence on the structure of potato tuber yield under sod-podzolic soil conditions, the organo-mineral fertilisation treatment proved to be the most effective. Narrow-leaved lupin green manure had an advantage over cruciferous crops due to nitrogen fixation and its deep root system. Similar conclusions were obtained in the studies of O.V. Yegorov *et al.* (2021), who established that the highest productivity in short-rotation crop rotations was ensured by an organo-mineral system combining the application of crop residues, green manure crops, and mineral fertilisers. The authors noted that the exclusive use of an organic system, although contributing to the maximum accumulation of humus, was inferior to the organo-mineral system in terms of crop rotation productivity by 12-15%. Based on this, the researchers concluded that, in order to ensure high yields while simultaneously restoring soil fertility, the optimal approach is an integrated strategy in which the deficit of manure is compensated by the incorporation of straw and green manure biomass against the background of moderate doses of mineral fertilisers.

It should be noted that the analysis of the results obtained by A. Arlauskienė *et al*. (2019) demonstrated that the agronomic efficiency of straw directly depends on accompanying factors. The authors established that the incorporation of straw without compensatory measures does not ensure yield increases. However, the introduction of catch crops (white mustard, buckwheat) into the fertilisation system radically changed the situation. Green manure crops accumulated 14.6-25.4 kg/ha of nitrogen in their biomass, preventing its leaching during the autumn-winter period (reducing nitrate content in the soil by 40-50%). This made it possible to obtain a significant increase in the yield of the subsequent crop (spring barley) at the level of 0.35-0.53 t/ha precisely due to the reutilisation of nutrients from green manure biomass. Assessing the productive potential of sod-podzolic soils, U. Karbivska *et al*. (2025) pointed to a direct correlation between the level of mineral nutrition and biomass yield. The natural fertility of these soils ensures the production of only 1.4-1.9 t/ha of grass dry matter, which is economically inefficient. The potassium situation is particularly critical: even with the application of mineral fertilisers, its balance remained deficient (from −38 to −112 kg/ha) due to intensive leaching from light-textured soils. This confirms the necessity of involving additional sources of fertility restoration, particularly green manuring, in order to mobilise potassium from deeper soil horizons.

Table 1 presents potato yield data under different fertilisation treatments. The lowest potato yields were naturally recorded in the unfertilised control treatment, amounting to 7.5 and 6.8 t/ha in 2013 and 2014 respectively. The use of organic, mineral, and organo-mineral fertilisers significantly increased potato yield. Yield increases in these treatments compared with the control amounted to 7.5-8.2 t/ha, with a slight advantage observed for the narrow-leaved lupin treatment. The application of considerable amounts of nutrients to the soil in the mineral fertiliser treatment contributed to a substantial increase in potato yield to 19.3 t/ha.

Table 1. *Yield of the 'Nevska' potato variety on sod-podzolic soil under biologised fertilisation treatments, 2013-2014*

No.	Experimental treatment	Potato yield, t/ha			
		2013	2014	Average for 2013-2014	±compared with the control
1	Without fertilisers (control)	7.5	6.8	7.2	-
2	$N_{120}P_{90}K_{120}$	20.4	18.2	19.3	12.1
3	Narrow-leaved lupin green manure	16	14.8	15.4	8.2
4	Crop residues (rye straw)	15.4	14	14.7	7.5
5	Oilseed radish green manure	15.5	13.8	14.7	7.5
6	Narrow-leaved lupin green manure + $N_{120}P_{90}K_{120}$	24.8	21.4	23.1	15.9
7	Crop residues (rye straw + $N_{120}P_{90}K_{120}$)	24.6	20.6	22.6	15.4
8	Oilseed radish green manure + $N_{120}P_{90}K_{120}$	22.6	20.8	21.7	14.5
	$LSD_{0.05}$	0.28	0.21		

Source: compiled by the authors

The combination of green manure crops and rye straw with mineral fertilisers increased potato productivity to 22.6-24.8 t/ha, that is, threefold compared with the control, and increased yield gains to 14.5-15.9 t/ha. The treatments involving narrow-leaved lupin and the application of rye straw showed an advantage, where yield increases amounted to 15.4-15.9 t/ha, whereas the treatment with oilseed radish green manure showed slightly lower values. The application of complete mineral fertiliser ($N_{120}P_{90}K_{120}$) ensured a significant increase in yield: the increase compared with the control amounted to 12.1 t/ha, or 2.7 times. All biologisation treatments ensured a 2.0-2.2-fold increase in yield compared with the control. In general, it may be noted that all investigated treatments involving green manure crops and straw, both separately and in combination with mineral fertilisers, were effective and contributed to increased yield of the 'Nevska' potato variety.

Crop productivity largely depends on soil fertility. Under the current challenges facing the agricultural sector of Ukraine, fertilisation systems should comply with the principles of the biologisation of farming. The use of green manure crops as a source for replenishing the soil with fresh organic matter and plant nutrients is of particular importance. Under organic farming conditions, green manure crops are an effective component of crop cultivation technology, contributing to improved soil structure and quality, ensuring mineral nutrition, and creating favourable conditions for plant growth and development, thereby increasing yields.

At the same time, N. Zhao *et al*. (2024) concluded that the benefits obtained from increased crop yields through the use of green fertilisers were insufficient to compensate for the production costs associated with growing green manure crops, resulting in less favourable economic returns. To assess the nutritional value of potato tubers, it is important to investigate their biochemical composition. Starch is the main component of tubers; however, proteins, vitamins, and mineral elements are also important. According to V.S. Kravchenko & D. Akinchyts (2025), the nutritional value of potato tubers is primarily assessed by starch content. This indicator varies depending on the maturity group of the potato and the conditions of cultivation. Regarding starch distribution within the tuber, the greatest amount is concentrated in the cortex parenchyma, while the smallest amount is found in the core of the tuber. Starch content depends on a number of factors. Starch accumulation is influenced by soil type, soil fertility level, the amount of mineral and organic fertilisers applied, and the weather conditions during the growing season. R.O. Myalkovsky & P.V. Bezvikonnyi (2024) established that starch content in tubers is also determined by varietal characteristics, meteorological conditions, and cultivation technology. In order to obtain the maximum starch yield per hectare, it is necessary to apply agronomic cultivation practices in an integrated manner, namely an optimal level of plant nutrition combined with appropriate planting density, taking into account the intended processing of tubers for starch production.

The starch content in tubers of the 'Nevska' potato variety on sod-podzolic soil under different biologised fertilisation treatments is presented in Table 2.

Table 2. *Starch content in tubers of the 'Nevska' potato variety on sod-podzolic soil under biologised fertilisation treatments, 2013-2014*

No.	Experimental treatment	Starch content, %			
		2013	2014	average	±compared with the control
1	Without fertilisers (control)	14.2	12.4	13.3	-
2	$N_{120}P_{90}K_{120}$	14.0	12.0	13.0	-0.3
3	Narrow-leaved lupin green manure	14.5	12.8	13.7	+0.4
4	Crop residues (rye straw)	14.6	13	13.8	+0.5
5	Oilseed radish green manure	14.2	12.8	13.5	+0.2

Table 2. *Continued*

No.	Experimental treatment	Starch content, %			
		2013	2014	average	±compared with the control
6	Narrow-leaved lupin green manure + $N_{120}P_{90}K_{120}$	14.5	13.0	13.7	+0.4
7	Crop residues (rye straw + $N_{120}P_{90}K_{120}$)	14.6	13.2	13.9	+0.7
8	Oilseed radish green manure + $N_{120}P_{90}K_{120}$	14.4	13	13.7	+0.4
	$LSD_{0.05}$	0.27	0.21		

Source: compiled by the authors

The starch content in potato tubers is mainly determined by varietal characteristics and varies within quite wide limits, from 10-15% in table potatoes to 22-25% in industrial varieties. Its content may also vary depending on weather conditions, planting dates, agronomic practices, and especially fertilisation. At the same time, potato yield and starch content may exhibit a multidirectional relationship. In studies conducted on Albeluvisol soil, starch content in tubers increased under biologised fertilisation treatments and showed the lowest values in the control treatment and in the treatment with mineral fertilisers only. In the unfertilised treatment, the starch content in potato tubers averaged 13.3% over the two years. Under the application of mineral fertilisers alone, this indicator was lower compared with the control and amounted to 14.0 and 12.0% in 2013 and 2014 respectively, with the difference being significant as it exceeded the LSD value. High values were recorded in the organo-mineral fertilisation treatments, where the starch content averaged 13.7-13.9% over the two years. Tubers from the treatments involving rye straw application had the highest starch content among all treatments, both with and without mineral fertilisers. With increasing potato yield, starch content also increased slightly, except in the treatment involving mineral fertilisers only. In this treatment, the considerable increase in potato yield compared with the control was not accompanied by an increase in starch content. It should also be noted that there were significant differences in this indicator, as well as in yield, between the years of the study. The reduction in potato yield in 2014 was accompanied by a decrease in tuber starch content, which may be explained by unfavourable weather conditions. The considerably lower-than-normal precipitation and high air temperatures during the growing season led to a substantial reduction in both potato yield and starch content. This is explained by the stimulation of growth processes and the accumulation of water in the tubers, which reduces the concentration of dry matter.

The application of green manure crops and crop residues contributed to an increase in starch content compared with the control. In the treatments where lupin was used as green manure, the increase in starch content amounted to 0.4%; rye straw, 0.5%; and oilseed radish, 0.2%. However, the highest increase in starch content was obtained with the use of straw together with mineral fertilisers – 0.7%. Thus, the combination of crop residues with mineral fertilisers ensured the maximum starch content. In general, it may be noted that organo-mineral fertilisation was particularly effective for potato yield and starch content, increasing these indicators compared with the control by 13.5-15.9 t/ha and 0.4-0.7% respectively. According to the research results of A.V. Koval *et al.* (2022), plants of the early-maturing potato variety 'Ariia' were characterised by higher accumulation of dry matter and, accordingly, starch compared with the medium-maturing variety 'Hurman' in treatments involving the recommended dose of mineral fertilisers combined with micronutrient application at a planting density of 70 × 20 cm (17.1% versus 16.3%). The vitamin C content did not depend on the maturity group of the variety, planting density, or the level of mineral fertilisation combined with foliar feeding and amounted to 23.2% in the 'Ariia' variety and 23.1% in the 'Hurman' variety. The results of determining dry matter content in potatoes are presented in Table 3.

Table 3. *Changes in dry matter content in potatoes following the introduction of organic fertilisation systems, 2013-2014*

No.	Experimental treatment	Dry matter content			
		2013		2014	
		%	t/ha	%	t/ha
1	Without fertilisers (control)	19.16	1.44	19.8	1.35
2	$N_{120}P_{90}K_{120}$	19.21	3.92	20.8	3.79
3	Narrow-leaved lupin green manure	18.93	3.03	19.6	2.9
4	Crop residues (rye straw)	21.15	3.26	20.8	2.91
5	Oilseed radish green manure	21.05	3.26	21.6	2.98
6	Narrow-leaved lupin green manure + $N_{120}P_{90}K_{120}$	22.15	5.49	22.4	4.79
7	Crop residues (rye straw + $N_{120}P_{90}K_{120}$)	20.16	4.96	21.6	4.45
8	Oilseed radish green manure + $N_{120}P_{90}K_{120}$	21.65	4.89	22.1	4.6
	$LSD_{0.05}$	0.21		0.27	

Source: compiled by the authors

Analysis of the data presented in Table 3 allows the conclusion that the combination of organic fertilisers with mineral fertilisation ensures maximum accumulation of dry matter. In the control treatment, the dry matter content ranged from 19.16 to 19.8%. The application of mineral fertilisers alone ($N_{120}P_{90}K_{120}$) significantly increased the gross dry matter yield per hectare (3.79-3.92 t/ha). The use of rye straw and oilseed radish as green manure crops had a positive effect on tuber quality and resulted in an increase in dry matter content to 20.8-21.6%. The treatment involving oilseed radish combined with mineral fertilisers ensured a dry matter content at the level of 21.65-22.1%. Mineral fertilisers increased dry matter yield per hectare by 2.7 times, green manure crops and crop residues by 2.1-2.3 times, while green manure crops and crop residues applied against the background of mineral fertilisers increased it by 3.4-3.8 times. The highest dry matter content and dry matter yield per hectare were obtained under the following fertilisation treatment: narrow-leaved lupin green manure + $N_{120}P_{90}K_{120}$, amounting to 22.2-22.4%. Accordingly, dry matter yield per unit area in this treatment reached the highest values – 5.49 t/ha (2013) and 4.79 t/ha (2014), which almost fourfold exceeded the control values.

A.V. Bykin & T.V. Panchuk (2022) emphasised the increased requirements of potatoes for mineral nutrition. They also pointed out such disadvantages of mineral fertilisers as the limited availability of nutrients under moisture-deficient conditions and as a result of reactions with soil cations, as well as their influence on the taste and nutritional qualities of potato tubers. In the studies of N.N. Usmanov (2023), it was also found that under the use of green manure crops, the growth and development of medium-early potato varieties occurred more intensively than that of early-maturing varieties, as tall, multi-stemmed plants with a powerful root system and broad leaf surface were formed; as a result, productivity amounted to 617.5-998.8 g per plant, the number of tubers ranged from 6.7 to 11.2, and the average weight of one tuber was 76.2-93.1 g. According to R. Hernández Maqueda *et al.* (2025) and G. Martirosyan *et al.* (2023), green manure crops increase the content of organic matter in the soil and improve its nutrient regime. The application of biologised fertilisation systems based on legume straw, moderate doses of NPK, and humic preparations ensures a substantial increase in the productivity of winter cereals. The integration of biostimulants into cultivation technology makes it possible to obtain additional yield increases and to produce grain with high quality characteristics, which indicates the promise of such agronomic measures for the Polissia zone (Dubitska, 2024). Fertility management is a complex task for organic crops with intensive nutrient requirements, such as potatoes. For this reason, it is important to create optimal nutritional conditions for their cultivation. T. Klymenko *et al*. (2020) established that the combination of green manure crops and straw significantly increased potato yield by 15% compared with the control. Organic fertilisers, namely green manure crops and straw, produced a statistically significant increase in yield, contributing to economic advantages.

The obtained results indicate a significant influence of different fertilisation systems on the formation of yield, yield structure, and quality indicators of potato tubers, confirming the expediency of applying biologised approaches in combination with mineral nutrition under the conditions of Albeluvisol soils of the Ukrainian Polissia. It was established that the combination of green manure crops and crop residues with mineral fertilisers ensures the fullest realisation of the productive potential of the crop and contributes to the improvement of the biochemical indicators of tubers, which is important both for increasing yield and for ensuring product quality. At the same time, the differences identified between fertilisation treatments make it possible to assess their agronomic efficiency and justify the selection of optimal technological solutions for specific soil and climatic conditions.

Conclusions

As a result of studies conducted on Albeluvisol soils of the Ukrainian Polissia, it was established that fertilisation systems had a significant effect on the yield and quality of potato tubers. The application of mineral fertilisers $N_{120}P_{90}K_{120}$ ensured a 4.7-4.9-fold increase in the proportion of tubers weighing more than 80 g compared with the control; however, the highest content of this fraction was observed in the narrow-leaved lupin + $N_{120}P_{90}K_{120}$ treatment, which confirms the expediency of applying such a fertilisation system in commercial potato production, since this treatment produced the highest potato tuber yield, amounting to 21.7-23.1 t/ha. The application of complete mineral fertiliser ($N_{120}P_{90}K_{120}$) increased potato yield by 2.7 times compared with the control. The biologisation of farming (green manure crops, straw) ensured an increase in yield to 14.7-15.4 t/ha; however, greater agronomic efficiency was established when organic fertilisers were combined with mineral fertilisers. The starch content in tubers of the 'Nevska' potato variety depended significantly on fertilisation systems and the weather conditions of the year. The application of complete mineral fertiliser ($N_{120}P_{90}K_{120}$) reduced the average starch content by 0.3% compared with the control, whereas the use of green manure crops and crop residues increased starch content by 0.2-0.5%. The highest starch content was obtained in the "rye straw + $N_{120}P_{90}K_{120}$" treatment, exceeding the control by 0.7%, which was statistically significant. As with starch content and yield, the best treatment in terms of dry matter content was the combination of narrow-leaved lupin with mineral fertilisation, making this fertilisation system the most promising for farms in the region. The combination of biological measures with moderate doses of mineral fertilisers is a promising approach for increasing the productivity and quality of potato tubers under the conditions of the Ukrainian Polissia. Further research into the economic efficiency of different fertilisation systems under the biologisation of potato cultivation technology is considered promising.

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Conflict of Interest

None.

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Вплив біологізації систем удобрення на урожайність та якість картоплі

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Анотація. В умовах деградації дерново-підзолистих ґрунтів удосконалення систем удобрення картоплі із застосуванням біологізації є необхідним для збереження та відновлення їх родючості. Метою статті була оцінка структури врожаю, урожайності та якості бульб картоплі за використання мінеральних добрив, соломи жита, сидератів люпину та редьки олійної. Дослід було проведено господарстві «Прогрес» Козелецького району Чернігівської області Інституту сільськогосподарської мікробіології на дерново-слабкопідзолистому супіщаному ґрунті на водно-льодовикових відкладах. У результаті проведених досліджень встановлено, що за впливом на розподіл бульб картоплі різної ваги, показники урожайності, вмісту сухої речовини найкращим був варіант із вирощуванням люпину вузьколистого в якості сидерату та внесенням $N_{120}P_{90}K_{120}$. Зокрема, урожайність у цьому варіанті становила 23,1 т/га, що на 15,9 т/га більше порівняно з контролем (7,2 т/га), а застосування мінеральних добрив забезпечило приріст урожайності на рівні 12,1 т/га. Використання редьки олійної для сидерації сумісно з $N_{120}P_{90}K_{120}$ дещо поступалося варіанту з люпином і мінеральним удобренням, але мало переваги над варіантом соломи і $N_{120}P_{90}K_{120}$ за структурою врожаю і вмістом сухої речовини, водночас на цьому варіанті отримали найвищий приріст вмісту крохмалю – 0,7 %. Водночас застосування біологізованих систем удобрення забезпечило підвищення врожайності у 2,0-3,0 рази порівняно з контролем. За вирощування сидератів показники врожайності, розподілу бульб та їх якості були нижчими порівняно з варіантом $N_{120}P_{90}K_{120}$. Практичне значення роботи полягає у тому, що варіант поєднання люпину як сидерату з мінеральним удобренням може бути рекомендований як для вирощування в промислових насадженнях картоплі, так і для присадибних господарств, оскільки він забезпечує формування стійких врожаїв високої якості

Ключові слова: сидерати; солома; мінеральні добрива; вміст крохмалю; бульби картоплі; дерново-підзолистий ґрунт; Полісся

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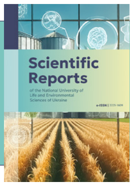

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Carbon sequestration and restoration of soil organic matter under giant miscanthus under different fertilisation systems in the Forest-Steppe conditions of Ukraine

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Abstract. The aim of the study was to determine the patterns of carbon sequestration by a perennial plantation of giant miscanthus (*Miscanthus × giganteus*), cultivar 'Osinniy Zoretsvit', in the soil and above-ground biomass under different fertilisation systems and foliar application of potassium humate in the Forest-Steppe conditions of Ukraine. Field experiments were conducted during 2021-2025 on strongly eroded podzolised chernozem with a light loamy texture. The two-factor experiment included three fertilisation systems (control; organic – Leonardite, 100 kg/ha; mineral – $N_{60}P_{16}K_{80}$) and four foliar potassium humate application regimes. It was established that the cultivation of giant miscanthus ensured a progressive accumulation of soil organic carbon (SOC) from 0.829 to 0.876-0.934% over five years. The greatest increase in SOC (+11.9%) was recorded under the organic fertilisation system combined with double foliar application of potassium humate (A_2B_4). Carbon stocks in the arable layer (0-30 cm) increased from 2.91 to 3.28 t C/ha. The maximum annual carbon sequestration by above-ground biomass reached 8.25 t C/ha (A_3B_4), while the cumulative amount over five years ranged from 16.74 to 29.25 t C/ha, equivalent to 61.4-107.3 t CO_2/ha in CO_2-equivalent terms. The study confirmed the statistically significant effects of fertilisation systems (LSD_{05} = 0.012%; $p < 0.01$) and foliar feeding (LSD_{05} = 0.008%). The organic fertilisation system using Leonardite in combination with double foliar application of potassium humate proved to be the most effective for restoring soil organic matter, whereas the mineral system $N_{60}P_{16}K_{80}$ ensured the highest carbon sequestration in above-ground biomass. A functional differentiation of agrotechnological effects was established: the organic system optimises the soil carbon pool, while the mineral system enhances the bioenergy carbon pool. The practical significance of the study lies in the scientific substantiation of a differentiated choice of

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fertilisation systems for miscanthus cultivation, serving as a basis for the implementation of climate-smart agriculture and the certification of carbon credits

Keywords: soil organic carbon; carbon balance; potassium humate; climate-smart agriculture; bioenergy crops

Introduction

Global climate change, caused by rising concentrations of greenhouse gases in the atmosphere, has become one of the most significant challenges facing modern civilisation. In this context, agricultural soils have been viewed as a strategic reservoir for storing atmospheric carbon and, at the same time, a significant source of its release due to inefficient agricultural practices. The development and implementation of scientifically sound carbon sequestration strategies in agroecosystems have become an integral part of measures to mitigate climate change and ensure food and energy security. This area has become particularly relevant for Ukraine, where significant areas of agricultural land have been degraded as a result of intensive exploitation, erosion and military action, accompanied by a substantial loss of soil organic matter. Perennial energy crops, primarily giant miscanthus (*Miscanthus × giganteus*), have been regarded as both an efficient producer of biomass for biofuel production and a powerful tool for long-term carbon sequestration. Through C_4 photosynthesis, a robust root system and the absence of annual tillage, miscanthus has demonstrated a unique combination of high productivity with soil-protection and climate-regulating functions. However, for the conditions of the Ukrainian Forest-Steppe, the patterns of soil organic carbon (SOC) accumulation and carbon sequestration by biomass during the initial five-year period (2021-2025) of plantation cultivation under various fertilisation systems (particularly in combination with humic-like biostimulants) remain poorly understood.

Recent international studies have confirmed the high potential of perennial energy crops as tools for climate-smart agriculture. I.A. Siddique *et al.* (2023) found that switching from annual crops to perennials increases SOC stocks by 16.6-23.1% in the 0-30 cm layer. Y. Wu *et al.* (2024) demonstrated that the rates of SOC sequestration under perennial energy crops depend on climate, soil type and plantation age, with the effect being most pronounced in the first 10 years after establishment. C. Bertola *et al.* (2024), using data from a European network of field experiments, showed that in the first 4 years after establishing miscanthus on marginal lands, there was a statistically significant increase in C stocks in the 0-30 cm layer, and the contribution of aboveground and belowground biomass to the formation of the soil C pool became decisive. J.L. Kane *et al.* (2023) demonstrated that miscanthus acted as an "ecosystem engineer", increasing soil bacterial diversity and organic matter content on marginal lands through specific rhizodeposition of C. Researchers N. Namoi *et al.* (2024) found that nitrogen fertilisation of mature miscanthus plantations (60-168 kg N/ha) increased individual shoot mass by 34-77% and biomass yield by 23-106%, with the effects of pre-fertilisation persisting for a long time. N. Namoi *et al.* (2026) confirmed in the Sun Grant Trial that adequate nitrogen nutrition was a key factor in maintaining the productivity of perennial miscanthus plantations.

The role of humic biostimulants in the formation of soil carbon pools and crop productivity has been actively studied. J. Shen *et al.* (2024) demonstrated that foliar application of potassium humate significantly increased photosynthetic activity, chlorophyll content and grain yield. J. Izquierdo *et al.* (2025), in a study across

448 production plots in Uruguay, demonstrated that a single foliar application of a humic biostimulant increased the yield of major cereal crops by 7.6-15.7%. A.M.S. Elshamly & S.M.A. Nassar (2023), in a study of the stimulating effect of potassium humate on crops under water stress, demonstrated that this biostimulant improved nutrient use efficiency and antioxidant protection in plants. V. Pidlisnyuk *et al*. (2025) found that miscanthus produced a productive biomass of 25-35 t/ha on marginal heavy clay soils under a low-intensity management system, whilst simultaneously sequestering CO_2 and producing oxygen. Despite a significant number of studies, there was a lack of systematic data for the conditions of the Ukrainian Forest-Steppe regarding the dynamics of SOC and carbon sequestration by Miscanthus under different fertilisation regimes in combination with foliar application of potassium humate. Furthermore, the CO_2 equivalent sequestration potential under different agricultural practices had not been established, which complicated the integration of this crop into climate compensation schemes. The aim of the study was to establish the patterns of carbon sequestration by a perennial giant miscanthus plantation in soil and above-ground biomass under different fertilisation regimes and foliar application of potassium humate during the first five years (2021-2025) of vegetation in the Forest-Steppe zone of Ukraine.

Materials and Methods

The field study was conducted between 2021 and 2025 at the Separate Subdivision of the National University of Life and Environmental Sciences of Ukraine "Velykosnitynske named after O.V. Muzychenko", Fastiv District, Kyiv Region. The soil of the experimental site was strongly eroded podzolised chernozem with a light loamy texture formed on loess-like loam. The agrochemical characteristics of the arable layer (0-30 cm) at the time of experiment establishment were as follows: humus content – 1.43%, bulk density – 1.17 g/cm^3, pH_{KCl} – 5.76, available phosphorus – 105 mg/kg, exchangeable potassium – 78 mg/kg. Climatic conditions of the region were characterised by a mean annual temperature of +7.8°C and annual precipitation of 540-620 mm. The experiment was a two-factor design with three replicates, and the treatment arrangement was randomised in blocks. The area of the sowing plot was 50 m^2, and the plot area was 35 m^2. The crop was giant miscanthus (*Miscanthus* × *giganteus*), 'Osinniy Zoretsvit' variety. Planting was carried out in April 2021 using rhizomes weighing 25-35 g at a density of 10,000 plants/ha (1.0 × 1.0 m spacing). Factor A (fertilisation system): A_1 – control (no fertiliser); A_2 – Leonardite (0-2 mm fraction, humic acid content ⩾75%), 100 kg/ha (applied once during spring tillage in the year the plantation was established); A_3 – mineral fertiliser $N_{60}P_{16}K_{80}$ (ammonium nitrate + ammonium phosphate + potassium chloride, localised application, annually). Factor B (foliar feeding with potassium humate 2 l/ha, solution concentration 1%): B_1 – no feeding (control); B_2 – during the tillering stage; B_3 – during the stem elongation stage; B_4 – both stages (twice). In total, the design comprised 12 treatments × 3 replicates = 36 plots. The analysis was conducted on the extreme variants for factor B (B_1 – no fertilisation; B_4 – double fertilisation), as it was precisely the contrastive comparison of these variants that allowed the nature of the effect of foliar fertilisation with potassium humate on C-sequestration indicators in each fertilisation system of factor A to be identified as clearly as possible.

Soil samples were collected annually throughout the study period at the end of the growing season (third decade of October) from three depths: 0-10 cm, 10-20 cm and 20-30 cm at five points on each plot using the envelope method in accordance with DSTU 4287:2004 (2005). A single composite sample weighing 1 kg was prepared from each plot for analysis; in total, 540 soil samples were collected and analysed over the 5-year period. Above-ground biomass yield was recorded by completely mowing the plot areas (35 m^2) at the end of the growing season (December – plants in a state of physiological dormancy). The samples were dried to air-dry condition in a drying oven at 60°C for 48 hours, and moisture content was determined in accordance with DSTU ISO 11465:2001 (2003). A biomass sample for each treatment was formed from three replicates. SOC (%) was calculated using the formula:

$$\mathrm{SOC} = \mathrm{Humus}/1.724, \tag{1}$$

where 1.724 – is the Van Bemmelen coefficient accounting for the average carbon content in humus (~58%).

Organic carbon stocks in the soil layer (t C/ha) were calculated according to the formula:

$$\mathrm{SC} = \mathrm{SOC} \times \rho \times \mathrm{h} \times 100, \tag{2}$$

where SC – the stock of organic carbon, t C/ha; SOC – the SOC content, %; ρ – the soil bulk density, g/cm^3; h – the thickness of the soil layer, m (0.30 m for the 0-30 cm layer); 100 – the conversion coefficient.

For comparative analysis, the study used the contrasting variants of factor B – without foliar feeding (B_1) and double foliar feeding (B_4), which enabled the clearest assessment of the effect of potassium humate within each fertilisation system. The carbon content in above-ground biomass was determined using an Elementar Vario MAX elemental analyser (Elementar Analysensysteme GmbH, Germany) (CHNS mode, combustion temperature 1,150°C, carrier gas – helium, standard – sulfanilamide). The average carbon content in dry miscanthus biomass was 46.0 ± 0.5%, corresponding to literature data for the genus *Miscanthus*. Carbon sequestration by above-ground biomass (CB, t C/ha) was calculated as:

$$\mathrm{CB} = \mathrm{DBY} \times \mathrm{Cc}/100, \tag{3}$$

where DBY is the yield of absolutely dry biomass, t/ha; and Cc is the carbon content in biomass, %. Conversion of carbon into CO_2 equivalent was performed using the coefficient 3.667 (molar ratio $CO_2/C = 44/12$).

The dynamics of indicators during 2021-2025 were assessed using non-linear regression with approximation by a logistic function (for biomass carbon sequestration) and linear regression (for SOC and soil carbon stocks). The coefficient of determination R^2 was calculated to assess the degree of correspondence between the approximated curve and the experimental data. Statistical processing of the results was carried out using analysis of variance (two-factor ANOVA) for a split-plot design. In addition, the following were calculated: Fisher's F-test, significance level (p-value), least significant difference at the 5% significance level (LSD_{05}), and coefficient of variation (CV, %). The normality of distribution was verified using the Shapiro-Wilk test, while homogeneity of variances was assessed using Levene's test. Differences were considered significant at $p \leqslant 0.05$. Multiple comparisons of means were performed using Tukey's HSD test. Correlation and regression analyses were conducted with calculation of pairwise correlation coefficients (r) and coefficients of determination (R^2). Calculations were performed using Statistica 13.3 (TIBCO Software, USA) and R Studio (package "agricolae"). Graphical data processing was carried out in the Python 3.11 environment (matplotlib, seaborn) with a resolution of 300 dpi. The study was conducted in accordance with the requirements of the Convention on Biological Diversity (1992).

Results and Discussion

The assessment of SOC dynamics during the initial years of miscanthus plantation establishment was important for clarifying the directions of carbon balance formation within the agroecosystem. It was precisely at the early stage of cultivation that the differences between fertilisation systems became most clearly apparent in terms of the rate of organic compound input into the soil and their subsequent stabilisation. Analysis of the research results (Table 1) indicated a progressive accumulation of organic carbon in the arable soil layer under the perennial plantation of giant miscanthus across all investigated treatments.

***Table 1.** SOC content under giant miscanthus, % (selected treatments, 2021-2025)*

No.	Factor A	Factor B	Years of study					Average
			2021	2022	2023	2024	2025	
1	Control	No foliar feeding (B_1)	0.829	0.829	0.835	0.847	0.858	0.840
2		Both growth stages (B_4)	0.829	0.835	0.847	0.858	0.876	0.849
3	Leonardite, 100 kg/ha	No foliar feeding (B_1)	0.835	0.847	0.864	0.887	0.905	0.868
4		Both growth stages (B_4)	0.835	0.858	0.882	0.911	0.934	0.884
5	$N_{60}P_{16}K_{80}$	No foliar feeding (B_1)	0.829	0.835	0.841	0.853	0.864	0.844
6		Both growth stages (B_4)	0.829	0.841	0.858	0.870	0.887	0.857

Note: soil layer 0-30 cm. LSD_{05} (factor A) = 0.012; LSD_{05} (factor B) = 0.008; LSD_{05} (A × B) = 0.015; Cv = 1.9%
Source: compiled by the author

Three distinct trends in SOC dynamics were identified depending on the fertilisation system (factor A). On the control plots (A_1), SOC content increased from 0.829% in 2021 to 0.858-0.876% in 2025, corresponding to an increase of 3.5% (B_1) and 5.7% (B_4) over 5 years. Under the mineral fertilisation system (A_3), the increase in SOC was 4.2% (B_1) and 7.0% (B_4), indicating a moderate contribution of mineral fertiliser to the formation of the soil carbon pool due to the predominant flow of assimilated carbon into above-ground biomass. The highest SOC increase was achieved by the organic fertilisation system (A_2, Leonardite) – 8.4% (B_1) and 11.9% (B_4), with the absolute SOC value in the A_2B_4 treatment in 2025 (0.934%) being the highest among all combinations studied. Analysis of variance confirmed a significant effect of factor A on SOC ($F = 87.3$; $p < 0.01$), factor B ($F = 24.6$; $p < 0.01$) and their interaction A × B ($F = 5.8$; $p < 0.05$). The smallest significant difference was $NIP_{05} = 0.012\%$ for factor A and $NIP_{05} = 0.008\%$ for factor B. The linear regression for SOC dynamics in variant A_2B_4 was described by the equation $SOC = 0.0245 \times t + 0.810$ ($R^2 = 0.994$), indicating high predictive reliability of the model and stable rates of organic carbon accumulation in the topsoil under the organic fertilisation system. For variant A_3B_4, the corresponding equation was $SOC = 0.0145 \times t + 0.815$ ($R^2 = 0.989$). This dynamic is associated with a complex of physico-chemical processes: the direct entry of humic acids from Leonardite into the soil carbon pool, stimulation of microbial activity, intensification of the humification of fresh plant residues, and stabilisation of organo-mineral complexes. Figure 1 shows the dynamics of SOC content and the hierarchy of fertilisation systems in terms of their impact on the soil carbon pool: $A_2B_4 > A_2B_1 > A_3B_4 > A_3B_1 > A_1B_4 > A_1B_1$. This pattern was consistent with the findings of C. Bertola *et al*. (2024), who established that the application of organic fertilisers to miscanthus plantations significantly accelerated the formation of stable carbon pools in the topsoil. Figure 1 showed that, as early as the second year of vegetation, the curves for the Leonardite treatments diverged from the control and the mineral fertiliser system, and in 2024-2025 the gap between A_2B_4 and the control reached its maximum. Importantly, two foliar applications of potassium humate enhanced the positive effect of each fertilisation system; however, the most pronounced additional increase in SOC was observed specifically with the organic system. The calculated organic carbon stocks in the 0-30 cm soil layer for the various fertilisation treatments are presented in Table 2.

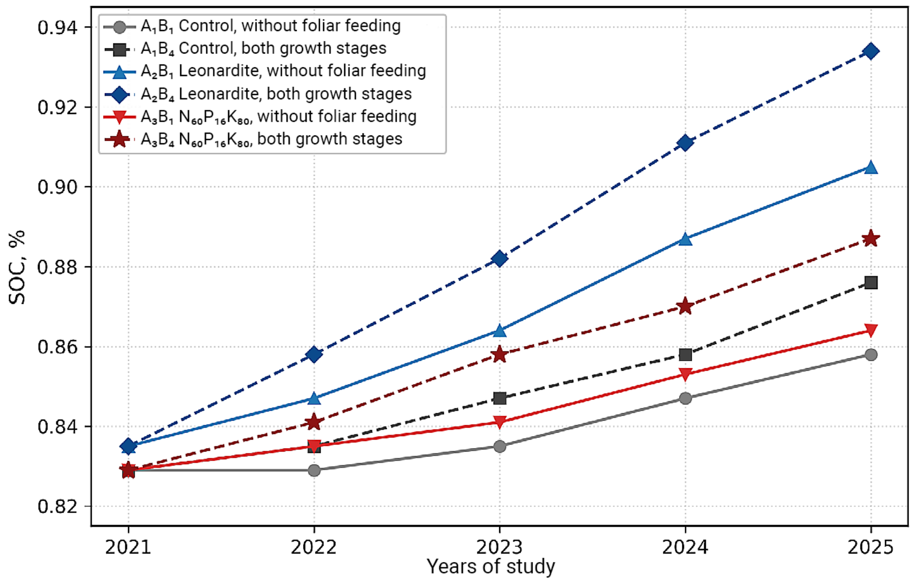

Figure 1. *Dynamics of SOC content under giant miscanthus under different fertilisation systems (2021-2025)*

Source: compiled by the author

Table 2. *Carbon stocks in the arable layer (0-30 cm) under giant miscanthus, t C/ha (selected treatments, 2021-2025)*

No.	Factor A	Factor B	Years of study					Average
			2021	2022	2023	2024	2025	
1	Control	No foliar feeding (B_1)	2.91	2.91	2.93	2.97	3.01	2.95
2		Both growth stages (B_4)	2.91	2.93	2.97	3.01	3.07	2.98
3	Leonardite, 100 kg/ha	No foliar feeding (B_1)	2.93	2.97	3.03	3.11	3.18	3.04
4		Both growth stages (B_4)	2.93	3.01	3.10	3.20	3.28	3.10
5	$N_{60}P_{16}K_{80}$	No foliar feeding (B_1)	2.91	2.93	2.95	2.99	3.03	2.96
6		Both growth stages (B_4)	2.91	2.95	3.01	3.05	3.11	3.01

Note: initial soil bulk density ρ = 1.17 g/cm³; layer depth h = 0.30 m. LSD_{05} (factor A) = 0.043 t C/ha; LSD_{05} (factor B) = 0.028 t C/ha; Cv = 1.7%

Source: compiled by the author

Organic carbon stocks in the arable layer (0-30 cm) demonstrated patterns similar to SOC dynamics. In the control plots, the absolute increase in carbon stocks amounted to only 0.10 t C/ha (A_1B_1) to 0.16 t C/ha (A_1B_4) over five years. The mineral fertilisation system ensured an increase of 0.12-0.20 t C/ha, whereas the organic system provided an increase of 0.25-0.35 t C/ha. The maximum absolute carbon stock value, 3.28 t C/ha, was recorded in treatment A_2B_4 (Leonardite + double foliar application of potassium humate), exceeding the control treatment A_1B_1 by 0.27 t C/ha (+9.0%). Thus, the annual rate of carbon accumulation in the 0-30 cm soil layer ranged from 0.02 t C/ha/year (A_1B_1) to 0.07 t C/ha/year (A_2B_4), which generally corres-onded to the lower limit of the range reported for long-term miscanthus plantations (0.83-1.37 t C/ha/year). The lower absolute values observed in the present study were explained by the initial stage of plantation establishment and the characteristics of the eroded chernozem soil. Analysis of Figure 2 demonstrated that the dynamics of carbon stocks closely followed the SOC trends: under the organic fertilisation system, carbon accumulation in the soil occurred most intensively, while the effect of foliar potassium humate application in combination with Leonardite proved to be synergistic.

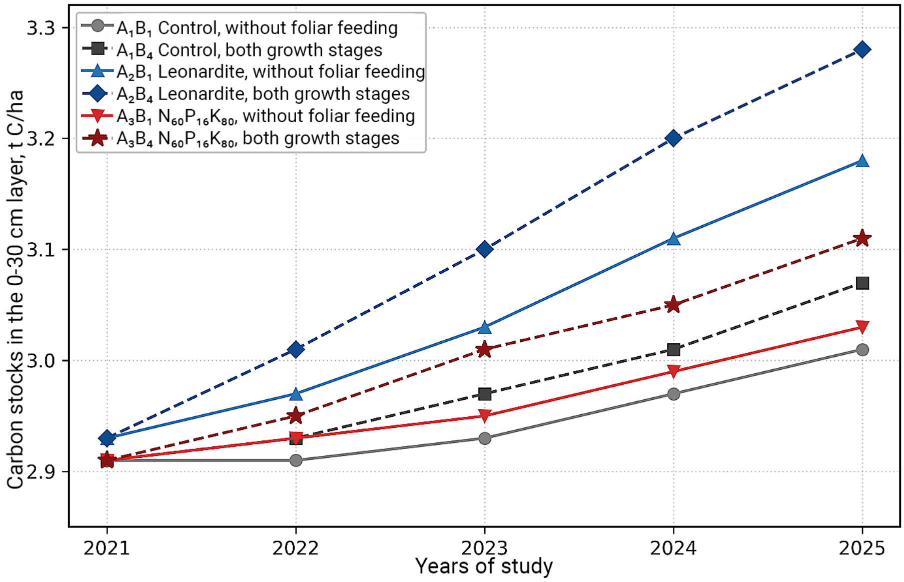

Figure 2. *Changes in carbon stocks in the topsoil (0-30 cm) under giant miscanthus under different fertilisation regimes (2021-2025)*

Source: compiled by the author

The graph shows that in 2023, treatment A_2B_4 exceeded the 3.10 t C/ha threshold, whilst the control treatments remained below 3.0 t C/ha almost until the end of the experiment. In 2025, the gap between A_2B_4 and A_1B_1 was 0.27 t C/ha, confirming the cumulative nature of carbon accumulation specifically under the organo-mineral-stimulated technology. Carbon sequestration by the above-ground biomass of miscanthus during 2021-2025 was characterised by steady annual growth across all fertilisation and foliar feeding treatments, with the highest values recorded in the $N_{60}P_{16}K_{80}$ treatment combined with foliar application in both phases (Table 3).

Table 3. *Carbon sequestration by above-ground biomass of giant miscanthus, t C/ha (selected treatments, 2021-2025)*

No.	Factor A	Factor B	Years of study					Average
			2021	2022	2023	2024	2025	
1	Control	No foliar feeding (B_1)	0.98	2.68	3.89	4.48	4.71	3.35
2		Both growth stages (B_4)	1.18	3.18	4.66	5.37	5.64	4.01
3	Leonardite, 100 kg/ha	No foliar feeding (B_1)	1.23	3.32	4.84	5.59	5.87	4.17
4		Both growth stages (B_4)	1.48	3.93	5.79	6.68	7.02	4.98
5	$N_{60}P_{16}K_{80}$	No foliar feeding (B_1)	1.44	3.89	5.69	6.57	6.89	4.90
6		Both growth stages (B_4)	1.72	4.61	6.81	7.86	8.25	5.85

Note: carbon content in dry miscanthus biomass – 46.0 ± 0.5% (determined using an Elementar Vario MAX elemental analyser). LSD_{05} (factor A) = 0.18 t C/ha; LSD_{05} (factor B) = 0.12 t C/ha; Cv = 3.4%

Source: compiled by the author

Thus, a logistic pattern characteristic of perennial C4 crops was observed: an intensive increase in carbon sequestration in 2021-2023, gradually levelling off in 2024-2025. In the first year after planting, C sequestration was minimal (0.98 t C/ha in A_1B_1 – 1.72 t C/ha in A_3B_4), due to the priority given to the formation of the root system and rhizome apparatus. By the fifth year of vegetation, C sequestration by biomass had increased to 4.71-8.25 t C/ha, with the maximum recorded under the $N_{60}P_{16}K_{80}$ mineral fertiliser regime with two foliar applications of potassium humate (A_3B_4). A two-factor analysis of variance confirmed the significance of the effects of both factors (A: $F = 142.8$; $p < 0.001$; B: $F = 38.1$; $p < 0.001$) and their interaction ($F = 4.2$; $p < 0.05$). The logistic regression for the A_3B_4 treatment was of the form $CB(t) = 8.55/(1 + 7.9 \cdot \exp(-0.86 \cdot t))$ with $R^2 = 0.996$, indicating a very high predictive ability of the model. Based on the mean values, the following hierarchy of fertilisation systems was established in terms of C sequestration by biomass: $N_{60}P_{16}K_{80}$ (4.90-5.85 t C/ha) > Leonardite (4.17-4.98 t C/ha) > control (3.35-4.01 t C/ha). The mineral fertiliser system provided a 46.3% increase in average C sequestration compared to the control, whilst the organic system provided a 24.5% increase. Two foliar applications of potassium humate further increased C sequestration by biomass by 16.4-19.4%. Figure 3 shows the dynamics of carbon sequestration by aboveground biomass for the period 2021-2025.

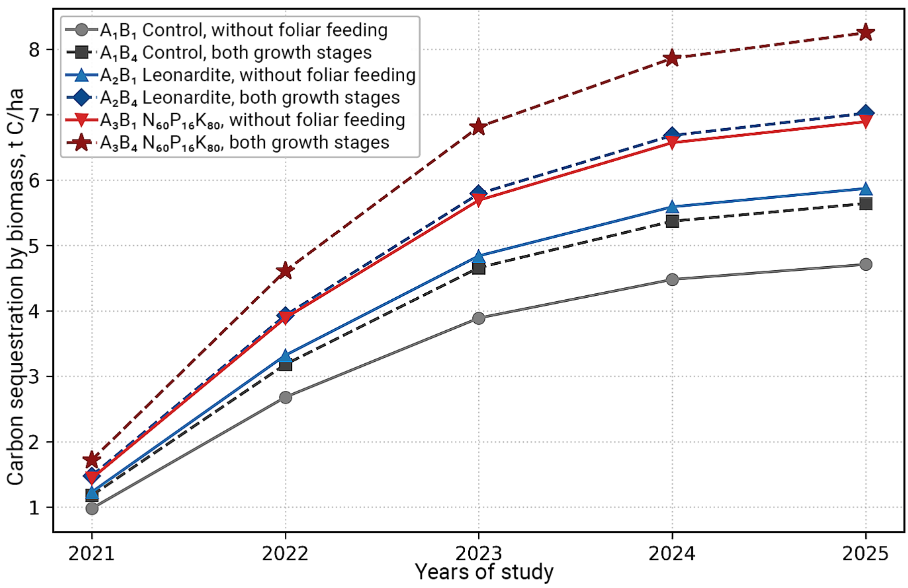

Figure 3. *Trends in carbon sequestration by the above-ground biomass of giant miscanthus under different fertilisation regimes (2021-2025)*

Source: compiled by the author

It was found that the mineral fertiliser regime produced the steepest rise in the curve as early as the second growing season, and the difference between A_3B_4 and A_1B_1 in 2025 reached 3.54 tonnes of carbon per hectare. Thus, it was the combination of full mineral nutrition with twice-yearly humic fertilisation that ensured the highest carbon sequestration in above-ground biomass. At the same time, although the organic system (Leonardite) lagged behind the mineral system in terms of absolute biomass sequestration, it demonstrated better carbon deposition in the soil, which shaped a specific carbon balance architecture. The cumulative carbon sequestration by above-ground biomass during 2021-2025 of miscanthus plantation vegetation varied widely depending on the fertilisation and feeding systems (Fig. 4).

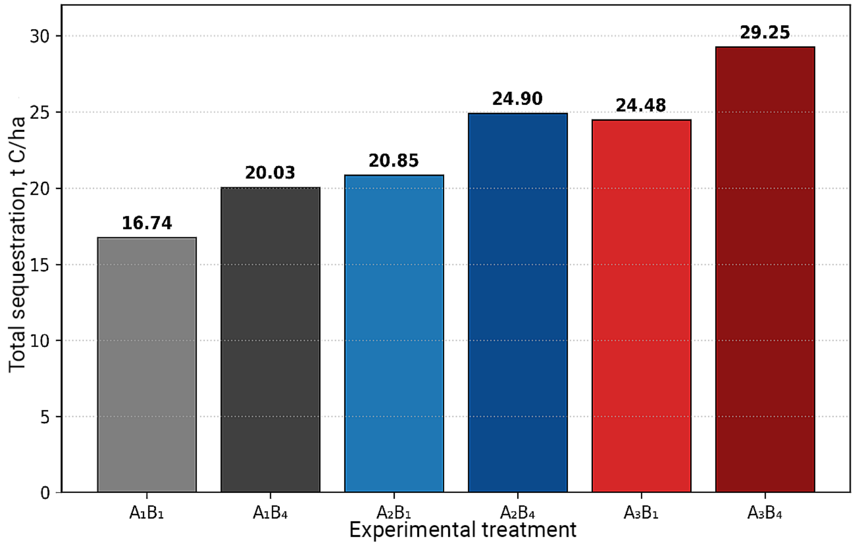

Figure 4. *Total carbon sequestration by the above-ground biomass of giant miscanthus over five growing seasons (ranking of variants, 2021-2025)*
Source: compiled by the author

The minimum value of cumulative carbon sequestration (16.74 t C/ha) was recorded in treatment A_1B_1 (control without foliar feeding), while the maximum value (29.25 t C/ha) was observed in treatment A_3B_4 (mineral fertilisation system with double foliar feeding). Of particular importance is the fact that treatment A_2B4 (Leonardite + double foliar feeding; 24.90 t C/ha) was practically equivalent to treatment A_3B_1 (mineral fertilisation system without foliar feeding; 24.48 t C/ha), indicating the high efficiency of combining an organic source of humic substances with a biostimulant as an alternative to intensive mineral technologies. According to O. Prysiazhniuk *et al.* (2022), miscanthus under low-intensity cultivation on heavy soils of Ukraine is capable of ensuring stable dry biomass production, which generally corresponds with the obtained results regarding the high productivity of the studied crop even on soil with reduced humus content. This confirmed that technological optimisation of fertilisation was a key factor in enhancing the sequestration function of miscanthus under specific soil and climatic conditions. The conversion of the obtained carbon sequestration values into CO_2 equivalent was performed using the standard coefficient 3.667 (molar ratio CO_2/C). The average annual CO_2 uptake by above-ground miscanthus biomass ranged from 12.28 t CO_2/ha/year (A_1B_1) to 21.45 t CO_2/ha/year (A_3B_4), exceeding the average values for the main C_4 crops of temperate climates – maize (8-12 t CO_2/ha/year) and sorghum (10-14 t CO_2/ha/year) – by 1.5-2.0 times (Fig. 5). The cumulative CO_2 uptake during 2021-2025 of giant miscanthus plantation vegetation ranged from 61.4 t CO_2/ha (A_1B_1) to 107.3 t CO_2/ha (A_3B_4). This corresponded to the upper range of values reported for the first five years of miscanthus cultivation. Double foliar application of potassium humate ensured additional CO_2 sequestration of 12.1-19.6% compared with treatments without foliar feeding. Figure 6 demonstrated the ranking of treatments according to their integrated climate-regulating effect: $A_3B_4 > A_2B_4 > A_3B_1 > A_2B_1 > A_1B_4 > A_1B_1$. The difference between the extreme treatments amounted to 45.9 t CO_2/ha, providing a substantial argument in favour of the differentiated selection of fertilisation systems depending on the objective – maximum carbon sequestration in biomass or in soil. The methodology for converting carbon into CO_2 equivalent complied with the recommendations of the IPCC (2019), which increased the suitability of the results for subsequent application in greenhouse gas accounting systems.

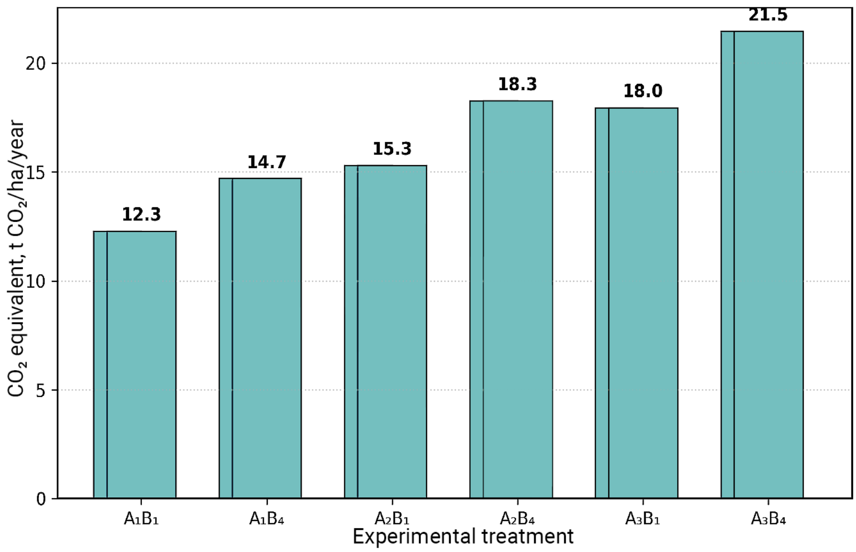

Figure 5. *Average annual CO_2 uptake by the above-ground biomass of giant miscanthus under different fertilisation regimes (2021-2025)*

Source: compiled by the author

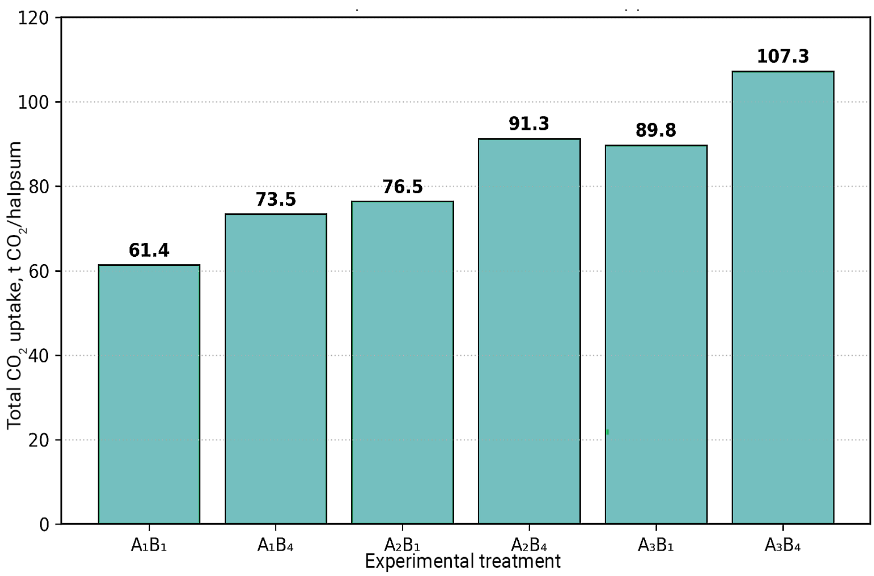

Figure 6. *Total CO_2 uptake by giant miscanthus crops over five growing seasons (2021-2025)*

Source: compiled by the author

Interpretation of the obtained results required the identification of two distinct pathways of carbon balance formation depending on the type of fertilisation system. The organic system (Leonardite, 100 kg/ha) ensured the greatest increase in SOC (+11.9% in treatment A_2B_4) at a relatively lower level of biomass carbon sequestration. To explain this pattern, a methodological understanding of the nature of the SOC indicator was essential: D.W. Pribyl (2010) demonstrated that the traditional coefficient used for converting humus into organic carbon represented a generalisation and required careful interpretation depending on soil type. In the present experiment, the use of the constant coefficient 1.724 was justified for comparing treatments within the same soil type, while the identified trends confirmed a genuine enhancement of organic matter sequestration following Leonardite application. At the same time, the IPCC (2019) approach to assessing carbon stocks in agroecosystems emphasised the need to combine data on soil and biomass carbon pools, which fully corresponded to the logic of the present experiment. The mineral fertilisation system ($N_{60}P_{16}K_{80}$) ensured the highest carbon sequestration by above-ground biomass (5.85 t C/ha/year) with a lower increase in SOC, indicating that assimilated carbon was predominantly directed towards biomass formation. In the work of O. Prysiazhniuk *et al*. (2022), it was shown that miscanthus under low-intensity agrotechnologies maintained high productivity stability on heavy low-productivity soils, and therefore additional mineral nutrition naturally contributed to an even fuller realisation of the crop's bioenergy potential. T. Nakajima *et al*. (2018) established that *Miscanthus* × *giganteus* was characterised by higher carbon accumulation and biomass productivity compared with *Miscanthus sinensis* under various growing conditions. The authors also demonstrated that perennial miscanthus plantations were capable of efficiently accumulating organic carbon in the soil, confirming the crop's potential for bioenergy and climate-oriented agriculture. Yu. Hayda *et al*. (2024) identified the high potential of miscanthus plantations for carbon dioxide sequestration and oxygen production under the conditions of Ukraine. The researchers noted that increasing plant biomass directly influenced the intensity of CO_2 uptake, confirming the environmental efficiency of crop cultivation. R. Teteriuk & M. Kulyk (2024) assessed miscanthus cultivars in terms of adaptability, yield, and energy productivity under the conditions of the Left-Bank Forest-Steppe of Ukraine. According to their results, certain cultivars demonstrated high stability of yield formation and significant potential for use as an energy feedstock. I. Beznosko *et al*. (2022) investigated the quantitative composition of micromycetes in chernozem soils of the Left-Bank Forest-Steppe of Ukraine under cereal crops. The researchers established that agroecological conditions and cultivation characteristics significantly influenced the structure of the soil mycobiota and soil microbiological activity. M. Gumentyk & M. Kharytonov (2018) developed and evaluated technologies for miscanthus cultivation under the Forest-Steppe conditions of Ukraine, taking into account crop productivity and energy efficiency. Their study demonstrated that optimisation of cultivation technology elements contributed to increased biomass yield and the economic feasibility of energy crop utilisation.

The synthesis by D. Rakhmetov (2011) concerning the introduction and economic-biological characteristics of energy crops in Ukraine also confirmed that miscanthus productivity was determined by cultivar adaptability and the level of resource provision, explaining the observed superiority of the mineral system regarding the biomass carbon pool. V. Yakovenko *et al*. (2024; 2025) established that in the chernozems of the Ukrainian Steppe, the key indicators of soil condition were SOC content, humus form, and the intensity of bioturbation, and that these indicators responded distinctly to the type of land use. A. Holder *et al*. (2026) proposed the concept of a cell-wall ideotype of miscanthus aimed at maximising soil carbon sequestration, opening new perspectives for breeding. The overall potential of CO_2 sequestration by miscanthus under Bioenergy with Carbon Capture and Storage systems reached 26 t CO_2-equivalent/ha per year. Foliar application of potassium humate provided an additional synergistic effect through activation of photosynthetic processes, the enzymatic apparatus of carbon and nitrogen metabolism, and enhanced plant stress resistance (Terravesta, n.d.). In combination with different fertilisation systems, this resulted in the formation of two practically significant scenarios for carbon balance management: the organic system combined with potassium humate proved more effective for the restoration of soil organic matter, whereas the mineral system combined with potassium humate was more effective for maximising carbon accumulation in harvestable biomass. Consequently, the choice of agrotechnology should be determined not only by productivity but also by which particular carbon pool is prioritised by the farm or climate reporting system.

Conclusions

As a result of the study, the patterns of carbon sequestration by a perennial plantation of giant miscanthus, cultivar 'Osinniy Zoretsvit', under the Forest-Steppe conditions of Ukraine were established. It was demonstrated that the cultivation of giant miscanthus under all investigated fertilisation treatments ensured a progressive accumulation of SOC: SOC content increased from 0.829% in 2021 to 0.858-0.934% in 2025, while carbon stocks in the arable layer (0-30 cm) rose from 2.91 to 3.28 t C/ha. The highest increase in SOC (+11.9%) and soil carbon stocks (+0.35 t C/ha over five years) was achieved under the organic fertilisation system (Leonardite, 100 kg/ha) combined with double foliar application of potassium humate (treatment A_2B_4), which was associated with the direct input of Leonardite humic acids into the soil carbon pool and the intensification of humification processes. The highest carbon sequestration by above-ground biomass was ensured by the mineral fertilisation system $N_{60}P_{16}K_{80}$ combined with double potassium humate application (treatment A_3B_4): the maximum annual sequestration reached 8.25 t C/ha, while the cumulative amount over five years totalled 29.25 t C/ha. In CO_2-equivalent terms, the average annual atmospheric CO_2 uptake by miscanthus crops ranged from 12.28 to 21.45 t CO_2/ha/year, with the cumulative five-year uptake ranging from 61.4 to 107.3 t CO_2/ha, corresponding to the highest carbon sequestration efficiency indicators among agricultural crops of temperate climates. Analysis of variance confirmed the significant effects of the fertilisation system ($p < 0.01$) and foliar feeding ($p < 0.01$) on all investigated parameters, while the coefficients of variation (1.7-3.4%) indicated high reproducibility of the results. The obtained findings served as a methodological basis for the implementation of climate-smart agriculture principles, the calculation of carbon credits in accordance with national and international greenhouse gas accounting standards, and the development of national decarbonisation policy for the agro-industrial sector. Prospects for further research include the long-term assessment of the stability of sequestered carbon, quantification of the total carbon footprint throughout the complete miscanthus cultivation cycle, and an integrated economic evaluation of the proposed agrotechnologies.

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Conflict of Interest

None.

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Секвестрація вуглецю та відтворення органічної речовини ґрунту під міскантусом гігантським за різних систем удобрення в умовах Лісостепу України

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Анотація. Метою дослідження було встановити закономірності секвестрації вуглецю багаторічною плантацією міскантусу гігантського (*Miscanthus* × *giganteus*) сорту ‘Осінній зорецвіт’ у ґрунті та надземній біомасі за різних систем удобрення та позакореневого підживлення гуматом калію в умовах Лісостепу України. Польові дослідження було проведено впродовж 2021-2025 років на чорноземі опідзоленому сильнозмитому легкосуглинковому. Двофакторний дослід включав три системи удобрення (контроль; органічна – Леонардит, 100 кг/га; мінеральна – $N_{60}P_{16}K_{80}$) та чотири режими позакореневого підживлення гуматом калію. Встановлено, що вирощування міскантусу гігантського забезпечило прогресивне нагромадження органічного вуглецю в ґрунті (SOC) від 0,829 до 0,876-0,934 % за п’ять років. Найбільший приріст SOC (+11,9 %) зафіксовано за органічної системи удобрення з дворазовим позакореневим підживленням гуматом калію (A_2B_4). Запаси вуглецю в орному шарі (0-30 см) зросли від 2,91 до 3,28 т С/га. Максимальна щорічна секвестрація вуглецю надземною біомасою – 8,25 т С/га (A_3B_4), сумарна за 5 років – 16,74-29,25 т С/га, що в перерахунку на CO_2-еквівалент становить 61,4-107,3 т CO_2/га. У роботі було підтверджено достовірність впливу систем удобрення ($НІР_{05}$ = 0,012 %; $p < 0{,}01$) та позакореневого підживлення ($НІР_{05}$ = 0,008 %). Органічна система удобрення з Леонардитом у поєднанні з дворазовим позакореневим підживленням гуматом калію виявилася найефективнішою для відтворення органічної речовини ґрунту, тоді як мінеральна система $N_{60}P_{16}K_{80}$ забезпечила максимальну секвестрацію вуглецю в надземній біомасі. Встановлено функціональне розмежування агротехнологічних ефектів: органічна система оптимізує ґрунтовий пул вуглецю, а мінеральна – біоенергетичний пул. Практична цінність роботи полягає у науковому обґрунтуванні диференційованого вибору системи удобрення міскантусу, які слугують основою для впровадження кліматично-розумного землеробства та сертифікації вуглецевих кредитів

Ключові слова: органічний вуглець ґрунту; вуглецевий баланс; гумат калію; кліматично-розумне землеробство; біоенергетичні культури

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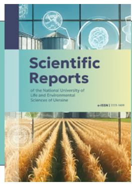

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Factors of technological stress in broiler chickens and strategies for its prevention

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Abstract. In intensive poultry farming, a range of technological stress factors affect the growth and development of broiler chickens and the quality of poultry products. The impact of acute or chronic stress is reflected in poultry behavioural reactions, eating disorders, feed conversion, daily weight gain, and product quality. The purpose of the study was to critically review and systematise scientific knowledge about changes in the bird's body under the influence of technological stress factors. This paper analysed scientific literature on physiological and behavioural changes in poultry caused by stress-related disorders, identifies clinical and laboratory markers for their diagnosis, and outlines methods of prevention. The review helped to identify the main criteria that help differentiate the manifestations of stress in poultry. It was determined that the main technological stressors for poultry are a violation of the temperature regime of poultry rearing, an increase in the density of its content, regrouping, weighing, sorting, forming groups, trapping, moving, transporting, technological noise. Clinical manifestations in the development of stressful phenomena in poultry were a decrease in feed consumption, excitability, anxiety, fear, fatigue, rapid shallow breathing, premature maturation and impaired reproductive functions, a decrease in egg laying, a weakening of muscle tone, and a decrease in the quality of poultry products. The criteria for determining the manifestation of stressful phenomena in poultry were behavioural changes and clinical indicators, in the blood – the ratio of

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heterophiles to neutrophils, the content of corticosteroids. To investigate oxidative stress in greater depth, it would be useful to measure the levels of malondialdehyde and 8-hydroxy-2-deoxyguanosine in the birds' blood, and the activity of glutathione and superoxide dismutase, and the heat shock proteins HSP70 and HSP90. Strategies for preventing the development of technological stress factors include optimisation of keeping conditions, correction of the diet with the addition of biologically active compounds, macro- and microelements, organic acids, probiotics and prebiotics to build the birds' resistance to both production-related and other environmental factors. The results of the study will be useful for developing effective means of preventing technological stresses in poultry

Keywords: poultry; stress factors; heat exposure; density of keeping; lighting; physiological changes; preventive measures

Introduction

Poultry farming is a highly effective method of animal husbandry, which makes a significant contribution to ensuring the food safety of a significant part of the global population. Market demand and the use of contemporary intensive farming methods contributed to a significant increase in global poultry production, which reached 151.4 million tonnes in 2024/2025. Ukraine produces 1 million 390 thousand tonnes of poultry meat, which is 1.3% of the world market for poultry products (Poultry meat production..., 2025). In the face of changing consumer preferences and biosafety threats associated with outbreaks of highly contagious diseases, poultry sustainability and adaptability are crucial to ensuring global food security. Furthermore, climate change, intensification and automation of poultry rearing processes almost inevitably leads to the emergence of various stressful factors, which has a significant impact on the physiological status, growth, development of poultry, and the quality of poultry products.

R.M. Bilal *et al.* (2021) noted that environmental changes pose significant threats to agricultural activities and negatively affect poultry health and productivity in the face of current climate change. They also change the immunological status of poultry, make them susceptible to various diseases, contribute to a decrease in productivity (reduced feed consumption, growth, feed efficiency, immunity and egg production), and a decrease in the quality of poultry products. L.I. Podobied (2022) argued that the problem of increasing seasonal temperatures is becoming increasingly important in Ukraine. Periods of heat, when the outdoor temperature in summer exceeds 30°C, and in some regions – 40°C, are becoming longer, which negatively affects the main production indicators of poultry farms, as it leads to increased death of poultry due to heat stress. For most bird species, the thermoneutral zone (the ambient temperature range in which the body can maintain body temperature) is between 15.6 and 23.9°C. When the ambient temperature rises to 29.4°C, the bird changes its behaviour and reduces its feed intake to prevent an increase in body temperature. When the air temperature rises to 37.8°C, the bird's body temperature rises to lethal limits. S.F. Olayiwola & S.A. Adedokun (2025) reported that the poultry are very sensitive to light conditions, air quality, humidity, poor ventilation, transportation and veterinary manipulations, and violations of the feeding regime, nutrient deficiencies, feed contamination, diseases, an imbalance of the intestinal microbiota, etc. Most often, the poultry are exposed to elevated ambient temperatures, to which it is particularly vulnerable due to the lack of sweat glands and the presence of feathers. On the other hand, in some regions, cold stress is very harmful, which also causes serious economic damage on a global scale. In general, according to K. Deshmukh *et al.* (2024), there are four main types of stress in poultry farming: technological, environmental, food, and internal. Under the influence of these stresses, it leads to harmful changes at the molecular, cellular, and physiological levels of the bird's body, which ultimately reduces the productive and reproductive indicators of industrial poultry farming.

The main technological stress factors affecting the physiological processes of poultry rearing are: deviations from the required temperature regime, increased stocking density, regrouping, weighing, sorting, grouping, catching, moving, transport, operational noise, prolonged storage of eggs, transport of eggs, inadequate storage conditions for eggs, and incorrect incubation regimes. Environmental stresses include temperature changes in the environment, insufficient ventilation, high levels of dust and ammonia in poultry premises, and insufficient lighting. Food stress is associated with impaired feed conversion, reduced average daily weight gain, immunosuppression, and increased mortality in growing poultry. In particular, these are the effects of mycotoxins, exposure to toxic metals (Pl, Kd, Hg), an imbalance of minerals (Se, Zn, Mn, Cu, etc.) and other nutrients, poor water quality, the use of coccidiostatics and other veterinary drugs. Internal stresses depend on the production cycle, including peak egg production and poultry vaccination, preventive treatments, microbial or viral diseases, and intestinal dysbiosis. Research, in particular by M. Shah Alam (2025), confirmed that most stresses in poultry farming occur at the cellular level. They are often linked to oxidative stress due to excessive production of free radicals or insufficient antioxidant protection. Furthermore, any stressful factors cause changes in regulatory mechanisms in the bird's body. This causes increased energy mobilisation and metabolic disorders that impair poultry productivity. Therefore, the development of effective ways to prevent stressful phenomena during intensive poultry rearing is an important task for researchers and practitioners.

The purpose of the study was to summarise and critically review existing scientific data on the occurrence of stresses in poultry during intensive farming, methods of diagnosis and strategies for preventing their occurrence. The focus of the research was on scientific data concerning the impact of various factors on poultry, the changes they induce, and their effect on growth, development, and the quality of poultry products, and a review of various strategies for preventing technological stress in poultry farming. The PubMed database, Elsiver, and specialised scientific journals were used to search for information. Descriptive, analytical, comparison, and synthesis methods were used. The comparison method was used to contrast different approaches to the diagnosis of stress conditions in poultry, described in scientific sources under the influence of various stress factors. This method helped to identify markers and criteria for the diagnosis of stress in poultry and approaches to prevention.

Characteristics of the main technological stress factors in poultry

In contemporary poultry farming, stress can be considered as an automatic reaction of the body caused by exposure to adverse environmental conditions. In poultry, stress manifests itself through a number of physiological responses, such as identification of a stress stimulus, activation of the sympathetic nervous system and adrenal medulla, and subsequent hormonal cascades, in particular, the production and release of corticosterone and catecholamines as a result of neuroendocrine-immune interaction. The secretion of epinephrine or norepinephrine increases respiratory and heart rate, blood pressure, and activates glycogenolysis and lipolysis. The release of corticosterone weakens the immune system, reduces feed intake, and impairs bowel function.

Y. Huang *et al.* (2024) highlighted that heat stress is becoming an increasingly serious problem for poultry. The degree of heat stress experienced by poultry is divided into mild, moderate, strong and depends mainly on heat radiation, humidity, thermoregulatory ability of the body, metabolic rate, age, intensity and duration of action of the heat factor. Due to the high metabolic rate that modern broiler chickens have, this leads to the production of more heat. When heat stress occurs, the neuroendocrine system in poultry is disrupted. It activates endocrine glands such as the pituitary gland, adrenal glands, thyroid gland, and sex glands through the action of the hypothalamus and sympathetic nerves. This causes changes in the level of hormones such as corticosterone, triiodothyronine, thyroxine, gonadotropin, decreased ovarian function, and promotes the release of catecholamines, which affects poultry productivity.

As indicated by S.F. Olayiwola & S.A. Adedokun (2025), in poultry, persistent corticosterone secretion is associated with cardiovascular disease, weakened immune systems, depression, muscle breakdown due to gluconeogenesis, and cognitive decline. This reaction can range from mild discomfort to serious consequences, including poultry mortality. Research data by C.M. Ncho (2025) indicated that heat stress significantly affects the digestive system of poultry. This leads to changes in the composition of microbial populations, disruption of specific functional pathways of microorganisms (tricarboxylic acid cycle, amino acid metabolism, antioxidant biosynthesis and fatty acid synthesis), disruption of intestinal barriers, which causes the appearance of increased intestinal permeability syndrome, which changes the pH of the intestinal environment, causes oxidative stress, and leads to inflammation. M.R. Farag & M. Alagawany (2018) indicated that acute or chronic heat stress negatively affects the well-being of poultry. In addition to inducing oxidative stress, heat stress can also cause cell damage. This, in turn, leads to endocrine disorders, immunosuppression, increased inflammatory processes, changes in the microbial population, deterioration of intestinal function, deterioration of meat quality, and the possible occurrence of intestinal diseases in poultry. Heat stress also causes hyperventilation, which disrupts the acid-base balance of the blood. This leads to respiratory alkalosis, the development of which interferes with the growth of chickens, egg production and eggshell quality of laying hens. Heat stress significantly reduces the egg production of laying hens and egg weight (Tesakul *et al.*, 2025).

Another factor that is important for ensuring the functioning, growth, and productivity of poultry is the lighting mode. As noted by C.M. Ncho *et al.* (2024), lighting plays a crucial role in triggering the secretion of various hormones that directly affect the growth, maturation, reproduction, and egg production of poultry. Therefore, to improve the production and efficiency of poultry farming, it is important to consider aspects of environmental management, such as regulating light intensity, duration, wavelength, and photoperiodic mode. Recent studies show that the wavelength of light affects a bird's ability to respond to stress, fear, and depression. In particular, exposure to green, red, and white light spectra can reduce fear and sensitivity in broilers, and irregular light exposure times can cause circadian rhythm disturbances that directly affect the bird's well-being and behavioural responses (El-Sabrout *et al.*, 2022). Currently, there is no conclusive data on the optimal lighting intensity to ensure the health and well-being of broiler chickens, which can be applied equally to all detention systems. With the exception of the first and last weeks of rearing, birds provide at least four hours of darkness every 24 hours. Four hours of darkness can be provided at one, two, or four-hour intervals.

Light intensity in poultry houses and other artificial environments is measured in lux or cd/m^2 and has a significant impact on the behaviour of chickens. According to the standard lighting mode for broiler chickens, a constant light intensity of 20 lux is maintained for the first 7 days (the early period after hatching), after which it is adjusted to a low intensity of 3-5 lux for the rest of the poultry rearing period. In addition, the density of poultry content is of great importance, which, if increased, can also cause violations of physiological and productive indicators, and affect the quality of the resulting products. As noted by E.T. Gül *et al.* (2022), the ideal keeping density for broilers should be 35 kg/m^2. Otherwise, there may be problems associated with reduced availability of feed and water in poultry, abnormal behaviour, lack of air, and poor flooring quality. In addition, the high density of poultry content can create a mild stress shock, increasing the temperature in the microenvironment around the bird and reducing heat transfer from the body. This leads to pathological reactions that may be associated with a violation of the function of the intestinal barrier.

Simultaneously, K. von Eugen *et al*. (2019) found that when the poultry content density is increased during the first 6 weeks, chickens have more anxious behaviour than when the poultry content density is low. Simultaneously, both high and insufficient keeping densities of chickens recorded a higher level of corticosterone in their blood, compared to those chickens that were kept in conditions of optimal density. At 10 weeks of age, plasma corticosterone levels in chickens decreased to optimal density levels in both groups, while feather corticosterone levels remained high only in the high density group. The researchers concluded that a threefold increase or decrease in broiler chicken keeping density affects corticosterone levels and anxiety behaviour in poultry, but within the adaptive capabilities of chickens. The increase in keeping density has led to a slowdown in their adaptation and an increase in the cost of its provision.

T. Shynkaruk *et al*. (2022) also evaluated the effect of poultry keeping densities at the rates of 31, 34.5, 38, and 41.5 kg/m^2 on the wellbeing and productivity of broiler chickens. They found that with a decrease in the density of content, the humidity of the litter, cases of dermatitis of the paw pads, poultry mortality from infections, fear, and stress decreased. The ratio of heterophiles/lymphocytes in the blood of poultry was the highest in the density variants of 31 and 41.5 kg/m^2. This suggests that the bird experienced more chronic stress at both low and high planting densities. The optimal body weight of poultry was highest in the density variants of 31 and 34.5 kg/m^2. The increase in body weight of the bird increased with a decrease in the keeping density, and feed consumption was greatest in the variant with a density of 34.5 kg/m^2. M.G. Anderson *et al*. (2021) in another experiment investigated the effect of environmental complexity and keeping density on determining broiler anxiety and tonic immobility (a measure of fear). The results of the study showed that the bird at the age of 3 weeks was less timid in high-density conditions than in low-density conditions. In addition, the results showed that a more challenging detention environment improved the well-being of broiler chickens by reducing anxiety. Thus, through behavioural testing, the researchers found that broiler chickens kept in higher-density environments reacted less fearfully than those kept in lower-density environments, contrary to the expectations and findings of other researchers.

Additionally, J. Son *et al*. (2022) indicated that the effect of high temperatures (33°C for 24 hours for 7 days and high content densities (23 and 26 birds/m^2) led to a significant decrease in the body weight and superoxide dismutase (SOD) activity in its blood, and an increase in rectal temperature and corticosterone levels. In addition, concentrations of the heat shock protein HSP 70 and malondialdehyde in the liver were higher in the group with 26 birds/m^2. Similarly, the uptake activity of 2,2-diphenyl-1-picrylhydrazyl radicals in breast tissue increased linearly with increasing bird landing density. Therefore, a lower planting density in broiler chickens can reduce oxidative stress caused by high temperatures and improve the antioxidant capacity of the bird's body and the quality of poultry products during the hot seasons of the year. Thus, technological stress factors such as temperature disturbances, lighting, and changes in content density lead to significant changes in the bird's body and affect metabolic processes on its growth and productivity.

Changes that occur in the bird's body under the influence of stressful factors

As noted by D. Diaz *et al*. (2022), prolonged heat exposure changes the course of anabolic processes in the poultry body to catabolic ones, which leads to a violation of the growth, muscle development, and quality of poultry meat. In addition, heat stress changes the composition of the gut microbiota, which leads to a decrease in microbial diversity and preference for opportunistic microorganisms. These changes impair the absorption of nutrients, disrupt energy metabolism, and impair immune system function, increasing physiological stress. In addition, significant haematological and biochemical changes occur in the bird's body, in particular, a decrease in the number of red blood cells, a decrease in the haemoglobin content and haematocrit value, which impairs oxygen transport and energy metabolism. An increase in the content of non-esterified fatty acids (NEFA) and β-hydroxybutyrate in the blood plasma of poultry indicates the development of catabolism, when lipids are mobilised for energy due to poor feed intake and increased metabolic needs.

L. Yang *et al*. (2010) experimentally proved that acute heat stress in the body of broiler chickens increases the production of reactive oxygen species (ROS), the activity of antioxidant enzymes (SOD, catalase (CAT), and glutathione peroxidase (GSH-Px)), and the synthesis of malonedialaldehyde (MDA). These parameters, which are caused by heat stress, gradually decrease during the first 12 hours after exposure to high temperatures. Therefore, acute exposure to high temperatures can inhibit the activity of the mitochondrial respiratory chain, which leads to excessive ROS production and, as a result, to lipid peroxide oxidation and oxidative stress. Therefore, an important consequence of heat stress is the development of oxidative stress. J.A.M. Prates (2025) also reported that prolonged exposure to heat leads to excessive production of ROS, including superoxide anions (O_2^-), hydroxyl radicals (OH-) and hydrogen peroxide (H_2O_2), which exceeds the capabilities of endogenous antioxidant systems of the bird's body. In addition, R. Farag & M. Alagawany (2018) argued that exposure to heat stress can increase salmonella colonisation in the gut and increase poultry susceptibility to *E. coli*, and alter the microbial background of the ileum.

According to M. Shah Alam (2025), oxidative stress is the driving force behind the development of mycotoxicosis in poultry, in which a cascade of pro-inflammatory cytokines is triggered by the activation of numerous cellular signalling pathways, such as nuclear transcription factor κβ and mitogen-activated protein kinase. It promotes apoptosis and dysfunction of tissues and organs, such as immunosuppression, hepatotoxicity, nephrotoxicity, neurotoxicity, and gastrointestinal toxicity. The effects of this toxicity lead to poor feed conversion, feed rejection, low growth rates, reduced fertility, and reduced poultry hatchability.

Heat stress also has a negative impact on the mineral balance of the bird's body, and the degree of this effect depends on the type of mineral and the severity of heat stress. Exposure to high temperatures on broilers negatively affects the metabolism of minerals and their elimination routes, and also reduces the delay in the bird's organism of certain mineral elements, such as P, Na, K, S, Mg, Mn, Zn, and Cu. It is known that heat stress worsens the productive parameters of broiler chickens. But there is no clear definition in the literature of neuroimmune involvement observed during heat stress in infectious processes. Furthermore, permanent damage or the presence of pathogenic agents causes a stressful process in the gastrointestinal tract of poultry, increasing inflammation and oxidative stress.

One of the critical functions of the poultry gastrointestinal tract is to prevent harmful substances from entering the body from the intestinal lumen, which is called the intestinal barrier function. The function of the intestinal barrier is often determined by intestinal permeability and is closely related to the integrity of the dense junction of mucosal enterocytes, which is a sealed protein complex between enterocytes in the gastrointestinal tract. They serve as the body's first line of defence against the penetration of microorganisms and antigens from the intestinal lumen. If the function of tight intestinal contacts is disrupted, then both local and systemic inflammation in the body increases.

This leads to a decrease in the health and productivity of the bird.

As noted by J.E. Shin *et al*. (2018), reduced expression of genes and proteins associated with tight contacts in the gastrointestinal tract often correlates with reduced intestinal barrier function under the influence of heat stress factors. Damage to the intestinal epithelial barrier increases permeability, promotes inflammation, and reduces the surface area available for nutrient absorption. The brain-gut-microbiota system is responsible for maturing and maintaining the balance of the immune and nervous systems, which improves poultry productivity and resistance to diseases. J.M. Lyte (2024) indicates that there is a close relationship between gastrointestinal neurophysiology and the gut microbiome, highlighting the intersection of neurochemistry and microbiome along the respiratory and reproductive pathways of poultry. It is known that thermal or cold stressors alter neurochemical concentrations in poultry intestines. In particular, heat stress modulates the expression of neurochemical enzymes in the gut, including monoamine oxidase. Transport stress, during 2-3-hour exposure, causes rapid changes in the number of microbiota strains, which affects the susceptibility to intestinal carriers of food pathogens in poultry.

Diagnostic markers of stress in poultry

Stress in poultry can be caused either by one factor or by a complex combination of several factors. In the occurrence of stress factors, local climatic conditions (sunlight, heat exposure, humidity, temperature and air movement), animal production systems (free range or intensive rearing), conditions of keeping (manual or automatic temperature control, ventilation systems), methods of keeping (planting density, feeding time, drinking water temperature), and breed characteristics of poultry (genetic background, gender, age, metabolic rate, and thermoregulatory ability) are important.

Understanding the biological mechanisms underlying the occurrence of stress in poultry, and using reliable biological markers (or biomarkers) are central to developing new approaches to mitigating the effects of stress on animals. Due to the lack of sweat glands, the bird cannot cool its body. To regulate their body temperature, chickens spread their wings, keep them away from their bodies by ruffling their feathers, collecting dust, and moving less. Poultry tends to drink more and increase their rest time, while reducing their feed intake. Based on the unique respiratory system, which includes air bags connected to the lungs, a continuous and unidirectional flow of air through the respiratory system is ensured, promoting efficient gas exchange and maintaining the high metabolic needs of poultry. It is important to note that increased peripheral blood flow reduces excessive heat production, and increased shortness of breath helps to cool the body by evaporation (Onagbesan *et al.*, 2023).

As noted by M.D. Kucheruk & D.A. Zasekin (2018), under the influence of heat stress factors, the bird moves little, sits with its beak open, and has shallow and rapid breathing (thermal respiration). Blood tests reveal reduced total protein levels, elevated haemoglobin levels against a background of dehydration, and an increased mean corpuscular haemoglobin (MCH) level. High levels of excitability in poultry have been observed in response to various technological stress factors, along with anxiety, fear, aggressive behaviour, loss of appetite, fatigue and behavioural disturbances in young chickens, quails, and ducks. Common signs of stress in mature chickens, quails, and ducks during the development of technological stress are premature mass maturation, decreased and complete cessation of egg laying, impaired reproductive function, and weakened muscle tone. In addition, young poultry have a slowdown in feather growth and tousling, depression of the clinical and physiological state, tachycardia, arrhythmia, slowing or increasing intestinal motility. In an adult bird, "pecking" or cannibalism, premature mass moulting is observed.

In poultry, as in animals of other species, a reliable indicator of stress is the content of corticosterone in the blood plasma. Corticosterone, which circulates in the blood, is extensively metabolised by the liver with a half-life of 10-15 minutes in chickens. Elevated levels of corticosterone in the blood plasma have been observed in poultry during the night. Restraining birds for blood sampling can also cause additional stress and, consequently, a surge in corticosterone levels within a few minutes. Thus, M. Dehnhard *et al*. (2003) suggested using the analysis of steroid metabolites in poultry faeces. They developed and validated a method for non-invasive measurement of glucocorticoid metabolites in chicken faeces. Non-invasive measurements of faecal metabolites are promising for monitoring stress in poultry.

In addition, the ratio of heterophiles to lymphocytes (H/L ratio) is considered an indicator of physiological stress in poultry. Heat stress in poultry significantly increases the ratio of heterophiles to lymphocytes (H/L), which is a standard indicator of physiological stress. During heat stress, the level of corticosteroids in the bird's blood increases, which leads to an increase in the number of heterophiles and a decrease in the number of lymphocytes. In the blood of poultry exposed to heat stress, a reduced number of lymphocytes is often found, which may correlate with a decrease in antibody production and immune suppression. Simultaneously, this ratio may change with the age of the bird, which is associated with the development of the immune system in broiler chickens, and may also differ between breeds. In particular, J.J. Danzaria *et al*. (2025) indicated that Cobb 500 cross chickens have the lowest H:L values both under natural heat stress and under experimental conditions, which indicates their thermal tolerance compared to Ross 308 cross.

Key indicators of oxidative stress in poultry include high blood levels of MDA, 8-hydroxy-2-deoxyguanosine (8-OHdG) and heterophyll/lymphocyte ratio, and reduced glutathione (GSH) and SOD activity (Weidong *et al*., 2023). M. Shanmugam *et al*. (2017) indicated that all living organisms respond to various stressors by synthesising a group of evolutionarily conserved polypeptides called heat shock proteins (HSP). Most HSPs serve as molecular chaperones, helping organisms overcome internal and external stresses. Changes in HSP expression after harmful stimuli suggest that these proteins can be used to assess physiological stress in birds of various species. Heat shock proteins, particularly HSP70 and HSP90, serve as critical, sensitive biomarkers for monitoring stress in chickens, especially under heat stress and high population densities.

Strategy for preventing technological stress in poultry farming

The strategy for preventing technological stress in poultry farming includes advanced methods of poultry management, including optimised feeding and control of environmental factors. Simultaneously, there is a constant search for ways to reduce the effects of stress in poultry and maintain the bird's vital activity at a high level. The main methods of combating stress in poultry include: cooling the premises with sprinklers, cooling water to 12-13°C with a constant flow from wells, changing the feeding regime of poultry (early in the morning and late in the afternoon), moistening the feed, rapid biocorrection of osmotic pressure in the cells of the protoplasm of the body with feed and water, etc.

S. Bhawa *et al*. (2023) argued that the negative effects of heat stress can be addressed through strategic management improvements, including the selection of poultry rearing systems with better ventilation, suitable conditions, and recommended proper planting densities. This is crucial for improving poultry productivity when exposed to high temperatures. The use of closed poultry keeping systems ensured optimal conditions for raising poultry, the ability to regulate temperature, humidity and lighting, which helped to reduce stress and promote egg production, minimising the influence of external factors. Instead, poultry raised in open systems is more exposed to environmental factors (Tesakul *et al*., 2025).

The use of ultraviolet (UV) light is also widely used in poultry farming. Since the bird can see in the UV spectrum with wavelengths of 320-400 nm, the inclusion of shorter wavelengths can be considered as more natural illumination. In addition, illumination with a wavelength of 280-310 nm has a physiological effect by stimulating the synthesis of vitamin D, which affects the skeletal development. As indicated by S.M. Rana & D.L.M Campbell (2021), UV light has a positive effect on reducing fear responses and stress responses, but it significantly increases the risk of feather biting with age. The addition of UV radiation may have a more pronounced effect on egg production and shell quality when hens are deficient in vitamin D_3 in their diet or if they are in the final stage of production. In addition, changes in the colour of light lead to changes in stress levels, which is manifested in a lower ratio of neutrophils to lymphocytes (H:L) in the blood of broiler chickens raised under blue light, compared to those raised under white light. Using blue light during the hatching and rearing phase of the bird leads to reduced stress and reduced fear. This suggests that blue light can improve emotional states of fear and stress, thereby improving the bird's well-being. However, the most powerful and cost-effective method of compensating for the negative effects of heat stress, as indicated by L.I. Podobied (2023), is the use of the effect of biocorrection of poultry body metabolism with the help of special feed additives and complete feeding of poultry.

A current trend in research is the optimisation of the gut microbial balance, metabolism, and intestinal integrity through the use of alternative products such as prebiotics, organic acids, plant extracts, essential oils, and trace elements, due to their anti-inflammatory, antioxidant and immunomodulatory effects. This is aimed at reducing the colonisation of various pathogens associated with the induction of inflammatory processes in the gastrointestinal tract. The complex interaction of intestinal microflora and these drugs plays a crucial role in their use and proper effect on the modulation of natural host microflora (Tellez-Isaias *et al.*, 2023).

Poultry's energy and amino acid needs during heat stress are the most studied, while vitamins and trace elements remain among the least studied nutrient groups. Therefore, knowing the nutritional needs of poultry for specific farming options under heat stress is of great importance and requires careful assessment of the nutrients in the diet. Feeding should include balancing the diet and providing the necessary trace elements to improve the productive and reproductive performance of poultry. Supplementation with fats, antioxidants, yeast, and electrolytes is one of the most common feeding strategies to ensure optimal poultry production. Replacing antibiotics in production systems with alternative products, improved management practices, strict biosafety, quality ingredients, the absence of diseases (mycoplasma/salmonellosis), and effective immunisation programs are effective strategies for achieving poultry health and productivity goals. Probiotics can improve the physiology, gut health, and immune system of poultry under heat stress. Therefore, it is believed that the use of probiotics together with proper management potentially helps to eliminate some of the negative effects of heat stress on poultry (Hernández-Coronado *et al.*, 2025).

Approaches to reducing the level of oxidative stress in poultry, according to O.E. Oke *et al.* (2024), include management techniques to reduce stress in the production environment, antioxidant supplements, and dietary supplements. Natural antioxidants, including plant extracts, minerals and vitamins, are effective in increasing the bird's resistance to oxidative effects. To improve antioxidant defence in poultry under stressful conditions, it is necessary to activate cellular homeostatic networks (vitagens/several key proteins encoded by genes that regulate a complex network of processes involved in longevity), to synthesise protective factors that can counteract the increased production of ROS and oxygen-saturated proteins. According to V. Calabrese *et al.* (2014) vitagens include heat shock proteins: HSP70 and hemoxygesnase-1 (HO-1); SOD; thioredoxin system (Trx, Trx peroxidase (peroxiredoxins), sulphiredoxin and TrxR); glutathione system (GSH, glutathione reductase (GR), glutaredoxin (Grx), GPx); sirtuins.

K. Deshmukh (2024) noted the role of antioxidants in reducing oxidative stress and the ability to maintain effective antioxidant protection and redox balance in the body. Antioxidants such as vitamin E or trace elements (such as Se, Mn, Cu, and Zn) are essential components of poultry premixes and, in most cases, provide the bird's physiological need for these elements. However, under stress, there is a need for additional support of the antioxidant system in poultry. Based on the activation of a number of vitagens (through mechanisms associated with Nrf2: SOD; haemoxygenase-1, HO-1; GSH and thioredoxin, or other mechanisms: heat shock proteins (HSP)/heat shock factors (HSF), sirtuins, etc.), the internal protection of the antioxidant system, and the maintenance of redox balance was carried out.

Vitamin E is the main antioxidant in biological systems. It has been found that the optimal addition of vitamin E to poultry feed improves feed conversion efficiency, body weight gain, productivity and egg quality, nutrient absorption, immune response, and antioxidant status in poultry (Khan *et al.*, 2011). Vitamin C can also prevent oxidative stress by scavenging ROS. Although poultry can synthesise vitamin C, its amount is limited under heat stress. Vitamin C supplements (250 mg/kg of feed) improved growth rate, nutrient utilisation, egg production and egg quality, immune response, and antioxidant status in poultry exposed to heat stress.

Changes in the acid-base balance in blood plasma and the development of respiratory alkalosis can be prevented by adding electrolytes such as NH_4Cl, $NaHCO_3$, and KCl. During respiratory alkalosis, the bird releases more bicarbonate ions with the kidneys to restore normal blood pH. A high level of electrolyte balance in the diet, 200-300 meq/kg, is considered effective in mitigating the adverse effects of heat stress in poultry (Mangan & Siwek, 2024). Selenium is widely recognised as an essential trace element for humans and animals of various species, including poultry. Adding selenium to the diet in appropriate doses plays a crucial role in promoting the growth and reproduction of poultry. A sufficient amount of selenium is necessary to ensure that selenium performs its biological functions. It is worth noting that both a deficiency and an excess of selenium in the diet can seriously threaten the health of poultry. As noted by X. Zi-Jian *et al.* (2025), for broiler chickens during the growth period, the required amount of selenium in the diet should be 0.15 mg/kg.

Effects of stress caused by high poultry keeping density, according to E.T. Gül *et al.* (2022), it is possible to correct for an increase in dietary concentrations of amino acids such as lysine, methionine, tryptophan, and arginine, minerals such as selenium and chromium, and vitamins such as C and E. The addition of supplements such as probiotics, humates, phytophenolic compounds and propolis to the diet effectively reduces or eliminates the negative effects caused by high livestock density. As a result, regulating animal feeding effectively reduces or prevents negative consequences, thereby improving animal welfare and ensuring a better food yield. In addition, there is a positive effect of organic acids (citric, formic, fumaric, sorbic, butyric, propionic) against pathogenic bacteria, due to their antimicrobial activity, reducing the pH in the gastrointestinal tract by acidification, which leads to an increase in the use of feed nutrients and, thus, affects the growth and development of broiler chickens (Kim *et al.*, 2015). P.M. Karkach & Y.L. Obraztsova (2019) highlighted the positive effect of drinking solutions of ascorbic acid (vitamin C) and potassium chloride (KCl) on reducing the effect of heat stress during fattening broiler chickens. J.L. Campo & S.G. Dávila (2002) showed that tocopherol and niacin effectively reduce the effects of heat stress, while lactic acid increases stress in poultry caused by high fever.

Trace elements, in particular zinc (Zn), selenium (Se), copper (Cu), manganese (Mn) and chromium (Cr), act as essential cofactors for antioxidant enzymes, significantly reducing physiological and heat stress in broiler chickens. Organic chelated forms are more bioavailable than inorganic sources. They effectively reduce HSP70 expression, reduce pro-inflammatory cytokine levels, and improve immune function. T. Mohammadizad *et al*. (2025) noted that the addition of oorganic trace elements in the diet of poultry exposed to heat stress have a positive effect on growth, intestinal structure, and physiological responses associated with stress. In particular, poultry fed with chelated forms of trace elements showed significantly higher body weight gain, production efficiency index, serum T_4 concentration and duodenal villus height compared with poultry fed a basic diet. In addition, poultry fed micronutrients had a lower feed conversion rate, mortality rate, blood heterophyll/lymphocyte ratio, alkaline phosphatase activity, and corticosterone concentration, and heat shock protein HSP-70 expression levels in the liver and hypothalamus, indicating better resistance to stress.

Technological innovations

K. Olejnik *et al*. (2022) argued that the use of technological solutions in various livestock systems that can help to better control environmental conditions and poultry health is a prospect for poultry farming. Various types of technologies allow collecting data about poultry in real time, which can help to prevent a decline in animal welfare by identifying the early stages of diseases in stressful situations, and also allows quickly taking measures to limit the negative consequences. I. Fontana *et al*. (2015) noted that a combination of audio and video information in automated herd management systems can be used in early warning systems to detect health or well-being problems in poultry. Due to the use of audio and video information in the technological process in online mode, it is possible to monitor and model the behaviour, health, and productivity of poultry. According to the researchers, the frequency of sounds made by a bird is inversely proportional to its age and body weight. Thus, the more they gain body weight, the lower the frequency of sounds. The non-invasive nature of audio and video equipment allows it to be used for long-term monitoring without disturbing the bird. The bird's body weight provides information about feed growth and conversion in the herd.

A. Goel (2021) pointed out that an important innovative development is technologies for influencing embryos for breeding heat-resistant poultry crosses. During incubation, various nutrients such as carbohydrates, trace elements, and amino acids are added to the developing embryos *in ovo*, or the embryos are subjected to heat treatment involving slight changes in incubation temperature. This increases thermal tolerance in hatched chickens, increases feed intake, and affects the resistance and adaptation of poultry immunity. G.D. Vandana *et al*. (2021) noted that the use of advanced biotechnological tools helps to identify relevant genetic markers in poultry. In the future, this will help in the development of new strains with higher thermal tolerance by developing an appropriate breeding programme that includes selection using markers. Thus, such strategies can help to optimise and support poultry production in the face of climate change.

Conclusions

Intensive conditions for raising poultry are inevitably associated with the occurrence of technological stress factors, such as temperature violations, insufficient lighting, changes in the density of keeping, regrouping, sorting, and transportation of poultry. Such factors have a significant impact on the growth, productivity, and metabolic processes in the bird's body due to hormonal imbalance, changes in the functioning of the cardiovascular and respiratory systems, immune response, and weakening of resistance to infectious agents, which increases the risk of poultry morbidity and causes significant economic damage to poultry farms.

Despite the growing amount of knowledge about the effects of technological stressors on the body, the literature lacks a comprehensive review of biomarkers that comprehensively characterise changes in the poultry. Currently, the diagnosis of stress in poultry is based on physiological and behavioural indicators. In particular, these are changes in behaviour, apathy, disorders of sleep, breathing, and heartbeat, reduced feed intake, aggressive behaviour, excessive pecking and cannibalism. In the blood of poultry, the content of glucocorticoids changes, the activity of white blood cells decreases, the ratio of heterophiles to lymphocytes changes, which is an important indicator of the physiological state and stress level in poultry. More thorough studies need to determine the indicators of oxidative stress – the activity of SOD, CAT and GSH-Px, MDA, and heat shock proteins HSP70 and HSP 90. Simultaneously, there is no clear substantiation for the indicators of the neuroimmune system, especially when exposed to pathogens against the background of stressful factors.

Developing strategies to reduce the impact of stress factors on the bird's body during intensive rearing is an important task for researchers and practitioners. Effective prevention of technological stress in poultry involves a comprehensive approach, which includes optimisation of the conditions of its maintenance, including ensuring a proper microclimate in poultry houses, ventilation and lighting systems, rational balanced feeding of feed in accordance with the regulatory age needs of poultry, the use of vitamins, trace elements, antioxidants that reduce the response to adverse stimuli or increase the resistance of the bird's body. In strategies for the prevention and mitigation of technological stress, it is advisable to thoroughly study the molecular changes caused by the use of probiotics, prebiotics, adaptogens, organic acids, vitamins, minerals, and their impact on the gut microbiota both under the influence of stress factors and pathogens.

Important for the study are advanced innovative implementations, research on which is still ongoing, such as gene mapping and genetic breeding during breeding to breed heat-resistant breeds, the use of thermal conditioning during embryonic development, which increases the ability of birds to tolerate high temperatures. And technologies for introducing nutrients *in ovo,* which contributes to the thermal tolerance of poultry, increasing its feed consumption, and increasing adaptogenicity. The use of advanced monitoring systems allows detecting stress manifestations in poultry in time and effectively correcting them. The timely diagnosis of stress in poultry ensures their welfare, guarantees high-quality poultry products, determines operational efficiency and minimises economic losses in the poultry industry.

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Conflict of Interest

None.

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Фактори виникнення технологічного стресу у курчат бройлерів та стратегії його профілактики

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Анотація. За інтенсивного вирощування птиці на ріст, розвиток птиці бройлерів та якість продукції птахівництва впливає цілий ряд технологічних стресових факторів. Вплив гострого чи хронічного стресу відображається на поведінкових реаціях птиці, розладах харчової поведінки, конверсії корму, добових приростах та якості отриманої продукції. Метою роботи був критичний огляд та систематизації наукових знань, щодо змін в організмі птиці за впливу технологічних стресових факторів. У статті проаналізовано наукові публікації щодо змін в організмі птиці на фізіологічному та поведінковому рівні за стресових розладів, визначені клінічні та лабораторні маркери їх діагностики та методи профілактики. Огляд допоміг виокремити основні критерії, які допомагають диференціювати прояви стресу у птиці. Визначено, що основними технологічними стресорами для птиці є порушення температурного режиму вирощування птиці, збільшення щільності її утримання, перегрупування, зважування, сортування, формування груп, відлов, переміщення, транспортування, технологічні шуми. Клінічними проявами за розвитку стресових явищ у птиці були зменшення споживання нею корму, збудливість, тривожність, страх, втома, прискорене поверхневе дихання, передчасне дозрівання та порушення репродуктивних функцій, зниження яйцекладки, ослаблення м’язового тонусу та зниження якості продукції птахівництва. Критеріями встановлення прояву стресових явищ у птиці є зміни поведінки та клінічні показники, у крові – співвідношення гетерофілів до нейтрофілів, вміст кортикостероїдів. Для більш глибокого дослідження оксидативного стресу корисним буде визначення вмісту в крові птиці малонового діальдегіду, 8-гідрокси-2-дезоксигуанозину, активності глутатіону та супероксиддисмутази, білків теплового шоку HSP70 і HSP90. Стратегії профілактики розвитку технологічних стресових факторів включають оптимізацію умов утримання, корекцію раціону із додаванням до його складу біологічно активних сполук макро – мікроелементів, органічних кислот, пробіотиків, пребіотиків для формування стійкості організму птиці до дії як технологічних, так і інших факторів зовнішнього середовища. Результати дослідження будуть корисними для розробки дієвих засобів профілактики технологічних стресів у птиці

Ключові слова: птиця; стресові фактори; тепловий вплив; щільність утримання; освітлення; фізіологічні зміни; превентивні заходи

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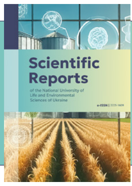

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Morphometric indicators of grain sorghum depending on sowing dates and stage of development

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Abstract. In the context of climate change and frequent droughts, the study of optimal sowing dates for grain sorghum as a drought-resistant crop with a C4 type of photosynthesis is highly relevant for ensuring the stable progression of critical growth stages and crop formation. The aim of the study was to determine the influence of sowing dates on the formation of morphometric indicators and the yield performance of grain sorghum plants under the conditions of the Forest-Steppe zone of Ukraine. The study employed a combination of field, analytical, morphometric, visual-instrumental, recording and statistical methods. It was established that the recommended sowing date ensured the maximum values of plant height (at BBCH stages 81-82) and the largest leaf surface area, which resulted in the highest photosynthetic potential of the crops. A tendency was identified whereby early sowing led to slower growth at the initial stages, whereas late sowing was accompanied by reduced growth intensity during the second half of the growing season and more rapid leaf senescence. The following tendency was observed: between the stage of plant development (BBCH 21-22 and BBCH 81-82) and both plant height and leaf surface area, there was a positive linear relationship ($r > 0$) with high values of the coefficient of determination (R^2), indicating a consistent increase in morphometric indicators throughout the growing season and varying intensity of their accumulation depending on the sowing date. It was established that the highest yield was obtained under the recommended sowing date,

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while the earliest sowing date resulted in the lowest yield, and the late sowing date produced yields at the level of the recommended one. It was also found that late sowing promoted more uniform seedling emergence and more rapid initial development and, provided sufficient moisture was available, made it possible to maintain a yield level close to that of the recommended sowing date. The obtained results may be used for the scientifically grounded selection of sowing dates for grain sorghum in order to improve the stability of productivity and the efficiency of its cultivation under the conditions of the Forest-Steppe zone of Ukraine

Keywords: productivity; leaf surface area; plant height; hybrid; grain sorghum

Introduction

Sorghum (*Sorghum bicolor* L.) is among the world's five most important cereal crops and is characterised by a strong capacity for growth under arid and semi-arid climatic conditions (Choudhary *et al.*, 2025). Sorghum is a major global crop possessing the C4 type of photosynthesis, which makes it highly resistant to abiotic stresses. Grain sorghum is cultivated for the production of high-quality feed. It is also used as a bioenergy crop, with sweet sorghum providing the highest ethanol yield per unit area among agricultural crops (Anija Hari Kumar *et al.*, 2025). Sorghum grain occupies an important place in human nutrition, which has led to growing interest in ancient cereal crops and the demand for gluten-free grain. Sorghum grain is used in food production as a nutrient-rich baking grain with a lower glycaemic index than wheat, high resistant starch content, and significantly greater antioxidant levels (McGinnis & Webster, 2024). B. Chen *et al.* (2025) note that climate change is increasingly affecting crop yields in various regions of the world due to rising temperatures, reduced precipitation, and frequent extreme weather events. Water deficiency affects plant functions ranging from transpiration, photosynthesis, and leaf and root growth to reproduction and other key physiological processes. At the same time, increasing sorghum productivity is largely determined by the improvement of cultivation technology elements (plant density, sowing methods and dates, etc.), as well as crop management systems, which has been confirmed by domestic studies (Pravdyva, 2021; Titarenko & Karpuk, 2021).

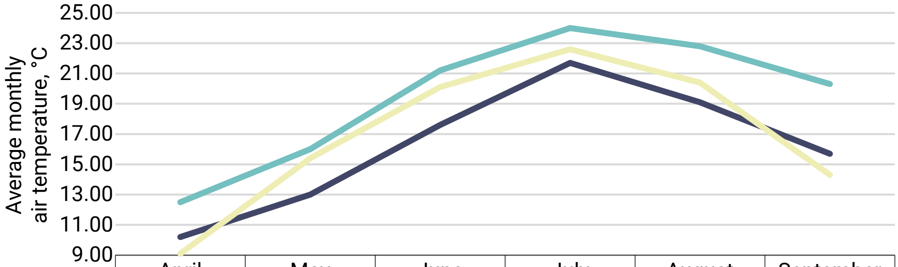
Grain sorghum may serve as a substitute for fodder maize in arid regions, as it adapts to changing conditions throughout ontogenesis. Its drought stress avoidance mechanism is based on the specific structure of the root system, which exploits the entire soil profile, and on the efficient use of moisture during photosynthesis (Otwani *et al.*, 2024). Due to the accumulation of carbon in the form of organic acids and their utilisation through the C4 photosynthetic pathway, sorghum is capable of continuing dry matter synthesis even when stomata are closed, while the stomata themselves can continue functioning without damage even under prolonged drought conditions (Fontanet-Manzaneque *et al.*, 2025). F. Fardin *et al.* (2023) emphasised that the cultivation of resilient and stress-tolerant crops is an alternative strategy for reducing crop yield losses caused by climate change. In particular, drought stress negatively affected sorghum plant height, leaf number, biomass, leaf-to-stem ratio, seed yield and yield components, as well as crude protein and hydrocyanic acid content in sorghum. However, different genotypes did not exhibit the same response to drought stress. O.P. Kibalnik *et al.* (2023) indicate that the biometric indicators of sorghum plants, including plant height, are among the principal parameters comprehensively reflecting plant responses to agronomic growing conditions, particularly sowing dates, temperature regime, and moisture availability. Numerous studies have confirmed that plant height is the most sensitive indicator to changes during cultivation, and its dynamics are associated with the progression of the main developmental stages.

L. Porčová *et al.* (2025), in their study, noted that sowing parameters, particularly sowing dates, determine the initial growth conditions of plants, which in turn define different intensities of stem growth throughout ontogenesis. Early sowing dates increase the duration of the growing season, thereby creating favourable conditions for the formation of sorghum plant height, whereas late sowing dates, conversely, lead to shorter interphase periods and reduced growth parameters. Sowing dates also influence the intensity of leaf surface formation and its values, especially during the tillering and stem elongation stages. Early sowing creates conditions for a longer period of leaf surface development, which in turn increases the photosynthetic potential of crops. Late sowing dates have the opposite effect, reducing the duration of interphase periods, which consequently decreases active leaf formation and results in a reduction of leaf surface area. Under early sowing dates, sorghum plants experience a longer period of active photosynthesis, whereas under late sowing dates accelerated leaf senescence is observed due to high temperatures and moisture deficiency during the period of active functioning. D. Otwani *et al.* (2024) stated that the selection of a sorghum hybrid is a key element of cultivation technology, since different genotypes respond differently to sowing dates, temperature regime, moisture availability, and the duration of phenological phases; the relevance of this issue is emphasised by contemporary reviews on breeding and the development of new sorghum hybrids (Lv *et al.*, 2024). The adaptive properties of hybrids to specific growing conditions determine the level of realisation of their productive potential, while sowing dates represent a critical factor that may either enhance or negate genetic advantages (Takanashi, 2023).

The aim of the study was to determine the influence of sowing dates on the formation of morphometric indicators of grain sorghum plants, particularly plant height, leaf surface area, and yield. The objectives of the study were to assess the influence of cultivation technology elements on the yield of the investigated crop and on the formation of morphometric indicators and productivity of grain sorghum plants.

Materials and Methods

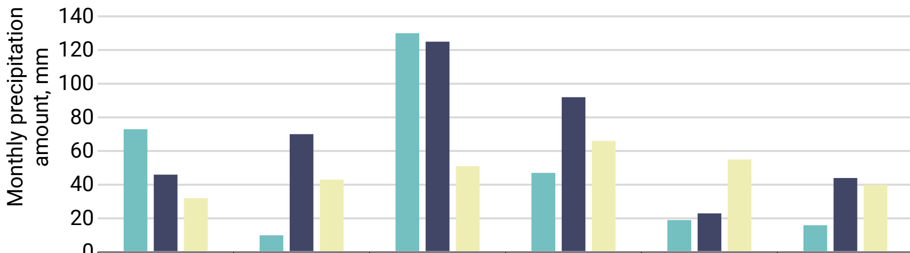
Field experiments were conducted during 2024-2025 at the farm enterprise "Promin", located in the western part of the Nizhyn district of Chernihiv region. The soil cover of the experimental site consisted of meadow-chernozem weakly solonetzic soil with a humus content of 2.84%. Soil nutrient reserves amounted to 107-110 mg/kg soil of mineral nitrogen, 135-140 mg/kg soil of available phosphorus, and 90-95 mg/kg soil of exchangeable potassium. Weather conditions in the Chernihiv region during the research period were characterised by elevated average monthly temperatures during the summer period compared with long-term averages, particularly in 2024 (Fig. 1). Air temperatures were especially higher in September, which promoted the ripening of grain sorghum – during this month temperatures were 6.0°C higher than normal, which is anomalous for this period. The 2024 growing season was characterised by a shortage of rainfall or its uneven distribution relative to long-term averages. Particularly unfavourable moisture conditions were observed during critical stages of sorghum development, which limited the intensity of growth processes and the formation of reproductive organs (Fig. 2). In contrast to 2024, in 2025 moisture conditions were more favourable for the growth and development of grain sorghum. Precipitation levels during the growing season were close to or exceeded long-term averages.



	April	May	June	July	August	September
2024 year	12.5	16	21.2	24	22.8	20.3
2025 year	10.2	13	17.6	21.7	19.1	15.7
pa – perennial	9.1	15.4	20.1	22.6	20.4	14.3

Figure 1. *Average monthly air temperature during the growing season of grain sorghum (April-September), 2024 and 2025*

Note: pa – perennial – perennial value
Source: developed by the authors



	April	May	June	July	August	September
2024 year	73	10	130	47	19	16
2025 year	46	70	125	92	23	44
pa – perennial	32	43	51	66	55	40

Figure 2. *Monthly precipitation during the growing season of grain sorghum (April-September), 2024 and 2025*

Note: pa – perennial – perennial value
Source: developed by the authors

The preceding crop in the experiment was winter wheat. Field tillage included two stubble cultivations with a disc harrow to a depth of 10-12 cm at two-week intervals. Following weed emergence, ploughing was carried out to a depth of 25-27 cm. The spring fieldwork commenced with early spring harrowing of the soil after reaching physical maturity. Subsequently, two cultivations were performed – the first to a depth of 10-12 cm and the second (pre-sowing) to 6-8 cm. Sowing was carried out according to the experimental design, with row spacing of 45 cm and a sowing rate of 170.0 thousand seeds/ha. The fertilisation system included the application of a complete mineral fertiliser at a rate of $N_{60}P_{40}K_{40}$. At BBCH stage 50-51, the crops were sprayed with the fungicide Impact T (application rate 0.8 l/ha) in a tank mixture with the insecticide Fosorgan Duo (application rate 0.3 l/ha). Grain sorghum was harvested by direct combining at full maturity.

The experiment was two-factorial: factor A – grain sorghum hybrids RGT Icebergg and Albanus (early-maturing); factor B – sowing dates: first sowing date (soil temperature 8-10°C at a depth of 10 cm); second sowing date (soil temperature

12-14°C at a depth of 10 cm); third sowing date (soil temperature 16-18°C at a depth of 10 cm). The total experimental plot area was 100 m^2, while the accounting plot area was 80.0 m^2. The experiment was conducted with three replications. Morphometric assessments were performed at BBCH stages 21-22, BBCH 36-37, and BBCH 81-82 (according to the experimental scheme), selecting representative plants from the accounting area of each plot in every replication; measurements were taken at the same time of day in order to minimise the influence of daily fluctuations in turgor and leaf position. Leaf surface area (S) was calculated using the linear method as the sum of the areas of all leaves on a single plant: for each leaf, length (L) and maximum width (B) were measured, after which leaf area was determined using the formula:

$$S = L \times B \times k, \tag{1}$$

where k – the coefficient of leaf blade shape; the leaf surface area per plant was obtained by summing the areas of all leaves; the results were expressed in cm^2/plant.

Field experiments were conducted in compliance with the national legislation of Ukraine, the principles of responsible scientific research, and the provisions of the Convention on Biological Diversity (1992) regarding the responsible use of plant resources. Grain yield was determined by the method of complete threshing of the accounting area of each plot followed by weighing of the obtained grain; the indicator was recalculated in t/ha taking into account the accounting plot area and adjusted to standard moisture content. Statistical processing was carried out using methods of statistics and comparative analysis; to assess the nature of changes in morphometric indicators throughout ontogenesis, linear regression analysis was applied with determination of the correlation coefficient (r) and coefficient of determination (R^2). The reliability of the results was evaluated taking into account the threefold replication of the experiment: primary datasets were formed separately for each replication, after which mean values of indicators and their variability were calculated. For all quantitative indicators (plant height, leaf surface area, yield, etc.), the mean value and standard error were presented, calculated using the R software environment (version 4.4.3) with the ggplot2 package. Similarity groups were calculated based on the results of Tukey's $HSD_{0.05}$ test. For linear regressions, 95% confidence intervals for model parameters were additionally calculated, ensuring correct interpretation of differences between treatments and the level of data variability.

Results

According to the results of the study conducted during 2024-2025, it was established that sorghum plant height depended significantly on both sowing date and plant developmental stage, as well as on the genetic characteristics of the hybrids. A clear dynamics of grain sorghum plant growth throughout the developmental stages was observed, and different responses of the studied hybrids to sowing dates were noted (Table 1).

Table 1. *Plant height of grain sorghum hybrids depending on sowing dates and stage of development*

Hybrid (factor A)	Sowing dates* (factor B)	Developmental stage		
		BBCH 21-22	BBCH 36-37	BBCH 81-82
Albanus	early	20.3 ± 0.1 c*	67.9 ± 0.3 c	106.9 ± 0.5 bc
	recommended	21.0 ± 0.2 abc	69.8 ± 0.2 b	108.0 ± 0.4 b
	late	20.5 ± 0.4 c	69.3 ± 0.3 bc	105.5 ± 0.5 cd
RGT Icebergg	early	20.8 ± 0.2 bc	69.6 ± 0.3 b	111.1 ± 0.1 a
	recommended	21.5 ± 0.2 ab	72.1 ± 0.4 a	112.6 ± 0.4 a
	late	21.9 ± 0.2 a	70.5 ± 0.4 b	103.8 ± 0.6 d

***Table 1.** Continued*

Hybrid (factor A)	Sowing dates* (factor B)	Developmental stage		
		BBCH 21-22	BBCH 36-37	BBCH 81-82
Mean for factor A		21.0	69.9	108.0
Mean for factor B	early	20.6	68.8	109.0
	recommended	21.3	71.0	110.3
	late	21.2	69.9	104.7

Note: *1. Early sowing date (soil temperature 8-10°C at a depth of 10 cm); 2. Recommended sowing date (soil temperature 12-14°C at a depth of 10 cm); 3. Late sowing date (soil temperature 16-18°C at a depth of 10 cm). Values are presented with the standard error of the mean (±SE); letters indicate membership of similarity groups according to Tukey's $HSD_{0.05}$ criterion for data within the column

Source: compiled by the authors

At BBCH stage 21-22 (beginning of tillering), the minimum height of sorghum plants was recorded, and only slight variability was observed. Depending on the studied hybrid and its sowing date, this indicator ranged from 20.3 to 21.9 cm. The average plant height was 21.0 cm, indicating a weak influence of the studied factors on the growth of grain sorghum plants at the early developmental stages. At BBCH stages 30-39, intensive growth of grain sorghum plants occurred. It was established that under the early sowing date, the average plant height was 68.8 cm, under the recommended sowing date – 71.0 cm, and under the late sowing date – 69.9 cm. Thus, the highest values were recorded under the recommended sowing date. At BBCH stage 81-82, growth processes were completed, and the differences between sowing dates became clearly expressed. The reduction in plant height under the late sowing date compared with the recommended sowing date amounted to 5.6 cm or 5.1%. A similar tendency was observed for the early sowing date, where plant height was 1.3 cm or 1.2% lower compared with the recommended sowing date, which was not significant. Among the studied hybrids, the tallest plants were obtained from sowing the RGT Icebergg hybrid under the recommended sowing date, where plant height reached 112.6 cm, exceeding the indicator of the Albanus hybrid by 4.6 cm. The results of the regression analysis confirmed that sowing dates influenced not only the absolute values of plant height, but also the intensity of their linear growth throughout the growing season (Fig. 3).

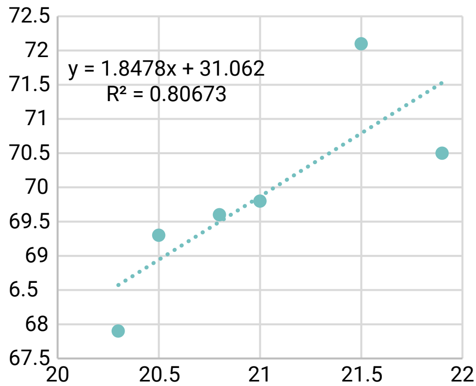


***Figure 3.** Linear relationship between the height of grain sorghum plants and the stage of development and sowing date*

Source: compiled by the authors

The linear regression models were characterised by positive regression coefficients (r > 0), confirming the natural increase in plant height during the growth process. Deviations in the values of the regression lines differed depending on the sowing dates, indicating unequal intensity of linear plant growth during the growing season. The highest values of the regression coefficient were established under the recommended sowing date, indicating higher rates of plant height increase at each developmental stage. Under the early sowing date, a slight reduction in growth processes was observed, whereas under late sowing dates the greatest decrease in the intensity of linear growth was recorded. High values of the coefficient of determination (R^2) indicate a strong linear relationship between developmental stages, plant height, and sowing dates.

Unlike the linear growth of the stem, leaf surface area responded more sensitively to changes in growing conditions. Thus, the next stage of the study involved determining the influence of sowing dates on the formation of leaf surface area in grain sorghum plants. At BBCH stage 21-22, the leaf surface area per plant varied within the range of 89.6-129.4 cm²/plant. On average, the leaf area per plant was 91.9 cm² under the early sowing date, 115.4 cm² under the recommended sowing date, and 121.3 cm² under the late sowing date (Table 2). Thus, at this developmental stage, the highest leaf area values were formed under the late sowing date, which in turn was associated with a more favourable temperature regime for leaf growth in grain sorghum plants.

Table 2. *Leaf area on grain sorghum plants depending on sowing date and stage of development*

Hybrid (factor A)	Sowing dates* (factor B)	Developmental stage		
		BBCH 21-22	BBCH 36-37	BBCH 81-82
Albanus	early	89.6 ± 1.7 d	468.3 ± 1.1 d	498.1 ± 2.6 e
	recommended	101.3 ± 1.8 c	523.2 ± 1.7 c	619.4 ± 2.9 c
	late	118.5 ± 2.9 b	518.4 ± 1.6 c	603.4 ± 2.3 d
RGT Icebergg	early	94.2 ± 1.0 cd	547.6 ± 1.3 b	684.3 ± 4.7 b
	recommended	129.4 ± 1.6 a	625.2 ± 2.7 a	769.5 ± 2.1 a
	late	124.1 ± 1.0 ab	617.1 ± 3.3 a	763.1 ± 1.2 a
Mean for factor A		109.5	550.0	656.3
Mean for factor B	early	91.9	508.0	591.2
	recommended	115.4	574.2	694.5
	late	121.3	567.8	683.3

Note: * – values are presented with the standard error of the mean (±SE); letters indicate membership of similarity groups according to Tukey's $HSD_{0.05}$ criterion for data within the column
Source: compiled by the authors

At BBCH stage 36-37, a rapid increase in leaf surface area occurred, corresponding to the period of intensive growth of grain sorghum plants. The maximum values were obtained under the recommended sowing date, where the leaf surface area reached 574.2 cm², exceeding the treatment with the early sowing date by 66.2 cm² and the late sowing date by 6.4 cm². This can be explained by the fact that optimal conditions for the formation of leaf surface area in grain sorghum plants were achieved under the recommended sowing date.

A similar tendency was maintained at BBCH stage 81-82, where the leaf surface area of sorghum plants increased to 591.2 cm² under the early sowing date, to 694.5 cm² under the recommended sowing date, and to 683.3 cm² under the late sowing date. Thus, the reduction in the assimilation surface of sorghum plants under the early sowing date was associated with delayed plant development at the beginning of the growing season, whereas under the late sowing date it was related to rapid leaf senescence caused by high temperatures and drought during the second half of the crop growing season. Among the studied hybrids, the RGT Icebergg hybrid formed a greater assimilation surface at all developmental stages and sowing dates compared with the Albanus hybrid.

A consistent pattern was established whereby all sowing dates were characterised by a positive linear tendency ($r > 0$), indicating a gradual increase in leaf surface area from BBCH stage 21-22 to BBCH stage 81-82. At the same time, the slope of the regression lines differed significantly, indicating unequal intensity of leaf apparatus formation under different sowing dates (Fig. 4). The greatest deviation of the regression line was observed under the recommended sowing date, indicating a rapid increase in leaf surface area during developmental stages. This was achieved due to the optimal combination of temperature and moisture regimes during the period of active growth of grain sorghum plants, which promoted prolonged functioning of the leaf apparatus and reduced premature leaf senescence. Under the early sowing date, the intensity of assimilation surface increase was lower, which was caused by the temperature regime at the initial stages of vegetation that slowed the formation of leaf blades. At the same time, under the late sowing date, despite the rapid initial growth of leaves, a subsequent decline in the rate of leaf surface formation was observed.

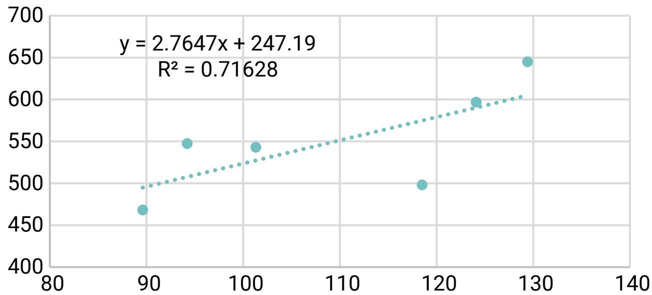


Figure 4. *Linear relationship between the leaf area of grain sorghum plants and their stage of development and sowing date*

Source: compiled by the authors

The high values of the coefficient of determination (R^2) for all variants studied indicate a strong linear relationship between the stage of plant development and leaf area. Thus, it has been confirmed that sowing dates have a significant influence not only on the absolute values of leaf area but also on the dynamics of its formation throughout the growing season, and the highest photosynthetic potential of grain sorghum was obtained at the recommended sowing date.

Analysis of the data indicates that yield levels varied significantly under the influence of both cultivation practices (sowing date) and the genetic characteristics of the hybrids, as well as the weather conditions during the growing seasons. In 2024, sorghum grain yield was lower compared to 2025 for all variants studied, which was due to the dry conditions during the growing season. On average, the yield in 2024 was 6.16 t/ha, whereas in 2025 it increased to 6.87 t/ha (Table 3).

Table 3. *Grain yield of grain sorghum depending on sowing dates, t/ha*

Hybrid (factor A)	Sowing dates* (factor B)	Year		
		2024	2025	Average for 2024-2025
Albanus	I	4.73 ± 0.07 c	5.20 ± 0.09 c	4.96 ± 0.12 c
	II	6.12 ± 0.08 b	6.89 ± 0.11 b	6.51 ± 0.18 b
	III	6.15 ± 0.08 b	6.84 ± 0.08 b	6.49 ± 0.16 b
RGT Icebergg	I	5.88 ± 0.04 b	6.53 ± 0.15 b	6.20 ± 0.16 b
	II	6.98 ± 0.21 a	7.91 ± 0.13 a	7.45 ± 0.24 a
	III	7.12 ± 0.09 a	7.83 ± 0.17 a	7.48 ± 0.18 a
Mean for factor A		6.16	6.87	6.51

Table 3. *Continued*

Hybrid (factor A)	Sowing dates* (factor B)	Year		
		2024	2025	Average for 2024-2025
Mean for factor B	I	5.30	5.86	5.58
	II	6.55	7.40	6.98
	III	6.64	7.34	6.99

Note: * – values are presented with the standard error of the mean (±SE); letters indicate membership of similarity groups according to Tukey's $HSD_{0.05}$ criterion for data within the column

Source: compiled by the authors

Among the sowing dates, the lowest grain sorghum yield was obtained under the early sowing date. On average for 2024-2025, it amounted to 5.58 t/ha, which was 1.40 t/ha or 20.1% lower compared with the recommended sowing date. The recommended sowing date ensured the highest grain yield level of 6.98 t/ha. Under the late sowing date, the yield was slightly lower and amounted to 6.99 t/ha; however, the difference compared with the recommended sowing date was minimal, indicating sufficient adaptability of the crop to shifts in sowing dates under favourable weather conditions. The RGT Icebergg hybrid was characterised by consistently higher grain yield compared with the Albanus hybrid across all sowing date treatments during 2024-2025. On average, the grain yield of the RGT Icebergg hybrid amounted to 7.04 t/ha, whereas the Albanus hybrid produced 5.99 t/ha, indicating a better realisation potential of the hybrid. The highest grain yield indicators were obtained when cultivating the RGT Icebergg hybrid under the recommended and late sowing dates, reaching 7.45 and 7.48 t/ha, respectively. This was achieved, in particular, due to plant height and leaf surface area, which were maximal under these treatments and ensured a high photosynthetic potential of grain sorghum crops.

It was established that the relationship exhibited a clearly expressed positive trend when transitioning from the early to the recommended sowing date, indicating an increase in grain yield. It was under the recommended sowing date that maximum yield was achieved, which in turn was associated with the most favourable conditions for the progression of critical developmental stages, particularly the formation of the leaf apparatus, reproductive organs, and grain filling (Fig. 5).

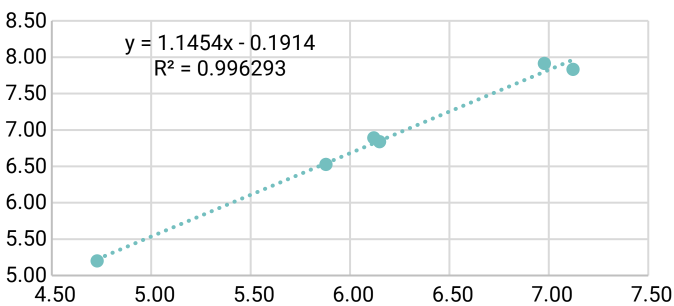


Figure 5. *Linear relationship between grain sorghum yield and sowing date*

Source: compiled by the authors

A further shift in sowing dates towards later periods is accompanied by a slight decrease in yield, as reflected in the less pronounced slope of the regression line. At the same time, the negligible difference between the recommended and late sowing dates indicates sufficient ecological plasticity of grain sorghum and its ability to adapt to climate change. Thus, optimising sowing dates is one of the key elements of grain sorghum cultivation technology, ensuring stability and increased yield. Recommended and late sowing dates create the most favourable conditions for the development of high photosynthetic potential in crops and the maximum realisation of the crop's potential.

Discussion

The results obtained confirm that sowing dates are one of the decisive factors influencing the morphometric characteristics of grain sorghum, as they determine the initial temperature conditions and the duration of the interphase periods. Thus, during 2024-2025, a consistent growth pattern was observed: at the early stages (BBCH 21-22), the difference in plant height between treatments was minimal, whereas during the period of intensive growth (BBCH 36-37) and at the end of the growing season (BBCH 81-82), the advantage of the recommended sowing date became evident. A similar pattern of crop response to sowing dates was also reported by other researchers, such as O.P. Kibalnik *et al.* (2023) and L. Porčová *et al.* (2025), who pointed to the relationship between plant height, temperature regime, moisture availability, and plant response to agronomic conditions.

The influence of sowing dates on leaf surface area was even more pronounced, which corresponds to the significant role of the assimilation apparatus in the formation of sorghum productivity. In the current experiment, the maximum leaf surface area values at the assessed developmental stages were predominantly formed under the recommended sowing date, which ensured a higher photosynthetic potential of the crops and, consequently, better conditions for grain formation and filling. Similar patterns regarding the role of assimilation surface in the formation of sorghum crop productivity were also reported by other authors (Gyrka *et al.*, 2021; Pravdyva *et al.*, 2023). Likewise, in the works of O.P. Kibalnik *et al.* (2023), devoted to the physiology and adaptation of sorghum to abiotic stresses, it is emphasised that temperature and moisture regimes significantly affect the intensity of leaf apparatus formation, the duration of leaf functioning, and the efficiency of photosynthesis (Behera *et al.*, 2022). In this context, the reduction in assimilation surface recorded under early sowing can be associated with slower initial growth at lower temperatures, while under late sowing it was related to rapid leaf senescence.

It is also important to note that the response of hybrids to sowing dates was not identical. This is confirmed by F.C. Gao *et al.* (2022) and D. Otwani *et al.* (2024), who state that morphometric indicators may be regarded as informative traits of potential productivity, particularly during stages of intensive growth when the basis of the crop photosynthetic potential is formed. The dynamics of plant height and assimilation surface in relation to yield indicate that the developmental stages from BBCH 30-31 to BBCH 82-85 are the most "sensitive" to sowing dates. P.P. Behera *et al.* (2022) and H.Y. Wu *et al.* (2025) note that it is during this period that the difference in leaf surface area between treatments is maximal; thus, treatments that ensure longer functioning of leaves create better conditions for dry matter accumulation and the realisation of hybrid potential. From a practical perspective, this means that assessment of the leaf apparatus condition at BBCH stage 36-37 may be useful for early forecasting of expected crop productivity.

The obtained results confirm that the influence of sowing date largely affects the progression of phenological phases and, accordingly, the differing probability of critical periods occurring under stress conditions. Under precipitation deficit, the early sowing date led to slower initial growth and the formation of a smaller leaf apparatus surface, which limited the compensatory capacity of the crop at later stages (Fardin *et al.*, 2023; Chen *et al.*, 2025). Under more favourable moisture conditions, the late sowing date did not cause a significant reduction in yield, as evidenced by the productivity indicators under the recommended and late sowing dates in the current experiment. The regression relationships obtained for plant height and leaf surface area demonstrate the systematic nature of changes in morphometric indicators throughout ontogenesis and confirm that sowing dates alter not only the value of the trait itself but also the rate of its increase. O. Brendel (2021) and P.P. Behera *et al.* (2022) note in their studies that higher values of the coefficients of determination for linear models indicate the predictability of growth processes within each sowing date, whereas differences in the slopes of the regression lines reflect variations in the rate of biomass accumulation and assimilation surface formation. From an applied perspective, this emphasises the expediency of selecting sowing dates as a tool for regulating crop growth rates with consideration of the typical weather risks of a particular region.

From an agronomic point of view, the early sowing date, despite the potential extension of the growing period, increases the risk of uneven emergence and slow growth under insufficient soil warming, which may subsequently affect the development of the leaf apparatus. L. Porčová *et al.* (2025) and D. Otwani *et al.* (2025) note that the late sowing date, on the contrary, shortens the duration of interphase periods and may increase the risk of grain filling occurring during periods of high temperatures and moisture deficit, thereby promoting premature leaf senescence. Thus, under the conditions of the Forest-Steppe zone of Ukraine, it is advisable to consider the recommended sowing date as the basic option, while shifting to a later sowing date may be regarded as acceptable under sufficient moisture availability and when using early-maturing and plastic hybrids.

At the same time, it should be taken into account that the results were obtained under the conditions of a specific soil cover and technological elements (preceding crop, replication, production conditions), while the range of weather conditions was limited to two years of research. In their studies, Y. Zhou *et al.* (2022), V.A. Tsygankova (2023) note that the interaction of sowing dates with plant density, mineral nutrition level, and crop protection may potentially alter the intensity of morphometric indicator formation and their relationship with yield. Thus, further studies should be aimed at expanding the temperature range, including years with more contrasting moisture conditions, and analysing complex technological combinations in order to refine optimal production decisions. F. Fardin *et al.* (2023) note that under drier conditions the yield level was lower in all treatments, which is consistent with data on the negative effect of drought on biomass, morphometry, and yield components of sorghum. At the same time, the higher average productivity indicators under the recommended and late sowing dates indicate the possibility of partially compensating for weather-related risks through optimisation of sowing dates and the selection of adaptive genotypes, as also highlighted by other authors analysing the "genotype × environment × sowing date" interaction (Gao *et al.*, 2022; Otwani *et al.*, 2024).

Overall, the comparison of the obtained data with literature sources indicates that the optimal sowing date for the conditions of the Forest-Steppe zone of Ukraine should be regarded as a compromise between ensuring a sufficient duration of vegetation and avoiding the occurrence of reproductive stages during periods of the highest stress load. The recorded yield levels under the recommended and late sowing dates may indicate the high ecological plasticity of sorghum and its potential for adaptation to changing weather conditions, which is consistent with current concepts of adaptability and yield plasticity in hybrids. Thus, further refinement of optimal sowing dates should be combined with assessment of the duration of leaf apparatus functioning and the photosynthetic productivity of specific hybrids in years differing in moisture availability.

Conclusions

Despite certain limitations of the study, particularly the lack of open access to some specialised scientific sources, the obtained results made it possible to establish that sowing dates are among the leading technological factors significantly affecting the growth, development, formation of morphometric indicators, and yield of grain sorghum under the conditions of the Forest-Steppe zone of Ukraine. It was experimentally proven that optimisation of sowing dates with consideration of the soil temperature regime contributes to a more complete realisation of the biological potential of the crop and enhances the stability of its productivity under variable agroclimatic conditions. The most favourable conditions for plant growth and development were observed under the recommended sowing date at a soil temperature of 10-14°C and the late sowing date at a temperature of 16-18°C at a depth of 10 cm.

It was established that under the recommended sowing date, the maximum indicators of plant height and leaf surface area were formed during the critical developmental stages (BBCH 81-82), which ensured the highest photosynthetic potential of the crops and created favourable conditions for the formation of high grain yield. The early sowing date negatively affected the initial stages of plant development, which was manifested in suppression and delay of growth processes and slower formation of the assimilation apparatus. The late sowing date was characterised by more uniform seedling emergence and more intensive initial growth; however, during the second half of the growing season, a decrease in growth intensity and accelerated leaf senescence were observed. At the same time, under sufficient moisture availability, grain yield under the late sowing date remained at the level of the recommended sowing date, indicating the ecological plasticity of the crop and the possibility of using this sowing period as an alternative under certain weather conditions.

Grain sorghum yield depended significantly on the combination of sowing dates and the meteorological conditions of the growing years. The highest productivity indicators were formed under the recommended sowing date, whereas the early sowing date provided the lowest yield. The identified patterns are confirmed by positive correlation-regression relationships between plant developmental stages, plant height, and leaf surface area, indicating a consistent increase in morphometric indicators throughout the growing season and differing intensities of their formation depending on the sowing date. Among the studied hybrids, RGT Icebergg formed greater morphometric indicators and grain yield under all sowing dates, indicating its higher adaptability and better capacity for realisation in the Forest-Steppe zone of Ukraine.

Further research should be directed towards a more detailed investigation of the adaptive responses of grain sorghum under variable agroclimatic growing conditions, particularly the study of sowing dates in combination with other technological elements and a deeper analysis of physiological and biochemical processes (duration of leaf apparatus functioning and photosynthetic intensity), which would make it possible to clarify the limits of the ecological plasticity of the crop and predict its productivity under climate change conditions.

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Conflict of Interest

None.

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Морфометричні показники сорго зернового залежно від строків сівби та стадії розвитку

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Анотація. В умовах кліматичних змін і частих посух актуальним є дослідження оптимальних строків сівби зернового сорго як посухостійкої культури з C4-типом фотосинтезу для забезпечення стабільного проходження критичних фаз росту та формування врожаю. Метою дослідження було встановити вплив строків сівби на формування морфометричних показників та урожайності рослин сорго зернового в умовах Лісостепу України. У дослідженні застосовано комплекс польових, аналітичних, морфометричних, візуально-інструментальних, облікових і статистичних методів. Встановлено, що рекомендований строк сівби забезпечував максимальні значення висоти рослин (на стадіях BBCH 81-82) і найбільшу площу листкової поверхні, що формувало найвищий фотосинтетичний потенціал посівів. Виявлено тенденцію, що ранній строк сівби зумовлював уповільнення росту на початкових етапах, тоді як пізній строк супроводжувався зниженням інтенсивності росту в другій половині вегетації та швидшим відмирання листків. Виявлено таку тенденцію між стадією розвитку рослин (BBCH 21-22 і BBCH 81-82) та їх висотою, і площею листкової поверхні простежується позитивна лінійна залежність ($r > 0$) з високими значеннями коефіцієнта детермінації (R^2), що свідчить про закономірне зростання морфометричних показників упродовж вегетації та різну інтенсивність їх наростання залежно від строку сівби. Було встановлено, що найвищу врожайність отримано за рекомендованого строку сівби, за раннього строку вона була найнижчою, а за пізнього − на рівні рекомендованого. Було встановлено, що пізній строк сівби сприяв появі дружнішим сходам і швидшому стартовому розвитку та, за наявності достатнього зволоження, дає змогу зберігати рівень урожайності, близький до рекомендованого. Отримані результати можуть бути використані для науково обґрунтованого вибору строків сівби сорго зернового з метою підвищення стабільності продуктивності та ефективності його вирощування в умовах Лісостепу України

Ключові слова: продуктивність, листкова поверхня, висота рослин, гібрид, сорго зернове

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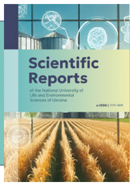

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Corrective efficacy of milk phospholipids in tetracycline-induced changes in the amino acid composition of blood in rats

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Abstract. The aim of the study was to establish the patterns of changes in the amino acid spectrum of whole blood in laboratory rats against the background of tetracycline-induced hepatopathy and the administration of milk phospholipids. To experimentally reproduce a model of acute hepatopathy, laboratory rats were intragastrically administered tetracycline hydrochloride at a dose of 250 mg/kg body weight for 7 days. Four groups were formed, including rats that were: clinically healthy; affected by tetracycline-induced hepatosis (without treatment); affected by tetracycline-induced hepatosis and administered milk phospholipids; clinically healthy and administered milk phospholipids. The amino acid profile of whole blood was determined using paper chromatography with ninhydrin detection. The development of an imbalance in the amino acid profile of whole blood in rats as a result of the cytotoxic effect of tetracycline hydrochloride on hepatocytes was experimentally established. In animals with experimental hepatopathy under conditions of spontaneous recovery, a significant increase in the level of the heterogeneous histidine/taurine/asparagine fraction (by 54%) was observed in whole blood, along with a tendency towards increased levels of the combined cystine/cysteine, glycine/serine, and ornithine cycle amino acid fractions. The concentration of individual amino acids remained relatively stable. Administration of the phospholipid dietary supplement to diseased animals was accompanied by a pronounced mobilisation of amino acids in the blood within the combined fractions of cysteine/cystine (by 161%), arginine/ornithine/lysine (by 69%), histidine/taurine/asparagine (by 196%), glycine/serine/

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glutamine/aspartic acid (by 92%), and valine/norvaline/tryptophan (by 86%), indicating stimulation of nitrogen metabolism and ureagenesis and reflecting activation of detoxification processes, antioxidant defence, and the hepatotropic effect of phospholipid correction. The obtained results may be used for the diagnosis of metabolic disturbances related to intermediate amino acid metabolism in drug-induced hepatopathies and as markers for evaluating the efficacy of newly developed medicinal products

Keywords: liver; hepatopathy; fatty hepatosis model; corrective therapy; paper chromatography; marker changes; drug-induced complications

Introduction

The problem of drug-induced liver injury (DILI) remains of considerable clinical and biomedical significance, since such lesions are accompanied by impaired functional status of the hepatobiliary system and, in some cases, may progress to acute liver failure. X. Yao *et al.* (2024) demonstrated that the commonly used antibiotics tetracycline and sulphadiazine exert a direct cytotoxic effect on liver cells: exposure in the Huh-7 cell model induced oxidative stress and membrane damage. Moreover, the toxicity of tetracycline proved to be significantly greater than that of sulphadiazine. These findings are consistent with the review by A. Borgne-Sanchez & B. Fromenty (2025), who emphasised that many drugs induce dysfunction of mitochondrial β-oxidation of fatty acids, leading to hepatic steatosis. In cases of marked inhibition of fatty acid β-oxidation, microvesicular steatosis developed with a risk of hypoglycaemia, whereas moderate inhibition resulted in macrovesicular steatosis, which over time progressed to steatohepatitis and cirrhosis. Within this pathogenesis, mitochondrial dysfunction and associated oxidative stress are key factors in the progression of hepatic lesions. Thus, excessive drug toxicity is capable of causing substantial disturbances in hepatic energy metabolism and provoking the accumulation of free radicals.

Oxidative stress necessitates activation of antioxidant defence mechanisms. L. Cesarini *et al.* (2024), in a systematic review, outlined the role of glutathione and other low-molecular-weight thiols in metabolic dysfunction-associated steatotic liver disease (MASLD). The researchers concluded that glutathione is critically important for maintaining redox balance in liver cells, and that disruption of glutathione homeostasis contributes to the development of steatosis. However, the authors noted that due to differences in measurement methodologies, it is currently impossible to define specific diagnostic threshold values for glutathione in MASLD. This indicates the need for standardised approaches to the investigation of hepatic redox status in future studies. Disturbances in oxidative metabolism are accompanied by alterations in the amino acid profile. L. Prechtl *et al.* (2024) identified a specific amino acid signature in coronary artery disease, characterised by decreased concentrations of ornithine cycle metabolites and antioxidant-related factors, namely histidine and lysine, which correlated with the severity of vascular lesions. The authors suggested that this signature reflected increased nitric oxide consumption and enhanced oxidative stress in affected patients.

Q. Tan *et al.* (2025) described another component of amino acid alterations, namely the tryptophan pathway. According to their review, metabolites of the kynurenine pathway (kynurenine and its derivatives) modulate immune response and hepatic fibrogenesis through activation of the aryl hydrocarbon receptor, suppression of T-cell activity, and inhibition of nuclear factor κB signalling pathways. Thus, alterations in this pathway may also influence the course of hepatopathology in both acute and chronic forms. Researchers Y. Yan *et al.* (2024) applied metabolomics and machine learning to a model of post-hepatectomy liver regeneration. The authors identified a number of amino acid markers indicative of disturbances in relevant metabolic pathways during tissue recovery. These studies demonstrated that alterations in intermediate amino acid metabolism, both in cardiovascular pathology and during liver regeneration, are closely associated with oxidative status and general metabolic dysfunction.

Liver dysfunction significantly affects the metabolism of proteins and macronutrients. A study by S.K. Han *et al.* (2025) highlighted that appetite disorders and sarcopenia are extremely common in patients with chronic liver disease, and these conditions worsen the prognosis. The researchers described the complex interaction between the metabolism of carbohydrates, lipids and amino acids in liver cirrhosis. In this context, muscle tissue partially compensated for impaired liver function by detoxifying ammonia. Attention was also paid to the importance of branched-chain amino acids (BCAAs). Their deficiency contributed to muscle atrophy and encephalopathy, whilst the addition of BCAAs in many studies improved muscle mass and quality of life in patients. Although the results of these clinical trials remain inconclusive. Overall, an analysis of the scientific literature suggests that protein metabolism disorders and muscle mass loss are actual manifestations of metabolic imbalance in liver injury.

Thus, the available studies point to a specific chain of cause-and-effect relationships: drug toxicity causes mitochondrial dysfunction and oxidative stress in hepatocytes, leading to changes in the metabolism of amino acids and proteins. At the same time, none of the cited sources detailed the specific tetracycline-induced effects on the metabolism of nitrogen-containing compounds in the liver. Furthermore, there are no data in the literature regarding the possible corrective effect of milk phospholipids on these changes. All these factors point to an under-researched aspect of the problem and justify the relevance of a study dedicated to the effect of tetracycline-induced hepatopathy on amino acid metabolism and the role of the milk phospholipid fraction in restoring impaired metabolism.

The aim of the study was to identify the main patterns of disruption in the blood amino acid profile in rats with tetracycline-induced hepatopathy and to determine the corrective effect of the milk phospholipid fraction.

Literature Review

Current understanding of the pathogenesis of liver diseases highlights their close association with metabolic dysfunctions. Non-alcoholic fatty liver disease (NAFLD) affects approximately 30% of the world's population and is associated with obesity and metabolic syndrome. Drug-induced liver injury can be hepatocellular, cholestatic or mixed, and often involves immune/inflammatory responses. The main mechanisms of DILI include mitochondrial dysfunction, overproduction of reactive oxygen species (ROS), activation of apoptosis/necrosis, and immune-mediated damage to the bile ducts. The complex pathogenesis of DILI – a combination of metabolic and inflammatory factors – drives the search for new biomarkers and therapeutic approaches.

In this context, the amino acid profile of whole blood is considered a potential source of biomarkers for steatosis and fibrosis. Researchers A.I. Amanatidou *et al.* (2023) found that patients with NAFLD and obesity had elevated plasma concentrations of essential amino acids, particularly BCAAs (leucine, isoleucine, valine), and aromatic amino acids (e.g., tyrosine), compared with patients with a lower degree of steatosis. These amino acids correlated with the degree of hepatic steatosis as assessed by magnetic resonance imaging and with markers of fibrosis (MRI-cT1), indicating their diagnostic significance. Similarly, R. Allison *et al.* (2023) reported that DILI is associated with changes in amino acid metabolism, which are reflected in the ratios of hepatocellular to cholestatic lesions and immune responses. In patients with chronic hepatitis and hepatocellular carcinoma, S.E. Ghanem *et al.* (2022) observed a significant reduction in plasma BCAA levels and an increase in aromatic amino acids (AAAs), which led to changes in the Fischer index (BCAA/tyrosine) – a potential non-invasive marker of liver disease severity. Together, these observations highlight the clinical value of assessing the blood amino acid profile as a marker of metabolic disturbances in hepatic steatosis.

Examining specific amino acid metabolic pathways is key to understanding the pathogenesis of steatosis and developing treatment strategies. The glutamate/serine/glycine (GSG) index was proposed by S. Leonetti *et al*. (2020) as a marker of NAFLD. The authors demonstrated that the GSG index was significantly higher in children with NAFLD and positively correlated with the percentage of fatty tissue in the liver and the level of aminotransferase activity. This suggests that metabolic imbalance in the glutamate and glycine cycles coincides with the progression of steatosis. Methionine and its metabolites also play an important role. In particular, Z. Li *et al*. (2020) noted in a review article that in chronic liver diseases, elevated concentrations of methionine and aromatic amino acids were observed, whilst BCAA levels decreased. At the same time, inhibition of the synthesis of S-adenosylmethionine (SAM), a key methyl group donor, was observed. SAM deficiency contributes to the progression of liver fibrosis and carcinogenesis.

The diagnostic relevance of the methionine cycle was confirmed by Y. Tang *et al*. (2022). The authors found higher levels of methionine metabolites, particularly S-adenosylhomocysteine (SAH) and homocysteine, in NAFLD, including a reduction in the SAM/SAH ratio. This suggested that disturbances in the metabolism of methionine, which accumulates during steatosis, may serve as a marker and a target for intervention. Taurine is another important factor which, according to a report by S. Baliou *et al*. (2021), acts as a potent antioxidant and cytoprotector – it supports mitochondrial function and glutathione stores, enhances antioxidant reactions, stabilises membranes and reduces the intensity of inflammation. This allows it to be considered a metabolic agent for mitigating oxidative stress in liver injury.

Tryptophan has also been found to have a protective effect on the liver. K. Gu *et al*. (2023) demonstrated that, in a model of lipopolysaccharide-induced liver failure, tryptophan helped to reduce the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), H_2O_2 and malondialdehyde levels, and increased the activity of antioxidant enzymes, whilst simultaneously inhibiting the inflammatory pathways of pyroptosis and necroptosis. Thus, tryptophan improved parameters relating to both the development of oxidative stress and the onset of an inflammatory response resulting from liver injury. Y. Sun *et al*. (2023) obtained innovative results regarding the role of asparagine in acute toxic liver injury. In particular, increased expression of asparagine synthetase was observed in pericentral hepatocytes, and intravenous administration of asparagine was associated with a reduction in tissue necrosis. Mice with genetic inactivation of the asparagine synthetase gene exhibited more pronounced liver injury. However, with the additional administration of asparagine to the animals, this damage was almost completely reversed. These data highlight the importance of asparagine as a hepatoprotective agent during acute stress.

The link between amino acid metabolism and the manifestation of oxidative stress and inflammation is evident. Most of the amino acids listed are substrates for the synthesis of glutathione or other antioxidants. Z. Li *et al*. (2020) noted that methionine is one of the main target molecules for ROS, and disruption of its metabolism is associated with severe liver injury. Taurine has pronounced anti-inflammatory and antioxidant properties, whilst tryptophan is associated with increased activity of antioxidant enzymes and a reduction in oxidative stress. Against a background of amino acid imbalance, depletion of glutathione stores and excessive ROS formation exacerbate inflammation and promote the development of apoptosis and necrosis. Thus, disturbances in amino acid metabolism should be considered a key link in the pathogenesis of DILI, and assessment of redox status is essential for a proper analysis of metabolic changes.

An experimental model of tetracycline-induced steatosis confirms the validity of metabolic correction. F. Aghaei *et al.* (2023) induced hepatic steatosis in rats via intraperitoneal administration of tetracycline (140 mg/kg). In these animals, significant lipid accumulation in hepatocytes and signs of oxidative stress were observed. In response, the application of physical exercise and the probiotic L. rhamnosus GG contributed to an increase in the activity of superoxide dismutase and high-density lipoproteins, and a reduction in the levels of triacylglycerols, ALT, AST and alkaline phosphatase (ALP). This indicates that combined therapeutic approaches are capable of mitigating tetracycline-induced liver injury. An analysis of existing studies suggests that a focus on metabolic and protective factors is promising, justifying the feasibility of evaluating the efficacy of the phospholipid fraction of milk as a potential therapeutic agent, which has already been partially demonstrated in studies of the amino acid profile of the liver and bile in experimental rats with tetracycline-induced hepatosis (Bryzhenko & Gryshchenko, 2026).

Despite the known changes in amino acid metabolism in NAFLD and DILI, there is a lack of direct data on the characteristics of the amino acid profile in tetracycline-induced steatosis. Studying these nitrogen metabolism metabolites in the blood of animals will enable the identification of specific markers of liver injury and the elucidation of its molecular mechanisms. This also highlights the need for new protective approaches, as amino acids that regulate redox and immune homeostasis may serve as criteria for determining the efficacy of therapeutic agents with hepatoprotective effects.

Materials and Methods

The experiment on laboratory rats was initiated on 15 January 2024 at the vivarium of the Educational and Scientific Institute of High Technologies of Taras Shevchenko National University of Kyiv (Kyiv, Ukraine), which is equipped with centralised water supply, sewage, ventilation, and air-conditioning systems. Chromatographic investigations of the amino acid profile of whole blood from experimental animals were conducted over the following two months (February-March 2024). Experimental procedures were carried out in accordance with the requirements of Directive 2010/63/EU (2010), Law of Ukraine No. 3447-IV (2006), and Order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 (2012), and were additionally approved by the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine (NULES of Ukraine) (Protocol No. 10 dated 10 October 2023).

Clinically healthy male laboratory rats aged 3 months and weighing 180-220 g were used in the experiment. All experimental animals were assigned to groups and housed in separate cages under a standard vivarium diet with free access to feed and drinking water. Prior to the scientific experiment, the rats underwent a two-week quarantine period with clinical examination and regular monitoring of body weight and feed consumption. The duration of the experiment was 9 days.

According to the methodology of D. Korolova *et al.* (2023), fatty hepatosis was artificially modelled in laboratory rats. To reproduce hepatopathology, a soft silicone probe was used for daily intragastric administration of a 4% tetracycline hydrochloride solution at a dose of 250 mg/kg body weight for 7 days. Intragastric administration of high doses of tetracycline hydrochloride induced the development of fatty hepatosis in rats. Animals receiving intragastric tetracycline hydrochloride without subsequent therapy were assigned to the “Self-rehabilitation” group (n = 6). Rats in the “Tetracycline + Supplement” group (n = 6) received intragastric administration of a 1% solution of the dietary supplement “FLP-MD”, based on the milk phospholipid fraction (experimental series, NULES of Ukraine), 1 hour prior to antibiotic administration. The dietary supplement was administered intragastrically for 9 days, one hour before antibiotic administration, and additionally for two days after completion of the intoxication course. The daily dose of "FLP-MD" was 13.5 mg/kg body weight (Melnychuk *et al.*, 2009). The next experimental group, the "Supplement" group, consisted of clinically healthy animals additionally receiving milk phospholipids as part of the "FLP-MD" dietary supplement (n = 6). Rats administered distilled water in a volume equivalent to that of the antibiotic and dietary supplement were included in the "Control" group (n = 6). Throughout the experiment, daily monitoring of body weight was performed using ORION OS-0K22 electronic scales (ORION ELECTRONICS LTD, Europe), allowing dose adjustment of the administered substances. On day 10, blood samples were collected from rats of all groups for subsequent investigation of their amino acid profile. The spectrum of free amino acids was determined using partition paper chromatography with ninhydrin detection, modified on the basis of the classical methods of R. Consden *et al.* (1944) and S.M. Partridge (1948), taking into account the approaches of S. Moore & W.H. Stein (1948) and R.J. Block *et al.* (1955), which enabled qualitative and quantitative investigation of the amino acid composition of biological material, with the exception of proline and hydroxyproline, which produce a non-specific reaction with ninhydrin.

Sample preparation. Since certain amino acids possess similar Rf coefficient values and are incompletely separated under one-dimensional chromatography conditions, preliminary concentration of the samples was performed prior to analysis. For this purpose, each sample was applied three times in 20 μL aliquots onto a 3.5 × 3.5 cm square of ash-free filter paper, with each previous layer dried before application of the next. After saturation with the sample, the paper was fragmented into small pieces and placed into a test tube containing 2 mL of extraction mixture consisting of acetone : ethanol : water (7:2:1, by volume). This procedure ensured desorption of amino acids into solution and partial separation from macromolecules and salts capable of complicating further analysis. Following extraction, the solution was evaporated to dryness in an air stream at room temperature, and the dry residue was dissolved in 15 μL of distilled water. The obtained concentrate was applied onto the starting line of chromatographic paper in three successive 5 μL droplets at intervals of approximately 15 minutes for drying, resulting in the formation of a compact concentrated spot. The principle of repeated sample application to improve chromatographic analysis accuracy corresponds to the approach described by R.H. Hackman & M. Lazarus (1956). Preliminary elution of amino acids from saturated filter paper was used as an authorial modification of the method.

Chromatographic separation. Chromatographic paper Filtrak FN-1 (Germany) was used as the stationary phase for chromatographic separation. The mobile phase consisted of a six-component solvent mixture: isoamyl alcohol (Carlo Erba, France), n-butanol (Carlo Erba, France), glacial acetic acid (Merck, Germany), formic acid (BASF, Germany), distilled water, and butyl benzyl ether (Thermo Fisher Scientific, USA), mixed in a ratio of 21:8:10:1:10:1 (by volume) with the addition of 0.2% ninhydrin (Thermo Fisher Scientific, USA). Chromatography was performed by ascending paper chromatography in a sealed chamber, pre-saturated with vapours of this mobile phase (Consden *et al.*, 1944; Williams & Kirby, 1948). The sample application zones were positioned on a starting line approximately 2-3 cm from the lower edge of the paper. Development of the chromatogram lasted approximately 7 hours, during which the solvent travelled almost the entire length of the paper strip. If necessary, repeated solvent runs or extension of the development time to 12-20 hours may be employed to improve separation. However, in the present study, a single chromatographic run of approximately 7 hours was sufficient for separation of most fractions.

Development of the chromatogram. Following chromatographic separation, the paper strip was removed from the chamber and dried in a fume hood at room temperature for approximately 1 hour until complete removal of residual solvent vapours. To visualise amino acids, the chromatogram was placed in a drying cabinet (Labexpert 3015, Ukraine) at 45°C for 120 minutes. During this period, ninhydrin (Thermo Fisher Scientific, USA), included in the mobile solvent, reacted directly with amino acids on the paper, forming coloured products. Consequently, additional application of the reagent was not required. The mild heating regime (45°C for 2 hours) ensured gradual development of amino acid spots. For comparison, standard methodologies involve brief immersion of the dried paper chromatogram in a ninhydrin solution, followed by repeated drying and heating for approximately 15 minutes at 60°C to produce purple spots (R.J. Block *et al.*, 1955). The approach applied in the present study, in which ninhydrin was incorporated directly into the eluent, simplified the visualisation procedure while providing an equivalent result.

Quantitative analysis. Following visualisation, amino acid spots were quantitatively evaluated using reflection densitometry. The intensity of spot coloration was measured in reflected light using a DO-1M densitometer (Ukraine). The use of spectral reflectometry for analysis of ninhydrin spots on chromatograms is a recognised approach for quantitative amino acid determination. Calibration curves were preliminarily constructed by applying known quantities of standard amino acids onto chromatographic paper, followed by the complete separation and visualisation procedure and measurement of the optical density of the resulting spots. Within the linear range of the ninhydrin reaction, optical density of coloration was proportional to the quantity of the corresponding amino acid. Optical density values obtained for experimental samples were compared with the calibration curve for the relevant amino acid, allowing determination of its concentration in the sample while taking into account dilution introduced during sample preparation. If individual amino acids were not separated because of similar Rf values and co-localised on the chromatogram, their quantities were recorded collectively, or additional separation approaches were applied, including solvent modification or two-dimensional chromatography.

Statistical analysis of the results. The obtained experimental data were processed using methods of variation statistics in Microsoft Excel 2016 software (Microsoft Corp., USA). For each parameter, the arithmetic mean (M), standard deviation (SD), and standard error of the mean (m) were determined. Statistical significance of differences between means was assessed using Student's t-test for independent samples with unequal variances (two-sample unequal variance t-test), with a significance level of $p < 0.05$.

Results and Discussion

The amino acid composition of blood was investigated as an informative marker of the systemic metabolic response of the organism to tetracycline-induced liver injury and to the corrective influence of the milk phospholipid fraction. Whole blood reflects integrated alterations in nitrogen-containing compound metabolism, providing grounds for considering its amino acid profile as a sensitive indicator of metabolic restructuring under conditions of toxic exposure in animals. The results present indicators of heterogeneous amino acid fractions in the whole blood of animals from different experimental groups in comparison with the control group. This approach made it possible to identify the most sensitive indicators of amino acid metabolism and to assess the direction of their alterations depending on the development of the hepatotoxic process and the application of the corrective factor. Collectively, this provides a basis for a more detailed interpretation of the role of individual amino acids in the mechanisms of liver injury and metabolic adaptation.

The heterogeneous cysteine/cystine fraction (Fig. 1) in the whole blood of rats with tetracycline-induced liver injury was considered one of the most sensitive indicators of redox homeostasis disturbance. In particular, rats with tetracycline-induced hepatopathology ("Tetracycline + Supplement" group) receiving intragastric administration of the milk phospholipid fraction demonstrated an increase in the content of the heterogeneous cysteine/cystine fraction components by 161%, whereas in healthy animals receiving milk phospholipids ("Supplement" group), their concentration increased by only 65% compared with the control group (0.51 ± 0.07 mg%). In contrast, rats in the "Self-rehabilitation" group exhibited a tendency towards a 20% decrease in cysteine/cystine levels compared with the control.

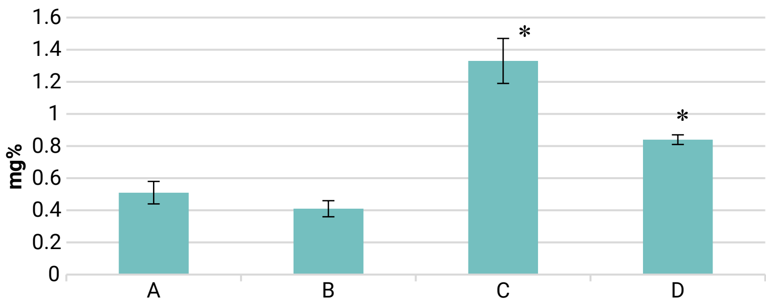

Figure 1. *Concentration of the cysteine/cystine fraction in the whole blood of rats with tetracycline-induced hepatosis following corrective therapy based on milk phospholipids (n = 6, M ± m)*

Note: * – $p < 0.05$, values significantly different compared with the control group of rats; A – clinically healthy animals, "Control" group; B – rats with tetracycline-induced hepatosis, "Self-rehabilitation" group; C – rats with tetracycline-induced hepatosis administered the milk phospholipid fraction, "Tetracycline + Supplement" group; D – clinically healthy rats administered the milk phospholipid fraction, "Supplement" group

Source: authors' own work

The peculiarities of the established changes may be associated with the fact that, under tetracycline-induced liver injury, a sharp increase in the content of cysteine/cystine amino acids may result from pronounced oxidative stress, activation of the methionine-homocysteine cycle, and "overloading" of the thiol reserve, when cysteine is massively mobilised for the resynthesis of glutathione and taurine, and is partly released into the bloodstream from damaged hepatocytes. In the group of animals receiving milk phospholipids, the marked increase in the concentration of amino acids of this fraction probably reflected moderate activation of their metabolism and enhanced synthesis and turnover of glutathione without pronounced liver injury. The established patterns are consistent with those described in the review by L. Cesarini *et al.* (2024), who emphasised that low-molecular-weight thiols, primarily cysteine and glutathione, are central components of redox homeostasis, and that their imbalance is closely associated with the severity of metabolic dysfunction-associated fatty liver disease. Y. Wang *et al.* (2018) additionally demonstrated that disruption of bile acid signalling alters cysteine catabolism and glutathione regeneration, thereby increasing liver susceptibility to oxidative injury, which corresponds to the findings of the present study showing a sharp increase in the total blood content of cysteine/cystine under tetracycline-induced hepatotoxic stress.

The experimentally established patterns indicate substantial activation of antioxidant defence mechanisms in the organism of rats with experimental hepatopathology, whereas further analysis of amino acids associated with the ornithine cycle allows a deeper assessment of alterations in the detoxification function of the liver (Fig. 2). In particular, the blood concentration of the arginine/ornithine/lysine amino acid fraction in rats of the "Self-rehabilitation" group remained within the control range, whereas in rats with tetracycline-induced fatty hepatosis treated with the milk phospholipid fraction, it increased by 69% relative to the control group (1.39 ± 0.19 mg%). In the group of healthy animals receiving the milk phospholipid fraction (the "Supplement" experimental group), the total level of these amino acids exceeded the control values by 57%.

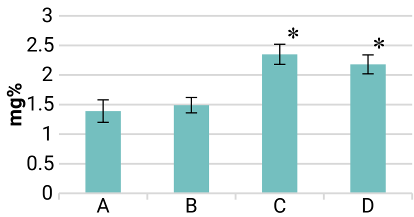

Figure 2. *Concentration of the arginine/ornithine/lysine fraction in the whole blood of rats with tetracycline-induced hepatosis following corrective therapy based on milk phospholipids (n = 6, M ± m)*

Note: * – $p < 0.05$, values significantly different compared with the control group of rats; A – clinically healthy animals, "Control" group; B – rats with tetracycline-induced hepatosis, "Self-rehabilitation" group; C – rats with tetracycline-induced hepatosis administered the milk phospholipid fraction, "Tetracycline + Supplement" group; D – clinically healthy rats administered the milk phospholipid fraction, "Supplement" group
Source: authors' own work

Thus, the most pronounced increase in the total blood content of arginine/ornithine/lysine in rats was observed specifically under tetracycline-induced injury ("Tetracycline + Supplement" group). In contrast, in the group of clinically healthy rats receiving the dietary supplement "FLP-MD" ("Supplement" group), this indicated moderate but persistent activation of nitrogen metabolism. From a biochemical perspective, arginine and ornithine are key metabolites of the ornithine cycle, which ensures ammonia detoxification, whereas lysine, as an essential amino acid, reflects the overall intensity of proteolysis and protein synthesis. The marked increase in the blood content of these amino acids in animals with tetracycline-induced fatty hepatosis treated with the dietary supplement "FLP-MD" ("Tetracycline + Supplement" group) may be interpreted as a consequence of their enhanced mobilisation in response to liver injury. In turn, the increase in blood arginine/ornithine/lysine levels in clinically healthy animals receiving the milk phospholipid fraction ("Supplement" group) was probably associated with stimulation of nitrogen metabolism and ureagenesis without pronounced cytolysis, which may reflect the anabolic and hepatotropic effects of phospholipid correction. Similar alterations in the arginine-ornithine pathway during liver injury have also been described by other authors. W. Saitoh *et al.* (2014), using models of acute liver injury in rats, demonstrated that the plasma arginine-to-ornithine ratio changes significantly, and that both amino acids may be regarded as specific biomarkers of liver injury. In a broader clinical context, L. Prechtl *et al.* (2024) showed that reduced concentrations of urea cycle amino acids (particularly arginine and ornithine) and lysine in circulating blood were associated with poorer outcomes in coronary heart disease, interpreting this as a sign of intensified ureagenesis and oxidative stress. Against this background, the multidirectional increase in the arginine/ornithine/lysine amino acid group in models of tetracycline-induced fatty hepatosis with and without phospholipid correction further highlights the sensitivity of urea cycle amino acids to the state of hepatic nitrogen metabolism and their potential use as biomarkers.

Alongside amino acids of the ornithine cycle, fractions associated with antioxidant defence are also of considerable importance. In this regard, quantitative changes in the total histidine/taurine/asparagine fraction were analysed in the whole blood of experimental rats (Fig. 3). In the whole blood of diseased rats from the "Self-rehabilitation" group, the content of the total histidine/taurine/asparagine fraction increased by 54% relative to the control group (0.94 ± 0.10 mg%), whereas in the group of animals with tetracycline-induced fatty hepatosis treated with the milk phospholipid fraction, its level increased by 196% compared with the control group. In healthy rats receiving milk phospholipids ("Supplement" group), this amino acid fraction showed only a tendency towards an increase of 18%.

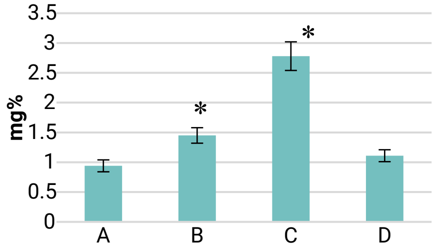

Figure 3. *Concentration of the histidine/taurine/asparagine fraction in the whole blood of rats with tetracycline-induced hepatosis following corrective therapy based on milk phospholipids (n = 6, M ± m)*

Note: * – $p < 0.05$, values significantly different compared with the control group of rats; A – clinically healthy animals, "Control" group; B – rats with tetracycline-induced hepatosis, "Self-rehabilitation" group; C – rats with tetracycline-induced hepatosis administered the milk phospholipid fraction, "Tetracycline + Supplement" group; D – clinically healthy rats administered the milk phospholipid fraction, "Supplement" group

Source: author's own work

The established patterns observed in animals of the "Self-rehabilitation" group may result from activation of protective mechanisms, since histidine exhibits buffering properties and participates in anti-inflammatory reactions (through carnosine and histamine), taurine is a key antioxidant and substrate for bile acid conjugation, and asparagine supports nitrogen and energy metabolism. In acute tetracycline-induced hepatocyte injury combined with the reparative effect of the milk phospholipid fraction, the sharp increase in the histidine/taurine/asparagine fraction compared with its content in animals of the "Self-rehabilitation" group probably reflected a combination of: enhanced synthesis of taurine and histidine-containing dipeptides and peptides in response to oxidative stress-induced inflammation; release of these amino acids into the bloodstream from damaged hepatocytes; and intensification of nitrogen metabolism, in which asparagine acts as an acceptor of amino groups. In healthy animals receiving phospholipids, only slight quantitative shifts in the amino acid parameters of this fraction were observed, indicating that phospholipid administration alone does not induce pronounced hyperaminoacidaemia of this amino acid group. A similar interpretation of these patterns was reported by S. Baliou *et al.* (2021), who described the marked hepatoprotective and antioxidant effects of taurine, contributing to the reduction of oxidative stress, steatosis, and mitochondrial dysfunction in various models of liver injury. This corresponds with the results described in the present study in response to tetracycline-induced hepatocyte damage. In turn, Y. Sun *et al.* (2023) indicated that activation of the asparagine synthetase pathway and increased asparagine availability protect pericentral hepatocytes during acute toxic liver injury, thereby reducing necrosis and improving cell survival. Against this background, the experimentally observed moderate increase in histidine/taurine/asparagine levels in the blood of rats from the "Self-rehabilitation" group and the sharp rise in their levels in animals of the "Tetracycline + Supplement" group may be interpreted as reflecting mobilisation of these amino acids in protective processes (antioxidant, anti-inflammatory, and detoxification), which is consistent with previously published experimental findings.

For further assessment of metabolic rearrangements in the organism of rats under the toxic effects of tetracycline on the liver, quantitative changes in the total glycine/serine/glutamine/aspartic acid fraction in whole blood were examined, as these amino acids are associated with nitrogen transport and detoxification processes (Fig. 4). Regarding the glycine/serine/glutamine/aspartic acid amino acid complex in whole blood, statistically significant changes were observed only in diseased rats of the "Tetracycline + Supplement" group, in which the concentration increased by 92% compared with the control group (1.75 ± 0.27 mg%). In animals of the "Self-rehabilitation" group and in clinically healthy rats receiving the milk phospholipid fraction ("Supplement" group), only a tendency towards increased values of this parameter was observed.

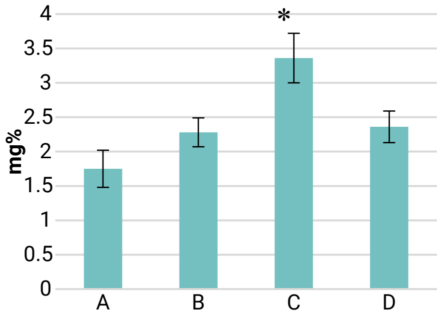

Figure 4. *Concentration of the combined glycine/serine/glutamine/aspartic acid fraction in the whole blood of rats with tetracycline-induced hepatosis following corrective therapy based on milk phospholipids (n = 6, M ± m)*
Note: * – $p < 0.05$, values significantly different compared with the control group of rats; A – clinically healthy animals, "Control" group; B – rats with tetracycline-induced hepatosis, "Self-rehabilitation" group; C – rats with tetracycline-induced hepatosis administered the milk phospholipid fraction, "Tetracycline + Supplement" group; D – clinically healthy rats administered the milk phospholipid fraction, "Supplement" group
Source: author's own work

The established patterns may indicate that, under tetracycline-induced liver injury, the sharp increase in the content of glycine/serine/glutamine/aspartic acid reflects activation of gluconeogenesis and alternative pathways of nitrogen detoxification. In particular, glutamine and asparagine serve as "reservoirs" for ammonia in cases of suppression of the urea cycle, whereas glycine and serine are mobilised as donors of one-carbon groups and substrates for glutathione synthesis. Against the background of tetracycline-induced mitochondrial dysfunction, the administration of milk phospholipids stimulates mobilisation of these amino acids in order to maintain adequate antioxidant, detoxification, and energy-dependent processes, which explains their accumulation in the blood. In clinically healthy animals receiving milk phospholipids ("Supplement" group), the absence of statistically significant differences in the levels of the studied amino acids compared with control values indicated a neutral effect of these phospholipids on nitrogen metabolism parameters and the implementation of antioxidant mechanisms. A similar role of glycine and serine in redox homeostasis during fatty hepatosis was described by S. Leonetti *et al.* (2020), who proposed the GSG index (glutamate/(serine + glycine)) as a non-invasive marker of paediatric NAFLD: decreased total serine and glycine levels and an increased GSG index were associated with greater hepatic fat content and elevated ALT activity. Thus, the substantial increase in the content of this heterogeneous fraction in the whole blood of rats under a model of acute tetracycline-induced hepatotoxic stress may be regarded as an early sign of strain in nitrogen metabolism and maintenance of antioxidant balance stability, which partly corresponds to changes described in the literature (primarily regarding quantitative changes in glutamine), but differs from the chronic metabolic context of NAFLD, where glycine and serine are often depleted.

At the next stage, quantitative parameters of the methionine/glutamic acid heterogeneous fraction, which characterises individual pathways of one-carbon and amino acid metabolism, were analysed (Fig. 5). In the case of the total methionine/glutamic acid amino acid fraction in the whole blood of rats, no statistically significant changes relative to the control group were recorded. In the "Self-rehabilitation" group, their level practically did not differ from the control value (1.86 ± 0.20 mg%), whereas in the group of animals with tetracycline-induced fatty hepatosis treated with the milk phospholipid fraction ("Tetracycline + Supplement"), only a tendency towards an increase in the parameter by 28% was observed, and in clinically healthy animals receiving the milk phospholipid fraction ("Supplement" group), the value exceeded the control level by 32%. Thus, the quantitative parameters of this amino acid fraction remained relatively stable even against the background of tetracycline-induced injury and administration of milk phospholipids.

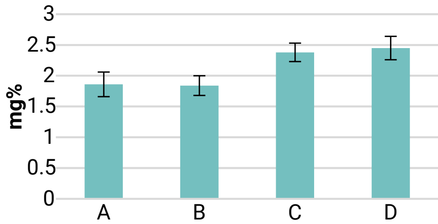

Figure 5. *Concentration of the combined methionine/glutamic acid fraction in the whole blood of rats with tetracycline-induced hepatosis following corrective therapy based on milk phospholipids (n = 6, M ± m)*

Note: * – $p < 0.05$, values significantly different compared with the control group of rats; A – clinically healthy animals, "Control" group; B – rats with tetracycline-induced hepatosis, "Self-rehabilitation" group; C – rats with tetracycline-induced hepatosis administered the milk phospholipid fraction, "Tetracycline + Supplement" group; D – clinically healthy rats administered the milk phospholipid fraction, "Supplement" group

Source: author's own work

The established findings may be interpreted as indicating the preservation of stability in one-carbon metabolism and transsulphuration pathways: methionine continued to be utilised for the synthesis of SAM, polyamines, and glutathione, whereas glutamic acid functioned as a key donor of amino groups in transamination reactions and as a substrate of the urea cycle. Clinical observations reported by Y. Tang *et al.* (2022) demonstrated that patients with NAFLD exhibited elevated levels of methionine metabolites, S-adenosylhomocysteine, and homocysteine, together with a reduced SAM/SAH ratio, confirming the presence of steatosis. Against this background, the absence of significant alterations in the present heterogeneous fraction in the conducted experiment indicates a relatively moderate degree of hepatocellular injury and partial preservation of methionine cycle regulation compared with the more severe or prolonged chronic processes described in clinical studies.

To assess possible alterations in gluconeogenesis and anabolic processes in the experimental rats, the quantitative parameters of the total alanine/tyrosine/threonine fraction were investigated (Fig. 6). In the heterogeneous fraction comprising this amino acid complex in the whole blood of rats, only tendencies towards quantitative changes relative to the control group (1.61 ± 0.28 mg%) were observed. In particular, in animals of the "Self-rehabilitation" group, the total concentration of these amino acids decreased by 43% compared with the control level, whereas in rats with tetracycline-induced hepatosis receiving the milk phospholipid fraction ("Tetracycline + Supplement" group), it increased by 49%. In clinically healthy animals receiving the milk phospholipid fraction ("Supplement" group), the concentration increased by 37% relative to the control group.

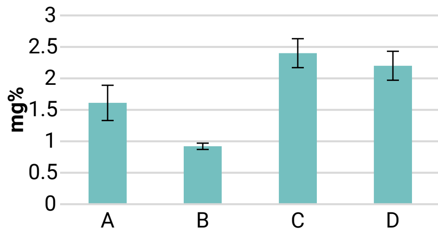

Figure 6. *Concentration of the combined alanine/tyrosine/threonine fraction in the whole blood of rats with tetracycline-induced hepatosis following corrective therapy based on milk phospholipids (n = 6, M ± m)*

Note: * – $p < 0.05$, values significantly different compared with the control group of rats; A – clinically healthy animals, "Control" group; B – rats with tetracycline-induced hepatosis, "Self-rehabilitation" group; C – rats with tetracycline-induced hepatosis administered the milk phospholipid fraction, "Tetracycline + Supplement" group; D – clinically healthy rats administered the milk phospholipid fraction, "Supplement" group

Source: author's own work

The identified tendencies in the quantitative changes of the investigated amino acid fraction indicated the relative stability of its parameters under conditions of acute liver injury in the experimental setting. The biological significance of this amino acid profile is consistent with the fact that alanine is a key gluconeogenic substrate and a marker of metabolic stress, whereas tyrosine belongs to the aromatic amino acids that are sensitive to impaired hepatic detoxification. In a clinical study by A.I. Amanatidou *et al.* (2023), patients with obesity and NAFLD demonstrated significantly elevated plasma levels of L-alanine, L-threonine, and L-tyrosine, together with other amino acids, in groups with more pronounced hepatic steatosis and fibrosis. The findings obtained by these researchers confirmed that activation of gluconeogenesis and accumulation of aromatic amino acids in the blood are characteristic features of progressive chronic liver pathology. At the same time, the results reported by S.E. Ghanem *et al.* (2022) showed that in chronic hepatitis and hepatocellular carcinoma, it was specifically the increase in tyrosine and other aromatic amino acids against a background of decreased BCAA levels that altered the Fischer ratio and reflected hepatic insufficiency, whereas no such pronounced alterations were recorded in the present experiment.

Further analysis of the amino acid pool of whole blood in the experimental rats focused on determining the quantitative parameters of the total valine/norvaline/tryptophan fraction, the components of which reflect the specificity of alterations in BCAA and tryptophan metabolism (Fig. 7). In particular, a significant increase in the total concentration of the components of this fraction was observed in the whole blood of rats from the "Tetracycline + Supplement" and "Supplement" groups, by 86% and 50%, respectively, compared with the control group (1.61 ± 0.18 mg%). At the same time, diseased rats of the "Self-rehabilitation" group showed only a tendency towards an increased concentration of this heterogeneous amino acid fraction (by 64%) relative to the control group, indicating incomplete spontaneous restoration of their amino acid profile. The identified pattern may be interpreted as evidence of profound disturbances in the metabolism of BCAA (primarily valine) and the aromatic amino acid tryptophan under tetracycline-induced hepatotoxic stress. The sharp increase in the total concentration of valine/norvaline/tryptophan in whole blood reflected a combination of enhanced proteolysis in skeletal muscle and reduced utilisation of BCAA by the damaged liver, which typically occurred against a background of energy deficiency in hepatocytes. Administration of milk phospholipids to the experimental rats reduced the amplitude of these alterations, which may be associated with partial restoration of mitochondrial BCAA oxidation, membrane integrity, and transamination efficiency. However, they did not ensure complete normalisation of the amino acid profile. Likewise, elevated tryptophan levels may reflect a shift in its metabolism towards the kynurenine pathway, which is activated during inflammation and oxidative stress and provides substrates for the formation of immunomodulatory and antioxidant metabolites. The obtained results are consistent with contemporary metabolomic studies in MASLD/NAFLD. Regarding tryptophan, the review by Q. Tan *et al.* (2025) noted that metabolites of the kynurenine pathway play a dual role in liver diseases: on the one hand, they modulate the immune response and reduce oxidative stress, while on the other hand, under chronic activation, they may promote fibrosis and disease progression. Experimental work by K. Gu *et al.* (2023) in piglets demonstrated that dietary tryptophan supplementation attenuated lipopolysaccharide-induced liver injury, enhanced antioxidant activity, and reduced the expression of necroptosis and pyroptosis markers. Overall, this suggests that in the tetracycline-induced model of hepatopathology, the increase in the total level of valine/norvaline/tryptophan, as well as its partial restoration against the background of milk phospholipid administration, reflects a balance between catabolic processes (proteolysis, insulin resistance, activation of the inflammatory response) and adaptive mechanisms associated with BCAA- and tryptophan-dependent pathways. Thus, the pronounced quantitative alterations in the valine/norvaline/tryptophan fraction confirm substantial restructuring of energy and protein metabolism during experimental tetracycline-induced liver injury in laboratory rats.

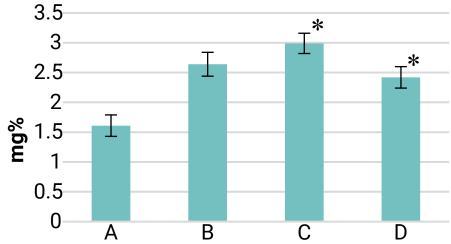

Figure 7. *Concentration of the combined valine/norvaline/tryptophan fraction in the whole blood of rats with tetracycline-induced hepatosis following corrective therapy based on milk phospholipids (n = 6, M ± m)*

Note: * – $p < 0.05$, values significantly different compared with the control group of rats; A – clinically healthy animals, "Control" group; B – rats with tetracycline-induced hepatosis, "Self-rehabilitation" group; C – rats with tetracycline-induced hepatosis administered the milk phospholipid fraction, "Tetracycline + Supplement" group; D – clinically healthy rats administered the milk phospholipid fraction, "Supplement" group
Source: authors' own work

The next stage of the analysis of the blood amino acid profile involved investigating the quantitative characteristics of the total leucine/norleucine/phenylalanine fraction in the whole blood of the experimental rats. The obtained results made it possible to assess the degree of stability of individual components of protein metabolism under conditions of medicinal toxic stress on the liver and the applied corrective intervention (Fig. 8). In particular, only moderate deviations relative to the control group (2.77±0.29 mg%) were recorded for the total concentration of leucine/norleucine/phenylalanine amino acids in the whole blood of animals from all groups. In rats of the "Self-rehabilitation" group, the total concentration of these amino acids showed a tendency to increase by 3%, in animals of the "Tetracycline + Supplement" group it changed by 5%, and in clinically healthy animals of the "Supplement" group by 9% compared with the control group. Thus, this amino acid complex may be regarded as relatively stable even under conditions of tetracycline-induced liver injury and the corrective influence of milk phospholipids. The obtained results differed from the pattern described by researchers in chronic liver diseases, where decreased BCAA levels (particularly leucine) and increased phenylalanine concentrations with a reduced Fischer ratio were considered typical. In the study by B. Kinny-Köster *et al.* (2016), alterations in plasma phenylalanine levels and the BCAA/AAA ratio were associated with mortality in patients with end-stage liver disease. In the work of M. Yamakado *et al.* (2017), the combination of BCAA with phenylalanine enabled differentiation between patients with fatty liver disease and individuals without steatosis, with amino acid alterations being considerably more pronounced than those observed in the present experiment. Against this background, the absence of significant quantitative changes in the leucine/norleucine/phenylalanine amino acid complex in the experimental rats rather indicates preservation of BCAA metabolism and the absence of pronounced decompensation in this metabolic direction, which is consistent with a model of acute rather than chronic toxic liver injury, as well as with the investigation of the amino acid pool in whole blood rather than plasma.

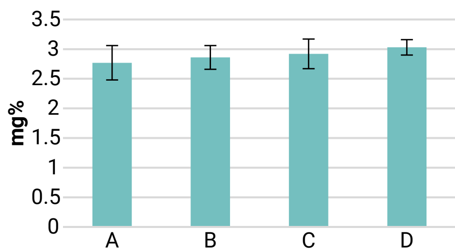

Figure 8. *Concentration of the combined leucine/norleucine/phenylalanine fraction in the whole blood of rats with tetracycline-induced hepatosis following corrective therapy based on milk phospholipids (n = 6, M ± m)*

Note: * – $p < 0.05$, values significantly different compared with the control group of rats; A – clinically healthy animals, "Control" group; B – rats with tetracycline-induced hepatosis, "Self-rehabilitation" group; C – rats with tetracycline-induced hepatosis administered the milk phospholipid fraction, "Tetracycline + Supplement" group; D – clinically healthy rats administered the milk phospholipid fraction, "Supplement" group
Source: authors' own work

Generalisation of the presented results demonstrated that the blood amino acid profile in tetracycline-induced hepatosis changed unevenly, with predominant involvement of those metabolic pools responsible for adaptation to oxidative stress, maintenance of nitrogen balance, and compensation for impaired liver function. The most pronounced response was observed in clusters associated with thiol metabolism, ureagenesis, antioxidant-related amino acids, and certain energetically important substrates, indicating the selective nature of metabolic restructuring during toxic liver injury of medicinal origin. Comparison of the results regarding alterations in the amino acid pool in animals from different experimental groups demonstrated that, against the background of administration of milk phospholipids in the form of the dietary supplement "FLP-MD", quantitative shifts in heterogeneous amino acid fractions acquired a different directionality compared with animals undergoing spontaneous self-rehabilitation, indicating modification of the systemic biochemical response of the organism under the influence of different factors. Thus, the identified alterations reflected not only the presence of hepatotoxic stress, but also the specific features of metabolic adaptation under conditions of corrective intervention.

Conclusions

The study demonstrated that tetracycline-induced hepatosis in rats is accompanied by pronounced alterations in the amino acid profile of whole blood, reflecting the systemic metabolic response of the organism to toxic liver injury. The amino acid fractions most sensitive to the effects of tetracycline were those associated with redox homeostasis, ammonia detoxification, nitrogen metabolism, and energy metabolism, namely cysteine/cystine, arginine/ornithine/lysine, histidine/taurine/asparagine, glycine/serine/glutamine/aspartic acid, and valine/norvaline/tryptophan fractions. Animals in the "Self-rehabilitation" group were characterised by signs of metabolic imbalance with accumulation of certain amino acid fractions involved in antioxidant defence and nitrogen metabolism, indicating delayed spontaneous recovery following tetracycline exposure. In particular, this group demonstrated a significant increase in the histidine/taurine/asparagine fraction by 54% compared with the control group, whereas changes in other amino acid fractions were predominantly trend-related in nature. The character of the detected alterations indicates that under conditions of tetracycline toxicity, metabolic stress develops alongside activation of nitrogen binding and excretion mechanisms, enhancement of antioxidant defence, and restructuring of amino acid supply to hepatocytes in response to cytotoxic injury.

The increased levels of certain amino acid fractions in whole blood reflected mobilisation of substrates required for maintenance of redox balance, detoxification reactions, and compensation for impaired hepatic functional status. At the same time, the absence of statistically significant changes in the heterogeneous methionine/glutamic acid, alanine/tyrosine/threonine, and leucine/norleucine/phenylalanine fractions indicated the selective nature of the metabolic response during acute tetracycline-induced injury. Administration of milk phospholipids as part of the dietary supplement "FLP-MD" was accompanied by pronounced restructuring of the blood amino acid profile. In particular, a significant increase was observed in the heterogeneous cysteine/cystine fraction by 161%, arginine/ornithine/lysine by 69%, histidine/taurine/asparagine by 196%, glycine/serine/glutamine/aspartic acid by 92%, and valine/norvaline/tryptophan by 86% compared with the control group, indicating a corrective and modulatory effect under conditions of drug-induced hepatosis. At the same time, administration of the milk phospholipid fraction to clinically healthy animals did not produce signs of severe metabolic distress, further confirming the adaptive rather than damaging nature of the observed alterations. In the group of healthy animals receiving milk phospholipids (the "Supplement" group), significant increases were detected in the heterogeneous cysteine/cystine fraction by 65%, arginine/ornithine/lysine by 57%, and valine/norvaline/tryptophan by 50% relative to the control.

The obtained findings collectively demonstrate that the amino acid spectrum of whole blood sensitively reflects the severity of metabolic disturbances in tetracycline-induced hepatosis and may be used as an informative criterion for evaluating the systemic response of the organism to drug-induced toxic exposure. The practical significance of the study lies in substantiating the feasibility of further investigation of milk phospholipids as a promising means of metabolic correction in drug-induced hepatopathies in veterinary medicine, as well as in the use of amino acid fractions as potential markers for the preclinical assessment of the efficacy of corrective interventions. The obtained results expand current understanding of the metabolic mechanisms underlying tetracycline-induced liver injury and provide a foundation for further experimental validation of amino acid parameters as early biomarkers of toxic hepatopathy. Future studies are planned to determine the patterns of changes in the protein spectrum of the liver and whole blood in laboratory rats, taking into account the amino acid profile alterations described in the present study during the development of drug-induced fatty liver disease.

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Conflict of Interest

None.

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Коригувальна ефективність фосфоліпідів молока за тетрациклініндукованих змін амінокислотного складу крові в щурів

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Анотація. Метою дослідження було встановити закономірності змін амінокислотного спектра нативної крові в лабораторних щурів на тлі розвитку тетрациклініндукованої гепатопатології та застосування фосфоліпідів молока. Для експериментального відтворення моделі гострої форми гепатопатії лабораторним щурам упродовж 7 діб внутрішньошлунково вводили тетрацикліну гідрохлорид у дозі 250 мг/кг маси тіла. Було сформовано чотири групи, які включали щурів: клінічно здорових; хворих на тетрациклініндукований гепатоз (без лікування); хворих на тетрациклініндукований гепатоз, яким вводили фосфоліпіди молока; клінічно здорових, яким застосовували фосфоліпіди молока. Амінокислотний профіль нативної крові визначали методом паперової хроматографії з нінгідриновим проявленням. Експериментально встановлено розвиток дисбалансу в амінокислотному профілі нативної крові щурів внаслідок цитотоксичного впливу тетрацикліну гідрохлориду на гепатоцити. У хворих на експериментальну гепатопатологію тварин за умов самореабілітації відзначалося достовірне підвищення в нативній крові рівня гетерогенної фракції гістидину/таурину/аспарагіну (на 54 %) та тенденцією до зростання вмісту сумарних фракцій цистину/цистеїну, гліцину/серину й амінокислот орнітинового циклу. Концентрація окремих амінокислот залишалася відносно стабільною. Введення хворим тваринам фосфоліпідної біодобавки супроводжувалось вираженою мобілізацією в крові амінокислот у складі сумарних фракцій цистеїну/цистину (на 161 %), аргініну/орнітину/лізину (на 69 %), гістидину/таурину/аспарагіну (на 196 %), гліцину/серину/глутаміну/аспарагінової кислоти (на 92 %) та валіну/норваліну/триптофану (на 86 %), що вказувало на стимуляцію азотного обміну й уреагенезу та відображало активацію процесів детоксикації, антиоксидантного захисту та гепатотропний ефект фосфоліпідної корекції. Отримані результати можуть бути використані для діагностики метаболічних порушень, які стосуються проміжного обміну амінокислот, за гепатопатології медикаментозного ґенезу та як маркери для оцінки ефективності новостворених препаратів

Ключові слова: печінка; гепатопатологія; модель жирового гепатозу; коригувальна терапія; паперова хроматографія; маркерні зміни; медикаментозні ускладнення

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Formation and utilisation of alfalfa sward under the soil and climatic conditions of the Right-Bank Forest-Steppe

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Abstract. The aim of the study was to determine the optimal combination of sowing rate and complex mineral fertilisers for the formation of a productive alfalfa (*Medicago sativa* L.) sward of the 'Radoslava' variety under the soil and climatic conditions of the Right-Bank Forest-Steppe. The experiment was conducted on grey forest soils during 2024-2025 using a three-factor design: sowing rates of 6.0 and 8.0 million viable seeds per hectare, two harvesting periods, and three levels of mineral nutrition. It was established that in the year of sowing (2024), the crop reached the beginning of flowering stage (BBCH code 61-62) after 69-70 days, with a daylight duration of 14:53-16:12 hours, and provided three cuts with a total green matter yield of up to 33.13 t/ha. The greatest plant height in the first cut was 66.1-66.3 cm at a sowing rate of 6.0 million/ha and maximum fertilisation (Background + $K_{60}S_{20}Ca_{30}Mg_{20}$), whereas in the third cut it decreased to 21.2-30.3 cm due to reduced daylight duration and increased temperature. On average across the treatments, plant height ranged from 36.00 to 42.93 cm, while leafiness ranged from 33.74 to 40.77%. In the second year (2025), across three cuts at the beginning of flowering stage (BBCH code 64), the average plant height was 61.4-61.8 cm, leafiness was 53.0-54.3%,

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and green matter yield reached 75.8 t/ha. The statistical significance of factor effects was confirmed by $LSD_{0.05}$. It was determined that the optimal combination was a sowing rate of 6.0 million/ha with increased fertilisation (Background + $K_{60}S_{20}Ca_{30}Mg_{20}$), which ensured the highest total green matter yield with minimal risks of moisture deficiency under the unstable hydrothermal conditions of the Right-Bank Forest-Steppe

Keywords: sowing rate; mineral fertilisers; plant height; leafiness; green matter yield; mowing time

Introduction

Alfalfa is regarded as one of the principal perennial legume crops from which forage production systems are expected to obtain simultaneously high yields, stable quality of leaf-stem biomass, and long-term productive utilisation of the sward (Antoniv *et al.*, 2023). Under the conditions of the Right-Bank Forest-Steppe, this crop was of particular importance due to the combination of summer temperature stress, unstable moisture supply, and increased requirements for the economic efficiency of each technological component. In contemporary scientific literature, leading attention has been devoted to studies of the role of potassium nutrition. Consequently, it became relevant to clarify the technological combination of sowing rate, level of complex mineral nutrition, and mowing time for the alfalfa variety 'Radoslava' under the conditions of grey forest soils of the Right-Bank Forest-Steppe, taking into account not only yield but also biometric indicators, sward quality, and economic and energy efficiency. Data from E.A. Appiah *et al.* (2024) showed that optimising potassium nutrition was one of the key factors in realising the production potential of alfalfa. Scientists D.J. Heuschele *et al.* (2023) established that potassium fertilisation influenced not only the yield of leaves and stems, but also forage quality, nutrient uptake and the phytosanitary condition of the sward. M.M. Baidoo & M.A. Islam (2024) demonstrated that the effect of potassium must be assessed in relation to the harvest date, as it is the combination of these factors that determines the productivity and profitability of alfalfa cultivation. At the same time, H. Ren *et al.* (2025) concluded that the most stable increase in alfalfa yield was not achieved by an isolated increase in a single nutrient dose, but by a balanced ratio of N, P and K, adapted to the specific soil and climatic conditions. Phosphorus nutrition was also considered one of the determining factors for early crop development, winter survival and yield stabilisation. The results of studies by Y. Wang *et al.* (2024) showed that phosphorus fertilisation enhanced the overwintering of late-sown alfalfa, stimulated root system development and increased forage yield on saline soils. Based on a review of scientific studies, researchers L. Zhang *et al.* (2025) noted that optimising phosphorus nutrition improved not only yield but also forage quality. H. Ma *et al.* (2024) confirmed that, over a multi-year cycle, the combination of irrigation and phosphorus fertilisation resulted in a more stable level of productivity. Researchers J. Zhao *et al.* (2023) found that the combined application of nitrogen and phosphorus significantly altered the protein fractions and the content of non-structural carbohydrates in alfalfa, and thus directly influenced the quality of the plant material.

A separate group of contemporary studies concerned plant density, sowing rate, and the adaptation of alfalfa to environmental stress conditions. J. Liu *et al.* (2024) demonstrated that on saline soils, the interaction between plant density and nitrogen nutrition altered the growth and quality of the forage. P. Álvarez-Vázquez *et al.* (2020) demonstrated that optimising row spacing and sowing rates allowed yield to be maintained without excessive crop density. A group of researchers led by A. Liatukienė *et al.* (2025)

established that it was the combination of soil and climatic conditions that determined the growth pattern and yield of alfalfa. A synthesis of these studies provided grounds for concluding that, for the conditions of the Right-Bank Forest-Steppe, it is insufficient to assess either the fertiliser rate or the sowing rate in isolation – an integrated determination of their combination is required, one that ensures high green matter yield, sufficient foliage, rational use of moisture, and an energy-efficient technology. The aim of the study was to investigate the influence of sowing rates and various combinations of macro- and microelements on the formation of the alfalfa sown variety 'Radoslava', as well as on the components of green matter yield and the quality indicators of plant raw material during the first two years of vegetation (2024-2025). To achieve this aim, the following objectives were set: 1) to determine the effect of sowing rates and complex mineral fertilisers on the formation of the sward, plant height, leafiness and green matter yield of alfalfa during the first two years of growth; 2) to determine the characteristics of yield distribution across swaths and mowing times in the second year of sward utilisation.

Materials and Methods

Alfalfa (*Medicago sativa* L.) is a perennial legume forage crop of the 'Fabaceae' family that develops a deep root system, is characterised by a high regenerative capacity after mowing, a substantial proportion of leaf mass in the yield, and the ability for symbiotic fixation of atmospheric nitrogen. The combination of these characteristics determined its high forage value and suitability for repeated utilisation throughout the growing season. The study was conducted during 2024-2025 at the experimental field of Vinnytsia National Agrarian University on grey forest soils according to a staged experimental scheme. At the first stage, visual observations of the growth and development of alfalfa plants were carried out throughout the growing season, with recording of the timing of the main developmental stages and the formation of cuts depending on weather conditions. At the second stage, before each cut at the budding and beginning of flowering stages, plant height was measured on 20 typical plants in each treatment. Leafiness was determined by the gravimetric method as the ratio of leaf mass to the total mass of the plant sample. At the third stage, green matter yield was calculated by complete weighing of the accounting plot area and converted into t/ha. Yield calculation was performed using the following formula:

$$Y = \frac{m}{S} \times 10, \quad (1)$$

where Y – yield, t/ha; m – mass of green matter from the sample plot, kg; S – sample plot area, m^2. Leafiness index was determined using the formula:

$$L = \frac{m_1}{m_2} \times 100, \quad (2)$$

where L – leafiness index, %; m_1 – leaf mass, kg; m_2 – total mass of the plant sample, kg. To assess the compliance of the forage mass with the requirements for legume hay, the provisions of DSTU 4674:2006 (2007) were used.

Field germination was determined by counting the number of plants within the accounting area after the emergence of full seedlings, while shoot density was determined by counting the number of shoots per 1 m^2 before mowing. According to the results of the agrochemical analysis of the arable soil layer (0-30 cm), the humus content was 2.69%, alkaline hydrolysable nitrogen – 81 mg/kg, available phosphorus – 218 mg/kg, exchangeable potassium – 72 mg/kg, available sulphur – 5.3 mg/kg, available copper – 0.29 mg/kg, available zinc – 0.45 mg/kg, available manganese – 14.6 mg/kg, and pH of the salt extract – 6.1. The experiment was conducted with three replications, systematic arrangement of treatments, a total plot area of 30 m^2, and yield accounting after excluding border rows. The weather conditions of 2024 and 2025 were characterised by elevated temperatures and uneven distribution of precipitation during the active growing season. Spring regrowth began earlier than the long-term average dates, which accelerated the initial stages of organogenesis; however, during summer this intensified the risk of moisture deficiency and shortened the intervals between the stages of cut formation. The alfalfa variety 'Radoslava' was sown on 8 April 2024 using a HEGE 80 (Hege Maschinenbau) precision seed drill in combination with a MITSUBISHI GX 50 tractor (Mitsubishi Agricultural Machinery) with row spacing of 12.5 cm. The experimental design included two sowing rates – 6.0 and 8.0 million viable seeds/ha – and three mineral nutrition treatments: Background $N_{35}P_{90}K_{90}S_{3.5}$, Background + $K_{30}S_{10.5}Ca_{15}Mg_{10}$ and Background + $K_{60}S_{20.5}Ca_{30}Mg_{20}$. Mineral fertilisers were applied before sowing, full emergence was obtained 10 days after sowing, and mowing dates were determined according to the crop reaching the budding or beginning of flowering stages. The study was conducted in accordance with the requirements of the Convention on Biological Diversity (1992).

Results and Discussion

Observations showed that during the formation and utilisation of the first-cut sward, the most favourable weather conditions were created with an average monthly air temperature in May of 16.3°C and total atmospheric precipitation of 205.4 mm. Under such conditions, the height of alfalfa plants reached 66.3 cm under the high mineral nutrition background (Background + K_{60} $S_{20,5}Ca_{30}Mg_{20}$) and a sowing rate of 6.0 million seeds/ha, with leafiness of 53.53% and field germination of 87%. On average across the three cuts, the treatments demonstrated a decrease in plant height and leafiness with increasing sowing rate (Table 1).

Table 1. *Biometric indicators of alfalfa at the beginning of the flowering stage (first cut, 2024), M ± m*

Fertilisation	Sowing rate, million seeds/ha	Field germination, %	Height, cm		Leafiness, %	
			first cut	average over 3 cuts	first cut	average over 3 cuts
Background $N_{35}P_{90}K_{90} + S_{3.5}$	6.0	84 ± 3.6	64.4 ± 6.60	37.03 ± 5.07	48.24	35.55 ± 12.36
	8.0	80 ± 6.4	63.4 ± 6.16	36.00 ± 4.78	46.62	33.74 ± 21.44
Background + + $K_{30}S_{10}Ca_{15}Mg_{10}$	6.0	84 ± 2.6	65.3 ± 4.89	39.23 ± 4.19	50.45	37.66 ± 11.53
	8.0	82 ± 2.6	64.4 ± 4.44	38.37 ± 3.92	48.05	35.50 ± 10.91
Background + + K_{60} $S_{20.5}Ca_{30}Mg_{20}$	6.0	87 ± 2.6	66.3 ± 5.24	42.93 ± 4.27	53.53	40.77 ± 11.32
	8.0	84 ± 4.2	66.1 ± 3.54	41.67 ± 2.94	51.29	39.04 ± 11.15

Note: M ± m – confidence interval of the arithmetic mean at the 5% significance level
Source: developed by the authors

At a sowing rate of 6.0 million seeds per hectare, the best values for plant height (42.93 cm) and leafiness (40.77%) were obtained when the maximum dose of mineral fertilisers was applied, resulting in an increase in these parameters of 5.9 cm (15.9%) and 5.22%, respectively, compared with the Background + $N_{35}P_{90}$ + $K_{90}S_{3.5}$. The formation of subsequent cuttings took place in summer with an increase in the average monthly air temperature to 24.6°C and above, accompanied by an uneven distribution of precipitation and a reduction in the length of daylight hours in autumn to 12:48 hours. Changes in temperature and moisture supply influenced the growth processes of alfalfa and green matter yield indicators. The average height of alfalfa plants in the year of sowing varied depending on the fertilisation level within the range of 36.00-42.93 cm. The lowest values were recorded in the background treatment (36.00-37.03 cm), whereas with the application of increased doses of mineral fertilisers (Background + $K_{60}S_{20.5}Ca_{30}Mg_{20}$) plant height increased to 41.67-42.93 cm. The increase in plant height compared with the background treatment averaged 4.7-5.9 cm. The sowing rate had only a minor influence on plant height, since the difference between the 6.0 and 8.0 million seeds/ha treatments did not exceed 1.0-1.5 cm. Analysis of the dynamics of plant height formation by cuts showed that the maximum values were formed in the first cut (66.1-66.3 cm), whereas in the second cut they decreased to 23.4-32.2 cm and in the third cut to 22.3-30.3 cm. The reduction in height in the later cuts was explained by the physiological characteristics of the crop and the influence of weather conditions in the year of sowing, particularly the elevated temperature regime and unstable moisture supply. At the same time, the application of increased fertiliser doses contributed to an increase in plant height in the second and third cuts by 6.7-7.6 cm compared with the background treatment. Variations in biometric indicators between the first and third cuts across the experimental treatments resulted in a yield reduction ranging from 5.2 to 25.3%. Despite the difficult hydrothermal conditions, the crop formed a vigorous sward and entered the winter period in satisfactory condition. In the second year of growth (2025), utilisation of the alfalfa sward was planned at the budding stage (BBCH code 49) and the beginning of flowering stage (BBCH code 62). According to the data presented in Table 2, plant height at the budding stage ranged from 52.7 to 55.7 cm at a sowing rate of 6.0 million seeds/ha and from 54.1 to 55.0 cm at a sowing rate of 8.0 million seeds/ha.

***Table 2.** Plant height and leafiness of alfalfa in the second year of growth (2025) depending on fertilisation and the timing of green matter harvesting (average over three cuts), M ± m*

Fertilisation	Sowing rate, million seeds/ha	Height, cm		Leafiness, %	
		BBCH code 49	BBCH code 62	BBCH code 49	BBCH code 62
Background $N_{35}P_{90}+K_{90}S_{3.5}$	6.0	52.7 ± 7.36	59.7 ± 5.22	56.1 ± 10.66	51.5 ± 11.30
	8.0	54.1 ± 5.87	61.2 ± 4.95	54.3 ± 8.66	51.4 ± 15.30
Background + $+K_{30}S_{10}Ca_{15}Mg_{10}$	6.0	54.4 ± 5.48	61.8 ± 4.72	55.4 ± 2.47	54.0 ± 16.60
	8.0	54.3 ± 5.95	61.4 ± 5.26	52.3 ± 3.75	54.6 ± 14.35
Background + $+K_{60}S_{20.5}Ca_{30}Mg_{20}$	6.0	55.7 ± 4.93	62.7 ± 4.87	51.9 ± 6.21	53.6 ± 18.17
	8.0	55.0 ± 4.06	62.7 ± 5.18	53.2 ± 7.79	56.9 ± 12.41
Average	6.0	54.3 ± 5.92	61.4 ± 4.94	54.5 ± 5.43	53.0 ± 5.18
	8.0	54.5 ± 5.29	61.8 ± 5.13	53.3 ± 5.21	54.3 ± 5.17

Note: M ± m – confidence interval of the arithmetic mean at the 5% significance level
Source: developed by the authors

At the beginning of the flowering stage, plant height increased to 59.7-62.7 cm and reached its maximum in the treatments with enhanced mineral nutrition (Background + $K_{60}S_{20.5}Ca_{30}Mg_{20}$), where it attained 62.7 cm regardless of sowing rate. On average across the experimental treatments, plant height was 54.3-54.5 cm at the budding stage and 61.4-61.8 cm at the beginning of flowering, indicating a stable tendency towards increased growth during the transition to the generative phase of development. Plant leafiness at the budding stage ranged from 51.9 to 56.1%, while at the beginning of flowering it ranged from 51.4 to 56.9%. On average, this indicator amounted to 54.5-53.3% (BBCH code 49) and 53.0-54.3% (BBCH code 62), indicating a slight reduction in leafiness during the transition to the flowering stage. The highest leafiness (56.9%) was recorded at a sowing rate of 8.0 million seeds/ha under the maximum fertilisation treatment (Background + $K_{60}S_{20.5}Ca_{30}Mg_{20}$), which was accompanied by the formation of tall plants (62.7 cm). In the year of sowing, the total seasonal green matter yield reached 33.13 t/ha at a sowing rate of 6.0 million viable alfalfa seeds/ha under mineral fertilisation at the dose of Background + $K_{60}S_{20.5}Ca_{30}Mg_{20}$, which was 29.7% higher than the background. It is worth noting that, owing to the uniform stem density and plant height within the established sward, green matter yield maintained a tendency to increase according to the studied factors (Table 3).

Table 3. *Green matter yield of alfalfa at the beginning of flowering by cuts, t/ha, 2024*

Fertilisation (factor A)	Sowing rate, million/ha (factor B)	Cuts			Total
		1st	2nd	3rd	
Background $N_{35}P_{90}+K_{90}S_{3.5}$	6.0	8.80	8.76	8.34	25.90
	8.0	10.94	8.70	8.17	27.81
Background + $K_{30}S_{10.5}Ca_{15}Mg_{10}$	6.0	10.86	10.55	9.87	31.28
	8.0	11.44	9.88	9.63	30.95
Background + $K_{60}S_{20.5}Ca_{30}Mg_{20}$	6.0	11.58	11.21	10.34	33.13
	8.0	11.78	10.70	10.07	32.55
$LSD_{0.05}$	*A*	0.69	0.63	0.59	1.90
	B	0.56	0.51	0.48	1.55
	AB	0.97	0.89	0.84	2.69

Note: A – factor A (fertilisation), B – factor B (sowing rate), AB – interaction of factors A and B
Source: developed by the authors

The data obtained led to the conclusion that, regardless of changes in hydrothermal conditions throughout the growing season, during the formation of the sward, the studied variety of alfalfa 'Radoslava' was adapted to stressful environmental conditions and yielded three cuts by the time the crop reached the early flowering stage. In the second year of growth, the hydrothermal conditions during the growing season enabled the plants to reach the early flowering stage throughout the season and produce three full cuts, starting with the first cut in the first ten days of June and the third in the first ten days of September. The highest green matter yield was observed with maximum fertilisation, amounting to 83.8 t/ha with a sowing rate of 8.0 million seeds/ha and a shoot density of 2,096 shoots/m². In total, across the three cuts, the gross green matter yield varied between treatments, with an average of 45.5 t/ha over the years, due to tillering and plant growth against a background of sufficient nutrients and moisture. The increase attributable to sowing rates was 2.1 t/ha. Increasing the application rates of mineral fertilisers ensured an increase in green matter yield compared to the background treatment. In particular, when applying additional $K_{30}S_{10.5}Ca_{15}Mg_{10}$ the average yield increased by 10.1%, and with the application of $K_{60}S_{20.5}Ca_{30}Mg_{20}$ – by 18.8% compared with the background (48.4 t/ha), amounting to 53.3 and 57.5 t/ha respectively (Table 4).

Table 4. *Green matter yield of alfalfa at the beginning of flowering over two years (2024-2025) of vegetation, t/ha*

Fertilisation (factor A)	Sowing rate, million/ha (factor B)	Years			Average by	
		2024	2025	Mean	Factor B	Factor A
Background $N_{35}P_{90}+K_{90}S_{3.5}$	6.0	25.9	67.3	46.6	52.0	48.4
	8.0	27.8	72.4	50.1	54.1	
Background + $K_{30}S_{10.5}Ca_{15}Mg_{10}$	6.0	31.3	73.8	52.6		53.3
	8.0	31.0	77.0	54.0		
Background + $K_{60}S_{20.5}Ca_{30}Mg_{20}$	6.0	33.1	80.4	56.8		57.5
	8.0	32.6	83.8	58.2		
Average over the years		30.3	75.8	+45.5	2.1	
$LSD_{0.05}$	*A*	1.90	2.11			
	B	1.55	1.34			
	AB	2.69	2.86			

Source: developed by the authors

The method of sowing alfalfa with a row spacing of 12.5 cm, combined with the provision of nutrients to the plants, created favourable conditions for the formation of a dense sward across the area. Under these conditions, the alfalfa, with its upright bush type, plant height of 44-86 cm (1st-3rd cuts) and leafiness of 53.0-54.5%, ensured consistent green matter yield figures during the formation of the second and third cuts. The data obtained indicated the adaptability of this variety to changing weather conditions during the growing season and its ability to maintain consistent plant height and leafiness within standard sowing rates and mineral fertiliser application rates. With a leaf content of over 50%, the forage mass obtained during the budding to early flowering stages met the requirements for high-quality legume hay as per DSTU 4674:2006 (2007).

It is advisable to consider the research results not in isolation, but within the broader context of contemporary research into resource-saving alfalfa cultivation technologies. In contrast to the introduction, where the main focus was on the role of mineral nutrition, the Discussion section analysed studies that investigated the combined effects of water regime, fertilisation levels, harvest timing, varietal characteristics and environmental constraints. This approach made it possible to better explain why, specifically under the conditions of the Right-Bank Forest-Steppe, a moderate sowing rate of 6.0 million seeds/ha combined with more intensive mineral nutrition was preferred, and why the greatest effect was observed not so much in the formal increase in crop density as in the stabilisation of the yield structure, foliation and green matter yield. L. Liu *et al.* (2025) found that a moderately managed water deficit in alfalfa crops can reduce water consumption without significantly compromising forage quality and may even enhance the economic return of the technology. The data obtained were consistent with these findings: under conditions of an unstable hydrothermal regime, the advantage lay not with maximum plant density, but with the option that best balanced the plants' water requirements with the actual resource capacity of the agrocenosis. This is precisely why a sowing rate of 6.0 million seeds/ha proved more productive in terms of overall economic efficiency, as it did not intensify competition for water but allowed the benefits of increased mineral nutrition to be maximised. The results were further supported by studies on water management systems for alfalfa crops. Z. Jia *et al.* (2024) demonstrated that optimising the drip irrigation regime in semi-arid conditions improved the crop's seed yield through better regulation of water supply during critical growth stages. U. Cholula *et al.* (2026) demonstrated that under water-deficient irrigation, not only yield but also the nutritional value of various alfalfa varieties changes significantly. Although the experiment in this study was conducted without artificial irrigation, the general pattern was consistent: under limited water availability, the most effective approach was one that did not cause excessive grass stand density but created conditions for stable leaf mass formation. This explained why the advantage of maximum fertilisation was most clearly evident precisely in combination with a lower sowing rate.

S. Tlahig *et al.* (2024) established that even under similar yield levels, varieties and growing seasons differed significantly in forage quality indicators, which were critical for adapting production to climate change. In the present study, the variety 'Radoslava' demonstrated relatively stable indicators of leafiness, while a leaf proportion exceeding 50% at the budding and beginning of flowering stages indicated the preservation of high forage value. Importantly, increasing the sowing rate did not provide an advantage in terms of quality; instead, it intensified competition among plants. This once again confirmed that, under practical production conditions, not every increase in crop density is technologically justified. When interpreting mowing dates, it is important to consider the phytosanitary aspect as well. J.S. Herreid *et al.* (2024) demonstrated that the timing of alfalfa harvest in commercial crops significantly influenced the population dynamics of the alfalfa weevil. Although this study did not set out to conduct a specific phytosanitary assessment, the very fact of a clear difference between the yield of the cuts and the timing of developmental stages indicated that the management of the grass stand has broader implications than simply a change in forage yield. The first cut, which accounted for more than half of the annual yield, proved critical not only for gross yield but also for the subsequent stability of the agro-phytocenosis (Bélanger *et al.*, 2020). A study by X. Li *et al.* (2023) found that sustained irrigation and the application of phosphorus fertilisers over a six-year period significantly increased alfalfa yield and the stability of green matter formation. The authors also noted that optimal phosphorus nutrition promoted better root system development and more efficient water use by plants. R.M. Sulc *et al.* (2021) investigated changes in the nutritional value of low-lignin alfalfa during regrowth after mowing. The researchers demonstrated that reducing lignin content improved feed digestibility and maintained high-quality green matter even at later stages of plant development.

Thus, optimising mowing times should be considered as part of integrated management of crop productivity, quality and sustainability. Z. Ben & H. Yang (2025) noted that alfalfa's response to fertilisation varied significantly depending on the combination of agrochemical and weather factors; consequently, universal recommendations not tailored to the specific site had limited practical value. The maximum dose of compound fertiliser yielded the best results not on its own, but in a specific combination with a moderate sowing rate, grey forest soils and unstable moisture supply. It was precisely this combination of conditions that allowed the Background + $K_{60}S_{20}Ca_{30}Mg_{20}$ treatment to demonstrate its superiority in terms of plant height, leafiness, gross green matter yield and energy efficiency. A.G. Desta (2026) summarised data on the role of alfalfa in improving soil condition and reducing greenhouse gas emissions in integrated crop-livestock systems. This approach was important for interpreting the results of this study, as the choice of cultivation technology must take into account not only the current year's profit but also the long-term stability of the agroecosystem. The advantage identified in the variant with a sowing rate of 6.0 million seeds/ha and increased complex fertilisation can be assessed as more rational not only from an economic but also from an agroecological perspective: it ensured high productivity without an excessive increase in plant density and the associated risks of resource depletion. In particular, it is important to note the crop's adaptability to changing weather conditions and the ability to produce a consistent green matter yield, which was adjusted by the timing of grass cutting and the level of mineral nutrition. Thus, the results of the study confirmed that for alfalfa in the conditions of the Right-Bank Forest-Steppe, the effectiveness of the technology was determined not by the maximum intensification of a single element, but by a balanced combination of sowing rate, mineral nutrition, the timing of grass stand utilisation, and a resource-saving yield formation regime. It was precisely this logic that explained why, in the experiment, the combination of 6.0 million/ha of germinating seeds with the Background + $K_{60}S_{20}Ca_{30}Mg_{20}$ treatment proved to be the best: it ensured a high gross yield of green matter, sufficient foliage, better economic indicators and technological stability under variable hydrothermal conditions. Thus, the data obtained were not only of local practical significance but also complemented current understanding of adaptive yield management in perennial leguminous grasses.

Conclusions

The alfalfa variety 'Radoslava' under the conditions of grey forest soils of the Right-Bank Forest-Steppe demonstrated stable productivity and adaptability to fluctuations in the hydrothermal regime, providing three full cuts during the growing season both in the year of sowing and in the second year of use. In the year of sowing (2024), the optimal combination was a sowing rate of 6.0 million/ha with the fertiliser dose Background + $K_{60}S_{20}Ca_{30}Mg_{20}$, which ensured the highest total green matter yield of 33.13 t/ha, exceeding the background treatment by 27.9% ($LSD_{0.05}$ for factor A = 1.90 t/ha). At the same time, the average plant height over three cuts was 42.93 cm, while leafiness reached 40.77%, representing the highest values among all studied fertilisation treatments. In the second year of vegetation (2025), mowing at the beginning of the flowering stage (BBCH code 62) provided a total yield of 75.8 t/ha, which was 2.5 times higher than in the year of sowing (30.3 t/ha). The average plant height was 61.4-61.8 cm, and leafiness ranged from 53.0 to 54.3%. In the total green matter yield during the vegetation period of alfalfa, the first cut accounted for 54.4-56.2%. Increasing the sowing rate from 6.0 to 8.0 million/ha had no significant effect on green matter yield (the difference remained within the $LSD_{0.05}$ limits), although it was accompanied by a reduction in plant height and leafiness. The decrease in leaf mass under the higher sowing rate was considered undesirable in terms of the qualitative characteristics of the plant material. Taking this into account, the sowing rate of 6.0 million viable seeds/ha proved to be the more rational choice for the conditions of the Right-Bank Forest-Steppe. Plant material harvested at the budding to early flowering stages was characterised by a leaf content exceeding 50%, corresponding to the high forage value of legume hay. Prospects for further research include studying the chemical composition and nutritional value of green matter depending on the investigated factors, assessing the impact of agronomic measures on soil fertility and sward structure, as well as determining the longevity of the use of the alfalfa variety 'Radoslava' during the third and subsequent years of vegetation under conditions of an unstable hydrothermal regime.

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Формування та використання травостою люцерни посівної за ґрунтово-кліматичних умов Лісостепу Правобережного

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Анотація. Метою дослідження було визначити оптимальне поєднання норми висіву та комплексних мінеральних добрив для формування продуктивного травостою люцерни посівної (*Medicago sativa* L.) сорту ‘Радослава’ в ґрунтово-кліматичних умовах Лісостепу Правобережного. Дослід було проведено на сірих лісових ґрунтах у 2024-2025 роках за трифакторною схемою: норми висіву 6,0 і 8,0 млн/га схожих насінин, двох строків збирання та трьох рівнів мінерального живлення. Встановлено, що у рік сівби (2024 рік) культура досягла фази початку цвітіння (код 61-62 BBCH) через 69-70 діб за тривалості світлової доби 14:53-16:12 години та забезпечила три укоси із валовим збором зеленої маси до 33,13 т/га. Найбільша висота рослин у першому укосі становила 66,1-66,3 см при нормі висіву 6,0 млн/га і максимальному удобренні (Фон + $K_{60}S_{20}Ca_{30}Mg_{20}$), тоді як у третьому укосі – знизилась до 21,2-30,3 см внаслідок скорочення тривалості світлової доби та підвищення температури. В середньому за варіантами висота рослин коливалась від 36,00 до 42,93 см, облиствленість – від 33,74 до 40,77 %. На другий рік (2025 рік) за трьома укосами у фазі початку цвітіння (код 64 BBCH) середня висота рослин становила 61,4-61,8 см, облиствленість – 53,0-54,3 % з урожайністю зеленої маси 75,8 т/га. Статистична значущість впливу факторів підтверджена $НІР_{05}$. Встановлено, що оптимальним поєднанням стала норма висіву 6,0 млн/га із підвищеним удобренням (Фон + $K_{60}S_{20}Ca_{30}Mg_{20}$), яке забезпечувало найбільший валовий збір зеленої маси за мінімальних ризиків дефіциту вологи в умовах нестійкого гідротермічного режиму Лісостепу Правобережного

Ключові слова: норма висіву; мінеральні добрива; висота рослин; облиствленість; урожайність зеленої маси; строк скошування

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Dendrochronological assessment of common ash stands in areas of intensive dieback

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Abstract. The widespread dieback of the common ash (*Fraxinus excelsior* L.) is one of the most pressing issues, caused by both global climate change and the spread of dangerous infectious diseases. Analysis of how the combination of hydrothermal stress and various types of pathogens affects the viability of stands is critical for developing strategies to conserve forest ecosystems. The study aimed to conduct a comprehensive dendrochronological assessment of the condition of ash stands and to analyse the dynamics of tree radial growth under conditions of varying degrees of pathogen infestation and the influence of climatic factors. The study was based on the collection of growth cores using age-specific borers from trees in various condition categories: healthy (control), affected by sulphur-yellow tinder fungus, canker tumours, and in areas of intensive dieback. Analysis of annual ring width was conducted by scanning the samples and subsequently processing them in the specialised software ImageJ with the ObjectJ plugin. Climatic analysis was conducted using archived temperature and precipitation data for the period from 1979 to 2025. A steady trend towards climate warming (an increase in the mean annual temperature of 2.5-3.0°C) and moisture deficit was identified, creating conditions of chronic

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hydrothermal stress for ash trees. The study established that trees in areas of intense dieback show a sharp reduction in radial growth, which is no longer linked to the climatic trend due to pathological weakening. Infection by tinder fungi and canker tumours leads to a gradual decline in growth or high variability in growth rates; however, it has a less critical impact on viability over short time periods compared with halar necrosis. The obtained data can be used to apply radial growth indicators as an accurate indicator of stand condition for the early diagnosis of forest degradation. The results can be utilised by forestry enterprises to optimise sanitary measures and predict the resilience of ash forests under changing environmental conditions

Keywords: radial growth; climate change; forest pathogens; halar necrosis; annual rings; stand vitality; hydrothermal stress

Introduction

Forest dieback and degradation are complex processes driven by numerous factors, including the long-term weakening of forest stands, adverse weather conditions, and subsequent infestation by pests and pathogens. Climate change, prolonged droughts and unstable groundwater levels are central. As a result, trees become less resilient, and stress factors contribute to their decline, which is further accelerated by the activity of harmful organisms (Tkach & Kobets, 2025).

Trees of the ash genus (*Fraxinus* sp.) have significant ecological and economic potential; they are typical mesotrophs and occur as subdominants in forest ecosystems. K. Davydenko *et al.* (2022) established that climate change caused the issue of ash dieback, the main causes of which are climatic factors, halar necrosis caused by the invasive pathogen *Hymenoscyphus fraxineus* L., and the emerald ash borer *Agrilus planipennis Fairmaire* L. (Emerald ash borer (EAB)), which in the future may cause the complete disappearance of ash trees from forest ecosystems. In the publication by N. Puzrina *et al.* (2025), the authors investigated the distribution and extent of damage to ash trees caused by the emerald ash borer. They monitored trees of the genus *Fraxinus* in 2021-2023 and concluded that *Agrilus planipennis* poses a substantial threat to ash trees in the study area, and that further research is needed to develop control measures for this invasive pest. S. Klesse *et al.* (2021) established a negative correlation between growth energy and the susceptibility of *Fraxinus excelsior* to *Hymenoscyphus fraxineus*: trees with narrower annual rings and smaller sapwood vessel diameters exhibit more severe crown dieback symptoms and higher mortality. The authors demonstrated that defoliation directly reduces the growth and maximum diameter of earlywood vessels, creating a positive feedback loop that accelerates tree decline.

Tree growth in a stand is determined both by external factors (microclimatic, soil-hydrological, etc.) and by the physiological characteristics of the tree species. Tree diameter growth depends on the genotype's response to precipitation and heat levels and their distribution throughout the year (Netsvetov *et al.*, 2021). Weather conditions are substantial in the deterioration of trees' physiological condition, as they determine the level of stress experienced by stands during the growing season. Prolonged droughts, rising average annual temperatures, sharp fluctuations in weather conditions, and soil moisture deficits lead to a disruption of the plants' water balance, a reduction in photosynthetic activity, and the suppression of growth processes. As a result, tree vitality declines, creating conditions for subsequent infection by pathogens. Weather events are particularly dangerous – summer droughts, late spring and early autumn frosts, as well as abnormally warm winters. Under such conditions, trees lose their natural resistance to biotic factors, in particular to xylophagous insects and pathogens of infectious diseases. Weakened trees are more heavily infested by pests, notably ash bark beetles, and are also affected by disease-causing agents, particularly those responsible for halar necrosis (Shvidenko *et al.*, 2018).

In the field of forest ecology research, tree ring growth is widely used as a comprehensive indicator that can provide a relatively rapid and objective assessment of the condition of forest stands in both spatial and temporal terms, as well as determine the extent of damage to tree stands caused by natural or anthropogenic stress factors.

I.M. Koval (2021) investigated the dendrochronological basis for assessing pine and oak stands in Ukraine, in which the author analysed the response of tree rings to climate change and anthropogenic stressors in various natural zones of the country. The study showed that the sensitivity of radial growth to climatic factors and air pollution varies depending on tree species and growing location, which is substantial for assessment of the condition of forest ecosystems and predicting their response to environmental changes. O. Lesnik *et al.* (2022) assessed the growth and physiological resilience of pine forests in the Ukrainian Polissya. Their study demonstrated that various environmental factors and anthropogenic influences shape trees' adaptive mechanisms and affect forest productivity. V. Myroniuk *et al.* (2026) proposed a method for estimating the current annual increment of stands based on single measurements of trees on temporary sample plots. The results of their study were used for the rapid determination of growth volume and the spatio-temporal characteristics of forest stands.

Despite the considerable body of research devoted to assessing tree radial growth and the resilience of forest stands to pathogens and climatic stresses, a number of unresolved issues remain. In particular, the complex interaction of chronic hydrothermal stress and various types of pathological damage on the long-term growth and viability of common ash in natural stands has not been sufficiently studied.

The study aims to identify patterns in changes to radial growth under the combined influence of chronic hydrothermal stress and pathogenic factors, to assess their impact on tree vitality, and to identify indicators of early stand degradation.

Materials and Methods

The study was conducted on an area of 4.5 hectares in a stand with a stocking level of 0.5, aged 120 years, comprising 7As2O1Hb (As – common ash, O – common oak, Hb – common hornbeam) of natural origin. The average stand height was 31 m, the average diameter 44 cm, site class I, forest type D_2HO (D_2 – fresh fertile site, HO – hornbeam-oak forest), and volume 280 $m^3 \cdot ha^{-1}$ (Fig. 1).

AshF AshC AshDB AshK

Figure 1. *Categories of tree condition from which cores were taken*

Note: AshF – trees bearing tinder fungus fruiting bodies, AshC – trees with cancerous growths, AshDB – trees in areas of severe dieback, AshK – trees showing no signs of drying out (control group)

Source: photo by the authors

The dieback of trees in this stand began in 1997, as evidenced by data from the Monitoring forest pathologies (n.d.) resource, which notes the sanitary felling operations and the reasons for the dieback. To determine the current growth of forest stands in this study, a method was used to estimate the percentage of growth based on the results of measuring the width of annual rings on increment cores This method was used to directly assess the intensity of radial growth in the trees of the stands under study, enabling the characterisation of their current growth without the use of growth tables (Myronuk *et al.*, 2019; Lesnik *et al.*, 2022). The advantage of this approach over the use of growth tables is the direct study of tree growth in the study area, as well as the relative simplicity of collecting field data. In Ukraine, this approach was scientifically substantiated by K.E. Nikitin in the 1960s and is the primary among the approximate methods for estimating growth, which does not require the felling of sample trees.

The spatial framework for tree selection was designed based on the characteristics of damage to common ash trees caused by various pathogens, namely infection by the sulphur-yellow polypore *Laetiporus sulphureus* (AshF), trees showing signs of canker (AshC), and trees in areas of severe dieback (AshDB). Core samples were also taken from healthy ash trees showing no signs of damage (AshK). Annual growth cores were collected from sample trees of common ash; four sample plots were established for core sampling. At each plot, measurements of the diameters and heights of 3-4 inventory trees were taken, from which increment cores were collected at a trunk height of 1.3 m using Haglöf (Sweden) age-determining augers (Fig. 2). To measure tree diameters, two mutually perpendicular measurements were taken using Haglöf aluminium measuring callipers. Tree heights were measured using a Halgöf Electronic Clinometer (HEC, Sweden).

Figure 2. *Selected growth cores from monitored common ash trees with various types of damage*

Note: AshF – trees bearing tinder fungus fruiting bodies, AshC – trees with cancerous growths, AshDB – trees in areas of severe dieback, AshK – trees showing no signs of drying out (control group)

Source: photo by the authors

The annual growth cores from the sample trees were placed in wooden holders, and the collected data were processed in the office. The data on the diameter and height of the sample trees in the test plots were used to calculate the average values of the stand's inventory parameters from which the cores were taken (average diameter, average height, and evenness).

The selected growth cores were first sanded using fine-grit sandpaper and then scanned in *.jpg format at a resolution of 3200 dpi (Fig. 3). The images obtained were processed using the ImageJ software with the ObjectJ plug-in installed, which is designed for dendrochronological research. Growth cores were analysed, and annual growth rings were marked. Based on this, data on the annual dynamics of radial growth in the sample trees were obtained. A fragment of the cores processed in the ObjectJ programme is shown in Figure 4.

AshK 

Figure 3. *A scanned sample with growth rings mounted on a wooden strip*

Source: photo by the authors

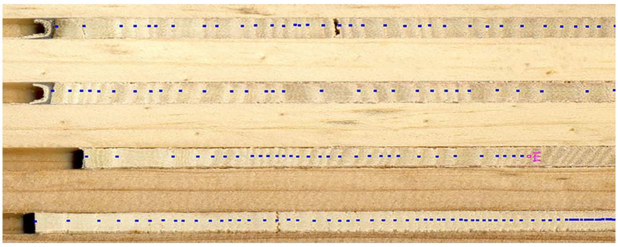
AshDB 

Figure 4. *A screenshot showing the processing of a sample with embedded cores in the ObjectJ programme*

Source: photo by the authors

Archival data on weather conditions in the study region for the period 1979-2025 were analysed using data from Meteoblue (n.d.). The desk-based work involved processing the collected data and analysing the results obtained. Experimental plant studies complied with national and international guidelines and adhered to the standards of the Convention on Biological Diversity (1992).

Results and Discussion

Changes in climatic conditions contribute to the expansion of the ranges of harmful organisms and an increase in the number of their generations during the growing season, which significantly exacerbates their harmful effects. Combined with anthropogenic pressure, this causes a disruption of the ecological balance of forest ecosystems, a decline in stand productivity and their gradual degradation; therefore, an analysis of changes in weather conditions over the study period was first conducted. Archival weather data for the study region from 1979 to 2025 demonstrate a marked warming of the climate (Fig. 5). The average annual air temperature has risen from approximately 7.5-8°C in the late 1970s and early 1980s to 10.5-11.5°C in 2015-2025; the overall increase is approximately +2.5-3.0°C. The trend line corresponds to an increase of approximately 0.004-0.05°C per year (or about 0.4-0.5°C per decade). Until 1995, negative temperature anomalies prevailed (up to -1+1.5°C below normal), whereas since 2000, positive deviations have dominated, reaching +1-+2°C in 2015-2025. Such quantitative changes indicate a significant warming of the region's climate, accompanied by increased evaporation and a moisture deficit (Fig. 6).

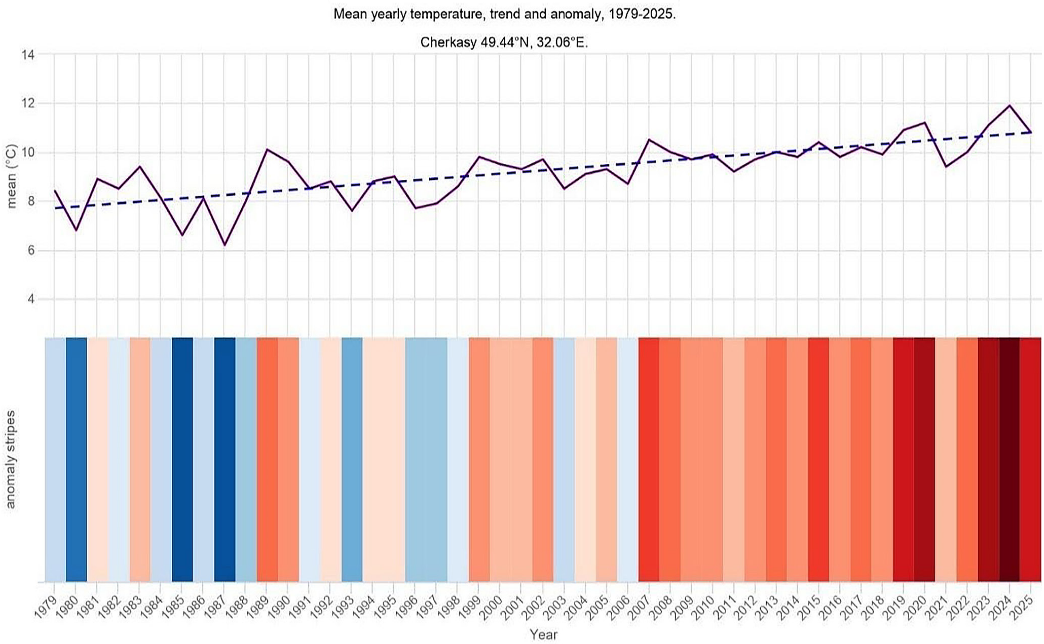


Figure 5. *Trends in the average annual temperature, 1979-2025*

Source: compiled by the authors based on Meteoblue (n.d.)

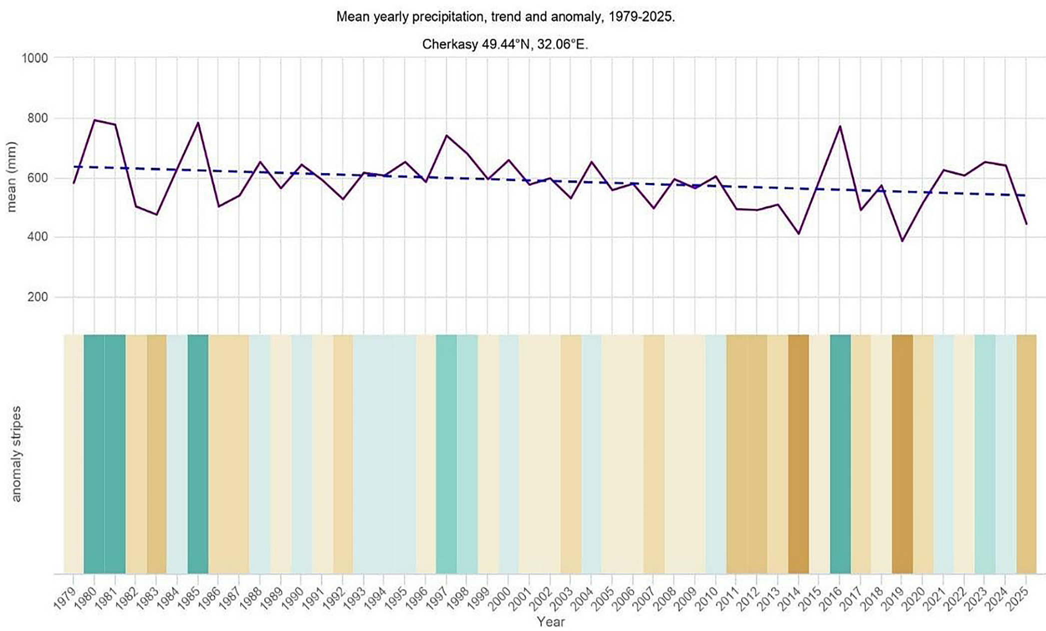


Figure 6. *Trends in average annual precipitation, 1979-2025*

Source: compiled by the authors based on Meteoblue (n.d.)

An analysis of annual precipitation in the region for the period 1979-2025 shows that, in the context of significant interannual variability, a slight but consistent trend towards decreasing moisture levels is emerging. Whilst in the 1980s the average annual precipitation was predominantly 600-700 mm, with peaks of up to 750-800 mm, between 2000 and 2025 these figures fell to 550-600 mm, and in some particularly dry years to 400-450 mm. The overall decrease over the period is around 80-100 mm, which corresponds to approximately 15-20 mm per decade. Combined with an increase in the average annual temperature of +2.5-3°C, this leads to a sharp rise in evaporation and the development of a moisture deficit. Analysis of monthly temperature and precipitation anomalies indicates a change in weather patterns (Fig. 7).

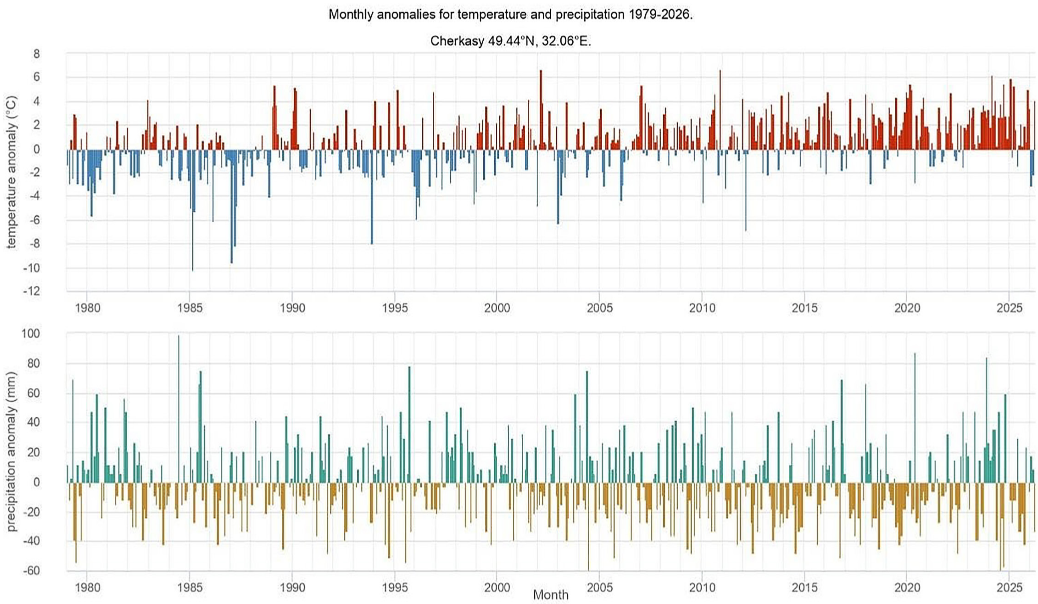


***Figure 7.** Trends in temperature and precipitation anomalies*

Source: compiled by the authors based on Meteoblue (n.d.)

The data show that temperatures have risen significantly: until the mid-1990s, colder-than-average temperatures predominated, whereas since 2000 warmer-than-average temperatures have been the norm, with peaks of +4 to +6°C and above, and severe cold spells have become rare. Precipitation remains variable, but since the 2000s, moisture deficits (30-60 mm) have been observed more frequently. A correlation is observed: rising temperatures are often accompanied by a lack of precipitation, creating arid conditions and exacerbating the hydrothermal imbalance. As a result, chronic hydrothermal stress develops, which negatively affects ring width, leading to narrower annual rings, and increases the trees' susceptibility to pathogens, a key factor in dieback.

An accurate assessment of forest stand growth is a key foundation for sustainable forest management. It can be used to analyse the productivity of forest stands, identify patterns in their growth, determine how trees develop under the influence of various factors (biotic and abiotic), and optimise the use of forest resources. Stand growth can be estimated using models of forest stand growth and productivity (growth tables);

however, such estimates require field verification and comparison with actual data.

Following the processing and analysis of data from increment cores, graphs of radial growth in millimetres were produced for each year in plots with various types of damage and in the control plot. The radial growth of common ash in plots showing no signs of damage demonstrates the typical growth pattern of ash under these forest conditions (Fig. 8).

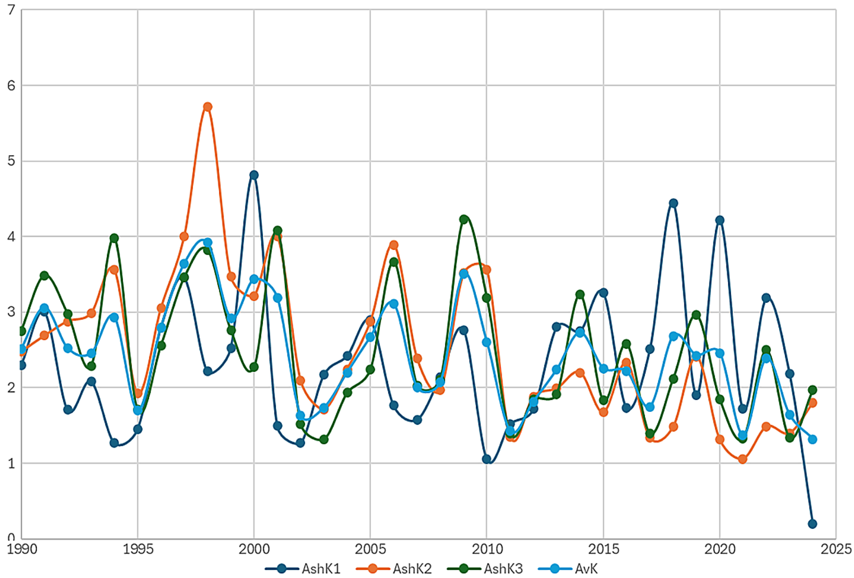


Figure 8. *Radial growth of common ash without signs of damage (control)*
Note: AshK1-AshK3 – measurement results for model trees; AvK – average
Source: compiled by the authors

The graph shows that the model trees exhibit interannual variability in growth, with the highest values occurring in the late 1990s and early 2000s. The average growth rate (AvK) varied mainly within the range of 1.5-3.5 mm and showed relatively stable trends. Since 2010, there has been a slight decline in average growth rates and an increase in variation between individual trees.

The fluctuations in growth on the graph reflect the influence of climatic factors (temperature, precipitation). The synchronisation of the growth curves of different trees (AshK1-AshK3) indicates that the main limiting factor is the climate, rather than pathological factors. The sulphur-yellow polypore *Laetiporus sulphureus* causes heart rot. As the fungus destroys the central part of the trunk (the heart), the conductive function of the sapwood (the outer layers of wood) is often preserved for a long time (Fig. 9). Figure 9 shows a relatively stable growth rate over a long period of the disease. A sharp decline in growth on such graphs usually occurs only in the later stages, when the tree loses its mechanical stability, or the rot affects the sapwood, disrupting the water supply to the crown. At the same time, some model trees are characterised by significant interannual fluctuations in radial growth, which may be linked to the trees' individual response to the development of the pathological process and the influence of climatic conditions. The highest growth rates were observed in 2020-2024, particularly in tree AshF2, where growth exceeded 7-8 mm. The average growth rate (AvF) remained relatively stable throughout the study period, with a tendency towards gradual increase, indicating that growth processes were maintained even in the presence of infection by the sulphur-yellow bracket fungus.

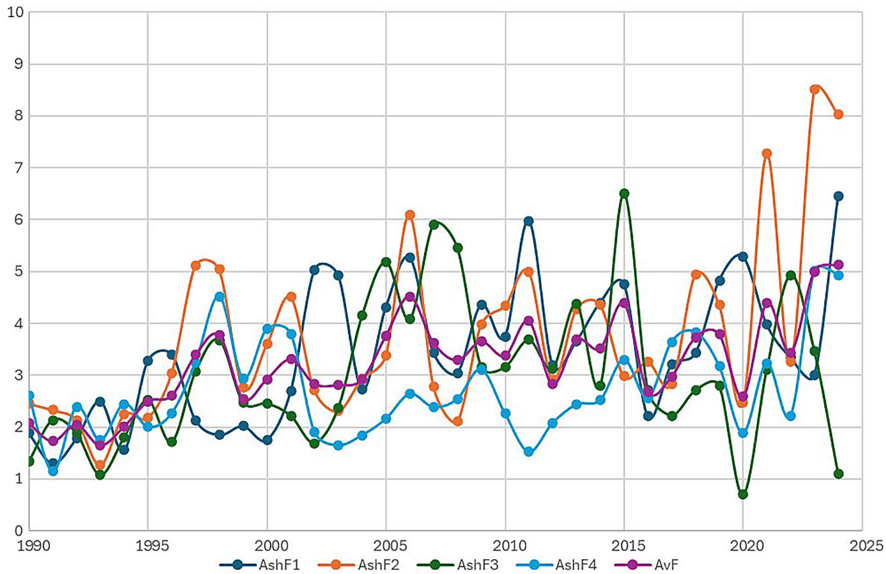


***Figure 9.** Radial growth of common ash affected by sulphur-yellow tinder fungus*
Note: AshF1-AshF4 – measurement results for model trees; AvF – average
Source: compiled by the authors

The pathogens that cause tumours to form on the trunk directly affect the bark and cambium; consequently, the growth rates shown on the graph may exhibit high variability. If the cancerous tumour does not completely encircle the trunk, the tree may compensate for the loss by increased growth on the other side of the trunk (eccentric growth) (Fig. 10).

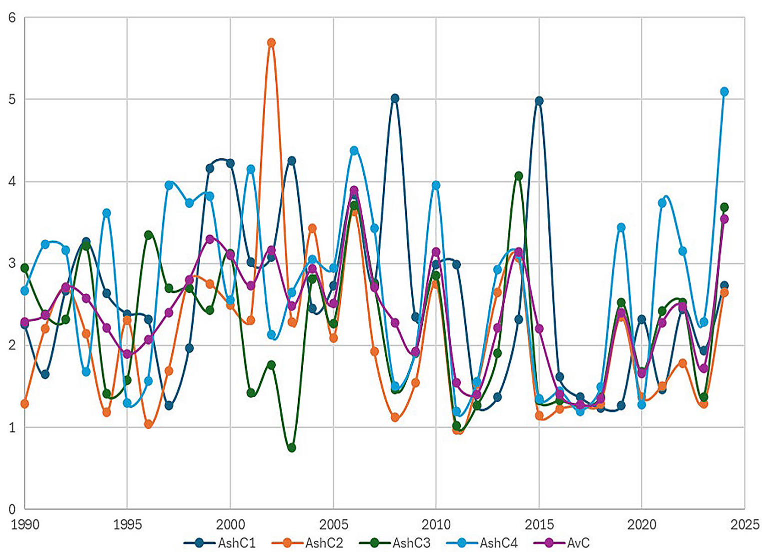


***Figure 10.** Radial growth of common ash with the presence of cancerous tumours*
Note: AshC1-AshC4 – measurement results for model trees; AvC – average
Source: compiled by the authors

A gradual, undulating decline in growth is typically observed, as the tree's vascular system is affected more slowly than in cases of vascular mycoses, but more rapidly than in cases of heart rot. The graph shows significant year-to-year variability in the radial growth of the model trees, with periodic increases and decreases in the figures. Average growth (AvC) during the study period varied mainly within the range of 1.5-3.5 mm, indicating that growth processes were partially maintained even in the presence of cancerous tumours. Since 2010, there has been a trend towards a decrease in average growth values and an increase in variation between individual trees.

The radial growth of common ash in areas affected by severe dieback, particularly ash dieback (caused by *Hymenoscyphus fraxineus*), has shown a sharp and irreversible decline in annual ring width over the last 10-20 years (Fig. 11).

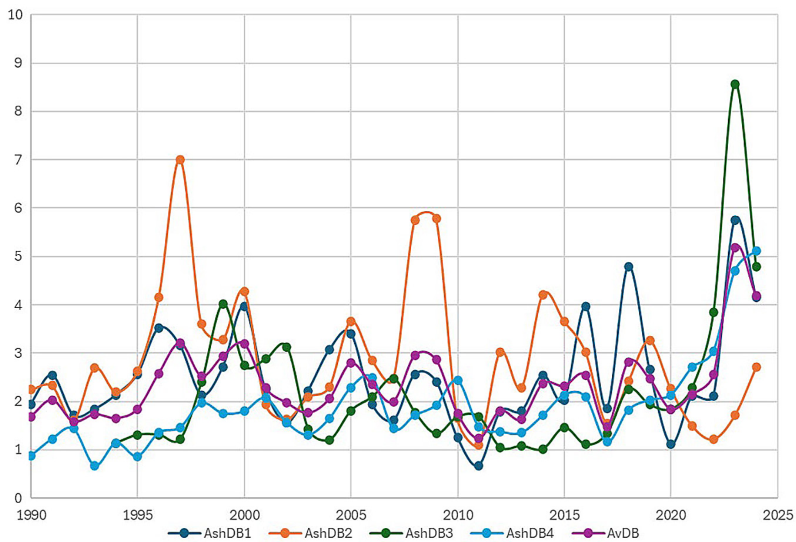


***Figure 11.** Radial growth of common ash in areas of severe dieback*

Note: AshDB1-AshDB4 – measurement results for model trees; AvDB – average

Source: compiled by the authors

At a certain point (the onset of infection), the growth curves "break away" from the climatic trend (as seen in the control) and drop sharply, regardless of whether the year was favourable in terms of precipitation. This is a clear indicator of pathological weakening leading to the death of the tree. The average values of radial growth for common ash, depending on the type of damage, are shown in Figure 12. The data in Figure 12 show that, in both control trees and those with canker tumours, negative growth trends have been observed since the stands were first affected, whereas trees with tinder fungus fruiting bodies and in areas of intense dieback show an increase in radial growth, confirming the conclusion that the conductive function of the sapwood is often preserved for a long time after infection. The most pronounced decline in growth is characteristic of trees with canker tumours, where a sharp suppression of growth processes has been observed since 2015. At the same time, trees with polypore fruiting bodies demonstrate the highest growth rates, particularly in 2023-2024. Trees in areas of intensive infection are characterised by significant interannual fluctuations in growth, which may be linked to the trees' stress response and reduced competition in damaged stands.

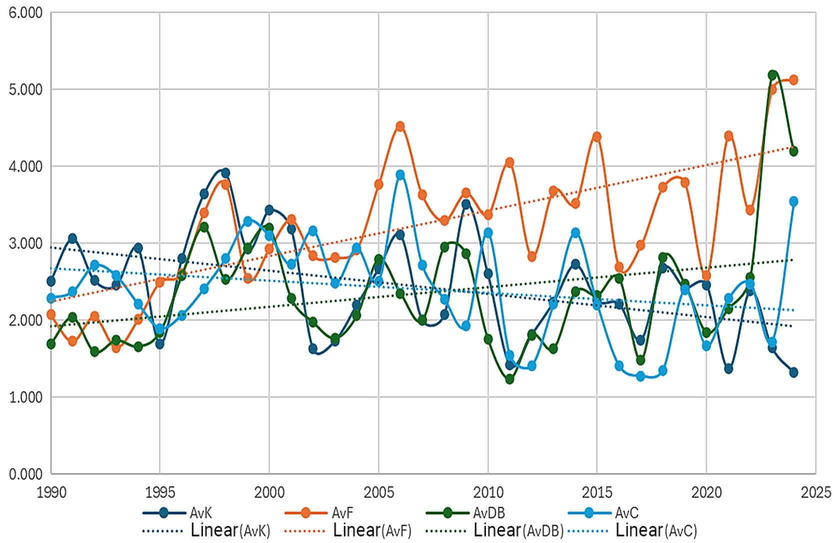


Figure 12. *Average values of radial growth across different types of lesions*

Note: AvF – trees bearing tinder fungus fruiting bodies, AvC – trees with cancerous growths, AvDB – trees in areas of severe dieback, AshK – trees showing no signs of dieback (control group) with linear change tendency

Source: compiled by the authors

According to the presented data, over the period 1979-2025, the average annual air temperature rose by 2.5-3.0°C, whilst the precipitation deficit increased by 80-100 mm. This is consistent with the findings of A.Z. Shvidenko *et al.* (2018), who indicated that climate change in Ukraine is becoming irreversible, creating conditions for the physiological weakening of broad-leaved tree species. Our results confirm that rising temperatures are often accompanied by a lack of precipitation (deficits of up to 30-60 mm), which causes chronic stress and is consistent with the findings of M. Netsvetov *et al.* (2021) regarding the potential for identifying the climatic sensitivity of species in their natural environment.

The results of the study indicate that the impact of short-term stress responses on the physiological resilience of tree stands is negligible; they achieve maximum resilience to adverse environmental factors at the age of 50-60 years and older. A comparison of the results of the study on the physiological resilience of stands with the data of O. Lesnik *et al.* (2022) revealed minor differences within the peak periods. A. Pliûra *et al.* (2011) established that, despite the widespread infestation of European populations by the pathogen *Hymenoscyphus fraxineus*, there are significant inter- and intra-population differences in the degree of crown defoliation and the intensity of necrosis formation. A substantial finding of the study is the identification of an inverse correlation between tree growth rate and their susceptibility to the pathogen: individuals with higher growth energy often demonstrate better survival capacity, although the overall condition of the stands remains highly dependent on environmental conditions. In the present study, control trees with an increment of over 2.0 mm showed synchronisation with the climate and an absence of visual signs of crown degradation. This confirms that maintaining high growth energy in stands is a prerequisite for their resilience. Consequently, these results indicate the need to select genetically resistant biotypes as a fundamental basis for the restoration of ash forests in regions with a high incidence of infection.

A.N. Drogvalenko *et al.* (2019) note that the highest incidence of infection was observed in stands up to 80 years old, with an ash proportion of 40-70% and a canopy cover of over 0.5. The disease was most prevalent in fertile, moist growing conditions, which are also the most favourable for ash growth. According to M. Keßler *et al.* (2012), a correlation between disease severity and location has been confirmed. These results indicate a high prevalence of ash dieback and a complex interplay of factors influencing the development of the disease, highlighting the need for further research to develop effective measures for the conservation of ash stands in Ukraine. The results of the present study are consistent with the research by Z. Vacek *et al.* (2017), which investigated changes in the productivity of ash stands in the Krkonoše Mountains (Czech Republic) under the influence of H. fraxineus and climatic factors during 2009-2015. The authors recorded a 15.2% increase in tree mortality and an intensification of dieback symptoms, particularly in stunted individuals in waterlogged areas. C.C. Roibu *et al.* (2020) developed the first differentiated dendrochronological series (earlywood, latewood, total ring width) for ash from Eastern Europe (Moldova) and found a significant negative correlation between growth and temperature and a positive correlation with precipitation and SPEI, confirming the increased sensitivity of ash to hydrothermal stress in the region.

A study by K. Davydenko *et al.* (2013) documented the presence of *Hymenoscyphus pseudoalbidus* in the leaves and shoots of common ash in eastern Ukraine, indicating a potential threat to tree stands in this region. Compared with the current study, which showed a sharp and irreversible decline in tree ring growth in areas of intense dieback, particularly due to halar necrosis, the findings of K. Davydenko *et al.* confirm that the presence of the pathogen directly correlates with reduced tree productivity. At the same time, the current data complement previous findings, demonstrating not only the fact of infestation but also its impact on the long-term growth dynamics of trees and the disruption of correlations with climatic factors, which underscores the importance of a comprehensive assessment of the condition of stands for predicting forest degradation. The study by H. Mazurchuk *et al.* (2025) on ash dieback in the Kyiv Polissya show that the rapid, critical dieback of ash trees is the result of a complex interaction between *Agrilus planipennis*, stem pests, as well as fungal and bacterial pathogens, which has a significant impact primarily on large-diameter trees. Dendrochronological series from the current study indicate that the critical decline in growth began approximately 10-20 years ago, which chronologically coincides with the phases of active spread of the Asian longhorned beetle in Ukraine. I.P. Matsiakh & V.O. Kramarets (2014) investigated the dieback of common ash in western Ukraine and found the disease to be widespread across various types of stands, depending on age, density and site conditions. Compared with the current study, which recorded a sharp decline in radial growth in areas of intense dieback, their results confirm the influence of local conditions on the intensity of the pathological process; however, the current data add a spatio-temporal perspective and demonstrate the interaction between climatic and pathogenic factors.

The health status of ash stands in the Forest-Steppe region requires careful investigation to establish the cause-and-effect relationships that caused its deterioration. Studies of individual chronologies of meteorological conditions and ring growth can be used to determine the physiological resilience of ash trees in stands, i.e. assess the extent to which physiological stress affects the growth and development of the stand. T. Kowalski (2006) found that throughout the entire growth period, stands of various tree species are subject to physiological stress, as evidenced by data from other studies. V.V. Melnyk & O.V. Zborovska (2018) investigated the radial growth of Scots pine in the Zhytomyr Polissya on plots where no thinning had been conducted following the Chornobyl nuclear power plant accident. They demonstrated that the absence of management measures affects growth rates and the overall condition of the stands.

The dieback of ash trees in Ukraine is caused by the combined effects of the invasive fungus *Hymenoscyphus fraxineus*, the emerald ash borer, and associated pathogens. Damage is mainly observed in stands of a certain age and composition, whilst climatic stresses further exacerbate the degradation. This situation indicates a significant deterioration in the health of ash forests and necessitates measures for their conservation and restoration.

Conclusions

A study of the condition of common ash (*Fraxinus excelsior* L.) stands in the context of climate change and pathological influences has led to the following conclusions. An analysis of meteorological data for the period 1979-2025 confirmed a consistent trend towards climate aridification. An increase in the average annual air temperature of 2.5-3.0°C has been recorded (from 7.5-8°C at the start of the period to 10.5-11.5°C in the last decade). A moisture deficit is observed; the average annual precipitation has decreased by 80-100 mm, which, combined with the temperature regime, creates conditions of chronic hydrothermal stress.

A dendrochronological assessment revealed specific responses of trees to different types of damage. Healthy trees maintain relative stability (average growth of 1.5-3.5 mm), but since 2010, interannual fluctuations in growth have been observed due to climatic factors. In trees with canker tumours, a sharp decline in growth intensity has been observed since 2015. In areas of intense dieback, a reduction in the width of annual rings has been recorded over the last 10-20 years. A characteristic feature is the 'divergence' of growth curves from the climatic trend, indicating the dominance of pathological factors over abiotic ones. Infection by the sulphur-yellow polypore (*Laetiporus sulphureus*) in the early and middle stages does not significantly restrict radial growth (up to 7-8 mm in individual trees in 2020-2024), as the sapwood's conductive function is maintained for a long time.

The study established that in severely weakened trees, the correlation between radial growth and climatic factors is disrupted. This indicates that pathological processes become the dominant influencing factor, negating the tree's positive response to favourable weather conditions. The dendrochronological method using software (ImageJ, ObjectJ) is sufficiently accurate for retrospective assessment of the condition of forest stands and can identify the onset of tree degradation before visual signs of crown dieback appear. Further research addresses the genetic resistance of individual ash trees that maintain stable growth within infection foci.

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Conflict of Interest

None.

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Дендрохронологічна оцінка стану насаджень ясена звичайного в осередках інтенсивного всихання

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Анотація. Масове всихання ясена звичайного (Fraxinus excelsior L.) є однією з найгостріших проблем, зумовленою як глобальною зміною клімату, так і поширенням небезпечних інфекційних захворювань. Розуміння того, як поєднання гідротермічного стресу та різних типів патогенів впливає на життєздатність насаджень, є критично важливим для розробки стратегій збереження лісових екосистем. Мета роботи полягала у проведенні комплексної дендрохронологічної оцінки стану ясеневих насаджень та аналізі динаміки радіального приросту дерев за умов різного ступеня ураження патогенами та впливу кліматичних чинників. Дослідження базувалося на відборі прирісних кернів за допомогою вікових бурів у дерев різних категорій стану: здорових (контроль), уражених сірчано-жовтим трутовиком, раковими пухлинами та в осередках інтенсивного всихання. Аналіз ширини річних кілець виконувався шляхом сканування зразків та їх подальшої обробки у спеціалізованому програмному забезпеченні ImageJ з плагіном ObjectJ. Кліматичний аналіз проводився за архівними даними температури та опадів за період з 1979 по 2025 роки. Виявлено стійку тенденцію до потепління клімату (зростання середньорічної температури на 2,5-3,0°C) та дефіциту вологи, що створює умови хронічного гідротермічного стресу для ясена. Встановлено, що дерева в осередках інтенсивного всихання демонструють різке зменшення радіального приросту, яке втрачає зв'язок із кліматичним трендом через патологічне ослаблення. Ураження трутовиками та раковими пухлинами призводить до поступового зниження приросту або його високої варіабельності, проте менш критично впливає на життєздатність на коротких часових інтервалах порівняно з халаровим некрозом. Отримані дані дозволяють використовувати показники радіального приросту як точний індикатор стану насаджень для ранньої діагностики деградації лісів. Результати можуть бути застосовані лісогосподарськими підприємствами для оптимізації санітарних заходів та прогнозування стійкості ясеневих лісів у мінливих екологічних умовах

Ключові слова: радіальний приріст; кліматичні зміни; патогени лісу; халаровий некроз; річні кільця; життєздатність насаджень; гідротермічний стрес

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Characteristics of maize parental components according to yield structure and grain quality indicators

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Abstract. The relevance of the study is determined by the need to select maize source material capable of combining high productivity with high grain quality. In breeding processes, it is important to conduct complex studies on the morphometric traits of the ear, elements of productivity, grain filling, and the content of the main storage substances in the parental components. This study aimed to study the characteristics of the maize parental components on the basis of the elements of ear structure, grain quality parameters, and to reveal the relationships between the economically valuable traits. Field experiments were conducted during 2024-2025 in the Kyiv Region on typical low-humus chernozem soil. The experiment was arranged according to a randomised design with three replications. The studied characteristics are: weight of the ear with and without the cob, diameter and length of the ear, number of kernel rows, number of kernels per row, grain yield, 1,000-grain weight, test weight, content of proteins, starch, and oil in it. Statistical processing of the results was performed using analysis of variance and correlation analysis. Considerable phenotypic variability of the studied material was established. Ear weight with cob ranged from 30.06 g to 148.34 g, ear weight without cob from 18.48 g to 130.72 g, grain yield from 60.86% to 88.12%, and 1,000-grain weight from 174.2 g to 360.8 g. Genotype LLF 2983 was characterised by the highest values of ear weight, grain yield, and 1,000-grain weight. The highest content of proteins was revealed in LLN 2948, the highest starch content in LFH 1940 × LMH 3161, and the highest oil content in LGR 2038. Correlation analysis between the studied

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traits showed the presence of high positive correlations between the weight of the ear, dimensions of the ear, number of kernels in the row, and grain yield. Simultaneously, multidirectional relationships were established between productivity and grain quality indicators. The practical significance of the study consists of the substantiation of promising genotypes and signs for their selection in the process of obtaining high-yield hybrids with high grain quality

Keywords: inbred lines, 1,000-grain weight, protein, starch, oil, correlation analysis

Introduction

Maize (*Zea mays* L.) ranks among the most important cereal crops in the world, playing a vital role in food, feed, industrial and breeding applications. The grain is used to obtain products, feed, food, starch, oil, bioethanol, and many other types of processing products. Currently, one of the main directions in the breeding of maize is not only the increase in yield potential, but also the improvement of grain quality and technological properties. In the conditions of increasing demand for grain production stability, the breeding of hybrids with high yield, adaptation to the changing environment, grain quality and technological properties of the grain is especially relevant.

Modern maize breeding development is based on a synthesis of traditional selection methods with molecular, genomic, and biotechnological approaches. B. He *et al*. (2024) noted that current trends in maize breeding encompass marker-assisted selection, genomic selection, haploid induction, genetic engineering, genome editing, and synthetic biology. Such approaches are aimed at improving yield potential, quality of grain, increasing resistance to biotic and abiotic factors, and improving the efficiency of nitrogen use. At the same time, in all approaches, the effectiveness of breeding largely depends on the quality of the source material, in particular, inbred lines and parental components with valuable characteristics for obtaining new hybrids. P. Kaushik & W. Grichar (2024) consider maize as one of the most important crops for ensuring food security and advancing innovation in the agricultural sector. The authors noted that the role of genetic and varietal diversity, genomics, and biotechnological approaches in the direction of new research on maize breeding, in particular for yield, and the grain nutritional quality improvement. Therefore, evaluation of parental components according to a set of productivity and quality traits remains an important stage in the development of competitive breeding material.

Maize displays an exceptional range of variability, both morphologically and in terms of productivity and quality indicators. High adaptability of the crop has led to the formation of huge genetic diversity and genetic resources with different usage, grain quality, adaptability and agronomic properties have emerged in the world according to P. Revilla *et al.* (2022). This is very important for breeding because different fields of maize usage require different target characteristics: for feed purposes, grain protein content and energy value are most important; for food purposes, nutritional value and technological properties are of particular importance; and in industry, starch, oil and the content of other components, as well as their composition, are of significant importance.

One of the trends of modern studies is the assessment of maize grain quality indicators (protein, starch, and oil content, amino acid composition, and the physical properties of the kernel). I.K. Amegbor *et al.* (2022b) established that hybrids developed using lines with enhanced grain quality may differ in tryptophan content and protein quality index. It has been shown that QPM lines crossed with non-QPM material can result in loss of quality protein advantages, while some QPM hybrids have good yield combined with improved nutritional value. Under Ukrainian conditions, the assessment of the source material of maize by grain quality indicators is also relevant. According to V.L. Jemeida *et al.* (2020), the creation of new hybrids is carried out on the basis of the formation of the necessary properties of the parental elements through the selection of those that serve as a source of improved biochemical and agronomically valuable traits. In their research, lines that were selfpollinated maize have been evaluated for protein, starch and oil content, which allowed the identification of lines that could be used in the tester-crossing scheme. Such an approach is close in methodological terms to the assessment of parental components by the set of yield structure and grain quality traits.

The assessment of the source material of maize for its grain quality traits should also include other important indicators, in particular, ear structure traits and yield components – ear weight, ear length and diameter, number of kernel rows, number of kernels per row, grain yield, 1,000-grain weight, and test weight. According to L. Yang *et al.* (2021), ear structure traits are among the key factors in the formation of maize grain yield. It was revealed in their study that the ear length, ear diameter, number of kernel rows, number of kernels per ear row and number of kernels per ear have significant QTLs, and there are high genetic and phenotypic relations between these traits. Thus, this research supports the use of indicators of ear structure traits as important indicators in breeding evaluation. Ear structure traits and productivity components are of particular importance. N.S. Khalaf & W.A. Hassan (2022) evaluated the yield in the introduced and local maize varieties and their components depending on plant density. Plant density significantly influenced almost all of the examined traits, namely ear length, row number, kernel number per row, kernel number per ear, and grain weight. Introduced varieties surpassed local ones in some productivity traits, which underlines the necessity to conduct the comparative analysis of genotypes at specific locations. These findings confirm that the productive potential of maize is formed under the combined influence of genotype, agronomic factors, and their interaction.

V.V. Bagatchenko *et al.* (2020) also demonstrated that the formation of the fractional composition and sowing qualities of maize parental seed components depends on genetic characteristics and plant density. Different parental components were shown to respond differently to changes in plant density, which affected the yield of valuable seed fractions, 1,000-grain weight, germination energy, and germination capacity. Thus, the quality of seed material and the productivity of parental components are determined by the interaction between genotype and growing conditions. Hence, they should be assessed on the basis of a complex of morphometric traits, productivity and grain quality characteristics. In research by V. Babić *et al.* (2024), the quality of maize grain depends on the genotype and year of cultivation, agrotechnical factors, as well as the relationships between the individual traits of the structural and biochemical content. According to V. Babić *et al.*, among elite maize lines and hybrids, there is great variability of quality traits of grain, as well as the year of the study and the influence of the components of parents, which are manifested for almost all quality characteristics. According to the authors, simultaneous improvement of some complexes of traits is possible, for instance, protein and starch or protein and oil, while the joint increase in oil and starch content is due to some negative relationships associated with the structure of the kernel.

There is a huge number of studies on the issues of breeding, productivity, and grain quality of maize. However, this aspect of the complex evaluation of the components of parents according to a combination of productivity, physical and mechanical properties of grain and biochemical composition, and depending on the peculiarities of the combination of soil and climatic conditions, was not sufficiently investigated. Of particular relevance is the identification of relationships between valuable agronomic traits, which makes it possible to determine indicators suitable for use as selection criteria in breeding programmes.

This study aimed to evaluate maize parental components according to ear structure traits, productivity components, physical grain properties, and grain quality indicators, as well as to establish relationships between valuable agronomic traits in order to identify promising source material for further use in breeding programmes.

Materials and Methods

Field experiments were conducted during 2024-2025 on the experimental plots of the Experimental Field unit of the Department of Genetics, Breeding and Seed Production named after Professor M.O. Zelenskyi at the National University of Life and Environmental Sciences of Ukraine (Agronomic Research Station), located within the Bila Tserkva District of Kyiv Region. The soil cover of the experimental area was represented by typical low-humus chernozem.

The source material consisted of 12 genotypes of maize developed by MAS Seeds, representing two groups of parents. The first group included lines of self-pollination for the development of twocomponent hybrids (LOR 3696, LNR 3495, LLN 2948, LIN 2456, LLF 2983, LGR 2038, LSH 369), and the second group included hybrid combinations that are used as the components of parents to obtain three-component hybrids (LIR 2232 × LIR 2234, LFH 1940 × LMH 3161, LAR 1490 × LBR 1558, LLR 2961 × LMR 3185, LGR 2038 × LSH 369).

The accounting plot area was 9.8 m^2. The experiment was arranged according to a randomised design with three replications. Determination of protein, starch, and oil content in the grain of maize parental components, as well as analysis of ear structure elements, was carried out in accordance with the Methodology for Conducting Qualification Examination of Plant Varieties for Suitability for Dissemination in Ukraine (Kienko *et al.*, 2016). Biochemical grain quality indicators were determined using a FOSS Infratec 1241 Grain Analyzer (Denmark).

Weather conditions during the years of the study were characterised by considerable inter-annual variability in temperature regime and uneven moisture supply. Air temperatures in 2024 were, in general, higher than the corresponding mean values over long-term average data. Together with moisture deficiency in the second half of July, partial suppression of growth processes was found during the tasselling period. The year 2025 was more typical in air temperature conditions, except in the month of May, when it was cooler than normal. Moisture conditions in these years were characterised by a moisture supply at higher levels compared to long-term averages; however, the rain distribution during the growing season was not uniform. The most satisfactory conditions for the moisture supply were during the first half of the summer of 2024; periods of moisture deficiency were determined in the second half of the growing season for both years. It is clear that high temperature and irregular distribution of rainfall were among the key reasons why the productive potential of the crop could not be realised.

Statistical processing of the obtained results was performed using methods of variation statistics and analysis of variance (ANOVA) in order to assess the influence of the studied factors on ear structure traits and maize grain quality indicators. For each treatment, the arithmetic mean (M) and standard error of the mean (SE) were determined. The significance of differences between treatments was evaluated using post hoc multiple comparison tests (LSD/Tukey HSD) at a significance level of $p \leq 0.05$. The results of the statistical analysis are presented in tables using letter indices to indicate homogeneous groups. The study was conducted in accordance with the international ethical requirements set out in the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1973). During the research process, the principles of these documents aimed at preserving biodiversity and preventing negative impacts on the studied species were observed.

Results and Discussion

As a result of the experimental studies, ear structure indicators in maize were evaluated depending on genotype. Analysis of the obtained data made it possible to determine the level of variation in the main ear structure traits and to identify statistically significant differences among the studied variants. Ear weight with cob ranged from 30.06 g to 148.34 g, whereas ear weight without cob ranged from 18.48 g to 130.72 g (Table 1). Significantly high values for both indicators were recorded in genotype LLF 2983 (148.34 g and 130.72 g, respectively), while the minimum values were observed in LLN 2948 (30.06 g and 18.48 g). Differences among the samples for these indicators were statistically significant ($p \leqslant 0.05$).

Table 1. *Morphometric parameters of maize ear structure depending on genotype (M ± SE)*

Maize sample	Ear weight with cob, g	Ear weight without cob, g	Ear diameter, cm	Ear length, cm	Number of rows, no.	Number of kernels per row, no.
LOR 3696	122.02 ± 17.12^{bc}	97.72 ± 16.63^{bc}	3.58 ± 0.38^{b}	14.60 ± 2.16^{cd}	14.40 ± 0.89^{b}	24.00 ± 2.35^{c}
LNR 3495	94.76 ± 15.57^{d}	77.78 ± 13.79^{c}	3.34 ± 0.48^{bc}	13.20 ± 1.10^{def}	16.40 ± 4.77^{a}	22.00 ± 3.16^{c}
LLN 2948	30.06 ± 7.86^{e}	18.48 ± 6.58^{d}	2.80 ± 0.27^{de}	12.00 ± 1.41^{fg}	11.60 ± 2.19^{d}	8.80 ± 2.28^{e}
LIN 2456	49.80 ± 14.63^{e}	35.12 ± 12.57^{d}	3.10 ± 0.22^{cd}	13.00 ± 1.22^{ef}	14.00 ± 0.00^{b}	16.80 ± 1.79^{d}
LLF 2983	148.34 ± 19.50^{a}	130.72 ± 19.40^{a}	3.82 ± 0.19^{a}	18.14 ± 1.14^{a}	14.40 ± 0.89^{b}	29.00 ± 3.32^{ab}
LGR 2038	51.66 ± 15.38^{e}	31.44 ± 10.17^{d}	2.43 ± 0.15^{f}	13.76 ± 2.16^{cde}	12.40 ± 0.89^{cd}	16.20 ± 2.59^{d}
LSH 369	53.42 ± 15.58^{e}	38.02 ± 10.88^{d}	2.50 ± 0.35^{ef}	11.40 ± 0.96^{g}	8.00 ± 0.00^{e}	16.4 ± 1.52^{d}
LIR 2232 × × LIR 2234	116.70 ± 25.90^{bcd}	93.52 ± 20.80^{bc}	3.12 ± 0.47^{c}	14.58 ± 1.79^{cd}	12.00 ± 1.41^{cd}	30.80 ± 3.77^{a}
LFH 1940 × × LMH 3161	127.20 ± 22.14^{abc}	106.66 ± 20.16^{b}	3.34 ± 0.22^{bc}	15.10 ± 0.55^{bc}	16.80 ± 3.35^{a}	30.40 ± 6.39^{ab}
LAR 1490 × × LBR 1558	138.70 ± 11.31^{ab}	95.92 ± 45.00^{bc}	3.30 ± 0.27^{bc}	18.4 ± 1.67^{a}	14.40 ± 0.89^{b}	32.80 ± 3.63^{a}
LLR 2961 × × LMR 3185	109.34 ± 22.64^{cd}	81.34 ± 22.05^{c}	3.38 ± 0.30^{bc}	16.14 ± 1.02^{b}	12.40 ± 1.67^{cd}	25.80 ± 1.64^{bc}
LGR 2038 × × LSH 369	103.00 ± 29.13^{cd}	76.86 ± 21.30^{c}	3.40 ± 0.86^{bc}	15.04 ± 2.22^{bc}	13.20 ± 2.28^{bc}	26.00 ± 1.87^{bc}
$\bar{x}$	95.42 ± 39.46	73.63 ± 35.01	3.18 ± 0.42	14.61 ± 2.17	13.33 ± 2.34	23.25 ± 7.36

Note: values are presented as arithmetic mean (M) ± standard error (SE). Different letters (a, b, c, d, e, f, g) within a single indicator denote statistically significant differences between genotypes according to the multiple comparison test at $p \leq 0.05$

Source: developed by the authors on the basis of their own research

The ear diameter varied within 2.43-3.82 cm, and the ear length varied from 11.40 to 18.40 cm. Genotypes LLF 2983 and LAR 1490 × LBR 1558 had the largest ear diameter and length (3.82 cm and 18.14-18.40 cm, respectively), which were statistically reliably greater than others. The number of kernel rows was in the range of 8.0-16.8, and the number of kernels per row ranged from 8.8 to 32.8. The largest numbers of kernel rows were detected in genotypes LFH 1940 × LMH 3161 and LNR 3495 (16.8 and 16.4, respectively), the maximum number of kernels per row (30.4-32.8) was in genotypes LAR 1490 × LBR 1558, LIR 2232 × LIR 2234 and LFH 1940 × LMH 3161, and minimum values of these indicators were determined for genotypes LLN 2948 and LSH 369.

Depending on the genotype, the grain yield ranged within 60.86%-88.12%, and the 1,000-grain weight ranged from 174.2 to 360.8 g (Table 2). The highest grain yield was recorded in genotype LLF 2983 at 88.12%, which was also characterised by the maximum 1,000-grain weight of 360.8 g. This indicates that the genotype combines a high proportion of grain in the ear structure with the formation of large kernels, which is an important characteristic of its productive potential. A similar tendency was observed in the self-pollinated lines LNR 3495 and LOR 3696, in which high grain yield values of 82.08% and 80.09%, respectively, were combined with relatively high 1,000-grain weights of 312.0 g and 311.2 g. This may indicate a more balanced formation of ear productivity components in these genotypes.

Table 2. *Productivity parameters of the ear and physical grain properties of maize depending on genotype (M ± SE)*

Maize sample	Grain yield, %	1,000-grain weight, g
LOR 3696	80.09 ± 2.66^{b}	311.20 ± 24.16^{b}
LNR 3495	82.08 ± 2.29^{b}	312.00 ± 37.97^{b}
LLN 2948	61.48 ± 7.24^{e}	258.00 ± 16.28^{d}
LIN 2456	70.52 ± 9.18^{cd}	174.20 ± 50.36^{h}
LLF 2983	88.12 ± 10.98^{a}	360.80 ± 56.02^{a}
LGR 2038	60.86 ± 6.11^{e}	205.00 ± 69.20^{gh}
LSH 369	71.17 ± 1.174^{cd}	222.00 ± 44.50^{fg}
LIR 2232 × LIR 2234	80.14 ± 1.30^{b}	267.60 ± 50.22^{cd}
LFH 1940 × LMH 3161	83.85 ± 1.678^{ab}	208.20 ± 43.41^{fgh}
LAR 1490 × LBR 1558	69.16 ± 31.88^{d}	275.00 ± 22.92^{cd}
LLR 2961 × LMR 3185	74.39 ± 5.13^{cd}	301.4 ± 15.65^{bc}
LGR 2038 × LSH 369	74.62 ± 0.80^{c}	240.00 ± 68.56^{ef}
$\bar{x}$	74.71 ± 8.55	261.28 ± 54.26

Note: values are presented as arithmetic mean (M) ± standard error (SE). Different letters (a, b, c, d, e, f, g) within a single indicator denote statistically significant differences between genotypes according to the multiple comparison test at $p \leq 0.05$

Source: developed by the authors on the basis of their own research

At the same time, the samples did not display a direct correlation between grain yield and 1,000-grain weight. For example, for LFH 1940 × LMH 3161, one of the high grain yield values was observed (83.85%), yet the 1,000-grain weight was low (208.2 g). This suggests that in this genotype, such high values for grain yield were determined not by the kernel size, but probably by the greater number of kernels or less content of cob tissue in the ear mass. For LIR 2232 × LIR 2234, a similar pattern was also observed. Despite the high grain yield of this combination (80.14%), 1000-grain weight was only average (267.6 g), while 1,000-grain weight of LAR 1490 × LBR 1558 was relatively large (275.0 g); however, it was characterised by a relatively lower grain yield (69.16%). As for the samples with the lowest grain yield values – LGR 2038 (60.86%) and LLN 2948 (61.48 %), they also differed from each other by the indicator of 1,000-grain weight. In LGR 2038, it reached 205.0 g, and in LLN 2948, 258.0 g, which confirms that low grain yield does not always go together with the lowest grain weight and may depend on the morphological structure of the ear and the relationship between the grain mass and the cob. The lowest 1,000-grain weight was recorded in LIN 2456 at 174.2 g; however, the grain yield of this genotype was 70.52%, which was higher than that of LGR 2038 and LLN 2948.

Test weight of maize grain varied widely depending on genotype, ranging from 390 to 754 g/L, indicating substantial genotypic differentiation in physical grain properties (Fig. 1). The best results in test weight were shown by

LIN 2456 – 754 g/L, LLR 2961 × LMR 3185 – 752 g/L, LSH 369 – 750 g/L, and LGR 2038 × LSH 369 – 743 g/L; they formed group "a". This indicates that there are no statistically significant differences between them in this respect. High test weight of these genotypes is evidence of better kernel filling, higher grain density, and potentially greater technological value. For food-grade maize, this indicator is particularly relevant, as a higher test weight implies a more uniform kernel, its density and better suitability for processing.

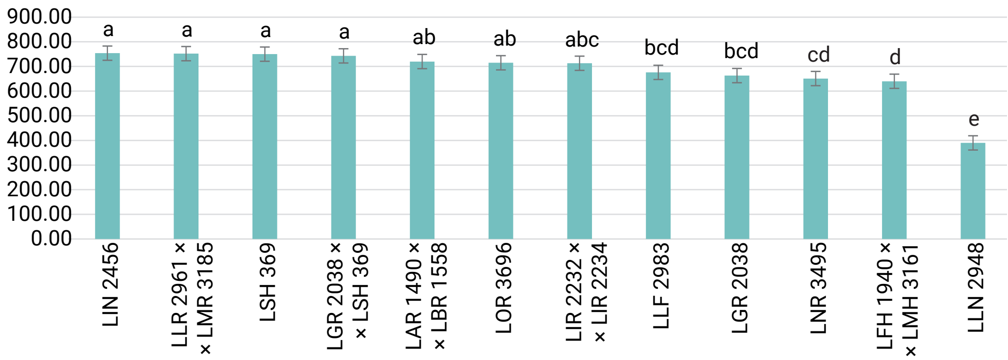

***Figure 1.** Test weight of maize grain depending on genotype*

Note: different letters above the bars (a, b, c, d, e) indicate statistically significant differences between genotypes at $p \leqslant 0.05$

Source: developed by the authors on the basis of their own research

Another group was made up of genotypes with relatively high, although slightly lower values, of test weight: LAR 1490 × LBR 1558 – 720 g/L, LOR 3696 – 715 g/L, LIR 2232 × LIR 2234 – 713 g/L. These fell into transitional statistical groups "ab" and "abc", indicating that their values were close to those of the maximum test weight group. In other words, this suggests that these genotypes may also be considered promising for this quality feature, as values in this group exceeded 700 g/L. This is sufficiently high for the formation of high-quality grain mass, especially in conjunction with other good agronomic features such as the 1,000-grain weight, grain yield per ear and yield stability. An intermediate group for test weight was formed by LLF 2983 – 676 g/L, LGR 2038 – 663 g/L, LNR 3495 – 651 g/L, and LFH 1940 × LMH 3161 – 640 g/L. Their values were much lower than those of the leading genotypes, yet remained within a range that may be acceptable for grain utilisation. In addition, comparing these indicators with other characteristics of productivity, it is possible to see that the highest 1,000-grain weight and the best grain yield did not necessarily indicate the greatest test weight values. For example, LLF 2983 had the highest 1,000-grain weight, and also showed the greatest grain yield, but the test weight for this genotype was within the intermediate range. In turn, LFH 1940 × LMH 3161 had the high grain yield and low values of test weight (640 g/L), which indicates that test weight depends not only on the size and weight of an individual kernel but also on its density, morphology and degree of filling.

The lowest test weight was shown by the genotype LLN 2948 – 390 g/L, and it considerably differed from the others. These values are very low, and they cannot be considered an indicator of high-quality grain for technological use, which can be evidenced by poor filling of grain, lower kernel density or other features of the grain morphology of this genotype. Additionally, it is worth noting that LLN 2948 showed one of the lowest grain yield values, confirming its comparative modesty in productive and technological potential among the tested samples.

The content of grain protein in maize ranged from 9.0% to 11.7% (Table 3). The highest indicator was obtained for the LLN 2948 genotype (11.7%), which significantly surpassed other variants. High values were also identified for the LGR 2038 (11.5%) and LNR 3495 (11.0%) genotypes. The lowest results were noted for LLF 2983 and LAR 1490 × LBR 1558 genotypes (9.0% each) and for the LIN 2456 genotype (9.2%).

Table 3. *Maize grain quality indicators depending on genotype (M ± SE)*

Maize sample	Protein content, %	Starch content, %	Oil content, %
LIR 2232 × LIR 2234	10.1 ± 0.2[de]	69.8 ± 0.2[c]	3.9 ± 0.0[ef]
LFH 1940 × LMH 3161	9.3 ± 0.3[fg]	72.1 ± 0.2[a]	3.8 ± 0.0[fg]
LAR 1490 × LBR 1558	9.0 ± 0.2[g]	68.2 ± 0.2[fg]	4.0 ± 0.0[de]
LLR 2961 × LMR 3185	10.7 ± 0.1[cd]	68.8 ± 0.2[def]	4.1 ± 0.0[d]
LGR 2038 × LSH 369	9.6 ± 0.1[efg]	69.8 ± 0.3[c]	4.1 ± 0.0[d]
LOR 3696	10.2 ± 0.2[de]	68.5 ± 0.3[efg]	4.3 ± 0.0[bc]
LNR 3495	11.0 ± 0.3[bc]	69.1 ± 0.2[cde]	3.7 ± 0.0[gh]
LLN 2948	11.7 ± 0.1[a]	67.8 ± 0.3[g]	4.4 ± 0.0[ab]
LIN 2456	9.2 ± 0.1[g]	70.6 ± 0.3[b]	3.8 ± 0.0[fg]
LLF 2983	9.0 ± 0.1[g]	69.2 ± 0.14[cde]	3.6 ± 0.0[h]
LGR 2038	11.5 ± 0.13[ab]	68.3 ± 0.16[fg]	4.5 ± 0.0[a]
LSH 369	9.8 ± 0.2[ef]	69.4 ± 0.42[cd]	4.1 ± 0.1[cd]
$\bar{x}$	10.1 ± 0.2	69.3 ± 0.3	4.0 ± 0.0

Source: developed by the authors on the basis of their own research

Starch content fluctuated from 67.8% to 72.1%. The highest value was recorded in the LFH 1940 × LMH 3161 genotype (72.1%), which significantly surpassed other variants. High values were also identified for the LIN 2456 (70.6%) and LIR 2232 × LIR 2234 (69.8%). The lowest value for this indicator was established for the LLN 2948 genotype (67.8%). The oil content of grain ranged from 3.6% to 4.5%. The highest value was recorded in the LGR 2038 genotype (4.5%), as well as in the LLN 2948 (4.4%) and LOR 3696 (4.3%) genotypes, which significantly surpassed other variants. The lowest result was recorded for LLF 2983 (3.6%) and LNR 3495 (3.7%) genotypes.

A set of very high and strong positive correlations was identified between the traits related to ear structure (Table 4). Thus, a very high correlation coefficient (r = 0.98) was obtained for the relationship between the weight of the ear with cob and the weight of the ear without cob. In this case, the weight of the ear with cob also showed strong positive correlations with the number of kernels in rows (r = 0.94), length of the ear (r = 0.86), and diameter of the ear (r = 0.82), as well as a good correlation with grain yield (r = 0.76). Almost the same picture was observed with the weight of the ear without cob, which shows a good correlation with the number of kernels in rows (r = 0.90), the diameter of the ear (r = 0.85), and grain yield (r = 0.85). Grain yield showed strong positive correlations with the diameter of the ear (r = 0.78) and the number of kernels in rows (r = 0.67), as well as a moderate correlation with 1,000-grain weight (r = 0.54). At the same time, a strong negative correlation was identified for the grain yield and oil content (r = -0.77), which indicates a contrasting nature of formation of these traits. Statistically significant relationships were also found between some structural traits of the ear: the diameter of the ear was in correlation with its length (r = 0.69), the number of rows with kernels (r = 0.66), as well as with the number of kernels in rows (r = 0.67). Ear length had a high correlation with the number of kernels per row (r = 0.81) and a medium correlation with the number of kernel rows (r = 0.45). The 1,000-grain weight was mainly associated with moderate positive correlations with ear structure traits (r = 0.52-0.66), a weak one with the number of kernel rows (r = 0.22) and almost zero with test weight (r = -0.05). The test weight was correlated in a majority of cases weakly to moderately with the examined traits, including moderately positive with the number of kernels per row (r = 0.51), and weakly with ear mass traits (r = 0.33-0.39). Protein content showed medium negative correlations with ear structure traits (r = -0.49...-0.57) and with test weight (r = -0.57), while a medium positive correlation was found with oil content (r = 0.63). Starch content was characterised by a medium positive correlation with grain yield (r = 0.50), medium negative with 1,000-grain weight (r = -0.43) and medium negative with oil content (r = -0.57). The results obtained show large genotypic diversity among parental components of maize regarding main ear structure traits, productivity and grain quality traits. Significant variation was established in the weight of the ear with and without the cob, ear diameter and length, number of rows, and number of kernels per row, which confirms the broad range of morphological variability among the genotypes studied.

Table 4. *Correlation matrix of relationships between agronomically valuable traits of maize*

	Ear weight with cob, g	Ear weight without cob, g	Grain yield, %	Ear diameter, cm	Ear length, cm	Number of rows, no.	Number of kernels per row, no.	1,000-grain weight, g	Test weight, g/L	Protein content, %	Starch content, %	Oil content, %
Ear weight with cob, g	1.00											
Ear weight without cob, g	0.98	1.00										
Grain yield, %	0.76	0.85	1.00									
Ear diameter, cm	0.82	0.85	0.78	1.00								
Ear length, cm	0.86	0.79	0.41	0.69	1.00							
Number of rows, no.	0.54	0.56	0.52	0.66	0.45	1.00						
Number of kernels per row, no.	0.94	0.90	0.67	0.67	0.81	0.47	1.00					
1,000-grain weight, g	0.61	0.64	0.54	0.66	0.52	0.22	0.38	1.00				
Test weight, g/L	0.39	0.33	0.32	0.22	0.30	-0.03	0.51	-0.05	1.00			
Protein content, %	-0.57	-0.56	-0.50	-0.49	-0.52	-0.24	-0.63	0.02	-0.57	1.00		
Starch content, %	0.20	0.27	0.50	0.19	-0.04	0.35	0.33	-0.43	0.30	-0.55	1.00	
Oil content, %	-0.52	-0.59	-0.77	-0.59	-0.36	-0.51	-0.54	-0.27	-0.30	0.63	-0.57	1.00

Source: developed by the authors on the basis of their own research

Similar patterns were reported by L. Yadesa *et al.* (2022), who, in their evaluation of maize inbred lines with high-quality traits, established significant differences between genotypes for almost all traits studied. The authors noted that traits such as kernel number per ear, ear diameter, and 1,000-grain weight are of considerable breeding importance, as they combine moderate to high heritability with a positive effect on grain productivity. The variability for ear structure traits also corresponds to the results of B.T. Magar *et al.* (2021), who reported high genetic variability in maize genotypes for growth and yield traits. In that study, the highest values for heritability and genetic gain were recorded for grain yield and 1,000-grain weight, providing the authors with grounds to consider these indicators as effective selection criteria. In this regard, genotypes with higher values for ear weight, grain yield and 1,000-grain weight could be used as promising germplasm for breeding programmes.

The efficiency of maize hybrid production depends on high-value inbred lines and the mode of inheritance of traits, according to R.M.M. Habiba *et al.* (2022). In their study, for the majority of yield and yield component traits, the variance of general combining ability exceeded the variance of specific combining ability, suggesting a significant role for additive genetic variability. This suggests that the significant differences identified among the studied genotypes may be utilised in further selection of parental forms. An important component in assessing productive potential is grain yield and 1,000-grain weight. B.T. Magar *et al.* (2021) noted that 1,000-grain weight is one of the most indicative traits for assessing maize productivity due to its high heritability and correlation with yield. In a similar vein, L. Yadesa *et al.* (2022) reported on the importance of 1,000-grain weight, ear diameter and kernel number to grain yield determination in maize inbred lines.

The correlation analysis conducted in this study revealed a close relationship between the main ear structure traits and maize productivity. A high positive correlation was found between ear weight with cob and ear weight without cob. In addition, ear weight had close correlations with the number of kernels per row, ear length and ear diameter. Grain yield also showed a positive correlation with ear diameter, number of kernels per row and 1,000-grain weight. This agrees with the research of B.T. Magar *et al.* (2021), who found that grain yield was positively and significantly directly associated with test weight, ear length, ear diameter and number of kernel rows per ear. These results suggest that ear structure traits can be used as a valuable indirect indicator for the selection of productive maize forms. Similar results have been reported by A. Khatibi *et al.* (2023), who studied the relationships between maize yield and its components under different levels of drought stress. They found that under optimal growing conditions, grain yield directly depends on plant height, ear length and number of kernels per row. Under conditions of stress, the type of correlation changes as well as its strength. These findings are of special importance for the interpretation of the results of the current study, as the weather in 2024-2025 was characterised by uneven distribution of precipitation and periods of moisture deficit, which could affect the manifestation of the yield potential of maize. Hence, the differences observed between genotypes can be caused both by different genetic backgrounds and by different abilities to form the ear under changing environmental conditions.

Of particular concern is the physical properties of grain, especially test weight. Test weight is an important indicator of kernel filling and technological quality. C. Dorsey-Redding *et al.* (1991) reported that significant relationships between individual grain quality traits of maize are also possible. Among them: a positive relationship was found between protein content, ash content and test weight, and a negative relationship between starch and protein. This implies that test weight should be interpreted not just as a physical characteristic, but also as another trait indicating the quality of grain. S. Chander *et al.* (2008) also investigated genotypic differences in protein, starch, and oil. They concluded that genotypes significantly differed in the biochemical composition of grain. The authors also demonstrated a high level of heritability for most quality indicators, indicating the potential for their effective improvement within breeding programmes. S. Cao *et al.* (2024) revealed that in cereals, a trade-off is frequently noted between yield and quality due to the interaction of storage proteins and starch biosynthesis with a complicated system. D.P. Chaudhary *et al.* (2012) also identified complicated connections between some nutritional components of maize grain. As a result, the inverse association between starch and oil suggests a likely competition between different storage compounds within the kernel.

The data presented by I.K. Amegbor *et al.* (2022a) for QPM and non-QPM maize hybrids also verify that there may be complicated relationships between yield and quality traits. In particular, their study found positive links between grain yield and protein and between tryptophan and oil and negative associations between yield and tryptophan as well as protein and tryptophan. This suggests that the improvement of one quality trait is not always accompanied by the improvement of others; therefore, selection assessment should be carried out using a complex of productive and biochemical parameters. Q. Ying *et al.* (2025) confirmed the relevance of breeding high-protein maize forms for improvement in the nutritional value of maize grain. However, an increase in protein and essential amino acid content in grain was observed to cause problems with obtaining high yield and other agronomic characteristics, which limit their breeding. In this context, genotypes with increased protein or oil content should be considered as potential sources of quality traits, while highly productive forms with greater ear weight, higher grain yield, and 1,000-grain weight may be utilised as sources of productivity.

Research by H. Wang *et al.* (2023) indicates that the quality of maize grain can vary significantly not only under the influence of genotype but also depending on mineral nutrition. It was revealed that the introduction of sulphur improved the yield due to the increase in the number of kernels in the ear and weight of grain, as well as improved the nutritional quality of the grain due to the regulation of amino acid composition. These findings are crucial for interpreting the results because the accumulation of proteins, oils, and starches is governed by the interplay of genetic potential, growing environments, and plant nutrition. This conclusion is corroborated by general breeding patterns found in other cereal crops: an integrated evaluation of both yield and quality is essential. As I. Havryliuk & H. Kovalyshyna (2024) have shown, the traits that constitute a crop's yield structure, its grain quality, and its 1,000-grain weight are all significantly affected by the genotype itself, the specific year's environmental conditions, and the interaction between the two.

The results of B. Mazurenko *et al.* (2025) and M. Voitovyk *et al.* (2023) further confirm the broader agrobiological context of crop productivity formation. In particular, B. Mazurenko *et al.* (2025) showed that the application of biostimulants in mixed cropping systems increased yield and certain productivity components, which were associated with anti-stress effects and stimulation of rhizosphere processes. M. Voitovyk *et al.* (2023) found that fertilisation systems and tillage practices influence the content and composition of humic substances in typical chernozem soils, thereby indirectly determining soil fertility and the conditions for realising crop yield potential. Taken together, these data confirm that effective breeding and agronomic evaluation of crops should take into account genotype, environmental conditions, adaptability, and product quality.

It has, therefore, been demonstrated that the parental maize lines used in this investigation exhibit pronounced differences in ear structure, yield capacity, physical grain characteristics, and grain quality. The most valuable traits for breeding high-yielding lines are the weight of the ear, diameter and length of the ear, number of kernels per row, grain yield, and 1,000-grain weight. Simultaneously, the concentrations of proteins, starch, and oils retain independent breeding significance, as they are primary determinants of the grain's nutritive, feeding, and technological value. Negative relationships between a number of yield indicators and grain quality components detected in this study underline the importance of comprehensive breeding strategies targeting the aggregation of these traits.

Conclusions

The genetic variability of the maize genotypes under study showed a significant distinction in indicators of ear structure, yield capacity and quality of grain. Ear weight with the cob ranged from 30.06 g to 148.34 g, ear weight without the cob from 18.48 g to 130.72 g, ear diameter from 2.43 cm to 3.82 cm, ear length from 11.40 cm to 18.40 cm, number of kernel rows from 8.0 to 16.8, and number of kernels per row from 8.8 to 32.8. The LLF 2983 genotype was characterised by the highest values for most morphometric parameters, while the lowest values for certain traits were established for LLN 2948 and LSH 369.

Regarding productivity indicators, the LLF 2983 genotype was identified as having the highest grain yield and the maximum 1,000-grain weight. High values of specific yield indicators were observed in LNR 3495, LAR 1490 × LBR 1558, LFH 1940 × LMH 3161, and LIR 2232 × LIR 2234. Significant differences between genotypes were established for test weight. A group of genotypes was identified that were similar in this trait – LIN 2456, LLR 2961 × LMR 3185, LSH 369 and LGR 2038 × LSH 369 – with high test weight values that indicated better physical grain quality.

Regarding the biochemical composition of the grain, protein content ranged from 9.0% to 11.7%, starch from 67.8% to 72.1%, and oil from 3.6% to 4.5%. Maximum protein content was observed in LLN 2948, with similar indicators of LGR 2038 and LNR 3495. The starch content reached maximum values in LFH 1940 × LMH 3161, and the percentage of oil in LGR 2038. These parental genotypes can be considered the most appropriate for breeding with specific grain quality.

Analysis of correlation relationships proved a positive relationship between the structural parameters of ears and productivity. Ear weight with cob showed a very strong correlation with ear weight without cob, number of kernels per row, ear length, and ear diameter. The grain yield was associated with the parameters of ear diameter, number of kernels per row and 1,000-grain weight. Consequently, the weight of the ear, diameter and length of the ear, number of kernels per row, and 1,000-grain weight are informative criteria for selecting productive maize parental components. The relationship between the productivity indicators and grain quality indices turned out to be divergent. For the protein content, it was negative in relation to the ear structure, whereas the percentage of oil was inversely proportional to the grain yield. A positive relationship existed between protein and oil content. In this respect, balanced breeding for maize is desirable as an increase in yield, and maintaining its desirable biochemical composition and quality of the product is required.

Further research should focus on a multi-year study of the identified genotypes in various soil and climatic conditions, determination of their combining ability, as well as the assessment of the stability of productivity and quality of grain. Further investigation of the heredity of protein, starch and oil content, test weight and 1,000-grain weight in the grain of hybrids is recommended.

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Conflict of Interest

None.

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Характеристика батьківських компонентів кукурудзи за показниками структури врожаю та якості зерна

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Анотація. Актуальність дослідження зумовлена необхідністю добору вихідного матеріалу кукурудзи, здатного поєднувати високу продуктивність із високими показниками якості зерна. Для селекційної роботи важливим є комплексне оцінювання батьківських компонентів за морфометричними ознаками качана, елементами продуктивності, виповненістю зерна та вмістом основних запасних речовин. Метою дослідження було оцінити батьківські компоненти кукурудзи за показниками структури качана, продуктивності, показниками якості зерна, а також встановити взаємозв'язки між господарсько-цінними ознаками. Польові дослідження проводили протягом 2024-2025 рр. у Київській області на чорноземі малогумусному типовому. Дослід закладали за рандомізованою схемою з триразовою повторністю. Визначали масу качана зі стержнем і без стержня, діаметр і довжину качана, кількість рядів зерен, кількість зерен у ряді, вихід зерна, масу тисячі зерен, натуру зерна, вміст білка, крохмалю та олії. Статистичну обробку результатів здійснювали із застосуванням дисперсійного та кореляційного аналізу. Встановлено істотну фенотипову мінливість досліджуваного матеріалу. Маса качана зі стержнем варіювала від 30,06 до 148,34 г, маса качана без стержня – від 18,48 до 130,72 г, вихід зерна – від 60,86 до 88,12 %, маса тисячі зерен – від 174,2 до 360,8 г. Генотип LLF 2983 вирізнявся найвищими значеннями маси качана, виходу зерна та маси тисячі зерен. Найвищий вміст білка встановлено у LLN 2948, крохмалю – у LFH 1940 × LMH 3161, олії – у LGR 2038. Кореляційний аналіз підтвердив тісні прямі зв'язки між масою качана, його розмірами, кількістю зерен у ряді та виходом зерна. Водночас виявлено різноспрямовані зв'язки між показниками продуктивності та якості зерна. Практичне значення роботи полягає у виділенні перспективних генотипів і критеріїв добору для подальшого створення гібридів із поєднанням високої продуктивності та якості зерна

Ключові слова: інбредні лінії; маса тисячі зерен; білок; крохмаль; олія; кореляційний аналіз

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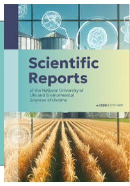

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Phytocenotic analysis of segetal flora as an indicator of agroecosystem stability under long-term herbicide application

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Abstract. The study aimed to identify patterns in the restructuring of the segetal flora and changes in the functional stability of the cereal agrocenosis under the influence of various herbicide control systems. Methods of square plots, geobotanical description, biometric and weight measurements, morphological and biological grouping, vegetation testing, soil chemical analysis, plot-by-plot yield recording and analysis of variance were used. The study determined that in the control plot, the total number of species was 24, the number of families was 10, the average number of species per 1 m^2 was 8.6, and the number of dicotyledonous species was 17. Under the integrated system, these figures were 22, 9, 7.8 and 16, respectively, i.e., they remained closer to the control values. The Shannon index ranged from 2.42 in the control to 1.31 following prolonged application of the acetolactate synthase inhibitor, whilst under the integrated system it was 2.28. The cover of the dominant species increased to 36.7%, whilst the number of rare species decreased to 1, indicating increased dominance and a narrowing of the community's reserve pool. Quantitative indicators also changed significantly: weed density decreased from 128 to 36 plants/m^2, fresh weight from 486 to 142 g/m^2, dry weight from 148 to

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47 g/m^2, and projective cover from 62 to 18%. With prolonged application of the acetolactate synthase inhibitor, the Morishita aggregation index reached 1.74, the number of herbicide-tolerant species was 8, the average root depth of the dominant species was 24.8 cm, and the weight of 1,000 seeds was 2.76 g. Functional indicators confirmed this pattern: the density of the soil seed bank decreased from 42.8 to 21.7 thousand seeds/m^2, nitrogen removal by weeds decreased from 31.5 to 9.6 kg/ha, and crop yield losses decreased from 27.4 to 6.9%. Practical significance of the study lay in the fact that its results can be used by agronomists, researchers and grain producers to select weed control systems that not only reduce weed infestation but also preserve the structural stability of the agroecosystem

Keywords: segmental flora; herbicides; dominant species; herbicide-tolerant species; agrocenosis; herbicide control systems; floristic structure

Introduction

The research relevance is determined by widespread and prolonged use of herbicides in cereal crops and the accumulation of changes affecting not only the level of weed infestation but also the structure of the weed flora. Such research is necessary, as long-term use of chemical control systems affects the species composition, dominance, spatial distribution of weeds and the functioning of the agrocenosis. Phytocenotic analysis makes it possible to track changes in the organisation of weed communities under the influence of different herbicide application regimes and to establish a link between the structure of the flora and indicators of agroecosystem stability. The problem addressed in this study arises from interrelated difficulties in studying the segetal flora of cereal crops. The abundance or biomass of weeds was prioritised, whilst floristic structure, coenotic indicators, biological and ecological characteristics of dominant species, and the functional consequences of their presence were reported separately. This complicates the comparison of herbicidal pressure with changes in species composition, seed regeneration, resource utilisation and losses in crop productivity. Such an approach requires a careful consideration of the segetal flora as a structural and functional component of the agroecosystem.

In the context of climate change, A.M. Lishchuk *et al.* (2025) examined the segetal phytobiota as a factor of ecological risk in agro-phytocenoses. Changes in species composition, the spread of more resilient components and an increase in the phytosanitary burden were linked to environmental transformation and the growing difficulty of controlling weed flora. From the perspective of ecological safety, A.M. Lishchuk *et al.* (2024) analysed the impact of segetal flora on the condition of agrophytocenoses under the influence of external stress factors. The spread of adventitious and competitively active species was accompanied by a disruption of the structural balance of crops and a reduction in the resilience of the cultivated component. Using data from winter wheat crops, S. Okrushko (2023) compared phytocenotic and chemical approaches to weed control. Dicotyledonous species predominated in the segetal flora, and varying intensities of weed control were accompanied by a restructuring of the weed layer and changes in competitive interactions within the crop. In line with the agroecological approach, R. Gómez-Gómez (2024) proposed a distinction between the concepts of weeds and segetal vegetation. The segetal component was regarded not merely as a target for suppression, but as an element of the agroecosystem linked to biodiversity and the choice of control methods.

Under conditions of water stress, M. Özkil *et al.* (2025) assessed the impact of various weed control strategies on the status of associated soil organisms within the crop phytocenosis. Different levels of weed cover suppression were accompanied by changes in the density of phytoparasitic nematodes and revealed a link between the control system and the biotic background of the agrocenosis. In a comparison of crop rotation systems, H.T. Nguyen & M. Liebman (2022) investigated the composition of weed communities in simpler and more diverse farming systems. Greater crop diversity was accompanied by changes in the composition, density and aboveground biomass of weeds, which made it possible to consider the structure of the community as an indicator of the stability of the agrosystem.

In the context of the transition to conservation agriculture, D. Derrouch *et al*. (2022) observed functional shifts in the composition of weed communities following the introduction of conservation agriculture. An increase in species richness, α-functional diversity and the proportion of gramineous forms was accompanied by an increase in average seed weight and the spread of species with a summer-winter germination pattern. Within integrated weed management systems, S. Cordeau *et al*. (2022) assessed the long-term consequences of contrasting approaches to weed control following many years of application. More integrated systems were associated with higher species richness, greater weed density and changes in the taxonomic and functional structure of the flora.

In the context of the Mediterranean agrolandscape, A. Boutagayout *et al*. (2025) summarised agroecological practices for sustainable weed management. Intensive land-use patterns were associated with the spread of resistant ecotypes and a simplification of vegetation cover, whilst agroecological approaches were linked to the preservation of biodiversity and the ecological balance of agrosystems. In modelling the response of weed communities, F.H. Oreja *et al*. (2022) traced how changes in the density of individual species altered community diversity under varying intensities of herbicide use. Changes in the abundance of dominant and associated components explained the impact of herbicide programmes on community structure and its subsequent restructuring.

Regarding effects of herbicide exposure, H. Wang *et al*. (2024) investigated the impact of herbicide residues on soil multifunctionality and the microbial community. Increased concentrations of herbicide residues were accompanied by a reduction in microbial diversity, changes in the composition of the microbiome, and a decline in soil functions related to nutrient cycling. In a comparison of herbicide strategies and crop rotations, M. Mayerová *et al*. (2022) analysed the effects of forty years of applying various chemical control schemes and crop rotations on weed communities at two sites in the Czech Republic. The composition of the flora depended not only on the herbicide load but also on the structure of the crop rotation, which made it possible to track changes in weed communities under the influence of combined agronomic factors.

The relationship between prolonged herbicide exposure, phytocenotic restructuring of the segetal flora and functional indicators of agroecosystem stability in cereal crops remained understudied. The study aimed to determine the impact of various herbicide control systems on the floristic structure, coenotic organisation, biological and ecological characteristics of dominant species, and functional indicators of agroecosystem stability in cereal crops. The objectives of the study were to determine changes in the floristic composition of the segetal flora, to assess the coenotic, quantitative, spatial and biological-ecological characteristics of dominant species, and to compare the functional indicators of the agroecosystem under different herbicide control systems.

Materials and Methods

The study was conducted in 2025 on a winter wheat production plot in the village of Verkhnia Syrovatka, Sumy District, Sumy Oblast, Ukraine. The subject of the study was the agrocenosis of the "Shestopalivka" variety of winter wheat. This variety was selected due to its consistent crop establishment, good adaptation to the conditions of the north-eastern Forest-Steppe, and relatively uniform plant development, which made it possible to assess changes in the segetal flora without any significant influence from varietal heterogeneity. During the experiment, the principles of Good Experimental Practice were adhered to in accordance with standards EPPO PP 1/181 and EPPO PP 1/152, and field work was conducted following requirements to prevent unjustified disturbance of the biotic components of the agroecosystem (European and Mediterranean Plant Protection Organization, 2012; 2021). Weather conditions in 2025 were contrasting: the early spring period was characterised by sufficient moisture and moderate temperatures, whilst the second half of the growing season saw prolonged rainless periods, rising air temperatures and a decline in productive moisture reserves in the arable layer. This hydrothermal regime intensified competition between cultivated plants and weeds, whilst at the same time creating conditions for the establishment of species with greater ecological resilience. The plot was characterised by typical low-humus, medium-loam chornozem. In the 0-30 cm topsoil layer, the humus content was 3.21%, the pH of the salt extract was 6.7, the nitrate nitrogen content was 16.8 mg/kg, the available phosphorus content was 54.7 mg/kg, and the exchangeable potassium content was 88.9 mg/kg. The previous crop was soya. Primary tillage involved disc harrowing the stubble to a depth of 6-8 cm and ploughing to a depth of 20-22 cm, whilst pre-sowing tillage consisted of cultivation with levelling of the field surface. In all five treatments, the same background mineral nutrient level of N60P60K60 was maintained. Sowing took place in the third decade of September 2024 with a row spacing of 15 cm. The experiment was set up using a randomised block design with four replicates, as this number of replicates ensured a statistically reliable comparison of the treatments and reduced the influence of natural heterogeneity in the soil cover within the field. The area of a single plot was 50 m^2, and the survey area was 32 m^2. Five treatments were compared: a control without herbicides; long-term application of an ALS inhibitor (mesosulfuron-methyl + iodosulfuron-methyl-sodium); long-term application of synthetic auxins (dicamba + MCPA (2-methyl-4-chlorophenoxyacetic acid)); herbicide rotation (prosulfocarb → clodinafop-propargyl + carfentrazone-ethyl); and an integrated control system (flufenacet + diflufenican + pre-emergence harrowing + agronomic measures). Agronomic measures referred to stubble ploughing and early spring harrowing. All herbicide applications were conducted using a HARDI Commander 3200 boom sprayer (HARDI International A/S, Denmark) in the morning between 06:00 and 09:00 or between 6:00 and 9:00 p.m., at wind speeds not exceeding 3-4 m/s and air temperatures up to 25°C; the application rate of the working solution was 200 l/ha.

The total number of species of the segetal flora was recorded at designated sample plots using a 1 m^2 metal frame and an Eschenbach Mobilux 6× LED handheld magnifying glass (Eschenbach Optik GmbH, Germany). All morphologically identified weed species found within the survey area were included in the final list. The number of families and the number of genera were determined following taxonomic analysis of the species list using a Leica EZ4 W stereomicroscope (Leica Microsystems, Germany) and a Dell Latitude 7030 Rugged Extreme field tablet (Dell Technologies, USA) for digital recording of the floristic matrix. The number of families was taken to be the number of botanical families represented by at least one identified species, and the number of genera was defined as the number of genera within which at least one species was recorded. The average number of species per 1 m^2 was calculated as the average number of species across all survey plots using formula (1):

$$\overline{\Sigma_1} = \frac{\sum \sigma_j}{\kappa}, \qquad (1)$$

where $\bar{\Sigma}_1$ – average number of species per 1 m^2, pcs; σ_j – number of species on j-th experiment plot, pcs; κ – number of experiment plots. Floristic richness index, units, was calculated as the ratio of the total number of species to the number of families, using formula (2):

$$K_{\phi} = \frac{\upsilon}{\varpi}, \qquad (2)$$

where K_{ϕ} – floral density coefficient, pcs; υ – total number of species of the segetal flora, pcs; ϖ – number of families, pcs. Number of monocotyledonous species, pcs., and the number of dicotyledonous species, were determined following the biomorphological grouping of the flora according to leaf venation pattern, seedling shape, tillering node morphology and the overall structure of the primary shoot, using an OPTIKA SZM-LED1 stereo microscope (OPTIKA Srl, Italy). Shannon species diversity index, units, was calculated based on the relative proportion of each species in the total weed population following digital processing of the floristic matrix in PAST 4.13 using formula (3):

$$\mathcal{H} = -\sum \rho_i \ln \rho_i, \qquad (3)$$

where *H* – Shannon's species diversity index, pcs; ρ_i – share of i-th species in the total number of the segetal flora; ln – natural logarithm; i – serial number of the species in the floristic list. The number of dominant species was determined by counting individual plants of each species using a Zebra TC57 field data collector (Zebra Technologies Corp., USA). Species were classified as dominant if their proportion of the total weed population exceeded 10%. The number of co-dominant species was determined using the same set of survey data. Co-dominant species were defined as those whose proportion of the total number was 5-10%. The cover of the dominant species, %, and the total cover of the three leading species, %, were assessed using a Braun-Blanquet Grid Frame 100 cells (Royal Eijkelkamp, Netherlands). These indicators were defined as the proportion of the survey grid's area vertically covered by the leaves and stems of a single dominant species or, collectively, by the three species with the highest proportion in the community. The calculation was performed using formula (4):

$$P_{\alpha} = \frac{\lambda_{\alpha}}{\lambda_{100}} \times 100, \qquad (4)$$

where P_{α} – dominant view coverage, %; λ_{α} – number of cells in the frame occupied by the vertical projection of the dominant feature; λ_{100} – total number of cells in the accounting grid. Average abundance, as measured on the Braun-Blanquet scale (score), was determined during the geobotanical survey, with the field scores subsequently converted into numerical values. The following gradations were used on the scale: 1 – numerous individuals with coverage of up to 5%; 2 – coverage of 5-25%; 3 – 25-50%; 4 – 50-75%; 5 – 75-100%. The assessment was conducted independently by two specialists with higher education in biology at each site; where discrepancies exceeded one point, a joint re-examination was conducted, after which the average value was calculated:

$$\overline{B_{BB}} = \frac{\sum \beta_t}{\mu}, \qquad (5)$$

where $\bar{B}_{BB}$ – average abundance on the Braun-Blanquet scale, points; β_t – abundance score at the t-th test site; μ – number of experimental plots; t – site number. Number of rare species was determined using a "species-by-plot matrix". This group included species accounting for less than 1% of the total number or with an occurrence frequency of no more than 20%.

Weed density, plants/m^2, was determined within a 1 m^2 Hummert Quadrat frame (Hummert International, USA) during the tillering stage of the cereal crop. This indicator characterised the number of weed plants per standard area at a single stage of crop development. The fresh weight of weeds, g/m^2, was recorded immediately after cutting the plants using CAS SW-20 field scales (CAS Corporation, Republic of Korea). The dry weight of weeds, g/m^2, was determined after drying the samples to constant weight in a Memmert UN55 drying oven (Memmert GmbH+-Co. KG, Germany) and weighing them on OHAUS Scout STX2202 laboratory balances (OHAUS Corporation, USA). Weed projective cover, %, was assessed using a 20 × 50 cm Daubenmayer frame (Forestry Suppliers Inc., USA). This indicator was defined as the proportion of the soil surface covered by the vertical projection of the above-ground parts of all weeds within the survey area. The Morishita aggregation index, units, was calculated based on weed counts in a series of microplots marked out using a 0.25 m^2 microframe (Seedburo Equipment Company, USA), using the formula:

$$I_M = \frac{\xi \sum \omega_j(\omega_j - 1)}{\Omega(\Omega - 1)}, \quad (6)$$

where I_M – Morishita's aggregation index, pcs; ξ – number of micro-plots; ω_j – number of weeds in the microplot, pcs; Ω – total number of weeds across all microplots, pcs; j – micro-plot serial number. Coefficient of variation in density, %, was used to assess the spatial heterogeneity of weed distribution across the field area (7):

$$V_q = \frac{s_q}{\bar{q}} \times 100, \quad (7)$$

where V_q – coefficient of variation of density, %; s_q – standard deviation of weed density; $\bar{q}$ – average weed density, pcs/m^2. Height of the weed layer, in cm, was measured using a 2 m Nedo mEssfix telescopic ruler (Nedo GmbH & Co. KG, Germany). The average height of the upper edge of the weed cover within the plot was taken as the measurement.

Number of annual species and the number of perennial species were determined following the biological grouping of dominant species by life cycle type, using a Peak 1961 10× hand-held magnifying glass (Peak Optical Co., Japan). Average depth of the root system of the dominant species, in cm, was measured after collecting soil cores using an AMS Regular Soil Probe 1 (AMS Inc., USA) and subsequently washing the roots by hand. This parameter characterised the average penetration depth of the bulk of the active roots of the dominant plants. The calculation was performed using formula (8):

$$R_{deep} = \frac{\sum r_k}{\psi}, \quad (8)$$

where R_{deep} – average depth of the root system of dominant trees, cm; r_k – depth of the root system of the k-th crop, cm; ψ – number of analysed plants. The average leaf area of a single plant, in cm^2, was determined after selecting typical dominant plants using a CI-202 portable leaf area analyser (CID Bio-Science Inc., USA). The mass of 1,000 seeds of dominant weeds, in g, was determined after preparing a standard sample and weighing it on a Sartorius Secura 225D-1S microbalance (Sartorius AG, Germany). The seed yield per plant, seeds/plant, was determined following the individual dissection of typical generative plants and the counting of seeds using a Pfeuffer Contador electronic counter (Pfeuffer GmbH, Germany). For each treatment, 20 typical generative plants of the dominant species, selected at the stage of full seed maturity, were analysed. The number of herbicide-tolerant species was determined in growth tests following treatment of the plants with standard rates of active ingredients in the Potter Precision Laboratory Spray Tower (Burkard Scientific, UK). Herbicide-tolerant species were defined as those in which the active growing point and viable foliage remained intact after treatment.

The seed bank density, in thousands of seeds per square metre, was determined in the 010 cm layer following the collection of soil cores and the separation of samples using a set of Retsch Test Sieves (Retsch GmbH, Germany). The field germination rate of seeds from the seed bank, %, was determined by vegetative germination of soil samples in a SANYO MLR-351H chamber (SANYO Electric Co., Japan). Nitrogen removal by weeds, kg/ha, was determined following mineralisation of the dry matter using a Kjeltec 8400 analyser (FOSS, Denmark); phosphorus removal, kg/ha, was determined spectrophotometrically using a UV-1900i (Shimadzu Corporation, Japan); and potassium removal, kg/ha, was determined using a Jenway PFP7 flame photometer (Bibby Scientific Ltd, UK). Calculations for each element were conducted using formula (9):

$$U_{el} = \frac{b_{wd} \cdot c_{el}}{100}, \quad (9)$$

where U_{el} – weed removal of the relevant component, kg/ha; b_{wd} – dry weight of weeds, kg/ha; c_{el} – content of the relevant element in the dry weight of weeds, %; el – chemical element for which the calculation was conducted. Reduction in available soil moisture reserves, in mm, was determined in the 0-20 cm layer following sampling with an Eijkelkamp Edelman Auger (Royal Eijkelkamp, Netherlands), followed by drying in a Thermo Scientific Heratherm OMS60 (Thermo Fisher Scientific, Germany), followed by conversion to moisture reserves. Permanent microplots measuring 0.25 m^2 in each replicate were used as reference plots; these were kept weed-free by manual weeding throughout the entire growing season. Crop yield losses, %, were determined based on the results of complete plot-by-plot harvesting using a Wintersteiger Classic mini-combine harvester (Wintersteiger AG, Austria), with the results converted to standard grain moisture content. This indicator characterised the relative reduction in yield due to weed competition compared with the reference weed-free microplots, using formula (8):

$$L_{crop} = \frac{y_{ref} - y_{exp}}{y_{ref}} \times 100, \tag{10}$$

where L_{crop} – losses in crop yield, %; y_{ref} – yield on the reference weed-free microplot, t/ha; y_{exp} – yield in the relevant experimental treatment, t/ha. For all parameters, the arithmetic mean, minimum and maximum values, range, variance, standard deviation, standard error of the mean, coefficient of variation and 95% confidence interval were calculated. Statistical analysis of the results was performed using Statistica 10.0 (StatSoft Inc., USA).

Results

The floristic structure of the segetal flora varied depending on the herbicide control system and reflected varying degrees of agrocenosis restructuring at the species, taxonomic and local-spatial levels. The most complete floristic spectrum was retained in the control treatment, where the total number of species was 24, whilst with prolonged application of an acetolactate synthase inhibitor, it decreased to 13. With prolonged application of synthetic auxins, the figure remained higher than in the previous scenario but was still noticeably lower than in the control. Under herbicide rotation and the integrated system, the number of species recovered to a level closer to the initial state, indicating a less severe reduction in floristic composition. Consequently, the most pronounced impoverishment of the segetal flora occurred under repeated, single-type chemical stress, whilst combined systems prevented the loss of a significant proportion of species and maintained a broader spectrum of components within the sown area.

A similar pattern was observed at the taxonomic level. The number of families in the control group was 10, whilst with prolonged use of the acetolactate synthase inhibitor it fell to 6. The number of genera followed the same trend: from 20 in the control group to 11 under the same treatment. With synthetic auxins, the reduction was also noticeable, though less pronounced, whilst under rotation and integrated control, the number of families and genera remained significantly higher. This indicated that the herbicidal effect affected not only individual species but also entire taxonomic groups sharing similar biological properties. Consequently, under the same level of pressure, the flora lost not only the number of taxa but also part of its internal taxonomic diversity. In contrast, under herbicide rotation and an integrated system, the taxonomic basis of the weed community remained broader, indicating a shallower selection pressure and the preservation of a greater number of morphologically and ecologically distinct lineages within the agrocenosis.

The average number of species per unit area also varied unevenly. In the control treatment, it was 8.6; with the acetolactate synthase inhibitor, it decreased to 4.2; and with synthetic auxins, to 5.1. Under the herbicide rotation and integrated management systems, this figure remained noticeably higher and was closer to that of the control. This distribution indicated that herbicide regimes of the same type restricted not only the total species list but also the number of forms capable of coexisting simultaneously within a small survey area. Within the micro-space of the crop, this meant a reduction in the number of ecological niches occupied by different components of the flora, and a decrease in the number of interspecific combinations that formed the small-scale mosaic of the weed layer. Crop rotation and integrated pest management, by contrast, supported higher local species richness and, consequently, greater complexity in the spatial organisation of the flora within the crop field.

The response of individual morphological groups also varied. The number of monocotyledonous species changed relatively moderately: from 7 in the control to 46 in the treatments with herbicide application. The dicotyledonous component proved to be significantly more sensitive: 17 species were recorded in the control, whereas only 8 were recorded under the acetolactate synthase inhibitor treatment. Under synthetic auxins, the number of dicotyledonous species was higher than in the previous treatment but lower than under rotation and integrated control. Thus, the reduction in floristic composition occurred primarily at the expense of the dicotyledonous group, which constituted part of the morpho-functional diversity of the segetal community. Consequently, the reduction in the number of dicotyledonous species reflected not isolated losses of sensitive forms, but a general narrowing of the spectrum of life strategies associated with growth rates, plant architecture and methods of space utilisation within the crop.

The floristic saturation coefficient complemented the comparative assessment. In the control treatment, it stood at 2.4; with prolonged application of the acetolactate synthase inhibitor, it fell to 2.17, and with synthetic auxins, to 2.14. Under the herbicide rotation regime, the index remained intermediate, whilst under the integrated system it reached 2.44 and exceeded the control value. This indicated that the integrated approach not only suppressed weed growth but also maintained a relatively more balanced ratio between the number of species and taxonomic groups. Consequently, different control systems altered the floristic structure unevenly: prolonged exposure to a single type of herbicide most severely impoverished the species, genus and family composition; synthetic auxins also reduced the diversity of the flora, but to a lesser extent; whereas herbicide rotation and the integrated system maintained their composition closer to that of the control variant (Table 1).

Table 1. *Floristic structure of the segetal flora within the agrocenosis*

Indicator	Control (no treatment)	Long-term use of an ALS inhibitor (mesosulfuron-methyl + + iodosulfuron-methyl-sodium)	Long-term use of synthetic auxins (dicamba + + MCPA)	Herbicide rotation (prosulfocarb → clodinafop-propargyl + + carfentrazone-ethyl)	Integrated control system (flufenacet + + diflufenican + + pre-emergence boron application + + agronomic measures)
Total number of species, pcs	24	13	15	20	22
Number of families, pcs	10	6	7	9	9
Number of species, pcs	20	11	12	17	19
Average number of species per 1 m^2, pcs	8.6	4.2	5.1	7	7.8
Number of monocotyledonous species, pcs	7	5	4	6	6

Table 1. *Continued*

Indicator	Control (no treatment)	Long-term use of an ALS inhibitor (mesosulfuron-methyl + + iodosulfuron-methyl-sodium)	Long-term use of synthetic auxins (dicamba + + MCPA)	Herbicide rotation (prosulfocarb → clodinafop-propargyl + + carfentrazone-ethyl)	Integrated control system (flufenacet + + diflufenican + + pre-emergence boron application + + agronomic measures)
Number of dicotyledonous species, pcs	17	8	11	14	16
Floristic richness index, units	2.40	2.17	2.14	2.22	2.44

Source: compiled by the authors

Floristic restructuring of the segetal flora took the form of a sequential change across several interrelated levels of organisation within the weed community. A reduction or preservation of species composition was accompanied by simultaneous shifts in taxonomic composition, local sown area density and the internal ratio of morphologically distinct components. Under prolonged exposure to a single type of herbicide, the flora lost some of the structural elements that had previously supported its branching, mosaic structure and capacity for multi-variant self-regeneration. With the rotation of active ingredients and the combination of chemical and mechanical methods, these processes developed in a different direction, as the community did not transition to a sharply narrowed model but retained a broader spectrum of floristic relationships. Consequently, different control systems determined not only the degree of suppression of the weed component, but also the nature of the subsequent organisation of the segetal flora within the agrocenosis.

The coenotic organisation and species dominance within the weed community varied depending on the herbicide control system and reflected varying degrees of restructuring of the segetal flora at the level of dominant, associated and rare components. The Shannon index was highest in the control treatment – 2.42; it remained close to this level under the integrated system (2.28) and herbicide rotation (2.15) but decreased noticeably with prolonged use of synthetic auxins (1.58) and an acetolactate synthase inhibitor (1.31). This pattern indicated that herbicide rotation and integrated management supported a more complex internal structure of the plant community, whereas the use of a single type of herbicide narrowed the range of species contributing to the weed layer. A similar pattern was evident in the cover of the dominant species. In the control treatment, this was 18.4%; under the integrated system, 19.1%; and under herbicide rotation, 21.3%; that is, the coverage concentration of a single species remained relatively moderate. With synthetic auxins, this figure increased to 30.9%, and with the acetolactate synthase inhibitor, to 36.7%. This meant that, under prolonged exposure to a single type of chemical treatment, a single species is disproportionately more substantial in the spatial organisation of the community. The same trend was observed in the total coverage of the three dominant species: ranging from 44.2% in the control to 45.8% under the integrated system, whilst under synthetic auxins and the acetolactate synthase inhibitor it reached 66.1% and 72.8% respectively. Consequently, under herbicidal pressure of the same type, the bulk of the cover was concentrated in a limited group of more resistant components.

Changes in the core of the community were reflected in the number of co-dominants and the mean abundance on the Braun-Blanquet scale. In the control treatment, there were 5 co-dominant species; under the integrated system, 6; and under the rotation system, 5, whereas under synthetic auxins their number decreased to

4, and under the acetolactate synthase inhibitor, to 3. At the same time, the average abundance in the control was 2.6 points, under the integrated system it was 2.5, under rotation it was 2.7, and under a single-type herbicidal treatment it increased to 3.43.8 points. This indicated that, under crop rotation and integrated management, several species remained substantial in the formation of the vegetation cover, whereas under monocultural herbicide treatment, some of the accompanying components were eliminated, whilst the abundance of a few species increased. The change in the reserve pool of the flora was evidenced by the number of rare species, which decreased from 6 in the control to 5 under the integrated system, 4 under crop rotation, 2 under synthetic auxins and 1 under the acetolactate synthase inhibitor. Thus, the greatest depletion of the community's reserve pool occurred with the prolonged, single-type application of herbicides. Herbicide rotation and the integrated system, by contrast, maintained a coenotic structure closer to that of the control, limiting the cover concentration of dominant species and supporting a broader participation of associated and rare species (Table 2).

***Table 2.** Cenotic characteristics and indicators of dominance of the segetal flora*

Indicator	Control (no treatment)	Long-term use of an ALS inhibitor (mesosulfuron-methyl + + iodosulfuron-methyl-sodium)	Long-term use of synthetic auxins (dicamba + + MCPA)	Herbicide rotation (prosulfocarb → clodinafop-propargyl + + carfentrazone-ethyl)	Integrated control system (flufenacet + + diflufenican + + pre-emergence boron application + + agronomic measures)
Shannon index, units	2.42	1.31	1.58	2.15	2.28
Number of dominant species, pcs	3	2	2	3	3
Number of co-dominants, pcs	5	3	4	5	6
Coverage of the dominant species, %	18.4	36.7	30.9	21.3	19.1
Total coverage of three leading types, %	44.2	72.8	66.1	49.5	45.8
Average abundance on the Braun-Blanquet scale, points	2.6	3.8	3.4	+2.7	2.5
Number of rare species, items	6	1	2	4	5

Source: developed by the authors of this study

Consequently, the coenotic organisation of the weed community responded to herbicide control systems not only by altering the growth rate of individual species, but also by restructuring the entire pattern of internal role distribution amongst dominant, co-dominant and rare components. Under integrated control, the contribution of species to canopy formation remained more balanced, which supported a more complex, multi-component structure of the community and preserved its spatial mosaic pattern. Under prolonged, uniform pressure, by contrast, the concentration of the vegetation cover in a few dominant species increased, the range of co-dominant species narrowed, and the representation of the peripheral part of the flora decreased. As a result, the weed community gradually shifted from a branched, multi-component organisation to a more compact system with a more clearly defined core. At the same time, the community's capacity for internal restructuring was reduced, its structural flexibility was weakened, and its potential for further self-recovery in response to changes in environmental conditions was diminished.

Quantitative development and spatial heterogeneity of the segetal flora varied significantly depending on the herbicide control system. The highest values of the main quantitative indicators were recorded in the control plot, where weed density was 128 plants/m^2, fresh weight was 486 g/m^2, dry weight was 148 g/m^2, projective cover was 62%, and the height of the weed canopy was 42.3 cm. This corresponded to the most fully developed weed cover, under which the segetal flora formed the most robust above-ground canopy and the highest spatial coverage of the crop. Under all herbicide control systems, these indicators decreased, although the extent of this reduction varied. The lowest values of the quantitative parameters were obtained under the integrated control system, where weed density decreased to 36 plants/m^2, fresh weight to 142 g/m^2, dry weight to 47 g/m^2, and projective cover to 18%. Herbicide rotation also significantly curbed the development of the weed component: density was 41 plants/m^2, fresh weight 158 g/m^2, dry weight 52 g/m^2, and projective cover 21%. Consequently, the integrated system and crop rotation were the most effective at reducing the quantitative development of the weed flora. The integrated approach was slightly more effective than crop rotation, indicating a greater suppression of both weed abundance and the development of their above-ground biomass.

Prolonged use of herbicides of the same type also reduced the number and biomass of weeds, but these changes were accompanied by other spatial effects. With the acetolactate synthase inhibitor, weed density was 54 plants/m^2, whilst with synthetic auxins it was 63 plants/m^2; correspondingly, the values for fresh weight, dry weight and cover remained higher. Thus, both schemes of the same type were inferior to the rotation and integrated systems in terms of their ability to limit the quantitative development of the weed community, and the synthetic auxin treatment proved less effective than the acetolactate synthase inhibitor. Spatial indicators revealed a different pattern. In the control treatment, the Morishita aggregation index was 1.28, and the coefficient of variation in density was 24.1%, which corresponded to a relatively more uniform distribution of weeds amidst high overall weed infestation. With prolonged application of the acetolactate synthase inhibitor, these indices increased to 1.74 and 41.3%, and with synthetic auxins – to 1.68 and 37.8%. This indicated that herbicides of the same type not only reduced the total number of weeds but also increased their aggregation and uneven distribution. Under the herbicide rotation and integrated management systems, the aggregation index and coefficient of variation remained lower, indicating a reduced formation of localised clusters of weed density. Thus, the control plot was characterised by the highest values for weed density, mass, cover and height; the integrated system and crop rotation were most effective at reducing these quantitative indicators, whereas the prolonged use of a single type of herbicide, despite lower weed abundance, was accompanied by greater aggregation and greater spatial heterogeneity of the weed community (Table 3).

Table 3. *Quantitative and spatial parameters of weed group*

Indicator	Control (no treatment)	Long-term use of an ALS inhibitor (mesosulfuron-methyl + + iodosulfuron-methyl-sodium)	Long-term use of synthetic auxins (dicamba + + MCPA)	Herbicide rotation (prosulfocarb → clodinafop-propargyl + + carfentrazone-ethyl)	Integrated control system (flufenacet + + diflufenican + pre-emergence boron application + + agronomic measures)
Weed density, plants/m^2	128	54	63	41	36
Fresh weight of weeds, g/m^2	486	214	246	158	142

***Table 3.** Continued*

Indicator	Control (no treatment)	Long-term use of an ALS inhibitor (mesosulfuron-methyl + + iodosulfuron-methyl-sodium)	Long-term use of synthetic auxins (dicamba + + MCPA)	Herbicide rotation (prosulfocarb → clodinafop-propargyl + + carfentrazone-ethyl)	Integrated control system (flufenacet + + diflufenican + pre-emergence boron application + + agronomic measures)
Dry weight of weeds, g/m^2	148	73	81	52	47
Projected weed cover, %	62	28	34	21	18
Height of the weed layer, cm	42.3	31.2	34.1	26.4	24.8
Morishita's Aggregation Index, unit	1.28	1.74	1.68	1.39	1.33
Coefficient of variation in density, %	24.1	41.3	37.8	29.4	27.2

Source: compiled by the authors

Comparison of quantitative and spatial parameters showed that the segetal flora responded to the control systems as a multi-level structure, in which a reduction in abundance was not always accompanied by the same pattern of plant distribution. Integrated control limited overall density and prevented the formation of a continuous cover, whereas prolonged, uniform pressure could combine lower abundance with increased aggregation and greater variability between plots. Consequently, quantitative development and spatial heterogeneity emerged as interrelated characteristics, reflecting the transition of the segetal flora from a more continuous coverage of the sown area to a more localised distribution, with plants concentrated in zones suitable for the survival of dominant and tolerant forms.

Biological and ecological characteristics of the dominant species varied depending on the herbicide control system and most clearly reflected the effects of prolonged exposure to a single type of chemical. In the control plot, there were 18 annual species and 6 perennial species, which corresponded to a broader mix of life forms within the dominant community. With prolonged application of an acetolactate synthase inhibitor, the number of annual species decreased to 9 and that of perennial species to 4; with synthetic auxins, these figures were 10 and 5, respectively. Under herbicide rotation, the number of annual species was 14 and that of perennial species 6, whilst under the integrated system these figures were 15 and 7. Thus, herbicide rotation and the integrated control system maintained a ratio of life forms closer to that of the control, whilst the use of a single type of herbicide caused a more pronounced shift in the structure of dominant species. A similar difference was observed in the average root system depth. In the control, it was 16.4 cm; under herbicide rotation, 18.7 cm; under the integrated system, 17.9 cm; whereas under the acetolactate synthase inhibitor it increased to 24.8 cm, and under synthetic auxins to 22.9 cm. A similar pattern was observed for the average leaf area of a single plant: in the control – 29.6 cm^2, with crop rotation – 31.5 cm^2, with the integrated system – 30.4 cm^2, with synthetic auxins – 37.8 cm^2, and with the acetolactate synthase inhibitor – 41.3 cm^2. This indicated that herbicide regimes of the same type selected more strongly for plants with a more developed root system and a larger leaf area, whereas under crop rotation and integrated pest management, these traits showed less variation.

The same trend was observed in terms of reproductive performance. The weight of 1,000 seeds in the control treatment was 1.84 g, in the herbicide rotation treatment 2.03 g, in the integrated system treatment 1.96 g, in the synthetic auxin treatment 2.51 g, and in the acetolactate synthase inhibitor treatment 2.76 g. Seed yield per plant in the control treatment was 5,620 seeds/plant, under the rotation treatment – 4,980 seeds/ plant, under the integrated system – 4,630 seeds/plant, under synthetic auxins – 6,810 seeds/plant, and under the acetolactate synthase inhibitor – 7,340 seeds/plant. The results showed that the acetolactate synthase inhibitor and synthetic auxins formed a dominant group with higher seed weight and greater seed yield, whilst the rotation and integrated system limited the enhancement of these traits. The most pronounced shift was confirmed by the number of herbicide-tolerant species. In the control treatment, there were 3; under herbicide rotation, 4; under the integrated system, 3; under synthetic auxins, 7; and under the acetolactate synthase inhibitor, 8. This indicated that herbicide regimes of the same type caused the most pronounced shift in the biological and ecological characteristics of the dominant species: fewer short-lived species, a deeper root system, greater leaf area, higher seed mass, greater seed productivity and a higher proportion of tolerant forms. Herbicide rotation and the integrated management system kept these indicators closer to the control levels, indicating a weaker enhancement of adaptive traits in the dominant segment of the segetal flora (Table 4).

Table 4. *Biological and ecological characteristics of the dominant species of the segetal flora*

Indicator	Control (no treatment)	Long-term use of an ALS inhibitor (mesosulfuron-methyl + + iodosulfuron-methyl-sodium)	Long-term use of synthetic auxins (dicamba + + MCPA)	Herbicide rotation (prosulfocarb → clodinafop-propargyl + + carfentrazone-ethyl)	Integrated control system (flufenacet + + diflufenican + pre-emergence boron application + + agronomic measures)
Number of juvenile specimens, pcs.	18	9	10	14	15
Number of perennial species, pcs.	6	4	5	6	7
Average depth of the root system of dominant trees, cm	16.4	24.8	22.9	18.7	17.9
Average leaf area of one plant, cm^2	29.6	41.3	37.8	31.5	30.4
Mass of 1,000 dominant seeds, g	1.84	2.76	2.51	2.03	1.96
Seed yield per plant, seeds/plant	5620	7340	6810	4980	4630
Number of herbicide-tolerant species, pcs.	3	8	7	4	3

Source: compiled by the authors based on formula (8)

Consequently, the biological and ecological characteristics of the dominant species shifted towards the formation of a new adaptive core of the segetal flora. The decline in the proportion of short-lived forms was accompanied by an increase in the role of species capable of persisting longer within the agrocenosis and utilising the resources of the soil profile more efficiently. The increased participation of deeper-rooted plants indicated a shift in the dominance structure towards components less dependent on short-term fluctuations in conditions within the surface soil layer. The increase in seed mass reflected a change in reproductive strategy, whereby population recovery increasingly relied on diaspores with higher initial resource endowment. The accumulation of herbicide-tolerant species demonstrated that prolonged selection altered not only the species composition of the weed community but also the very mechanism by which dominant species were maintained in the crop. Under these conditions, herbicide pressure determined not only the current level of suppression of the segetal flora, but also the morphobiological traits of those components that subsequently sustained the dominance, generative renewal and stability of the weed community within the agrocenosis.

Functional indicators of agroecosystem stability revealed clear differences between the herbicide control systems and showed that the segetal flora influenced the crop not only through its aboveground presence, but also through seed reserves, resource extraction and yield losses. The highest level of functional load was observed in the control plot, where the soil seed bank density was 42.8 thousand seeds/m^2, nitrogen leaching was 31.5 kg/ha, phosphorus leaching was 8.7 kg/ha, and potassium leaching was 37.2 kg/ha, a reduction in productive moisture reserves of 18.6 mm, and crop yield losses of 27.4%. This combination of indicators suggested that, in the absence of herbicide control, the weed community retained not only the largest reproductive reserve but also the highest capacity to compete for nutrients and moisture, directly translating this pressure into a reduction in crop productivity. The lowest values for most functional indicators were recorded under the integrated control system. Under this treatment, the density of the soil seed bank decreased to 21.7 thousand seeds/m^2, nitrogen removal to 9.6 kg/ha, phosphorus removal to 2.6 kg/ha, potassium removal to 11.5 kg/ha, a reduction in available moisture reserves to 5.7 mm, and yield losses to 6.9%. Herbicide rotation yielded similar results: 24.3 thousand seeds/m^2 for the seed bank, 11.2 kg/ha for nitrogen, 3 kg/ha for phosphorus, 13.1 kg/ha for potassium, 6.3 mm for moisture loss and 8.1% for yield loss. This demonstrated that an integrated approach and crop rotation were most effective in limiting the functional reserve of the weed flora, reducing its contribution to the depletion of soil resources and mitigating its impact on crop yield.

Prolonged use of herbicides of the same type also reduced weed pressure compared with the control but was less effective than crop rotation and integrated weed management. With the acetolactate synthase inhibitor, the soil seed bank density was 31.6 thousand seeds/m^2, whilst with synthetic auxins it was 34.9 thousand seeds/m^2; in other words, the latent reproductive reserve remained significantly higher than under the other two systems. Resource removal indicators showed a similar pattern: nitrogen removal was 16.4 and 18.8 kg/ha, phosphorus removal was 4.3 and 5.1 kg/ha, and potassium removal was 19.6 and 22.4 kg/ha, respectively. The reduction in productive moisture reserves under these treatments was 9.2 and 10.4 mm, whilst yield losses were 12.8 and 14.3%. Thus, single-type control schemes reduced the functional pressure of weeds compared with the control but did not provide the same level of suppression as crop rotation and the integrated system. Field germination of seeds from the bank was highest under single-type herbicide treatment: 46.8% for the acetolactate synthase inhibitor and 44.1% for synthetic auxins, whereas in the control it was 39.4%, under rotation – 35.2%, and 33.6% under the integrated system. This meant that, despite lower current weed infestation, prolonged application of the same type of herbicide in the soil preserved a functionally more active seed reserve, capable of transitioning more rapidly to a new wave of regrowth. Overall, control treatment was associated with the highest functional load, the integrated system with the lowest, and herbicide rotation was similar to the latter, whilst herbicides of the same type occupied an intermediate position, combining lower weed pressure with higher seed germination rates and a greater latent reserve of seedling flora (Table 5).

Table 5. *Functional indicators of agroecosystem stability under varying herbicide loads*

Indicator	Control (no treatment)	Long-term use of an ALS inhibitor (mesosulfuron-methyl + + iodosulfuron-methyl-sodium)	Long-term use of synthetic auxins (dicamba + + MCPA)	Herbicide rotation (prosulfocarb → clodinafop-propargyl + + carfentrazone-ethyl)	Integrated control system (flufenacet + + diflufenican + pre-emergence boron application + + agronomic measures)
Density of the soil seed bank, thousand seeds/m^2	42.8	31.6	34.9	24.3	21.7
Field germination rate of seeds from a jar, %	39.4	46.8	44.1	35.2	33.6
Nitrogen uptake by weeds, kg/ha	31.5	16.4	18.8	11.2	+9.6
Phosphorus uptake by weeds, kg/ha	8.7	4.3	5.1	3	2.6
Potassium uptake by weeds, kg/ha	37.2	19.6	22.4	13.1	11.5
Decrease in productive moisture reserves, mm	18.6	9.2	10.4	6.3	5.7
Loss of crop yield, %	27.4	12.8	14.3	8.1	6.9

Source: compiled by the authors

A comprehensive analysis of the reproductive reserve, seed dispersal, resource extraction and yield losses revealed that the stability of the agroecosystem was shaped by the interplay of several interrelated processes. The segetal flora influenced this stability through its ability to store a latent seed reserve, rapidly return to an active state, interfere with the crop's trophic regime, and transform this activity into yield losses. Under prolonged, uniform herbicidal pressure, some of these mechanisms did not disappear but took on a new form, related to the selection of more tolerant components and the maintenance of high field germination rates within the soil seed bank. When several control methods were combined, the functional pressure exerted by the weed flora was reduced at various levels simultaneously, which limited not only existing weed infestation but also the possibility of its recurrence. Consequently, the functional indicators proved suitable for assessing the stability of the agroecosystem as a process dependent on the weed complex's ability to interfere with regeneration, resource exchange and crop yield realisation.

Discussion

Results showed that different herbicide control systems altered the segetal flora not only in terms of overall weediness, but also in terms of its floristic, coenotic, spatial and functional organisation. Under prolonged exposure to a single type of chemical treatment, a narrowing of the species and taxonomic composition was observed, along with an increased role of a few dominant components and a reduction in the presence of rare species. This restructuring indicated a transition of the weed community towards a more compact model with less internal mosaicism and more limited capacity for self-regulation. At the same time, the integrated control system maintained a more complex structure of the segetal flora, in which the contribution of species to canopy formation remained more balanced. This was accompanied by a reduction in the compactness of the weed layer, a decrease in localised areas of dense growth, and a reduction in the functional load of the weed component on the agrocenosis. Consequently, it was not only the reduction in weed abundance that was significant, but also the nature of their spatial distribution and their participation in resource processes.

In the context of long-term management of weed communities, the studies by J.G. Guerra *et al.* (2022) and A.S. Westbrook *et al.* (2022) examined changes in the composition of plant communities under the influence of various weed control methods, but without revealing the full structural and functional profile of the segetal flora in the cereal agrocenosis. J.G. Guerra *et al.* demonstrated that weed management practices altered species composition, taxonomic diversity and yield, whilst the proportion of bare soil was associated with both yield and a reduction in species richness. A.S. Westbrook *et al.* linked living mulch systems to varying degrees of weed suppression and changes in community composition. Similar to these studies, in the present research the control system also determined changes in the floristic structure and the nature of dominance. At the same time, this study additionally demonstrated how prolonged, single-type herbicidal pressure narrowed the taxonomic basis of the flora, reduced the proportion of rare and co-dominant species, and restructured the spatial organisation of the community, whereas the integrated approach maintained a more complex mosaic structure. This multi-level interpretation extended the scope outside a general assessment of community composition and correlated changes in weed flora to the stability of cereal crops.

In the context of the dependence of agrosystem stability on the composition of plant communities, the studies by X. Rotllan-Puig *et al.* (2024) and K.A. Stahlheber & K.L. Gross (2025) focused on the role of community composition, dominant species and large-scale observations, but without a field-level analysis of the coenotic mechanisms underlying the restructuring of the semental flora under herbicide pressure. K.A. Stahlheber & K.L. Gross linked the stability of aboveground production primarily to community composition and dominant species, rather than solely to the number of sown species. X. Rotllan-Puig *et al.* used open-access data on weed occurrence in European Union countries as an indicator of agricultural intensification. This study also confirmed that community structure and the role of leading species were decisive for the state of the agrocenosis. At the same time, the results were analysed in much greater depth, as the study tracked not only the overall composition of the flora but also changes in co-dominance, the contribution of rare species, spatial aggregation, the coefficient of variation in density, and the biological and ecological characteristics of the dominant species. This level of detail demonstrated which elements of the weed community were the first to respond to various herbicide control systems and how these changes restructured the entire agroecosystem.

Regarding effects of chemical stress on the soil-plant system, the studies by Q. Li *et al.* (2025) and M. Gaylord *et al.* (2025) analysed the microbial and yield effects of weed control, but without a detailed description of how the sclerotial flora itself changed in the process. Q. Li *et al.* identified changes in arbuscular mycorrhizal fungal communities and increased wheat yields following long-term weed control. M. Gaylord *et al.* concluded that herbicides had minimal and variable effects on the structure and function of bacterial communities in agricultural soils. In the present study, as in these works, the impact of herbicides was considered not only in terms of weed infestation levels but also in terms of their broader functional consequences. The difference consisted in the fact that, in this study, the functional aspect was directly linked to indicators of the segetal flora – the soil seed bank, field germination, nitrogen leaching and yield losses. This made it possible to demonstrate a sequential transition from changes in the floristic, coenotic and spatial structure of the weed community to changes in the resource regime of the crop, seed regeneration and crop productivity.

In relation to the interplay between nutrient supply, soil processes and community structure, the studies by M. Karlsson *et al.* (2025) and Y. Guo *et al.* (2025) examined the effects of resource environment management on vegetation cover and soil biota, but without directly linking these changes to the coenotic organisation of the segetal flora of cereal crops. M. Karlsson *et al.* demonstrated that improved nutrient supply in organic farming was accompanied by a trade-off between yield and weed diversity, and that the response of the community was determined not only by fertilisation but also by the intensity of competition between the crop and weeds. Y. Guo *et al.* found that soil depth and fertilisation regimes altered the composition of fungal communities, with the most pronounced shifts occurring between the upper and lower layers and across different fertilisation treatments. This study also revealed a link between the resource regime of the agrocenosis and the structure of its biotic component. At the same time, the results were presented in a broader context, as changes in floristic composition, dominance, spatial heterogeneity, seed reserve and yield losses were analysed within a single system of indicators, which made it possible to directly link the restructuring of the segetal flora to the functional stability of the crop.

In terms of the interplay between nutrition, soil management and weed phytosociology, the studies by M. Esposito *et al.* (2023) and S. Kumar *et al.* (2022) addressed how agronomic factors altered the composition of weed communities and crop productivity; however, these studies did not provide a detailed analysis of the transition from floristic changes to functional consequences within a single field experiment. M. Esposito *et al.* demonstrated that the nutrient regime in rain-fed winter wheat differentiated weed communities, with excessive fertilisation reducing species richness and promoting the formation of more competitive assemblages, whilst optimal or reduced nutrient supply supported communities that were less harmful to the crop. S. Kumar *et al.* found that prolonged tillage and weed control systems altered the phytocenotic structure, caused a shift towards an increased role of monocotyledonous species, and affected the productivity of the crop rotation system. In the present study, management pressure was accompanied by a restructuring of the flora, a shift in dominance and consequences for productivity. At the same time, these processes were traced across a wider range of levels – from taxonomic narrowing and co-dominance to aggregation, field seed similarity, nitrogen removal and yield losses – which made the causal link between weed community structure and agroecosystem stability more comprehensive.

Beyond the weed layer, the studies by J. Tavella *et al.* (2025) and M. Aguiar *et al.* (2023) analysed the broader ecological consequences of agricultural management for adjacent plant, pollinator and soil systems, but without directly examining the internal restructuring of the segetal flora within the crop field. J. Tavella *et al.* demonstrated that herbicide programmes altered plant and pollinator diversity in non-ploughed areas and indirectly disrupted the structure and potential functioning of "plant-pollinator" networks, with more intensive programmes having a more pronounced effect. M. Aguiar *et al.* found that the edge zones between perennial grasslands and arable land formed a distinct plant community with a high proportion of undesirable annual species and were accompanied by changes in carbon, nitrogen and soil microbial communities. A common feature of this study was that agricultural pressure was considered a factor that altered not only weed abundance but also the broader ecological context. However, in this study, the analysis focused directly on the field weed community; consequently, it was possible to demonstrate how changes in species composition, mosaic pattern, dominance and reproductive reserve translated into changes in resource flows and the level of yield loss – aspects not covered by the approaches of J. Tavella *et al.* and M. Aguiar *et al.*

Concerning species selection under herbicide and soil stress, the studies by Z. Peng *et al.* (2024) and S. Humann-Guilleminot *et al.* (2025) examined the consequences of long-term agricultural impact on plant and soil communities, but without linking these changes to a complete coenotic characterisation of the segetal flora of cereal crops. S. Humann-Guilleminot *et al.* demonstrated that herbicides in cereal crops eliminated non-competitive species whilst simultaneously facilitating their replacement by more competitive weeds. Z. Peng *et al.* found that tillage and fertilisation altered arbuscular mycorrhizae, crop yield and soil functions, linking agricultural management to broader ecosystem consequences. This study found that prolonged, uniform pressure not only reduced overall diversity but also contributed to the establishment of a limited set of more resilient dominant species. At the same time, the results were interpreted more comprehensively, as the sequence of events was traced from taxonomic narrowing and shifts in dominance to changes in spatial heterogeneity, the biological and ecological characteristics of dominant species, seed reserves and yield losses.

Regarding soil resource availability and the restructuring of weed communities, the studies by M.A. Gannett *et al.* (2024) and E. Radicetti *et al.* (2025) addressed how soil conditions, fertilisation and tillage altered weed growth and community composition, but without presenting a comprehensive picture of floristic, spatial and functional shifts within a single experiment. M.A. Gannett *et al.* demonstrated that increasing the C:N ratio reduced weed growth, altered soil microbial parameters and, in soya, influenced the composition of the weed community. E. Radicetti *et al.* examined how fertiliser sources and tillage regimes affected the species composition and functional diversity of weeds in a durum wheat-potato crop rotation. The study also demonstrated that management of the agrocenosis altered not only weed abundance but also their structural organisation and functional load. The distinction lay in the fact that this study simultaneously covered floristic richness, co-dominance, aggregation, the depth of the root system of dominant species, seed mass, the soil seed bank and resource extraction; this made it possible to explain not merely individual responses of weeds, but the holistic mechanism underlying changes in the stability of the agroecosystem.

In the context of the seed bank and climate-driven variability in weed communities, the studies by T. Seipel *et al.* (2022) and Z. Ren *et al.* (2024) analysed either spatial differences in seed bank composition or the responses of weed communities to farming systems under different weather conditions, but without simultaneously combining these factors within a single field model. Z. Ren *et al.* found that region, cropping system and control strategy influenced species richness, functional traits and seed bank composition in herbicide-resistant agrosystems in the United States. T. Seipel *et al.* demonstrated that the farming system remained the primary factor shaping weed communities in winter wheat, whilst warming and reduced moisture levels altered these responses less markedly in the short term. In this study, these lines of research were integrated within a single approach: changes in floristic structure, dominance, spatial distribution, biological and ecological traits, and the seed bank were examined alongside nitrogen removal and yield losses. This demonstrated not only that the segetal flora responded to the control system, but also how this response translated into changes in the reproductive reserve, resource regime and productivity of the cereal crop. Thus, the results showed that this study provided a more accurate insight into the relationship between prolonged herbicide exposure, changes in the segetal flora and the functional manifestations of agroecosystem stability, as it combined floristic, coenotic, spatial, biological-ecological and productivity indicators within a single field analysis.

Conclusions

The study compared five weed control systems and found that the nature of the herbicidal load determined not only the level of weed infestation but also the direction of change in the segetal flora of the cereal agrocenosis. In the control treatment, the total number of species was 24, the number of families was 10, the number of genera was 20, the average number of species per 1 m^2 was 8.6, the number of monocotyledonous species was 7, the number of dicotyledonous species was 17, and the floristic richness index was 2.4. Under prolonged, single-type herbicidal pressure, the floristic structure became narrower, whereas crop rotation and the integrated system better preserved the species and taxonomic framework of the community. Coenotic indicators confirmed that herbicidal pressure altered the internal organisation of the weed layer. In the control plot, the Shannon index was 2.42, the number of dominant species was 3, the number of co-dominant species was 5, the cover of the dominant species was 18.4%, the total cover of the three leading species was 44.2%, the average abundance on the Braun-Blanquet scale was 2.6 points, and the number of rare species was 6. With prolonged, single-type application of herbicides, the dominance of a few species increased, and the proportion of rare species decreased, whilst integrated control maintained a more complex community structure. Quantitative and spatial parameters also reflected a marked differentiation between the control systems. In the control plot, weed density was 128 plants/m^2, fresh weight was 486 g/m^2, dry weight was 148 g/m^2, projective cover was 62%, weed canopy height was 42.3 cm, the Morishita aggregation index was 1.28, and the coefficient of variation in density was 24.1%. Under the integrated system, the quantitative weed pressure decreased, whereas under prolonged, single-type chemical treatment, the spatial concentration of plants and the heterogeneity of their distribution increased. The biological and ecological characteristics of the dominant species showed that herbicidal selection altered the adaptive profile of the leading species. In the control, there were 18 annual species and 6 perennial species; the average root system depth of the dominant species was 16.4 cm, the leaf area of a single plant was 29.6 cm^2, the mass of 1,000 seeds was 1.84 g, the seed production per plant was 5,620 seeds per plant, and the number of herbicide-tolerant species was 3. Under prolonged, uniform stress, the role of forms with deeper root systems, greater seed mass and higher tolerance increased. Functional indicators confirmed that the segetal flora influenced the crop through reproductive reserve and resource competition. In the control, the density of the soil seed bank was 42.8 thousand seeds/m^2, field germination was 39.4%, nitrogen removal was 31.5 kg/ha, phosphorus removal was 8.7 kg/ha, potassium removal was 37.2 kg/ha, a reduction in productive moisture reserves of 18.6 mm, and a loss in crop yield of 27.4%. A limitation of the study was that observations were conducted at a single agroecological site and over the course of a single growing season. Further research should conduct a multi-year analysis under various soil and climatic conditions, with an expanded list of crops and indicators.

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Conflict of Interest

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Фітоценотичний аналіз сегетальної флори як індикатор сталості агроекосистем при тривалому застосуванні гербіцидів

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Анотація. Метою дослідження було встановити закономірності перебудови сегетальної флори та змін функціональної стійкості зернового агроценозу під впливом різних систем гербіцидного контролю. У дослідженні було використано метод квадратних обліків, геоботанічного опису, біометричних і вагових вимірювань, морфолого-біологічного групування, вегетаційного тестування, ґрунтово-хімічного аналізу, поділянкового обліку врожаю та дисперсійного аналізу. Встановлено, що у контролі загальна кількість видів становила 24, кількість родин – 10, середня кількість видів на 1 м^2 – 8,6, а кількість дводольних видів – 17. За інтегрованої системи вони становили 22, 9, 7,8 і 16, тобто залишалися ближчими до контролю. Індекс Шеннона змінювався від 2,42 у контролі до 1,31 за тривалого застосування інгібітора ацетолактатсинтази, тоді як за інтегрованої системи дорівнював 2,28. Покриття виду-домінанта зростало до 36.7 %, а кількість рідкісних видів зменшувалася до 1, що вказувало на посилення домінування і звуження резервного фонду угруповання. Кількісні показники також істотно змінювалися: щільність бур'янів знижувалася зі 128 до 36 шт./м^2, сира маса – з 486 до 142 г/м^2, суха маса – з 148 до 47 г/м^2, проективне покриття – з 62 до 18 %. За тривалого застосування інгібітора ацетолактатсинтази індекс агрегованості Морісіти досягав 1,74, кількість гербіцидотолерантних видів – 8, середня глибина кореневої системи домінантів – 24,8 см, маса 1000 насінин – 2,76 г. Функціональні показники підтвердили цю закономірність: щільність ґрунтового банку насіння зменшувалася з 42,8 до 21,7 тис. шт./м^2, винос азоту бур'янами – з 31,5 до 9,6 кг/га, втрати врожайності культури – з 27,4 до 6,9 %. Практична значимість дослідження полягала в тому, що його результати можуть бути використані агрономами, науковцями та виробниками зерна для вибору систем контролю бур'янів, які не лише знижують забур'яненість, а й сприяють збереженню структурної стійкості агроекосистеми

Ключові слова: сегетальна флора; гербіциди; домінантні види; гербіцидотолерантні види; агроценоз; системи гербіцидного контролю; флористична структура

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Determining the effect of different aeration regimes on technological parameters of waste fermentation

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Abstract. The purpose of the study was to determine the optimal frequency of mechanical mixing of the substrate to intensify biothermal processes, stimulate microbiological activity, and increase the efficiency of bioconversion of organic matter. The study was performed under the conditions of an experimental farm using organic waste of plant origin and the biodestructor “Unikal-S”. The effect of aeration on the temperature regime and changes in moisture content of the compost mass was analysed during 150 days of fermentation. Different mechanical mixing regimes were used: once every 3, 5, 7, and 10 days. Compost temperature was monitored daily, and moisture content was determined once every 5 days according to applicable standards. The results demonstrate that regular aeration contributed to a more intensive course of biothermal processes in soybean waste and spoiled corn silage. The highest temperature values were recorded in the variants with mechanical mixing once every 3 days. The temperature reached 62°C in soybean waste and 66°C in spoiled corn silage, indicating active development of thermophilic microflora and intensive decomposition of organic matter. More frequent mixing ensured longer maintenance of the thermophilic phase of fermentation and contributed to a more effective decrease in substrate moisture. Aeration once every 3-5 days provided the best technological parameters of fermentation and intensified bioconversion of organic waste. The

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results can be used to improve plant waste composting technologies and increase the efficiency of fermentation processes in agriculture

Keywords: aeration; composting; soybean waste; biodestructor; compost temperature; moisture; bioconversion

Introduction

Rational utilisation of organic waste from agro-industrial production is one of the priority areas of modern biotechnology. Every year, agriculture generates substantial volumes of plant residues, feed waste, and by-products from the processing of plant raw materials. If stored improperly, these materials may become a source of environmental pollution, development of pathogenic microflora, and loss of organic matter. Fermentation and composting are among the most effective methods for processing such waste, since they ensure bioconversion of organic matter into a stabilised substrate suitable for use as organic fertiliser (Sánchez-Monedero *et al.*, 2018; Nasir *et al.*, 2025).

Particular attention is given to soybean waste and spoiled corn silage, as they accumulate in large quantities on farms in the agricultural sector. Soybean waste contains stems, leaves, pods, and other plant components rich in organic matter, cellulose, and hemicellulose. According to A. Asghar *et al.* (2022), waste from soybean processing is characterised by diverse microbiota and a considerable nutrient content, which makes it a promising substrate for fermentation processes. The researchers also noted the possibility of effective bioconversion of soybean waste with the involvement of microorganisms. The growing interest in the use of soybean processing by-products is confirmed by the review by M. Usman *et al.* (2025), which states that soybean waste is a valuable source of organic matter and can be used to produce bioproducts, organic fertilisers, and composts. Spoiled corn silage is also considered a promising raw material for fermentation because of its high content of organic matter and carbohydrates. When fodder preparation and storage technology is disrupted, the microbial composition changes and feed value decreases, which requires effective methods of utilisation. The paper of L.V. Mitiohlo *et al.* (2023a) indicates that composting spoiled corn silage converts waste into a valuable organic fertiliser and reduces the environmental burden.

D.M. Costa *et al.* (2023) established that the intensity of fermentation processes in plant raw material depends largely on the physicochemical properties of the substrate, its moisture content, degree of grinding, and storage duration. For corn silage, material structure and fermentation conditions are important factors, as they determine microbial activity and the rate of organic matter decomposition. According to M. Atauzzaman & Q.H. Bari (2023), the efficiency of composting organic waste depends largely on temperature, moisture, medium reaction, oxygen availability, and aeration intensity. The researchers established that forced or regular aeration activates aerobic microflora, accelerates biodegradation of organic matter, and supports a stable thermophilic phase of composting. During fermentation of organic waste, the mesophilic, thermophilic, and compost maturation stages follow one another. The thermophilic phase has the greatest importance, as substrate temperature reaches 55-70°C during this period, ensuring intensive decomposition of organic matter and sanitisation of the compost mass. Current research confirms that maintenance of an optimal temperature regime is one of the key factors in obtaining high-quality compost and increasing the efficiency of bioconversion of plant residues.

Biodestructors and microbiological preparations are increasingly used to intensify fermentation processes. L.V. Mitiohlo *et al.* (2023b) established that adding biodestructors during composting of spoiled corn silage prolongs the thermophilic phase, promotes active development of bacteria of the genus Bacillus, and reduces losses of nitrogen and phosphorus. This indicates the prospects of using microbiological preparations to accelerate fermentation and increase the agronomic value of the final product. Despite the large number of scientific publications, optimisation of aeration regimes during fermentation of soybean waste and spoiled corn silage remains insufficiently examined. Identifying the optimal frequency of mechanical mixing of organic matter is particularly relevant since it should maintain high compost temperature, activate microbiological activity, and ensure an optimal decrease in substrate moisture. In this context, the study aimed to determine the effect of different aeration regimes on the technological parameters of fermentation of soybean waste and spoiled corn silage, especially the temperature regime and moisture content during composting. The work also aimed to determine the optimal frequency of mechanical mixing of the substrate to intensify biothermal processes.

Materials and Methods

The effect of aeration regimes on the technological parameters (moisture content and compost temperature) of composting soybean waste and spoiled corn silage was examined in 2025 under the conditions of the experimental farm of the State Enterprise Experimental Farm "Nyva" in Uman District, Cherkasy Oblast. Soybean waste and spoiled corn silage were selected for composting, and four replicates were formed for the control and experimental groups. The mass of each sample was 120 kg. Before composting, the soybean waste and corn silage were mechanically ground to a homogeneous mass with a particle size of 2-5 cm and then thoroughly mixed. Composting was performed separately for each type of organic raw material without mixing them with each other. The initial moisture content of the substrates was 67.2% for soybean waste and 69.5% for spoiled corn silage, which provided favourable conditions for the development of microorganisms and biothermal processes. After substrate preparation, the biodestructor was added to the experimental samples, and mini-heaps were formed from the resulting mass for further composting. The Ukrainian biodestructor "Unikal-S" was added to each organic waste sample at a dose of 60.0 g/t. The preparation is a powdered biopreparation based on a complex of effective microorganisms and enzymes. Its active components are *Bacillus subtilis* and *Bacillus licheniformis*, which are characterised by high destructive activity towards organic matter. Before application, the preparation was activated in water and evenly applied to the organic substrate. Mini-heaps for composting were then formed from the prepared samples. The prepared samples were formed into mini-heaps of the same cylindrical shape, with a volume of about 0.10 m^3 (height – 0.50 m; base diameter – 0.50 m). The mini-heaps were placed on a concrete platform under a canopy, which protected them from direct precipitation and excessive solar radiation. The surface of the heaps was covered with polyethylene film to preserve moisture, stabilise the temperature regime, and minimise heat loss. The mini-heaps were arranged on the platform by randomisation to exclude the possible effect of microclimatic differences between individual areas on the composting process. Throughout the full research period (150 days), environmental parameters were monitored, including air temperature and relative humidity. The average air temperature was 18.5 ± 4.7°C (range 10.0-28.0°C), and relative humidity – 68.0 ± 9.0% (range 50-85%). The monitoring of these indicators was used to account for the effect of external factors on the intensity of biothermal processes and ensured comparability of composting conditions across all experimental variants. In the control, each sample of both soybean waste and spoiled corn silage was mechanically mixed once every 10 days (Table 1).

Table 1. *Scheme for examining the effect of aeration regimes on fermentation indicators of organic waste, n = 4*

Sample group	Aeration method	Frequency of operation
control	Mechanical mixing	Once every 10 days
experimental I	Mechanical mixing	Once every 3 days
experimental II	Mechanical mixing	Once every 5 days
experimental III	Mechanical mixing	Once every 7 days

Source: compiled by the authors

Compost mass temperature was monitored daily using an electronic probe thermometer that meets the requirements of DSTU OIML R 133:2019 (2019), with a measurement range from -50 to +150°C and an error not exceeding ±0.5°C. Measurements were taken in the central part of each mini-heap at a depth of 20-25 cm from the substrate surface, which corresponded to the zone of the most intensive biothermal processes. Temperature was determined at three points in each heap (in the central part and at two diametrically opposite points) to increase the representativeness of the results, after which the mean value was calculated for each sample. Moisture content in the compost was determined according to ISO 6496:1999 (1999) once every 5 days.

Results and Discussion

The results indicate that the use of the biodestructor in combination with different aeration regimes considerably influenced the temperature regime during fermentation of soybean waste. At the beginning of the experiment, on day 1, the temperature was identical in all groups and was 18°C, indicating equal initial fermentation conditions (Fig. 1).

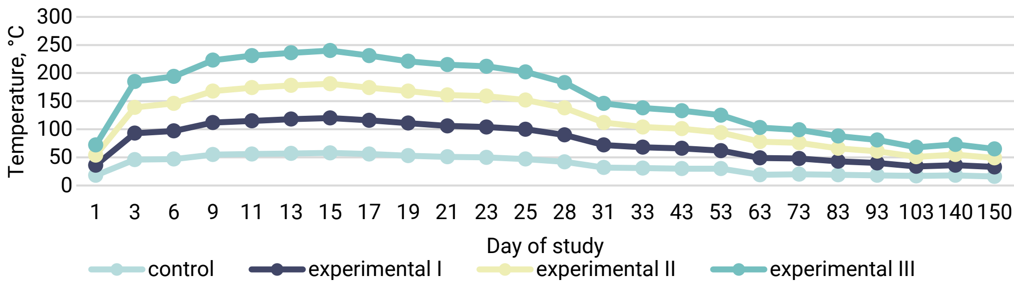

Figure 1. *Temperature of soybean waste under different aeration regimes*

Source: compiled by the authors

On day 3, a sharp temperature increase to 46-47°C occurred in all variants. This indicates rapid activation of microbiological processes and the beginning of intensive decomposition of organic matter. At this stage, the highest temperature was recorded in experimental group I, where aeration was performed once every 3 days. In the control, experimental group II, and experimental group III, the temperature of soybean waste was 2.1% lower than in experimental group I. From day 6 to day 15, the phase of the most intensive fermentation was observed. In the control group, the temperature increased from 47 to 58°C, whereas the temperature values in the experimental groups were higher. The maximum temperature was recorded in experimental group I on day 15 of composting. The value was 3.4% and 1.6% higher than in the control and experimental group II, respectively. Under aeration once every 7 days, the temperature difference compared with the control was the smallest and was 1.7%. Therefore, more frequent mixing once every 3 days ensured the most intensive heating of the mass, indicating better oxygen access and more active functioning of aerobic or facultative anaerobic microflora.

After day 15, the temperature gradually decreased, indicating a reduction in the intensity of biochemical processes. On day 17, the temperature in the control was 56°C. During this period, the compost temperature was highest under aeration once every 3 days. The differences compared with the control and experimental group II were 7.1% and 3.4%, respectively. On day 21, the temperature decreased to 51°C in the control group, whereas in experimental groups I and II, it remained 7.8% higher. This indicates that regular aeration contributed to longer maintenance of the thermophilic phase of fermentation. From day 23 to day 33, temperature values continued to decrease. In the control group, the temperature decreased from 50 to 31°C, in experimental group I from 54 to 37°C, in experimental group II – from 55 to 36°C, and in experimental group III – from 53 to 34°C. The slowest cooling occurred in experimental group I, confirming the effectiveness of more frequent mixing for maintaining active fermentation. On day 43, the temperature in the control group was 30°C, whereas in the experimental groups it was higher by 6.7-20.0%. At this stage, the highest temperature was recorded in the variant with the most intensive aeration.

Subsequently, from day 53 to day 93, the temperature gradually approached ambient temperature. On day 63, it was 19°C in the control group, whereas in experimental group I it was 30°C, in experimental group II – 29°C, and in experimental group III – 25°C. Even on day 73, the experimental variants maintained higher temperatures than the control value of 20°C. This indicates a longer course of fermentation processes under regular aeration. On day 103, the temperature in all groups equalised and was 17°C, indicating completion of the active fermentation phase. Later, on days 140 and 150, the temperature remained low, at 16-19°C, which corresponded to the final phase of organic matter stabilisation.

Thus, the most effective aeration regime was recorded in experimental group I, where mechanical mixing was performed once every 3 days. In this variant, the highest temperature value, 62°C, and prolonged maintenance of elevated temperature during fermentation were observed. Experimental group II, with mixing once every 5 days, also demonstrated high results close to those of experimental group I. Mixing once every 7 days was less effective, although it still ensured a better temperature regime than the control. The control group, where mixing was performed only once every 10 days, had the lowest temperature values in most observation periods, pointing to a less intensive course of fermentation. Analysis of the results presented in Figure 2 reveals considerable changes in the temperature of spoiled silage depending on fermentation duration and aeration regime. At the beginning of the study, the temperature was the same in all groups at 22°C.

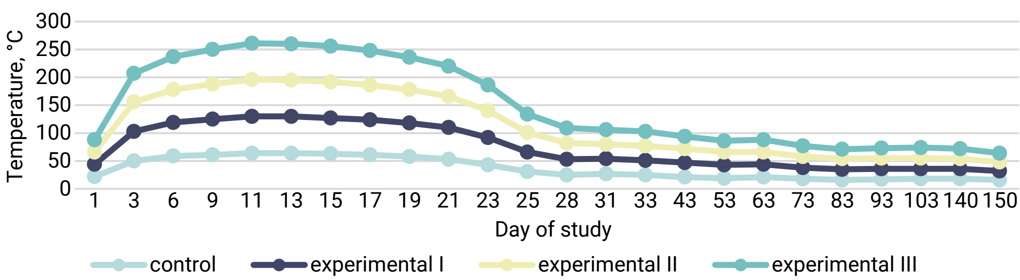

Figure 2. *Temperature of spoiled corn silage under different aeration regimes*

Source: compiled by the authors

On day 3, a sharp temperature increase to 50-53°C was observed in all experimental variants. This temperature rise is explained by the activation of microbiological processes and the intensive decomposition of organic matter in the silage. During the first two weeks, the temperature continued to increase and reached maximum values on days 11-13 of the study. In the control group, the temperature was 64°C. With two mixings per 10 days, the silage temperature in the samples was 1.6% higher than in the control. Similar results were obtained under aeration of waste once every 7 days (experimental group III). Mechanical mixing of spoiled silage once every 3 days increased the temperature by 3.1% compared with the control. Higher temperature values in the experimental variants indicate a more intensive course of biothermal processes under different aeration regimes. Oxygen supply activated aerobic microflora, which accelerated oxidation of organic compounds and was accompanied by the release of a substantial amount of heat.

It is particularly important that the temperature remained within 60-66°C for a rather long period – approximately from day 6 to day 17. This temperature regime is characteristic of the active thermophilic phase of bioconversion of organic raw material. During this period, readily available organic substances are intensively destroyed, the number of pathogenic microorganisms decreases, and partial sanitisation of the substrate occurs. High temperatures were maintained longest in experimental groups I and II, which may indicate optimal aeration conditions in these variants. From day 19, a gradual decrease in temperature was observed in all groups. This is explained by reduced microbial activity due to depletion of readily available nutrients. On day 25, the temperature in the control group decreased to 31°C, whereas in the experimental groups it remained higher by 6.4% and 12.9%. This indicates a longer course of organic matter mineralisation in the aerated variants.

Subsequently, the temperature gradually stabilised and approached ambient temperature. On days 43-63, the temperature in the control group ranged from 19 to 21°C, whereas in the experimental groups it remained 15.7-23.8% higher. During this period, the highest compost temperature remained in the variant in which the substrate was enriched with oxygen most intensively. This indicates continuation of weak microbiological processes in the aerated variants even after completion of the active thermophilic stage. At the end of the experiment, on days 140-150, the temperature in all groups almost equalised and was 16-18°C, indicating completion of the main biological decomposition processes and stabilisation of the substrate. Thus, the results indicate that different aeration regimes promoted more intensive development of biothermal processes in spoiled silage. The most pronounced temperature effect was observed in experimental groups I and II, where the highest temperatures and the longest maintenance of the thermophilic and mesophilic phases were recorded. This corresponds to a more effective course of organic matter bioconversion under optimised aeration conditions.

The main technological indicator, along with temperature, is moisture content in the biomass. The activity of bacterial enzymes is realised in moist waste. At low moisture content, enzymes do not display hydrolytic activity. Therefore, the task was to determine how the frequency of mechanical mixing affects the moisture content in soybean waste. The results prove that the mechanical mixing regime considerably influenced changes in moisture content in soybean waste during fermentation. In all experimental groups, moisture content gradually decreased throughout the research period, which was associated with active microbiological processes, heat release, and evaporation of water from the fermented mass (Table 2). On day 1, the moisture content was the same in all groups at 67.2%. This is due to equal initial conditions for the experiment. Already on day 5 of fermentation, a slight decrease in moisture was recorded in all variants. In the control group, the value was 67.0%. In experimental groups I and II, the moisture content was 0.2% and 0.8% lower, respectively, than in the control group. The greatest moisture decrease at the early stage was recorded in experimental group I, where mechanical mixing was performed once every 3 days. On day 10 of the research, the process of moisture loss intensified. In the control group, moisture content decreased to 65.4%. In the experimental groups, the moisture content in soybean waste was 0.4-2.2% lower than in the control. Compared with day 5 of fermentation, the smallest moisture losses were recorded in the control group and the largest in experimental group I, amounting to 1.6% and 3.0%, respectively. Thus, the lowest moisture value was observed in the variant with the most frequent mixing. This is explained by more intensive oxygen saturation of the substrate, activation of microbiological processes, and increased heat release, which promoted more active evaporation of water.

Table 2. *Moisture content in soybean waste under different mechanical mixing regimes during fermentation, %, M ± m, n = 4*

Day of study	Sample groups			
	control	experimental I	experimental II	experimental III
1	67.2 ± 1.23	67.2 ± 0.78	67.2 ± 1.33	67.2 ± 1.32
5	67.0 ± 0.23	66.2 ± 1.45	66.8 ± 1.08	67.0 ± 1.05
10	65.4 ± 0.67	63.2 ± 1.43	64.7 ± 0.76	65.0 ± 1.33
20	64.2 ± 1.21	61.2 ± 1.64	63.0 ± 0.86	63.8 ± 1.06
30	61.0 ± 1.11	58.1 ± 1.22	59.4 ± 0.58	60.5 ± 0.87
40	60.7 ± 1.43	57.2 ± 1.42	58.4 ± 0.77	60.0 ± 0.58
50	60.6 ± 1.53	55.8 ± 1.18	58.0 ± 0.86	59.8 ± 0.88
60	60.4 ± 1.25	55.0 ± 0.87	57.2 ± 0.93	59.1 ± 0.94
70	60.1 ± 1.55	54.3 ± 0.86	56.1 ± 1.23	58.0 ± 0.68
80	59.6 ± 0.56	53.3 ± 0.98	55.7 ± 1.04	57.3 ± 0.78
90	58.7 ± 0.64	52.7 ± 0.88	55.3 ± 1.45	56.4 ± 0.67
100	58.4 ± 0.37	52.4 ± 0.76	55.0 ± 1.06	56.0 ± 0.95
110	58.0 ± 0.49	51.3 ± 1.32	54.2 ± 0.88	55.1 ± 0.90
120	56.5 ± 0.76	50.0 ± 1.25	53.2 ± 0.78	54.0 ± 0.76
130	55.9 ± 1.09	49.2 ± 1.06	51.2 ± 0.94	53.3 ± 1.33
140	55.0 ± 1.04	48.2 ± 0.67	49.5 ± 0.75	51.2 ± 1.25
150	54.3 ± 0.77	46.3 ± 0.54	48.1 ± 0.59	50.4 ± 0.99

Source: compiled by the authors

During the first 30 days of fermentation, the moisture content decreased intensively in all groups. On day 20, in the control group, it was 64.2%. During this period, a relationship was established: the more active the aeration, the lower the moisture content in soybean waste. The moisture content in compost from experimental group I was 3.0% lower than in the control. On day 30, the values decreased to 61.0%, 58.1%, 59.4%, and 60.5%, respectively. The most intensive dehydration was observed in experimental group I, whereas the process was slowest in the control variant. From day 40 to day 70 of fermentation, the decrease in moisture continued, although its intensity declined slightly. On day 40, the moisture content in the control group was 60.7%. In experimental groups I, II, and III, this value was 4.8%, 2.3%, and 0.7% lower, respectively. On day 70 of fermentation, the moisture content in waste from the experimental groups was 0.7%, 1.1%, and 1.1% lower, respectively, than the data obtained on day 60 of the experiment.

The results confirm that the most frequent mechanical mixing ensured the most active reduction of moisture in the organic mass. In experimental group I, the difference from the control on day 70 was 5.8%. Experimental group II was also characterised by sufficiently intensive dehydration, whereas in experimental group III, where mixing was performed less frequently, the changes were less pronounced. In the second half of the experiment, from day 80 to day 120, moisture continued to decrease gradually in all variants. On day 80, the value in the control group was 59.6%. In the experimental groups, the values were lower by 2.3-6.3%. On day 100, moisture content was 58.4%, 52.4%, 55.0%, and 56.0%, respectively. On day 120 of the research, the intensity of moisture loss decreased, but the pattern of evaporation associated with aeration frequency remained stable. At the final stage of fermentation, moisture loss continued, although more slowly. The lowest moisture level was recorded on day 150 of fermentation: in the control group, the value was 54.3%. Under aeration once every 3 and 5 days, the moisture content in the compost during this period was 8.0% and 6.2% lower, respectively, than in the control.

Overall, during the whole period, moisture content decreased by 12.9% in the control group, by 21.2% in experimental group I, by 19.1% in experimental group II, and by 16.8% in experimental group III. Thus, moisture losses were highest under the most frequent mechanical mixing. More frequent mixing promoted better aeration of the substrate, more active development of aerobic microflora, and a more intensive course of fermentation processes. Mixing once every 5 days also ensured sufficiently effective dehydration of the mass, whereas mixing once every 7 days was less effective but still exceeded the control variant. Data in Table 3 present a gradual decrease in moisture content in spoiled corn silage throughout the fermentation period, regardless of the mechanical mixing regime. At the beginning of the experiment, moisture content was the same in all groups and was 69.5%, confirming equivalent initial experimental conditions.

Table 3. *Moisture content in spoiled corn silage under different mechanical mixing regimes during fermentation, %, M ± m, n = 4*

Day of study	Sample groups			
	control	experimental I	experimental II	experimental III
1	69.5 ± 1.32	69.5 ± 1.06	69.5 ± 1.06	69.5 ± 1.44
5	69.1 ± 1.11	67.9 ± 1.03	68.8 ± 1.07	69.2 ± 1.53
10	68.4 ± 1.08	66.3 ± 1.05	67.0 ± 1.23	68.0 ± 1.25
20	66.2 ± 1.04	64.3 ± 1.23	65.5 ± 1.52	66.1 ± 1.31
30	65.2 ± 1.05	63.5 ± 1.22	64.3 ± 1.11	64.9 ± 1.07
40	64.2 ± 1.23	61.4 ± 1.15	63.3 ± 1.27	64.0 ± 1.23
50	62.2 ± 1.51	59.2 ± 1.16	60.7 ± 1.53	61.8 ± 1.06
60	60.2 ± 1.17	56.5 ± 0.86	58.3 ± 1.04	59.5 ± 1.17
70	58.5 ± 0.87	55.5 ± 0.94	57.2 ± 1.32	58.0 ± 1.27
80	57.3 ± 0.96	54.7 ± 0.58	55.4 ± 0.96	56.7 ± 0.86
90	57.0 ± 0.59	54.0 ± 0.54	55.0 ± 0.58	56.2 ± 0.85
100	56.8 ± 0.68	53.8 ± 0.88	54.2 ± 0.88	55.5 ± 0.47
110	56.3 ± 0.83	51.4 ± 0.59	53.3 ± 0.57	55.6 ± 0.76
120	56.0 ± 0.96	50.8 ± 0.64	52.7 ± 0.86	55.1 ± 0.94
130	55.6 ± 0.59	49.0 ± 0.73	52.0 ± 0.45	54.4 ± 0. 82
140	55.3 ± 0.55	48.2 ± 0.74	51.5 ± 0.93	54.6 ± 0.76
150	55.1 ± 0.76	47.2 ± 0.93	51.0 ± 0.77	53.2 ± 0.77

Source: compiled by the authors

On days 5-10, a decrease in compost moisture was observed. In the control group, the value on day 10 was 68.4%. The most intensive decrease occurred in experimental group I, where the value was 2.1% lower than in the control. In experimental groups II and III, the moisture content was 1.4% and 0.4% lower than in the control, respectively. This demonstrates that mechanical mixing activated fermentation processes and promoted faster moisture evaporation due to increased aeration and heat release. During days 20-60, the downward trend in moisture content persisted in all groups. The lowest values were consistently recorded in experimental group I. Thus, on day 60, the moisture content in the control group was 60.2%, whereas in the experimental group I, it was only 56.5%, or 3.7% lower. In experimental groups II and III, this value was 1.9% and 0.7% lower than in the control, respectively. The results indicate that more intensive mechanical mixing ensured better contact between the organic mass and air, activated aerobic microflora, and accelerated biodegradation of organic components.

After day 70, the rate of moisture decrease slowed somewhat, which may be associated with a reduction in the intensity of microbiological processes and the transition of fermentation to the stage of organic mass stabilisation. Nevertheless, the difference between the experimental and control groups remained noticeable. On day 100, the moisture content in the control group was 56.8%. Under the most intensive aeration (experimental group I), the moisture content was 3.0% lower than in the control. At the end of the study, on day 150, the lowest moisture content was established in experimental group I, at 47.2%, which was 7.9% lower than in the control, where this value was 55.1%. In experimental groups II and III, it was 51.0% and 53.2%, respectively. Ultimately, mechanical mixing had a considerable effect on moisture loss during fermentation of spoiled corn silage. During the thermophilic phase of fermentation of spoiled corn silage, relative moisture losses in both the control and experimental samples were considerably higher than during the period of temperature decrease to the mesophilic or psychrophilic phase. Reduction of substrate moisture has important technological significance, as it improves the structure of the fermented mass, reduces the risk of anaerobic pathogenic microflora development, and stabilises organic matter. Excessively high moisture can suppress aerobic processes, whereas its gradual decrease creates favourable conditions for effective biothermal fermentation. Thus, the results indicate that mechanical mixing during fermentation of spoiled corn silage contributed to a more intensive decrease in substrate moisture. The greatest losses were recorded under the conditions of mixing once every 3 days.

The obtained results of the experiment demonstrate that aeration regimes majorly influenced the fermentation of soybean waste and spoiled corn silage. The most intensive development of biothermal processes was observed when the organic mass was mechanically mixed once every 3 days. This was accompanied by an increase in compost temperature to 62-66°C and prolonged maintenance of the thermophilic phase. Similar results were presented by C.T. Chang *et al.* (2022), who established that active composting of food waste and soybean curd residue is accompanied by intensive heat release and formation of a stable thermophilic phase, which ensures effective bioconversion of organic matter. Similarly, C. Sundberg (2005) noted that control of aeration and temperature regime is a determining factor in maintaining an active thermophilic phase during composting of organic waste. The study established that more frequent mechanical mixing promoted more intensive oxygen access to the substrate mass, which activated oxidative processes and accelerated bioconversion of organic matter. Similar patterns are described in the work of M. Atauzzaman & Q.H. Bari (2023), where aeration is identified as one of the key factors in maintaining active composting and increasing the temperature of the fermented mass. K.K. Patel *et al.* (2025) also reported that, during vermicomposting of soybean plant residues, intensive development of microbiological processes ensures active decomposition of organic components and transformation of cellulose-containing raw material into a stable organic product. M.P. Raut *et al.* (2008) established that the highest activity of microbial enzyme systems and the most intensive decomposition of organic compounds occur during the thermophilic period.

According to the data of the current study, under aeration once every 3 and 5 days, the temperature of the organic mass remained higher than in the control even in the second half of fermentation. This points to a longer course of microbiological processes and slower depletion of the nutrient substrate. Similar conclusions were reached by Y. Tang *et al.* (2025), who established that the intensity of fermentation processes in corn silage depends largely on microbial activity and fermentation conditions, which determine the quality of the final product. P. Zhou *et al.* (2025) also stated that corn silage and corn plant residues have high bioconversion potential due to the substantial content of organic matter and available carbohydrates. Similar findings were presented by M. Waqas *et al.* (2023) and Z.X. Keng *et al.* (2023), showing that optimal aeration prolongs the active phase of composting, supports intensive development of aerobic microflora, and increases the efficiency of organic matter mineralisation.

Moisture content in the substrate was an important technological indicator of fermentation. In the presented study, increasing the frequency of mechanical mixing increased the intensity of moisture loss. The lowest moisture content in soybean waste and spoiled corn silage was observed in experimental group I, where aeration was performed once every 3 days. This can be explained by increased water evaporation due to active heat release and a more intensive course of aerobic processes. During the thermophilic phase, moisture losses were considerably more intensive than during the mesophilic or psychrophilic phases of fermentation. Similar patterns were highlighted by C.T. Chang *et al.* (2022), who associated active reduction of substrate moisture with high temperature and intensive microbial metabolism during the thermophilic stage of composting. However, excessive moisture reduction can suppress microbial activity; therefore, maintaining an optimal water regime is an important technological task. Parallel conclusions on the effect of moisture on silage fermentation were obtained by K.G. Arriola *et al.* (2011), who found that the physicochemical parameters of the substrate directly affect the intensity of microbiological processes and the quality of the final product.

The use of the biodestructor "Unikal-S" combined with regular aeration promoted the activation of biodegradation of the organic components of the substrate. The results correspond to the conclusions of K.K. Patel *et al.* (2025), who established that using biologically active agents during the processing of soybean plant residues accelerates the destruction of organic matter and humification of the substrate. S. Panda & M.S. Jain (2025), in a systematic review, also emphasised that modern biotechnological methods for processing soybean waste ensure effective utilisation of organic raw material and production of valuable bioconversion products. V.V. Volkogon *et al.* (2019) established that the introduction of cellulolytic microorganisms accelerates decomposition of plant residues and optimises microbiological processes during composting. L.V. Mitiohlo *et al.* (2023b) reported a positive effect of biodestructors on the temperature regime and fermentation intensity of spoiled corn silage. The results on fermentation of spoiled corn silage also align with data from J.M. Wilkinson *et al.* (2015) and G. Santi *et al.* (2015), who noted that corn silage is a biologically active substrate with a high content of organic matter and considerable potential for aerobic and anaerobic bioconversion, provided that optimal technological process parameters are maintained. Thus, the results confirm that optimisation of aeration regimes is an important factor in intensifying the fermentation of organic waste. The most effective conditions were mechanical mixing once every 3 days, which ensured the highest temperature values, a more active course of biothermal processes, and more intensive reduction of substrate moisture.

Conclusions

The conducted study indicated that aeration regimes considerably influenced the technological parameters of fermentation of soybean waste and spoiled corn silage. Mechanical mixing of the organic mass activated biothermal processes, increased compost temperature, and ensured a more intensive reduction of substrate moisture. The most effective aeration regime was mixing once every 3 days, under which the highest temperature values and the longest maintenance of the thermophilic phase of fermentation were observed. Under intensive aeration, the temperature reached 62°C in soybean waste and 66°C in spoiled corn silage. Maintenance of these temperature regimes promoted active microbiological processes, more intensive decomposition of organic matter, and partial sanitisation of the substrate. In the control variants, where mixing was performed once every 10 days, temperature values were lower, indicating a less intensive course of fermentation.

More frequent mechanical mixing positively affected oxygen access to the depth of the organic mass, activated aerobic microflora, and accelerated bioconversion processes. Under aeration once every 3 and 5 days, the experimental groups maintained elevated temperatures for a longer period even after completion of the active thermophilic stage, indicating a prolonged course of microbiological processes. The research established a considerable effect of aeration regimes on changes in the moisture content of the fermented mass. The greatest moisture losses were characteristic of the variants with the most intensive aeration. On day 150 of fermentation, the moisture content in soybean waste under mixing once every 3 days was 8.0% lower than in the control, and in spoiled corn silage it was 7.9% lower. This indicates more active water evaporation due to increased heat release and a more intensive course of aerobic processes.

The use of the biodestructor "Unikal-S" combined with mechanical mixing contributed to the stabilisation of the fermentation process, acceleration of organic matter biodegradation, and improvement of technological composting parameters. The results confirm the feasibility of using aeration once every 3-5 days as an effective technological method during fermentation of soybean waste and spoiled corn silage to intensify biothermal processes and increase the efficiency of bioconversion of organic waste. Further research should determine optimal aeration parameters for different types of organic raw material, analyse the dynamics of microbial communities during fermentation, and evaluate the agronomic efficiency and environmental safety of the resulting product when used as organic fertiliser.

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Conflict of Interest

None.

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Встановлення впливу різних режимів аерації на технологічні показники ферментації відходів

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Анотація. Метою роботи було встановлення оптимальної кратності механічного перемішування субстрату для інтенсифікації біотермічних процесів, активізації мікробіологічної діяльності та підвищення ефективності біоконверсії органічної маси. Дослідження проводили в умовах дослідного господарства із використанням органічних відходів рослинного походження та біодеструктора «Унікал-с». У процесі дослідження вивчали вплив аерації на температурний режим та зміну вологості компостної маси упродовж 150 діб ферментації. Для цього використовували різні режими механічного перемішування: один раз на 3, 5, 7 та 10 діб. Температуру компосту контролювали щодоби, а вміст вологи визначали один раз на 5 діб згідно чинних стандартів. Встановлено, що застосування регулярної аерації сприяло інтенсивнішому перебігу біотермічних процесів у відходах сої та зіпсованому силосі кукурудзи. Найвищі температурні показники були зафіксовані у варіантах із механічним перемішуванням один раз на 3 доби. Температура у відходах сої досягала 62 °C, а у зіпсованому силосі кукурудзи – 66 °C, що свідчило про активний розвиток термофільної мікрофлори та інтенсивне розкладання органічної речовини. Виявлено, що частіше перемішування забезпечувало триваліше утримання термофільної фази ферментації та сприяло ефективнішому зниженню вологості субстрату. Доведено, що аерація один раз на 3-5 діб забезпечувала найкращі технологічні показники ферментації та сприяла інтенсифікації біоконверсії органічних відходів. Отримані результати можуть бути використані для удосконалення технологій компостування рослинних відходів та підвищення ефективності ферментаційних процесів у сільському господарстві

Ключові слова: аерація; компостування; відходи сої; біодеструктор; температура компосту; вологість; біоконверсія

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НАУКОВІ ДОПОВІДІ НАЦІОНАЛЬНОГО УНІВЕРСИТЕТУ БІОРЕСУРСІВ І ПРИРОДОКОРИСТУВАННЯ УКРАЇНИ

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