

## **SCIENTIFIC REPORTS OF THE NATIONAL UNIVERSITY OF LIFE AND ENVIRONMENTAL SCIENCES OF UKRAINE**

**Founder:**

National University of Life and Environmental Sciences of Ukraine

**Year of foundation: 2005**

*Recommended for printing and distribution  
via the Internet by the Academic Council  
of National University of Life and Environmental Sciences of Ukraine  
(Minutes No. 5 of November 27, 2025)*

**State Registration:**

**Media identifier - R40-02082.**

Decision of the National Council of Television and Radio Broadcasting of Ukraine  
No. 1471, Minutes No. 28, dated 23.11.2023.

**The journal is included in the list  
of Professional Scientific Publications of Ukraine**

Category "B". Specialties: 0511 – Biology, 0521 – Environmental Sciences, 0522 – Natural Environments and Wildlife, 0512 – Biochemistry, 0811 – Crop and Livestock Production, 0821 – Forestry, 0812 – Horticulture, 0841 – Veterinary (Order of the Ministry of Education and Science of Ukraine No. 409 of 14 March 2020), 0715 – Mechanics and Metal Trades, 0716 – Motor Vehicles, Ships and Aircraft (Order of the Ministry of Education and Science of Ukraine No. 886 of 02 July 2020).

**The journal is presented international scientometric databases, repositories  
and scientific systems:**

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# **НАУКОВІ ДОПОВІДІ НАЦІОНАЛЬНОГО УНІВЕРСИТЕТУ БІОРЕСУРСІВ І ПРИРОДОКОРИСТУВАННЯ УКРАЇНИ**

## **Засновник:**

Національний університет біоресурсів і природокористування України

**Рік заснування: 2005**

*Рекомендовано до друку та поширення  
через мережу Інтернет Вченою радою  
Національного університету біоресурсів і природокористування України  
(протокол № 5 від 27 листопада 2025 р.)*

## **Державна реєстрація:**

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№ 1471, протокол № 28 від 23.11.2023 р.

## **Журнал входить до переліку наукових фахових видань України**

Категорія «Б». Спеціальності: 091 – Біологія, 101 – Екологія, 162 – Біотехнології та біоінженерія, 201 – Агрономія, 204 – Технологія виробництва і переробки продукції тваринництва, 205 – Лісове господарство, 206 – Садово-паркове господарство, 211 – Ветеринарна медицина, 212 – Ветеринарна гігієна, санітарія і експертиза (наказ МОН України № 409 від 14 березня 2020 року), 131 – Прикладна механіка, 133 – Галузеве машинобудування (наказ МОН України № 886 від 02 липня 2020 року).

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UDC 633.111.1\*324":664.64.016

DOI: 10.31548/dopovidi/6.2025.09

## Evaluation of winter bread wheat (*Triticum aestivum* L.) varieties based on grain quality indicators

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**Abstract.** Increasing the gross yield of high-quality grain requires the improvement of breeding programmes through their continuous enrichment with new genetic plasma. Therefore, the aim of the study was to identify sources of improvement in grain quality indicators for use in the breeding process by differentiating between different varieties of winter bread wheat in the central part of the Forest-Steppe zone of Ukraine. Thirty-one varieties of winter bread wheat were evaluated for protein content, wet gluten content, and sedimentation index in the 2023 and 2024 harvests. The distribution of the obtained grain quality indicators was analysed. It was found that the degree of asymmetry and excess distribution did not exceed the critical value ( $|As/\sigma As| \leq 3$  and  $|Ex/\sigma Ex| \leq 3$ ). Thus, the experimental data on sedimentation, protein and wet gluten content during the years of testing were close to normal distribution. The variability of the quality indicators of winter bread wheat grain depending on the hydrothermal conditions of cultivation was established. A higher average protein content (13.3%) and wet gluten content (28.7%) were obtained in 2023, and a higher sedimentation index (74 ml) in 2024. The coefficient of variation revealed significant variability (CV = 10.2-13.5%) in the sedimentation index between winter bread wheat varieties during the years of testing, as well as weak and moderate

### Suggested Citation:

Pravdziva, I., & Khoroshko, N. (2025). Evaluation of winter bread wheat (*Triticum aestivum* L.) varieties based on grain quality indicators. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 9-21. doi: 10.31548/dopovidi/6.2025.09.

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variation in protein content (CV = 5.3-8.6%) and wet gluten content (CV = 3.9-5.8%). A decisive (40.7-63.8%) influence of the variety on the studied grain quality indicators was revealed. The conditions of the year had an insignificant effect on the sedimentation index (2.5%), but a significant effect on the protein (23.2%) and wet gluten (24.0%) content. The proportion of the interaction between the variety × year factors was significant (26.9-32.5%) for all three grain quality indicators. The following winter bread wheat varieties were identified as having the highest protein content: 'MIP Kniazhna' and 'Avrora Myronivska'; wet gluten content: 'MIP Kniazhna', 'Avrora Myronivska', 'Pokrovska', 'Hlad', 'Zolotokolosa', 'Kolumbiia', 'Optyma odeska', 'Doskonalist odeska', 'Perevaga', 'Nyva odeska', 'Kuialnyk', 'Kubok', 'Vahoma'; for sedimentation value – 'Pokrovska', 'Osнова odeska', 'Kuialnyk', 'Kubok'

**Keywords:** *Triticum aestivum* L.; protein content; wet gluten content; sedimentation index; hydrothermal conditions; coefficient of variation; ANOVA

## Introduction

Wheat – one of the principal food crops in both Ukraine and worldwide. The grain of *Triticum aestivum* L. is a staple food for a large proportion of the global population due to its high nutritional value. Owing to its production potential and technological value, wheat plays a crucial role in the global economy. According to the State Register of Plant Varieties Suitable for Dissemination in Ukraine, as of 2025, there are 776 registered varieties of winter bread wheat. However, not all of them are of high quality. The majority (38%) belong to the category of valuable wheats, while only 27% meet the criteria for strong wheats. A considerable proportion (23%) are fillers, and 12% belong to an undefined quality group (State Register..., 2025). To improve grain quality, both in Ukraine and globally, targeted breeding programmes are being carried out to combine in a single genotype a high yield potential with enhanced technological properties of grain and flour, as well as resistance to a complex of adverse abiotic and biotic environmental factors.

K. Wysocka *et al.* (2024) studied the effect of different cultivation systems for winter bread wheat (organic, integrated, and conventional) on flour quality. The authors found that the cultivation technology significantly affects baking quality parameters, particularly protein content, wet gluten content, and sedimentation value. Grain from organic systems had lower protein and wet

gluten contents, whereas integrated and conventional systems ensured higher values of the protein and wet gluten complex and better baking quality. T.P. Khumalo-Mthembu *et al.* (2025) analysed the current state and prospects of breeding bread wheat (*Triticum aestivum* L.) for pre-harvest sprouting resistance in South Africa, emphasising that this trait is crucial for maintaining baking quality, and that effective breeding requires combining traditional methods with modern molecular and genomic approaches. A.A. Melash & E.B. Ábrahám (2022) conducted a detailed analysis of innovative agronomic and breeding strategies to improve wheat productivity and grain quality under changing climatic conditions. The authors demonstrated that optimal nitrogen fertilisation and the selection of cultivars capable of efficiently utilising nutrients make it possible to obtain high yields with improved technological properties of the grain. T. Hao *et al.* (2023) examined the effect of different nitrogen application frequencies under drip irrigation on the quality indicators of winter wheat grain. They found that increasing the frequency of nitrogen application significantly increased protein content (by 2-8.6%) and wet gluten (by 4.5-22.1%) and had little effect on the sedimentation value, although it could lead to a reduction in flour quality.

H.A. Dutova *et al.* (2024), when evaluating new cultivars of winter bread wheat under various

soil and climatic conditions with respect to grain quality indicators, observed a trend of increasing protein and crude wet gluten content from the north to the south of Ukraine, specifically from the Polissya zone to the Steppe. A.M. Zvonar (2020) investigated the influence of cultivar and annual weather conditions on the quality of winter wheat grain and established that nitrogen accumulation in wheat grain depends more on the genetic characteristics of the cultivar than on hydrothermal conditions. Higher protein and wet gluten content were obtained under dry growing conditions. A. Szuba-Trznadel *et al.* (2024) compared winter bread wheat cultivars based on their chemical composition, biological value of proteins, and content of macro- and microelements, and found that the chemical composition of the grain was dependent on the genetic properties of the cultivars. They established that a higher protein content in the grain, even with a greater sum of essential amino acids, does not always correlate with a higher nutritional value of the protein, which depends on the specific amino acid composition. Knowledge and understanding of the various physical and chemical properties, protein quality, and information on the amino acid composition will be useful for a wide range of specialists, and conducting a targeted breeding process to create cultivars with the necessary nutrients will satisfy diverse dietary needs. H. Wieser *et al.* (2020) analysed the chemical composition of wheat grain in detail and concluded that the content and composition of proteins in the grain largely determine its baking qualities. The authors note that the variation in protein content in wheat grain ranges from 7% to 22%, but most commonly lies between 10% and 15%. Protein content, wet gluten content, and its quality play an important role in the food industry, as these indicators determine the quality of the final product. The quantity and quality of the protein-gluten complex primarily influence the formation of the commercial value of wheat samples. M.V. Radchenko *et al.* (2024) evaluated the potential of winter wheat cultivars created in

various breeding centres for the rational utilisation of agro-meteorological resources and identified cultivars with high adaptive potential and superior baking qualities. The authors noted that the successful choice of cultivar is one of the most important elements of winter wheat cultivation technology for realising the genetic potential.

Breeding programmes necessarily require constant replenishment with new genetic material – a condition without which progress in breeding is impossible. Evaluating new winter bread wheat cultivars based on grain quality indicators and selecting samples with higher content and better quality of the protein-gluten complex will provide breeders with high-quality starting material for further use in breeding programmes of various directions.

The aim of this study was to evaluate winter bread wheat cultivars under the conditions of the central part of the Forest-Steppe of Ukraine for protein content, wet gluten content, and sedimentation index, and to identify genotypes with higher grain quality indicators. To achieve this aim, the following tasks needed to be addressed:

- to determine the level of expression of grain quality indicators of winter bread wheat depending on the hydrothermal conditions of the year;
- to establish the pattern of distribution of grain quality indicators of winter bread wheat;
- to differentiate winter bread wheat cultivars according to protein content, wet gluten content, and sedimentation index.

## Materials and Methods

The study was conducted under the conditions of the V.M. Remeslo Myronivka Institute of Wheat of the National Academy of Agrarian Sciences of Ukraine (NAAS), located in the village of Tsentralne, Obukhiv District, Kyiv Region, Ukraine (49°38'41.5" N, 31°05'33.2" E). Thirty-one varieties of winter bread wheat (*Triticum aestivum* L.) harvested in 2023 and 2024 were evaluated, including four varieties of winter bread wheat bred by the V.M. Remeslo Myronivka Institute of Wheat NAAS (MIW) and the Institute of Plant Physiology and Genetics of the National Academy of Sciences

of Ukraine – 'Podolianka', 'Expromt', 'Kolumbiia', and 'Zolotokolosa'; three varieties bred by MIW – 'MIP Kniazhna', 'MIP Yuvileina', and 'Avrora Myronivska'; 24 varieties selected by the Plant Breeding and Genetics Institute – National Centre of Seed and Cultivar Investigation – 'Pokrovska', 'Heizer', 'Hlad', 'Optyma odeska', 'Doskonalist odeska', 'Spadshchyna odeska', 'Versiia odeska', 'Manera odeska', 'Perevaha', 'Pontiika', 'Vidpovid odeska', 'Virnist', 'Osnova odeska', 'Peremoha odeska', 'Zhuravka odeska', 'Zysk', 'Zorepad', 'Nyva odeska', 'Kantata odeska', 'Kualnyk', 'Mudrist odeska', 'Kubok', 'Lira odeska', and 'Vahoma'. The research was carried out in accordance with the Convention on Biological Diversity (1992).

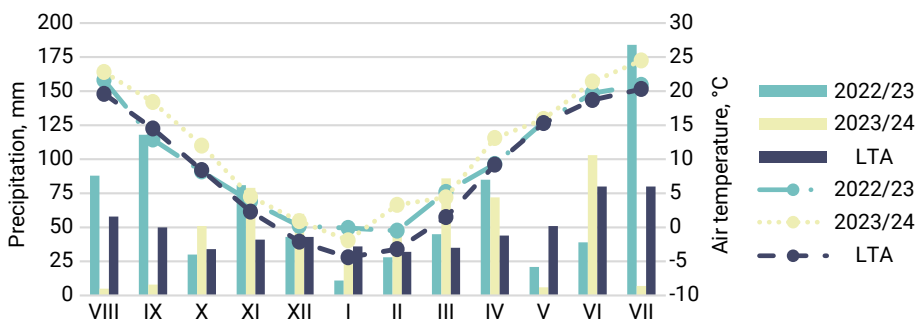
The soil cover of the MIW experimental fields is represented by deep low-humus slightly leached chernozem (38–42 cm). The humus content in the 20 cm soil layer is 3.6–4.0%, with readily available nitrogen – 0.006%, phosphorus – 0.025%, and exchangeable potassium – 0.011–0.018%. Soil pH ranges from 5.3 to 6.4, the sum of absorbed bases is 0.23–0.29 g-eq kg<sup>-1</sup> soil, and the base saturation degree is 86.2–94.4%. Winter bread wheat was cultivated according to the standard agronomic practices recommended for the Forest-Steppe zone of Ukraine (Sirosh-tan & Kavunets, 2023). The preceding crop was soybean. Sowing was performed on 10 October using a cassette drill SN-10Ts at a sowing depth of 4–5 cm and a seeding rate of 4.5 million viable

seeds per hectare, with an experimental plot area of 3 m<sup>2</sup>. Grain was harvested manually.

To obtain flour, winter bread wheat grains were ground in a Quadrumat Junior Brabender laboratory mill (Germany). The protein and wet gluten content was measured using a near-infrared reflectance spectrometer (spectral range 1,400–2,400 nm) on a SPECTRAN 119M Lomo Photonics (Russia) device. The sedimentation index was determined in a 2% acetic acid solution with the addition of bromophenol blue dye using the micro method of A.Ya. Pumpianskyi. Statistical processing of experimental data was performed using descriptive statistics and analysis of variance (ANOVA) (Rudakov & Tishkov, 2016; Koval, 2021).

## Results and Discussion

The years of research were characterised by variability in air temperature and precipitation by month. In 2022/23, the average annual air temperature exceeded the long-term average (LTA) by 1.4°C, and in 2023/24 by 3.3°C. Each year, there was a significant increase in the average monthly air temperature of 1.0–6.5°C above the LTA in August, November, December, January, February, March and June. September, October, April and July 2023/24 were also abnormally warm, exceeding the LTA by 3.6–4.2°C. A decrease in air temperature from the LTA was observed only in September and October 2022/23, by 1.6 and 0.2°C, respectively (Fig. 1).



**Figure 1.** Hydrothermal regime during the study period

**Note:** LTA – long-term average value for 1960/61–2021/22

**Source:** developed by the authors of this study based on data from the Department of Biotechnology, Genetics and Physiology of the Myronivka Institute of Wheat

During the 2022/23 growing season, precipitation amounted to 773 mm (132% of the LTA), and in 2023/24 – 544 mm (93% of the LTA). Critically low precipitation (<50% of the LTA) was observed in January, May and June 2022/23, and in August, September, May and July 2023/24. An abnormally high amount ( $\geq 150\%$  of the LTA) was recorded in August, September, November, April and July 2022/23, and in October, November, March and April 2023/24. It should be noted that torrential

rainfall (230% of the LTA) in July 2023 fell after the harvest from the experimental plots, so its excessive amount during that month did not have a negative impact on the accumulation of nitrogen-containing compounds in winter bread wheat grain.

The results of the studies showed significantly higher minimum, maximum and average values of protein (12.2, 15.5 and 13.3%, respectively) and wet gluten (26.7, 32.3 and 28.7%, respectively) content in 2023 than in 2024 (Table 1).

**Table 1.** Variation in grain quality indicators during the study period

Value	Protein content, %			Wet gluten content, %			Sedimentation rate, ml		
	2023	2024	X	2023	2024	X	2023	2024	X
Min	12.2	10.4	11.5	26.7	24.0	25.7	47	49	57
Max	15.5	14.3	14.4	32.3	29.2	29.9	89	89	87
X	13.3	12.3	12.8	28.7	27.0	27.9	71	74	73
CV, %	5.3	8.6	5.5	4.3	5.8	3.9	11.5	13.5	10.2
LSD <sub>05</sub>	0.4			0.6			3		

**Note:** Min, Max, X – minimum, maximum and average values, respectively, CV – coefficient of variation, LSD<sub>05</sub> – smallest significant difference at  $p \leq 0.05$

**Source:** developed by the authors of this study

The conditions of the trial years did not significantly affect the formation of the sedimentation index limits, but the above-average value of this characteristic (74 ml) was obtained in 2024. A slight variation ( $CV \leq 5.0\%$ ) in protein and wet gluten content was observed in 2023 and a moderate variation ( $6.0 \leq CV \leq 10.0\%$ ) in 2024. The variation in the sedimentation index was significant ( $11.0 \leq CV \leq 20.0\%$ ) for both years. A.Z. Ghafoor *et al.* (2024) also noted a slight variation between winter bread wheat genotypes in protein content ( $CV = 5.23\%$ ) and a significant variation in sedimentation index ( $CV = 12.95\%$ ). The authors showed that genotype is the leading factor in the formation of the protein-gluten complex, while growing conditions and fertilisation system can significantly affect the variability of quality indicators, in particular the wet gluten level, the variability of which was significant in their studies ( $CV = 12.95\%$ ). These results are consistent with current data and confirm that the combination of genotypic characteristics and hydrothermal

conditions is a key factor in the differentiation of varieties in terms of grain quality. Depending on the nature of the distribution of experimental data, appropriate statistical calculation methods should be applied. Analysis of distribution patterns involves assessing the degree of sample homogeneity, asymmetry, and excess distribution (Danilov, 2019). The obtained grain quality values are homogeneous, since the condition  $CV \leq 33\%$  is met (Table 1).

The asymmetry coefficient (As) characterises the symmetry of the distribution law of a random variable relative to its centre, i.e. the symmetry of the tails of the distribution (Koval, 2021). The statistical distribution of the sedimentation index sample, both annually and on average over the years of testing, had a negative (left-sided) asymmetry with the asymmetry coefficient (As) varying from -0.30 to -0.71 (Table 2). In 2023, positive (right-sided) asymmetry was obtained for the values of protein content ( $As = 0.95$ ) and wet gluten ( $As = 0.75$ ), however, in 2024, the distribution of

samples of these grain quality indicators was characterised by negative values ( $A_s = -0.10$  and  $-0.36$ , respectively) (left-sided asymmetry). The sample of protein and wet gluten content values averaged over the years had a slight right-sided asymmetry ( $A_s = 0.16$  and  $0.05$ , respectively).

Positive asymmetry indicates that the sample contains several values that greatly exceed the average value of a particular trait. Negative asymmetry indicates the presence of values in the sample that are significantly lower than the mean value (Rudakov & Tishkov, 2016).

**Table 2.** Characteristics of the distribution of quality indicators for winter bread wheat during the trial years

Numerical characteristics	Protein content			Wet gluten content			Sedimentation index		
	2023	2024	$X^*$	2023	2024	$X^*$	2023	2024	$X^*$
$A_s$	0.95	-0.10	0.16	0.75	-0.36	0.05	-0.44	-0.71	-0.30
$Ex$	1.64	-1.01	-0.12	1.22	-1.03	-0.26	1.78	-0.25	-0.49
$\sigma A_s$	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
$\sigma Ex$	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
$ A_s/\sigma A_s $	2.33	0.25	0.40	1.85	0.89	0.13	1.09	1.75	0.74
$ Ex/\sigma Ex $	2.22	1.36	0.16	1.65	1.40	0.35	2.40	0.34	0.66

**Note:**  $A_s$  – asymmetry coefficient,  $Ex$  – excess,  $\sigma A_s$  – standard deviation of asymmetry coefficient,  $\sigma Ex$  – standard deviation of excess,  $X^*$  – calculated based on the average values of experimental data for two years

**Source:** developed by the authors of current study

Excess ( $Ex$ ) characterises the degree of concentration of variants around the mean value, i.e. it is an indicator of the steepness (sharpness) of the statistical distribution graph compared to the normal distribution. For a normal distribution,  $Ex = 0$ . If the excess is positive, the curve has a higher and “sharper” peak than the normal distribution; if the excess is negative, the curve under study has a lower and “flatter” peak than the normal curve. In 2023, a sharp peak ( $Ex = 1.22$ - $1.78$ ) was observed in the distribution of samples of the studied flour quality indicators; in 2024 and on average over two years, a flat peak was observed with a variation in excess from  $0.12$  to  $1.03$ . Thus, the experimental data obtained for grain quality indicators differed somewhat from the normal distribution in terms of both the asymmetry coefficient and the excess. However, the degree of significance of asymmetry is assessed by the ratio of the asymmetry coefficient to the mean square error of this coefficient ( $|A_s/\sigma A_s|$ ), and the significance of the excess of distribution is assessed by the ratio of the excess to its mean square error ( $|Ex/\sigma Ex|$ ). The degree of significance of asymmetry

and excess of grain quality indicators corresponded to the condition  $|A_s/\sigma A_s| \leq 3$  and  $|Ex/\sigma Ex| \leq 3$  (Table 2), under which deviations in the asymmetry coefficient and excess are considered insignificant, and the distributions of the obtained data are recognised as close to normal distribution. Thus, the experimental data obtained for the sedimentation index, protein and wet gluten content during the years of testing were close to normal distribution. This made it possible to apply statistical methods designed for normal distribution.

N.V. Vasylenko *et al.* (2019), when establishing the nature of the distribution of wheat grain quality indicators, obtained a negative excess of protein content ( $Ex = 0.84$ ) and wet gluten content ( $Ex = 0.41$ ), however, according to the asymmetry coefficient, the protein content was characterised by left-sided asymmetry ( $A_s = 0.13$ ), and the wet gluten content by right-sided asymmetry ( $A_s = 0.26$ ). The degrees of significance of asymmetry and excess of both grain quality indicators were below the critical value ( $A_s/\sigma A_s \leq 3$ ;  $|Ex/\sigma Ex| \leq 3$ ). Thus, the authors also found a normal distribution of protein and wet gluten content.

Comparing the results of this study with current data, differences in the asymmetry coefficient of protein content were noted, but the flat-topped distribution of experimental data for both protein and wet gluten content, as well as their proximity to normal distribution, was confirmed. X. Yang *et al.* (2014), evaluating 330 Chinese wheat varieties for flour quality indicators, also observed a normal distribution of protein content, wet gluten content, and sedimentation index. To determine the normality of the distribution of experimental data, the authors used Z-statistics with the Kolmogorov-Smirnov (K-S) normality criterion. The statistical values of the sedimentation rate ( $Z_{0.05} = 0.502$ ), protein content ( $Z_{0.05} = 0.340$ ) and wet gluten content ( $Z_{0.05} = 0.891$ ) were lower than the critical value ( $Z_{0.05} = 1.63$ ), and their

asymptotic significance was greater than 0.05, indicating their normal distribution. These results are consistent with current data and confirm the normality of the distribution of protein content, wet gluten content and sedimentation index.

According to the results of the variance analysis, the maximum influence (40.7-63.8%) of the variety on the studied grain quality indicators was established (Table 3). The conditions of the year had the least influence on the sedimentation rate (2.5%). The formation of protein and wet gluten content significantly depended on both the year of cultivation (23.2 and 24.0%, respectively) and the interaction of the variety  $\times$  year factors (26.9 and 27.9%, respectively). A significant influence (32.5%) of the interaction between the variety  $\times$  year factors on the sedimentation index was also found.

**Table 3. Results of variance analysis of winter bread wheat grain quality indicators, 2023 and 2024**

Source of variation	df	SS	MS	F act.	Share of influence, %
Protein content					
Total	247	288.4722	-	-	-
Variety (A)	30	129.041	4.30	54.030*	44.7
Year (B)	1	66.99681	67.00	841.561*	23.2
A $\times$ B	30	77.62694	2.59	32.503*	26.9
Unaccounted factors	186	14.8075	0.08	-	5.1
Wet gluten content					
Total	247	767	-	-	-
Variety (A)	30	312.0387	10.40	33.975*	40.7
Year (B)	1	183.7994	183.80	600.372*	24.0
A $\times$ B	30	214.2194	7.14	23.325*	27.9
Unaccounted factors	186	56.9425	0.31	-	7.4
Sedimentation index					
Total	247	20,973.35	-	-	-
Variety (A)	30	13,389.35	446.31	329.421*	63.8
Year (B)	1	522.5806	522.58	385.714*	2.5
A $\times$ B	30	6,809.419	226.98	167.533*	32.5
Unaccounted factors	186	252	1.35	-	1.2

**Note:** df – number of degrees of freedom, SS – sum of squares, MS – mean square, F act. – Fisher's criterion actual value, \* –  $p \leq 0.001$

**Source:** developed by the authors of current study

The highest proportion of influence of the variety, compared to other factors, on the studied grain quality indicators indicates the decisive role of genetic potential in the formation of protein

content, wet gluten content and sedimentation index. This confirms that breeding work and the right choice of variety are of paramount importance for obtaining high-quality grain. The low

level of influence of annual conditions indicates relative stability to external conditions, i.e. the sedimentation index was least affected by growing conditions. The formation of protein and wet gluten content was influenced by a combination of both genetic and weather factors. In general, the results of the variance analysis prove that the quality of winter wheat grain is determined mainly by the genetic characteristics of the variety, but external factors also have a significant influence, especially in interaction with the genotype.

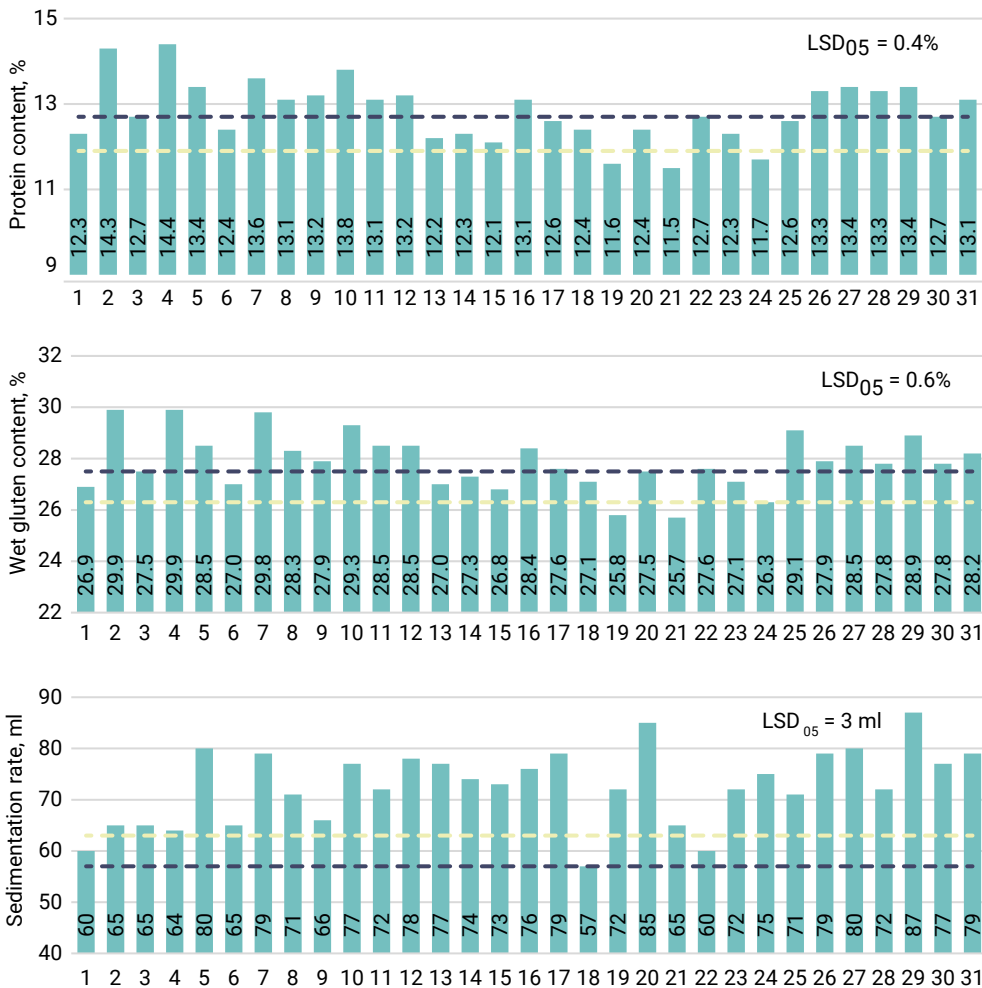
Researchers A.Z. Ghafoor *et al.* (2024) identified the influence of genotype, environment and farming methods (moderate and high) on the baking properties of wheat grain. The authors established a decisive influence of the genotype on the formation of the sedimentation index and a significant influence on the content of protein and wet gluten. They also noted that growing conditions have a significant impact on quality indicators. They emphasised the need for a comprehensive assessment of the influence of genetic and environmental factors, as well as their interactions on the formation of economically valuable traits. According to the results of studies by O. Demydov *et al.* (2023), the constitutive influence of the variety, compared to other factors (agrotechnical and environmental), was established only on the sedimentation index (30.2%). The wet gluten content was determined by the interaction of the variety  $\times$  year factors (40.3%), and the formation of the protein content depended both on the growing conditions (23.4%) and on the interaction of the variety  $\times$  year factors (21.8%). The authors note that genotype and genotype-environment interaction are the main factors influencing wheat grain quality. L.M. Prysiashniuk *et al.* (2022), when determining the influence of genotype, year conditions and growing area on the quality indicators of winter bread wheat grain, established the maximum influence of the growing area on protein (49.0%) and wet gluten (65.0%) content, while the impact of annual conditions was 42% for protein content and 28% for wet gluten content, and that of genotype was only 5% and 4%, respectively.

The authors emphasised that the synthesis and accumulation of nutrients in wheat grain depends on moisture supply and air temperature, so even minor changes in weather conditions and soil properties affect protein and wet gluten content. Some discrepancies were observed between the results obtained in this study and those reported by other researchers, as well as similar patterns in the influence of various factors on grain quality indicators, particularly the sedimentation index. However, all authors emphasise the reliable effect of genotype on the formation of wheat grain quality traits.

On average over the years of testing (Fig. 2), most winter bread wheat varieties significantly exceeded the Podolyanka standard variety: 60% of varieties in terms of protein content, 70% in terms of wet gluten content, and 93% in terms of sedimentation index. Winter bread wheat varieties were selected that, on average over two years, significantly exceeded the standard in terms of the studied flour quality indicators, namely: 'MIP Kniazhna', 'MIP Yuvileina', 'Avrora Myronivska', 'Pokrovska', 'Hlad', 'Zolotokolosa', 'Expromt', 'Kolumbiia', 'Optyma odeska', 'Doskonalist odeska', 'Perevaga', 'Kantata odeska', 'Kuialnyk', 'Mudrist odeska', 'Kubok', 'Lira odeska', 'Vahoma'. Of these, the varieties 'MIP Kniazhna', 'MIP Yuvileina', and 'Avrora Myronivska' significantly exceeded the 'Podolianska' variety in terms of protein and wet gluten content, while the varieties Pokrovska, Hlad, and Kolumbiia exceeded it in terms of three indicators. According to classification standards (Tkachyk, 2016), the following varieties of strong wheat were identified on average for 2023 and 2024 by protein content ( $\geq 14.0\%$ ) – MIP Kniazhna and Avrora Myronivska; based on wet gluten content ( $\geq 28.0\%$ ) – 'MIP Kniazhna', 'Avrora Myronivska', 'Pokrovska', 'Hlad', 'Zolotokolosa', 'Kolumbiia', 'Optyma odeska', 'Doskonalist odeska', 'Perevaha', 'Nyva odeska', 'Kuialnyk', 'Kubok', and 'Vahoma'; by sedimentation index ( $\geq 60$  ml) – all varieties except 'Vidpovid odeska'. Varieties Pokrovska, 'Osнова odeska', 'Kuialnyk', and 'Kubok' had a higher sedimentation index ( $\geq 80$  ml). The

varieties 'MIP Kniiazna' and 'Avrora Myronivska' were characterised by a complex of high grain quality indicators. Thus, the above-mentioned varieties

of winter bread wheat should be used in the breeding process as sources of appropriate grain quality indicators for the creation of high-quality material.



**Figure 2.** Grain quality indicators for winter bread wheat varieties, average for 2023 and 2024

**Note:** 1 – 'Podolianka', 2 – 'MIP Kniiazna', 3 – 'MIP Yuvileina', 4 – 'Avrora Myronivska', 5 – 'Pokrovska', 6 – 'Heizer', 7 – 'Hlad', 8 – 'Zolotokolosa', 9 – 'Expromt', 10 – 'Kolombiia', 11 – 'Optyma odeska', 12 – 'Doskonalist odeska', 13 – 'Spadshchyna odeska', 14 – 'Versiia odeska', 15 – 'Manera odeska', 16 – 'Perevaha', 17 – 'Pontiika', 18 – 'Vidpovid odeska', 19 – 'Virnist', 20 – 'Osнова odeska', 21 – 'Peremoha odeska', 22 – 'Zhuravka odeska', 23 – 'Zysk', 24 – 'Zorepad', 25 – 'Nyva odeska', 26 – 'Kantata odeska', 27 – 'Kuialnyk', 28 – 'Mudrist odeska', 29 – 'Kubok', 30 – 'Lira odeska', 31 – 'Vahoma'; dashed lines indicate the confidence interval of differences from the standard ( $p \leq 0.05$ )

**Source:** developed by the authors of current study

Over a period of 16 years, O.Yu. Leonov *et al.* (2025) evaluated 1,200 genotypes of winter bread wheat for a wide range of grain quality indicators. As a result of summarising many years of

data, sources of high protein and wet gluten content and quality, high rheological properties and other grain quality indicators were identified. Lines with a combination of high values of various quality

indicators for different areas of use were selected. It should be noted that the variation in protein (10.99-14.89%) and wet gluten (20.4-33.1%) content in this study was close to the current data. In studies by I. Havryliuk & H. Kovalyshyna (2024), when evaluating 53 varieties of winter bread wheat selected by four research institutes in Ukraine, significantly lower values of protein content (8.70-11.60%) and wet gluten content (15.57-20.73%) were noted compared to the results of the current study. However, the authors still identified varieties with higher grain quality indicators and recommended them for further use in breeding programmes. K.V. Kostetska *et al.* (2024), evaluating Ukrainian winter bread wheat varieties for their milling properties, obtained higher maximum values for protein (12.2-15.0%) and wet gluten (24.9-31.7%) content compared to results from the current study. However, a significantly lower sedimentation rate (27-58 ml) was noted. The authors emphasised that evaluating varieties for their milling properties makes it possible to justify their further use. Thus, the differences between the results obtained by different scientists and the current data depend on genotypes, specific cultivation factors and the environment.

The results of the studies confirmed the important role of both the genotypic characteristics of winter bread wheat varieties and hydrothermal conditions in determining grain quality indicators. It was established that the most stable factor is genetic potential, while external conditions significantly modify the manifestation of protein and wet gluten content. The differences identified between varieties made it possible to identify valuable sources for breeding work and the creation of high-quality starting material.

### Conclusions

The study revealed variability in the quality indicators of winter bread wheat depending on the hydrothermal conditions of cultivation. Higher average protein (13.3%) and wet gluten (28.7%) contents were observed in 2023, and higher sedimentation (74 ml) in 2024. It was established

that the experimental data on grain quality indicators obtained during the years of testing were close to normal distribution, since the degree of asymmetry and excess distribution did not exceed the critical value ( $|As/\sigma As| \leq 3$  and  $|Ex/\sigma Ex| \leq 3$ ). Weak and moderate variation in protein content (CV = 5.3-8.6%) and wet gluten content (CV = 3.9-5.8%) between winter bread wheat varieties during the years of testing and significant variability (CV = 10.2-13.5%) in the sedimentation index.

A decisive (40.7-63.8%) influence of the variety on the studied grain quality indicators was revealed. The conditions of the year had an insignificant effect on the sedimentation index (2.5%), but a significant effect on the protein (23.2%) and wet gluten (24.0%) content. The interaction between variety and year had a significant effect (26.9-32.5%) on three grain quality indicators. The results of the analysis of variance confirmed that the quality of winter wheat grain is mainly determined by the genetic characteristics of the variety, but external factors also have a significant influence, especially in interaction with the genotype. Winter bread wheat varieties that can be used in the breeding process as sources of increased protein content were identified: 'MIP Kniazhna' and 'Avrora Myronivska'; wet gluten content – 'MIP Kniazhna', 'Avrora Myronivska', 'Pokrovska', 'Hlad', 'Zolotokolosa', 'Kolumbiia', 'Optyma odeska', 'Doskonalist odeska', 'Perevaha', 'Nyva odeska', 'Kuialnyk', 'Kubok', and 'Vahoma'; sedimentation index – all varieties except the variety Vidpovid odeska. Winter bread wheat varieties that combine high values of the complex of studied grain quality indicators in one genotype have been identified – 'MIP Kniazhna' and 'Avrora Myronivska'.

The evaluation of winter bread wheat genotypes according to grain quality indicators and the selection of the best ones will help accelerate the breeding process in the creation of strong and valuable varieties. The further introduction of high-quality varieties into production will ensure: improvement in the quality of grain, flour and bakery products; improvement in the economic performance of agricultural enterprises, which

will contribute to the development of the grain processing industry in Ukraine.

None.

## Funding

## Acknowledgements

None.

## Conflict of Interest

None.

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## Оцінювання сортів пшениці м'якої озимої (*Triticum aestivum* L.) за показниками якості зерна

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**Анотація.** Підвищення валового збору високоякісного зерна потребує удосконалення селекційних програм шляхом їх постійного збагачення новою генетичною плазмою. Тому мета дослідження полягала у виявленні джерел поліпшення показників якості зерна для використання у селекційному процесі за рахунок диференціації різних сортів пшениці м'якої озимої в умовах центральної частини Лісостепу України. Оцінювали 31 сорт пшениці м'якої озимої за вмістом білка, вмістом клейковини та показником седиментації урожаю 2023 та 2024 рр. Проаналізовано характер розподілу отриманих значень показників якості зерна. Виявлено, що ступінь істотності асиметрії та ексцесу розподілення не перевищував критичного значення ( $|As/\sigma As| \leq 3$  і  $|Ex/\sigma Ex| \leq 3$ ). Таким чином, експериментальні дані за показником седиментації, вмістом білка та клейковини у роки випробувань були наближеними до нормального розподілу. Встановлено мінливість показників якості зерна пшениці м'якої озимої залежно від гідротермічних умов вирощування. Отримано вищий середній вміст білка (13,3 %) та клейковини (28,7 %) у 2023 р., а показника седиментації (74 мл) у 2024 р. За коефіцієнтом варіації виявлено значну варіабельність ( $CV = 10,2-13,5$  %) показника седиментації між сортами пшениці м'якої озимої у роки випробування, а також слабку та помірну варіацію вмісту білка ( $CV = 5,3-8,6$  %) та клейковини ( $CV = 3,9-5,8$  %). Виявлено визначальний (40,7-63,8 %) вплив сорту на досліджувані показники якості зерна. Умови року неістотно впливали на показник седиментації (2,5 %), однак суттєво на вміст білка (23,2 %) та клейковини (24,0 %). Частка впливу взаємодії чинників сорт  $\times$  рік була значною (26,9-32,5 %) для всіх трьох показників якості зерна. Виділено сорти пшениці м'якої озимої з вищими значеннями вмісту білка – 'МІП Княжна' та 'Аврора Миронівська'; вмісту клейковини – 'МІП Княжна', 'Аврора Миронівська', 'Покровська', 'Гладь', 'Золотоколоса', 'Колумбія', 'Оптіма одеська', 'Досконалість одеська', 'Перевага', 'Нива одеська', 'Куяльник', 'Кубок', 'Вагома'; показника седиментації – 'Покровська', 'Основа одеська', 'Куяльник', 'Кубок'

**Ключові слова:** *Triticum aestivum* L.; вміст білка; вміст клейковини; показник седиментації; гідротермічні умови; коефіцієнт варіації; ANOVA



UDC 632.4:633.63

DOI: 10.31548/dopovidi/6.2025.22

## Identification and species composition of pathogens of sugar beet storage-pile rot in short-rotation cropping systems of the Western Forest-Steppe of Ukraine

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**Abstract.** The relevance of the study is conditioned by the increase in phytosanitary risks due to the intensification of land use and reduction of crop rotation. The purpose of the study was to determine the species composition and population structure of fungal pathogens that affect beet roots during growth and storage. The study was conducted at the production fields of the private enterprise “Zakhidnyi Buh” using standard Mycological methods (isolation, morphological identification, calculation of the frequency of species occurrence). The species composition of the main pathogens of sugar beet storage-pile rot in short-rotation cropping systems of the Western Forest-Steppe of Ukraine, among which fungi of the genus *Fusarium* dominate, has been established. It was determined that five species of phytopathogenic fungi dominate short-rotation cropping systems: *Fusarium oxysporum* (30%), *F. solani* (18%), *Phoma betae* (19%), *Rhizoctonia solani* (13%), and *Botrytis cinerea* (10%). Total share of the genus *Fusarium* accounted for about 48% of all isolates, which indicates its leading role in the development of root and storage-pile rot. Significant morphological variability of isolates was found, in particular, variations in colony pigmentation and sporulation intensity, which confirms the high ecological plasticity of pathogens. It was shown that *F. oxysporum* and *Phoma betae* had the highest isolation frequency, while *B. cinerea* occurred mainly in conditions of high humidity. The results obtained indicate an increase in phytopathogenic load in short crop rotations and the development of stable fungal populations with high adaptive potential. The necessity for developing integrated sugar beet protection systems aimed at reducing the phytopathogenic load in short rotations was substantiated. The results obtained can be used in crop rotation planning, improving storage technologies, and introducing biological controls (*Trichoderma* spp. antagonists)

**Keywords:** storage of root crops; biological control; mycological analysis; *Fusarium oxysporum*; *Phoma betae*; *Rhizoctonia solani*

### Suggested Citation:

Kyselov, D. (2025). Identification and species composition of pathogens of sugar beet storage-pile rot in short-rotation cropping systems of the Western Forest-Steppe of Ukraine. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 22–30. doi: 10.31548/dopovidi/6.2025.22.

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## Introduction

Sugar beet (*Beta vulgaris* L.) is a strategic crop for the agro-industrial complex of Ukraine and Europe, providing raw materials for sugar production, bioenergy, and animal husbandry. However, the volatility of the price of products and the restriction of the number of processing enterprises, climate change lead to the intensification of agricultural production, in particular, the widespread use of short-rotation cropping systems (2-3 years of crop return to the field), contributes to soil fatigue, the accumulation of pathogens, and increased phytosanitary risks (Larney *et al.*, 2016).

The problem of intensification of crop rotations is systemic in nature and can be traced not only in beet, but also in forest and garden agrocoenoses. According to the findings of T. Bose *et al.* (2023), the use of monocultural plantings in short rotations in forestry leads to degradation of soil biota, a decrease in the proportion of beneficial microorganisms, and an increase in the frequency of phytopathogenic forms. The researchers emphasised that similar processes occur in agricultural systems where there is no microbial diversity that provides natural regulation of pathogens. Similar results were given by M. Li *et al.* (2020), who investigated the impact of sugar beet monoculture on highly productive soils in Northern China. Using metagenomic analysis, they found that long-term permanent cultivation leads to an imbalance of the microbiome, in particular, a decrease in populations of actinomycetes and saprotrophic fungi, which are natural antagonists of *Fusarium* spp. and *Rhizoctonia* spp. This increases the risk of accumulation of not only phytopathogenic fungi, but also highly virulent bacterial strains, in particular, *Pectobacterium* and *Burkholderia*.

V. Hanhur & V. Filonenko (2023) noted that in the Western Forest-Steppe of Ukraine, where high soil fertility and a favourable hydrothermal regime are combined, short crop rotations create conditions for the mass development of a complex of fungal diseases of sugar beet. The researchers proved that the reduction of

rotations is accompanied not only by an increase in the infectious load, but also by a decrease in the yield and technological qualities of raw materials. In further research, V. Hanhur & V. Filonenko (2024) showed that even the use of advanced tillage systems does not compensate for the negative effect of re-sowing beets. The complex of the main pathogens of beet root and storage-pile rot includes *Fusarium oxysporum*, *F. solani*, *Phoma betae*, *Rhizoctonia solani* and *Botrytis cinerea* (English, 2023). These pathogens are characterised by high ecological plasticity, the ability to form stable survival structures (chlamydospores, sclerotia, mycelial aggregates), and the versatility of damage – from field plants to root crops in storage facilities. According to W. English (2023), contamination during storage can lead to a loss of 10-15% of the mass of root crops during the first three months, and in years with high humidity – more than 20%.

R. Majumdar *et al.* (2024) reported that mechanical damage during beet digging creates conditions for secondary contamination with *Fusarium*, *Phoma*, and *Botrytis*, which reduces the sugar content and increases the content of inverse sugars. D. Wöber *et al.* (2025) found that microbial communities that form in storage pile determine not only mass stability, but also the rate of biochemical sugar loss. International experience shows the systemic effect of short crop rotations on the activation of soil pathogens. M. Roik & O. Yaholnyk (2024) noted that in Ukrainian agrocoenoses, the short-term crop system increases the manifestation of biotic stresses and increases the dependence of beet crops on weather fluctuations. The researchers suggest that optimising crop rotation and implementing biological controls are strategic for reducing crop losses. V.V. Ivanina & M.M. Gurska (2023) confirmed this trend in Ukrainian conditions, finding that excessive application of nitrogen fertilisers in crop rotations stimulates the development of fusarioses, changing the ratio between trophic groups of microorganisms in the soil.

A. Wolfgang *et al.* (2023) emphasised the importance of the “holobiont” concept in the “sugar beet microbiome” system. They considered the plant not in isolation, but as part of a microbial ecosystem, where the balance between pathogens and antagonists determines resistance to stress. This opens up prospects for integrated approaches to the biologisation of defence systems. In general, the analysis of the literature shows that short-term crop rotations are the main factor in the activation of the pathogen complex *Fusarium-Phoma-Rhizoctonia-Botrytis*, which determines the level of both field and post-harvest crop losses. The accumulation of inoculum in the soil, increased humidity in the microzone, and reduced microbial diversity create a closed cycle of infection. Advanced approaches to monitoring (qPCR, metagenomics) and biological control (use of antagonists of *Trichoderma spp.*, *Bacillus spp.*) open up new opportunities to prevent this phenomenon.

Contemporary studies both in Ukraine and abroad confirm that short-term crop rotations contribute to the accumulation of *Fusarium spp.*, *Phoma betae*, *Rhizoctonia solani* and *Botrytis cinerea*, which poses a threat to the stability of sugar beet production. Simultaneously, the development of molecular methods and the integration of biological control open up new prospects for reducing the risks of phytopathogenic pressure. Thus, the study of the species composition and population structure of fungal pathogens in beet agrocoenoses of short-rotation cropping systems in the Western Forest-Steppe is an urgent task that has both scientific and practical significance.

The purpose of the study was to identify the main pathogens of sugar beet storage-pile rot and analyse their population structure in the conditions of short-rotation cropping system in the Western Forest-Steppe of Ukraine.

## Materials and Methods

The object of research was samples of rhizospheric soil and sugar beet roots of the KWS Koncertina hybrid, which were grown in fields using short-rotation cropping system. The subject of

research was the species composition, morphological variability, and pathogenicity of fungal isolates isolated from these samples. 120 samples of sugar root crops and 40 samples of rhizospheric soil were selected for the study. Samples were taken 2 days after harvesting from field storage piles. The study was conducted in accordance with the ethical standards of the Convention on Biological Diversity (1992).

The research was conducted in 2024 at the production fields of the PE “Zakhidnyi Buh” (49°24'52”N; 24°18'31”E). The soil was characterised by a humus content of 3.2-3.5%, hydrolysed nitrogen 10-12 mg/100 g of soil, available phosphorus according to Chirikov 12-15 mg/100 g of soil, and exchange potassium 18-20 mg/100 g of soil. The reaction of the soil solution was slightly acidic (pH 6.2-6.4). The climate of the research zone was temperate continental, with an average annual precipitation of 580-620 mm, of which about 65% falls during the growing season.

Standard mycological approaches recommended in international practice were used to isolate pathogens (Leslie & Summerell, 2006; Misra *et al.*, 2023). Samples of affected sugar beet roots were pre-cleaned from the soil and superficially sterilised in a 1% sodium hypochlorite solution for 1-2 minutes, after which they were thoroughly washed three times in sterile distilled water. Sterilised fragments (3-5 mm) were transferred under sterile conditions to potato glucose agar (PGA), which was widely used for the cultivation of phytopathogenic fungi (Booth, 1971). The cups were incubated at 25-27°C for 5-7 days in the dark. For soil samples, the soil dilution method was used, which helped to determine the species composition and relative abundance of micromycetes in the rhizosphere. Soil suspension in serial dilutions was sown on PGA with the addition of streptomycin (50 mg/L) to suppress bacterial microflora. Primary colonies of pathogens were transferred by sowing hyphal tips or isolating monosporeous cultures to prevent mixing of species (Leslie & Summerell, 2006). This approach is standard when working with representatives

of the genus *Fusarium* and other fungi that form mixed colonies. Isolates were identified by morphological and cultural characteristics in accordance with modern atlases and keys (Booth, 1971; Summerell *et al.*, 2010). The main criteria were:

► for *Fusarium spp.*: shape and size of macro- and microconidia, presence of chlamydospores, pigmentation of colonies (Leslie & Summerell, 2006; Summerell *et al.*, 2010);

► for *Phoma betae*: characteristic dark concentric necrotic spots, slow sporulation, development of pycnidia with unicellular conidia (Aveskamp *et al.*, 2008);

► for *Rhizoctonia solani*: morphology of sclerotia, absence of sporulation, nature of hyphae with right-angled septa (Jash & Sarkar, 2025);

► for *Botrytis cinerea*: grey spore-bearing coating, multicellular oval-shaped conidia, rapid colony growth (Williamson *et al.*, 2007).

Microscopic studies were performed using a light microscope at magnifications of  $\times 400$ – $1,000$ . The isolation frequency of each species was determined as the percentage of positive seeding from the total number of samples. Relative abundance was calculated as the proportion of an individual species from the total number of isolates. The

$\chi^2$  test was used to test the significance of differences between soil types and crop rotations (Zar, 1999).

## Results

In the process of mycological analysis of root crops and rhizospheric soil of sugar beet grown in short-rotation cropping systems of the Western Forest-Steppe of Ukraine, 240 isolates of fungi belonging to the five dominant species were obtained: *Fusarium oxysporum*, *F. solani*, *Phoma betae*, *Rhizoctonia solani*, and *Botrytis cinerea*. In general, the genus *Fusarium* accounted for 48% of all selected crops, among which *F. oxysporum* was the leading species (30%), and *F. solani* – 18%. *Phoma betae* occupied 19% of the total number of isolates, *R. solani* – 13%, and *B. cinerea* – 10%.

The results show that short-term crop rotations contributed to the accumulation of pathogens, especially representatives of the genus *Fusarium* (Table 1). Among *Fusarium spp.* isolates, significant morphological variability was observed: variations in colony pigmentation (white-pink, orange, purple shades) and different rates of sporulation ( $2.5 \times 10^5$  -  $1.8 \times 10^6$  spores/mL). This indicates a high adaptive potential and ecological plasticity of pathogens.

**Table 1.** Species composition and frequency of occurrence of phytopathogenic fungi in sugar beet samples

Type of pathogen	Proportion of isolates, %	Range of variation, %
<i>Fusarium oxysporum</i>	$30.0 \pm 2.8$	25-35
<i>Fusarium solani</i>	$18.0 \pm 1.9$	16-21
<i>Phoma betae</i>	$19.0 \pm 2.1$	17-22
<i>Rhizoctonia solani</i>	$13.0 \pm 1.7$	11-15
<i>Botrytis cinerea</i>	$10.0 \pm 1.5$	8-12

**Source:** author's research

The results obtained (Table 1) indicate that the species composition of phytopathogenic fungi isolated from sugar beet roots was quite diverse, but it was characterised by a clearly defined dominant structure. The largest share was *Fusarium oxysporum* –  $30.0 \pm 2.8\%$ , which is on average 1.6–1.8 times higher than in other species. The high frequency of isolation of this pathogen indicates

its leading role in the development of a complex of root and storage-pile rot. Its variation in the range of 25–35% confirmed the stability of the species in conditions of short-rotation cropping systems. The second most common was *Fusarium solani* ( $18.0 \pm 1.9\%$ ), which together with *F. oxysporum* formed a single *Fusarium* block, covering about 48% of all isolates. This indicates the dominance

of *Fusarium* aetiology in the structure of the pathogenic complex, which is consistent with the findings of A. Farhaoui *et al.* (2023), who recorded the preference of these species in beet agrocoenoses with intensive soil use. Similar patterns were described by P. Kusstatscher *et al.* (2019), who showed that in sugar beet agrocoenoses, reduced microbial diversity correlates with an increase in the proportion of pathogenic forms *Fusarium oxysporum* and increasing their morphological variability. The researchers noted that it is the combination of stressful conditions and reduced rotations that contributes to the development of stable adaptive populations. The data obtained support these trends, demonstrating a predominance of *F. oxysporum* in short-rotation cropping systems. In addition, S. Misra *et al.* (2023) emphasised that the morphophysiological variability of pathogenic fungi is a key mechanism of their ecological adaptation. According to their data, variations in the rate of colony growth and sporulation are directly related to the ability of fungi to survive under the influence of environmental changes. Consistent with these findings, isolates of *Fusarium* from short-rotation cropping systems, an increased intensity of sporulation and pigmentation variation were shown, which indicates the formation of stable adaptive races of the pathogen. *Phoma betae* occupied  $19.0 \pm 2.1\%$  of the total number of isolates. Despite the lower frequency than *Fusarium* spp., this species is important as a necrotroph that actively affects weakened or mechanically damaged root crops. A wide range of variations (17-22%) can be caused by different humidity conditions over the years of observation.

Isolates of *Phoma betae* were characterised by a typical pycnidia development with dark concentric necrotic zones, whereas *Rhizoctonia solani* was manifested mainly due to the development of sclerotia and the low growth rate of colonies. *Botrytis cinerea* occurred less frequently, but was characterised by a high intensity of sporulation and a typical grey coating, which confirms its danger as a pathogen of storage-pile rot during storage. The results obtained confirmed that short-term crop

rotations in the Western Forest-Steppe are a factor of increasing the phytosanitary load. Domination *Fusarium* spp. (more than 45% of all isolates) indicates the development of populations resistant to agrotechnical influences that can persist in the soil for a long time due to chlamydospores. High detection rate of *Phoma betae* (19%) highlights its relevance as a causative agent of necrotic lesions and storage-pile rot, especially in wet years.

*Rhizoctonia solani* was detected in  $13.0 \pm 1.7\%$  of cases, which indicates a moderate but stable presence of the pathogen in the soil. Its distribution is closely related to its predecessors from the cereal family, in particular corn, which was confirmed by H.J. Koch *et al.* (2018). In areas with frequent cultivation of corn as a predecessor, a higher frequency of detection of rhizoctoniosis was recorded. Frequency of *Botrytis cinerea* in field samples was lower (10%), but the risk of its development increases significantly during storage, which was confirmed by C.A. Strausbaugh (2025). Thus, even a moderate infection in the field can be a source of massive damage in storage piles. Statistical comparison of the shares of identified species showed a significant prevalence of *Fusarium* spp. over other pathogens ( $\chi^2 = 12.43$ ;  $< 0.05$ ). The total proportion of fusarium isolates is almost twice as high as other groups of fungi, which indicates a stable trend of dominance of this genus in short-rotation cropping systems. The coefficient of variation between species was 22.6%, which indicates moderate heterogeneity of the pathogen population.

The results obtained confirmed the presence of a pronounced species differentiation of pathogens of sugar beet storage-pile rot in short-rotation cropping systems in the Western Forest-Steppe of Ukraine. According to the results of mycological analysis, five main types of fungi formed a stable complex of pathogens, among which *Fusarium oxysporum* (30%) and *F. solani* (18%) dominated. Total share of the genus *Fusarium* was 48%, which is almost twice the average for similar agricultural landscapes in Europe (Götze *et al.*, 2017). The statistical distribution of the isolation frequency

( $\chi^2 = 12.43$ ;  $p < 0.05$ ) showed a significant prevalence of *Fusarium* spp. over other species, which indicates their ecological competitive advantage in the conditions of short-rotation cropping systems. Similar patterns were established by A. Farhaoui *et al.* (2023), who proved that *F. oxysporum* was the main agent of root and post-harvest rot in North African regions, and its dominance increases with decreasing microbiota biodiversity. M. Lin *et al.* (2023) in China, short crop rotations (2-3 years) contributed to the accumulation of *Fusarium* spp. in the rhizosphere, while the longer rotations were dominated by saprotrophic species. This is consistent with the results of the current research and indicates a direct relationship between the frequency of crop return in crop rotation and the accumulation of pathogenic forms.

Compared to data by M. Bill *et al.* (2025) for northern US states, detection rate of *Phoma betae* in the current sample (19%) was higher, which is probably conditioned by a combination of high humidity and mild winters in the Western Forest-Steppe. The researchers pointed out that *Phoma betae* actively colonises mechanically damaged tissues and increases the manifestation of secondary infections. This is consistent with the current study of an increased incidence of necrotic lesions in years with excessive precipitation. Detected share of *Rhizoctonia solani* (13%) is consistent with the findings of H.J. Koch *et al.* (2018), who noted a significant spread of rhizoctoniosis in “corn-beet” cropping systems. Comparison of the average lesion rates indicates a moderate level of contamination ( $13 \pm 1.7\%$ ), which is statistically different from the control samples in the previously cited studies ( $t = 2.11$ ;  $p < 0.05$ ), which may be conditioned by the specifics of regional climatic conditions. Similar results were presented by S. Kalenska *et al.* (2025), who showed that pre-sowing seed treatment and foliar top dressing with chelated fertilisers contribute to increasing the physiological resistance of sugar beet plants and reducing the manifestation of fungal diseases. The obtained data are consistent with the results of this study, confirming the role

of increasing the immune potential of plants in reducing the activity of the pathogen complex of *Fusarium-Phoma-Rhizoctonia-Botrytis* in short-rotation cropping systems.

*Botrytis cinerea*, despite the low isolation rate (10%), has a high potential hazard during storage. According to W. English & H.L. Jönsson, (2023), this pathogen exhibits the ability to quickly activate in conditions of high humidity of storage piles and a decrease in temperature below  $+3^\circ\text{C}$ . In the current study, *B. cinerea* was recorded mainly in the late stages of storage, which indicates a similar mechanism for the development of secondary infection. The ecological stability of the pathogen complex was confirmed by the significant morphological variability of the isolated isolates. Changing colony pigmentation of *Fusarium* spp. (white-pink, purple, orange tones) and different intensity of sporulation ( $2.5 \times 10^5 - 1.8 \times 10^6$  spores/mL) indicate genetic plasticity of populations. Similar results were provided by P. Kusstatscher *et al.* (2019), who proved that a decrease in microbial diversity in the rhizosphere correlates with an increase in the frequency of pathogenic isolates. In this case, the coefficient of variation in the intensity of sporulation was 24.3%, which indicates the presence of several ecotypes adapted to different environmental conditions.

Summarising, the results of mathematical analysis ( $\chi^2$ , t-test, coefficient of variation) confirmed the reliability of dominance of *Fusarium* spp. and statistical differences between pathogen groups. This indicates the stability and adaptability of the pathogen complex in short-rotation cropping systems. Given environmental trends, further research should focus on identifying strains with high virulence, studying their genetic structure, and finding biological agents that can limit their development.

## Conclusions

As a result of the conducted studies in short-rotation cropping systems of the Western Forest-Steppe of Ukraine, the species composition,

frequency of occurrence, and morphological features of the main pathogens of sugar beet diseases were established. A total of 240 isolates belonging to the five dominant species were obtained from the affected root crops and rhizospheric soil: *Fusarium oxysporum*, *F. solani*, *Phoma betae*, *Rhizoctonia solani*, and *Botrytis cinerea*. The leading role in the structure of pathogens is played by the genus *Fusarium*, the share of which accounted for 48% of all isolates. Among them, *F. oxysporum* ( $30.0 \pm 2.8\%$ ) and *F. solani* ( $18.0 \pm 1.9\%$ ) dominated. Significant morphological variability of these isolates - variations in the colour of colonies from white-pink to purple and fluctuations in the level of sporulation in the range of  $2.5 \times 10^5 - 1.8 \times 10^6$  spores/mL – indicates a high adaptive ability of populations. *Phoma betae* was detected in  $19.0 \pm 2.1\%$  of cases, forming pycnidia with dark pigmentation and necrotic lesions characteristic of beet phomosis. *Rhizoctonia solani* occurred with a frequency of  $13.0 \pm 1.7\%$ , especially in areas where the predecessor was corn, which confirms the relationship of rhizoctoniosis with short rotations. *Botrytis cinerea* accounted for  $10.0 \pm 1.5\%$  of all isolates, but even a minor field infection can cause massive damage to root crops during storage. Statistical analysis ( $\chi^2 = 12.43$ ;  $p < 0.05$ ) confirmed a significant dominance of *Fusarium* spp. among

phytopathogenic complexes. The general structure of infectious load in short-rotation cropping systems indicates the predominance of *Fusarium* and *Phomopsis* aetiology.

The results highlighted the environmental risks of intensive use of short rotations and indicated the need for an integrated approach to sugar beet protection. It is advisable to combine crop rotation optimisation with the introduction of biological control methods, in particular, antagonistic fungi *Trichoderma* spp., application of sanitary measures during root crop storage and monitoring of the state of rhizospheric microflora to prevent the accumulation of pathogens.

The prospects for further research are to conduct molecular identification of isolated isolates, investigate their genetic variability and pathogenicity, and develop effective biological products for biological protection of sugar beet in the Western Forest-Steppe of Ukraine.

### Acknowledgements

None.

### Funding

Production programs of the PE “Zakhidnyi Buh”.

### Conflict of Interest

None.

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## Ідентифікація та видовий склад збудників кагатних гнилей цукрового буряка в короткоротаційних сівозмінах Західного Лісостепу України

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**Анотація.** Актуальність дослідження обумовлена підвищенням фітосанітарних ризиків через інтенсифікацію землекористування та скорочення ротачії культур. Мета роботи полягала у визначенні видового складу та популяційної структури грибних патогенів, що уражують коренеплоди буряка під час вегетації та зберігання. Дослідження проводили на базі виробничих полів приватного підприємства «Західний Буг» із використанням стандартних мікологічних методів (ізоляція, морфологічна ідентифікація, підрахунок частоти трапляння видів). Встановлено видовий склад основних збудників кагатних гнилей цукрового буряка у короткоротаційних сівозмінах Західного Лісостепу України, серед яких домінують гриби роду *Fusarium*. Визначено, що у короткоротаційних сівозмінах домінують п'ять видів фітопатогенних грибів: *Fusarium oxysporum* (30 %), *F. solani* (18 %), *Phoma betae* (19 %), *Rhizoctonia solani* (13 %) та *Botrytis cinerea* (10 %). Загальна частка роду *Fusarium* становила близько 48 % усіх ізолятів, що свідчить про його провідну роль у розвитку кореневих і кагатних гнилей. Виявлено значну морфологічну мінливість ізолятів, зокрема варіювання пігментації колоній та інтенсивності спороношення, що підтверджує високу екологічну пластичність патогенів. Показано, що найвищу частоту ізоляції мали види *F. oxysporum* та *Phoma betae*, тоді як *B. cinerea* траплялася переважно в умовах підвищеної вологості. Отримані результати свідчать про зростання фітопатогенного навантаження у коротких сівозмінах і формування стабільних популяцій грибів із високим адаптаційним потенціалом. Обґрунтовано необхідність для розробки інтегрованих систем захисту цукрового буряка, спрямованих на зниження фітопатогенного навантаження в коротких ротациях. Отримані результати можуть бути використані при плануванні сівозмін, удосконаленні технологій зберігання та впровадженні біологічних засобів контролю (антагоністів *Trichoderma* spp.)

**Ключові слова:** зберігання коренеплодів; біологічний контроль; мікологічний аналіз; *Fusarium oxysporum*; *Phoma betae*; *Rhizoctonia solani*



UDC 514.18

DOI: 10.31548/dopovidi/6.2025.31

## Formation a strip with a trapezoidal cross-section into a ring by rolling between conical rolls

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### **Suggested Citation:**

Kresan, T., Volina, T., Nesvidomin, V., Fedoryna, T., & Pylypaka, T. (2025). Formation a strip with a trapezoidal cross-section into a ring by rolling between conical rolls. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 31-42. doi: 10.31548/dopovidi/6.2025.31.

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**Abstract.** The formation of helical surfaces requires a ring that enables their continuous production without welded seams. The aim of this study was to develop an analytical description of the process of deforming a straight strip into a ring as a result of rolling it between conical rolls. The research methodology was based on analytical modelling of the process of plastic deformation of the strip using assumptions about the isotropy of the material and the constancy of its volume. As a result of the study, analytical dependencies were obtained between the dimensions of the strip cross-section before rolling and the parameters of the ring after rolling, which allow determining the optimal geometric characteristics for forming rings of a given radius. Generalised analytical expressions were developed to determine the elongation coefficient and ring radius, which take into account the change in the aspect ratio of the trapezoidal cross-section of the strip. A model of step-by-step rolling has been proposed, which describes the gradual reduction of the aspect ratio of the trapezoidal cross-section to a rectangular one, ensuring a uniform cross-section of the ring. The obtained expressions made it possible to solve both direct and inverse problems, determine the parameters of the initial strip for forming rings of different radii, predict the geometry of the ring based on the known dimensions of the strip, and use the obtained data to adjust the rolls before experimental verification. The proposed model of step-by-step rolling ensured a uniform cross-section of the ring, which increases the accuracy of manufacturing screw surfaces without welded seams. The results may be applied in mechanical engineering for the production of screws, helical surfaces, and conveyors without welded seams, particularly in the agricultural, food-processing, construction, and energy industries

**Keywords:** plastic deformation; volume; isotropic material; ring radius; relative elongation

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## Introduction

Deformation of a ring with a rectangular cross-section can form a helical surface, which is widely used in various fields of mechanical engineering. There were various technologies for manufacturing helical surfaces, including welding individual turns. This method has limitations due to the presence of welded joints, which reduce the strength of the structure. When forming a flat ring by rolling, it is possible to manufacture solid helical surfaces of the required length without welded joints, which increases their reliability. Existing technologies for winding helical surfaces from a straight strip with a rectangular cross-section require considerable effort to deform the material. In addition, this manufacturing method often leads to instability of the deformation process, which can cause metal breaks on the outer edge and the formation of corrugations on the inner edge. Thus, the manufacture of helical surfaces from a ring, which is an approximate development of the surface, is simpler, more

accurate and more cost-effective. That is why the formation of a ring of a given radius by rolling a straight strip remains a relevant problem that requires in-depth research.

Helical surfaces are an integral part of many devices and mechanisms, in particular for transporting bulk materials, soil cultivation and the production of high-precision parts. Despite their widespread use, existing manufacturing methods have limitations, particularly in terms of thickness uniformity and geometric accuracy. I. Hevko *et al.* (2021) proposed using elastic helical surfaces to prevent damage to bulk materials during transport. The authors focused on modifying the material but did not consider optimising the geometry of the blank for forming the ring, which limits the application of the method for heavy-duty structures. M.B. Klendii & A.P. Dragan (2021) investigated the use of helical surfaces for soil cultivation, focusing on the design features of the working bodies, but did not address the issue of forming

blanks with a trapezoidal cross-section, which could improve the strength characteristics of the parts. Various approaches, including both analytical and numerical methods, have been proposed to solve the problem of optimising surfaces that pass through a spiral curve with a variable pitch. For example, A. Nesvidomin *et al.* (2025) developed a method for optimising the model of a developing surface for such cases, which can be applied to rolling and surface forming processes in various fields of mechanical engineering.

Scientists O. Liashuk *et al.* (2019) conducted a technical and economic justification for the manufacture of screw working bodies, but did not propose analytical models for predicting the geometric parameters of rings during rolling. I. Oh *et al.* (2019) investigated defects that arise during the rolling of L-shaped rings and proposed methods for minimising them, but did not consider the forming of rings with a trapezoidal cross-section, which ensures a uniform thickness of the finished part. L. Liang *et al.* (2021) focused on the accuracy criteria for rolling rings with a profiled cross-section, and L. Liang *et al.* (2022) focused on the mechanisms of geometric defect formation. Although these works describe the deformation processes in detail, they do not offer analytical dependencies for calculating the parameters of the initial strip, which limits the possibility of optimising the process.

Researchers D. Bai *et al.* (2021) analysed intelligent forming technologies but did not address the specifics of rolling trapezoidal strips, which allows avoiding ring thickness irregularities. S. Yuan & X.B. Fan (2019) outlined the prospects for the precise forming of ultra-large integrated components, but did not consider the step-by-step rolling of strips with a trapezoidal cross-section, which allows the geometry of the ring to be controlled at each stage of deformation.

To summarise the above, the following general, previously unresolved issues can be formulated:

- the lack of analytical models for calculating the parameters of the trapezoidal cross-section output strip required to form rings of a given radius;

- insufficient attention by researchers to step-by-step rolling, which ensures a uniform cross-section of the ring and reduces the risk of defects;

- limited information on optimising the settings of conical rolls for forming rings with high precision.

It should be noted that the issue of forming helical surfaces from flat blanks can be considered from the point of view of the rational selection of these blanks. A flat ring can be cut from sheet material, but this method leads to significant material waste and costs for cutting the required contour from it. It is much easier to obtain this contour by rolling a straight strip. When forming a ring by rolling a strip of rectangular cross-section, it will have a variable thickness, which will be thinner at the periphery of the ring, i.e. at the outer edge of the screw surface, which wears out the most. In order for the thickness of the ring to be uniform, a straight strip with a trapezoidal cross-section must be used.

The aim of the study was to provide an analytical description of the transformation of a straight strip with a trapezoidal cross-section into a flat ring of a given radius by rolling it between conical rolls. This raises the following objectives:

- based on the plastic properties of the metal and the geometric properties of the shape, develop an approximate model of the redistribution of its mass under the action of deforming forces;

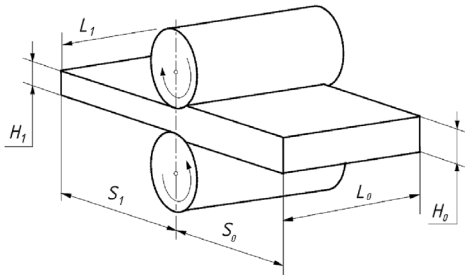
- find the dimensions of the cross-section of a straight strip for forming a flat ring of a given thickness and radius.

## Materials and Methods

The initial thickness  $H_0$  of a straight strip with a rectangular cross-section after rolling between cylindrical rolls (Fig. 1) decreased to a value  $H_1$ . It was assumed that the volume of the strip after rolling remained constant. The second condition was that the strip material was isotropic, so elongation occurred equally in all directions. An elongation coefficient  $p$  was introduced as the ratio of the corresponding sides of the strip element before and after deformation. In this case, the dimensions of the strip element after

deformation were recorded taking into account this coefficient  $p$ :  $L_1 = p \cdot L_0$ ,  $S_1 = p \cdot S_0$ . The thickness of the strip before deformation  $H_0$  and after deformation  $H_1$  were given values. Equating the volumes of the strip element before and after rolling gave the result  $L_0 H_0 S_0 = p^2 L_0 S_0 H_1$ , hence:

$$p = \sqrt{\frac{H_0}{H_1}}. \quad (1)$$



**Figure 1.** Diagram of rolling a straight strip with a rectangular cross-section between cylindrical rolls

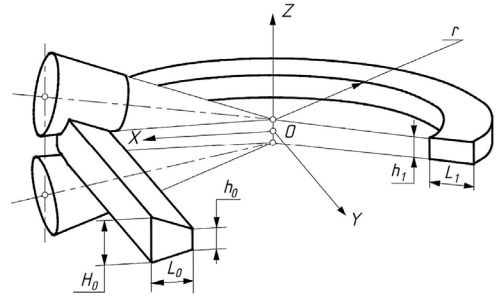
**Note:**  $H_0$  – strip thickness before deformation;  $H_1$  – strip thickness after deformation;  $L_0$  – strip width before deformation;  $L_1$  – strip width after deformation;  $S_0$  – strip length before deformation;  $S_1$  – strip length after deformation

**Source:** developed by the authors

With a known coefficient  $p$ , it is possible to find the longitudinal and transverse elongation of the strip. After rolling, the thickness of the strip decreased, while its width and length changed in proportion to the elongation coefficient. To describe the process, the assumption of material isotropy and volume constancy was used, which allowed the volumes of the strip before and after deformation to be equated. The elongation coefficient was defined as the ratio of the dimensions of the strip element after deformation to its initial values. Based on this, mathematical dependencies were introduced, which were used to further describe the transformation of a straight strip into a ring.

## Results

The rolling diagram of a straight strip with a trapezoidal cross-section between conical rolls is shown in Figure 2.



**Figure 2.** Diagram of rolling a straight strip with a trapezoidal cross-section into a flat ring  
**Note:**  $O_{xyz}$  – axes of the spatial coordinate system;  $L_0$  – height of the trapezoid of the strip cross-section before deformation;  $H_0$  – larger side of the trapezoid of the strip cross-section before deformation;  $h_0$  – smaller side of the trapezoid of the strip cross-section before deformation;  $L_1$  – width of the strip after deformation;  $h_1$  – thickness of the strip after deformation;  $r$  – radius of the inner arc of the ring

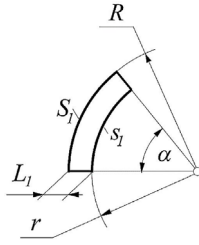
**Source:** developed by the authors

The rolls were adjusted so that the thickness of the ring after rolling the strip was equal to the shorter side of the trapezoid, i.e.  $h_1 = h_0$ . This means that the intensity of the strip deformation in the transverse direction will increase from the shorter side of the trapezoid to the longer side. Based on this, it can be assumed that during rolling, the material will also elongate in this direction. It follows that the length of the arc of the inner radius of the ring is equal to the length of the corresponding straight segment of the strip before deformation. This means that the outer arc of the ring elongates in the longitudinal direction, while the inner arc does not elongate and transforms into a circle with radius  $r$  (Fig. 2).

The volume  $V_0$  of a straight strip element with a trapezoidal cross-section of length  $s_0$  was determined by the following formula:

$$V_0 = \frac{H_0 + h_0}{2} L_0 S_0. \quad (2)$$

After deformation, the element of a straight line will turn into a corresponding element of a flat ring. An image for determining its volume is shown in Figure 3.



**Figure 3.** Projection of a flat ring element with its dimensions marked

**Note:**  $r$  – radius of the inner arc of the ring;  $R$  – radius of the outer arc of the ring;  $s_i$  – length of the inner arc;  $S_o$  – length of the outer arc;  $L_1$  – width of the strip after deformation;  $\alpha$  – central angle

**Source:** developed by the authors

Given that the thickness of the ring is  $h_1 = h_0$ , its volume can be found using the formula:

$$V_1 = \frac{R^2 - r^2}{2} h_0 \alpha. \quad (3)$$

It is assumed that the length of the inner arc  $s_i$  did not change during deformation and is equal to the length of the straight strip element, i.e.  $s_i = s_0$ . From this, an expression for the angle  $\alpha$  can be found:  $\alpha = s_0/r$ . The length of the outer arc  $S_o$  has increased, as has the width of the strip. The elongation of the strip width can be written in terms of the elongation coefficient  $p$ :  $L_1 = p \cdot L_0$ . In this case, for the radius  $R$  of the outer arc, the expression:  $R = r + p \cdot L_0$  can be written. The length of the arc  $S_o$  within the central angle  $\alpha$  can be found using the known formula:  $S_o = R \cdot \alpha = (r + p \cdot L_0) \alpha = (r + p \cdot L_0) s_0/r$ . On the other hand, the length of the arc  $S_i$  is the result of the elongation of the straight line strip, i.e.  $S_i = p \cdot s_0$ . Equating these two expressions, as well as the volume (2) before deformation and the volume (3) after deformation, allows to obtain a system of two equations with two unknown quantities  $p$  and  $r$ :

$$\begin{cases} \frac{H_0 + h_0}{2} L_0 s_0 = \frac{h_0 s_0}{2r} [(r + p L_0)^2 - r^2]; \\ p s_0 = (r + p L_0) \frac{s_0}{r}. \end{cases} \quad (4)$$

Reducing equations (4) by  $s_0$  and solving for  $r$  and  $p$  gives the result:

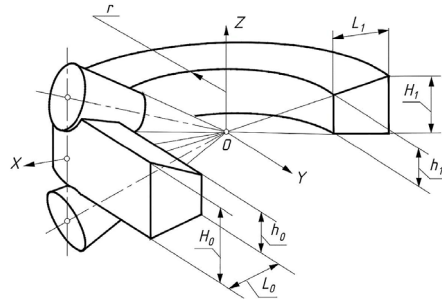
$$p = \frac{-h_0 + \sqrt{h_0(5h_0 + 4H_0)}}{2h_0}, \quad (5)$$

$$r = L_0 \frac{h_0 + 2H_0 + \sqrt{h_0(5h_0 + 4H_0)}}{2(H_0 - h_0)}. \quad (6)$$

In the case where  $H_0 = h_0$ , i.e. in the absence of plastic deformation, from (5)  $p = 1$ , can be obtained, and from (6)  $r = \infty$ , i.e. there is no elongation and the strip remains straight. The expressions obtained make it possible to solve the inverse problem, i.e. to find the dimensions of the cross-section of a straight strip to obtain a ring of a given radius. From equation (6), an expression for  $H_0$  can be found:

$$H_0 = h_0 \frac{L_0 r - L_0^2 + r^2}{(L_0 - r)^2}. \quad (7)$$

A model of step-by-step rolling of the strip can be developed, i.e. gradually reducing the larger side of the trapezoidal cross-section. Then the strip turns into a ring, the cross section of which will also be a trapezoid, but with a smaller difference between the longer and shorter sides (Fig. 4). In this case, the same deformation conditions remain as in the previous case, i.e.  $h_1 = h_0$ .



**Figure 4.** Diagram of rolling a straight strip with a trapezoidal cross-section into a ring with a smaller ratio of opposite sides of the trapezoid

**Note:**  $O_{xyz}$  – axes of the spatial coordinate system;  $L_0$  – height of the trapezoidal cross-section of the strip before deformation;  $H_0$  – larger side of the trapezoidal cross-section of the strip before deformation;  $h_0$  – the smaller side of the trapezoid of the strip cross-section before deformation;  $L_1$  – the height of the trapezoid of the strip cross-section after deformation;  $H_1$  – the larger side of the trapezoid of the strip cross-section after deformation;  $h_1$  – the smaller side of the trapezoid of the strip cross-section after deformation

**Source:** developed by the authors

The volume of the ring can be found by means of integral calculus as a body formed by the rotation of a trapezoid. Similarly, a system of two equations was obtained, the solution of which gave the following results:

$$p = \frac{-2h_0 - H_1 + \sqrt{16h_0^2 + 28H_1h_0 + H_1^2 + 12H_0(h_0 + 2H_1)}}{2(h_0 + 2H_1)}, \quad (8)$$

$$r = L_0 \frac{4h_0 - H_1 + 6H_0 + \sqrt{16h_0^2 + 28H_1h_0 + H_1^2 + 12H_0(h_0 + 2H_1)}}{6(H_0 - H_1)}. \quad (9)$$

When  $H_0 = H$  there is no deformation, and the values of  $p$  and  $r$  are similar to the previous case. If in expressions (8) and (9) the value  $H_1 = h_0$ , in expressions (8) and (9), new expressions are obtained that exactly coincide with (5) and (6). Thus, expressions (8) and (9) are generalised. They describe the process of gradual formation of a flat ring by reducing the ratio of the larger and smaller bases of an isosceles trapezoid to unity, i.e. to a rectangular cross-section. At the same time, the height of the trapezoid increases, which ultimately turns into the width of the ring.

Let the straight transverse cross-section of the strip have the following dimensions in millimetres:  $H_0 = 15$ ;  $h_0 = 5$ ;  $L_0 = 40$ . The elongation coefficient  $p$  can be found according to (5):  $p = 1,56$ . According to (6), the inner radius of the flat ring is  $r = 111$ . The width of the ring is accordingly  $L_0 = p \cdot L_0 = 62$ . The dimensions of the cross-section of the ring are  $5 \times 62$ . If the ring needs to be closed, the inner arc  $s$  must have a length of  $2 \cdot \pi \cdot r = 697$ . The straight strip must have the same length, since, according to the condition, the length of the inner arc of the ring is equal to the length of the strip. If  $r = 111$ , is substituted into formula (7), the result is  $H_0 = 15$ .

## Discussion

In metal pressure processing, a common method is to deform workpieces by rolling them between rolls. Precise analytical results regarding the relative position of the rolls to achieve the desired result are often difficult to obtain due to the complexity of the plastic deformation process. Roll

adjustment is usually based on accumulated experience and experimentation. However, to begin with, it is necessary to know at least the approximate value of the radius  $r$  (Fig. 2) in order to adjust the rolls correctly. Subsequently, the adjustment can be refined experimentally, but the availability of analytical dependencies significantly reduces the time and cost of equipment preparation.

Unlike the model proposed by L. Hua *et al.* (2023), which focuses on modelling the behaviour of metal during the rolling of thin-walled conical rings, the model proposed in the current study was based on two fundamental properties of plastic deformation of metal: volume constancy and material isotropy. These assumptions allow to obtain analytical dependencies that do not depend on experimental data, unlike the approaches described in the works of Z. Dong *et al.* (2019) and X. Han *et al.* (2023), where the emphasis is on numerical modelling and experimental settings. This is especially important for industrial applications, where the speed of equipment adjustment directly affects production productivity.

It can be seen that for the three rolling cases (Fig. 1, Fig. 2 and Fig. 4), the elongation coefficients  $p$  (1), (5) and (8) do not depend on the strip width  $L_0$ , but only on the aspect ratio  $H_0$  and  $H_1$ . This allows to calculate the dimensions of the transverse trapezoidal cross-section of a straight strip so that, as a result of rolling, flat rings of the same cross-section but with different radii  $r$  are obtained. For example, let the dimensions of the straight cross-section of the strip be:  $H_0 = 20$ ;  $h_0 = 5$ ;  $L_0 = 35$ . After its deformation, the value of the rectangular cross-section of the ring will practically not change ( $5 \times 62$ ), but the radius  $r$  will be different:  $r = 79$ . This indicates the universality of the proposed dependencies, which can be applied to different geometric parameters of the strip. Expressions (8) and (9) are generalised and describe the deformation process of a straight strip with a transverse cross-section that is an isosceles trapezoid. They describe the step-by-step deformation of the strip by reducing the larger side of the trapezoid's base until the bases

become equal, i.e. the trapezoid turns into a rectangle. It should be noted that at the intermediate stages of deformation, the vertices of the conical rolls are located in the centre of the ring (Fig. 4), and at the final stage, they are located on the axis of the ring and there is a distance between them equal to the thickness of the ring (Fig. 2). This distinguishes the present study from the work of Z. Dong *et al.* (2019), who focused on the rolling of large conical cylinders, and X. Han *et al.* (2023), who investigated the formation of cylindrical rings with high ribs. Unlike these works, the present study proposes a step-by-step rolling model that describes the reduction of the larger side of the trapezoid until it becomes a rectangle. This is particularly important for controlling the geometry of the ring at intermediate stages of deformation, which was not considered in the works of D.S. Qian *et al.* (2023) and D. Qian *et al.* (2024), where the emphasis was on controlling the rolling process using a closed loop.

It should be noted that X. Chang *et al.* (2023) investigated the influence of roller cross-sectional diameter on the rolling of double-curved surfaces. Like the study described above, this work concerns the processes of rolling and forming complex surfaces using metal pressure treatment methods. X. Chang *et al.* investigated the rolling of double-curved surfaces with a focus on the influence of roller diameter on forming quality. The authors focus on optimising roller parameters to minimise defects. In the study described, the emphasis is on the analytical description of the deformation process and obtaining generalised expressions for calculating the parameters of the strip and ring. This study complements the work of X. Chang *et al.*, proposing a new approach to forming rings from straight strips with a trapezoidal cross-section. Unlike the optimisation of roller parameters for rolling double-curved surfaces, the proposed method allows rings to be obtained directly from the strip, which is more technologically advanced and economical.

The work of W.Y.D. Yuen (2025) focused on the analysis of thermal and mechanical deformations

of strips during flat rolling, in particular on the influence of friction, heat generation and uneven stress distribution in the contact zone between the rolls and the strip. The author emphasised the importance of taking into account the thermoelastic deformations of the rolls to prevent defects in the flatness of the strip. As in the study described above, the author analyses the processes of plastic deformation of the material under the influence of external loads, but does not consider the specifics of forming rings with a trapezoidal cross-section, which requires taking into account the uneven elongation across the width of the strip and the gradual reduction of the trapezoid aspect ratio. The study by J. Miao *et al.* (2022) focused on the analysis of contact behaviour and thermal deformations in planetary roller screw mechanisms used to convert rotary motion into linear motion. Unlike the study described above, this work examines the effect of thermal deformations on the accuracy of motion transmission and load distribution in screw mechanisms, as well as the optimisation of tooth profiles to reduce contact stresses and wear. Common to the work of J. Miao *et al.* and the presented study is the emphasis on the importance of considering geometric parameters to achieve the desired characteristics of the final product.

The work of Y. Caoqi *et al.* (2024) is devoted to the issue of synchronous forming of the outer shape and inner hole of hollow shafts using cross-screw rolling with a mandrel. The authors focused on analysing the influence of process parameters (tension angles, roll rotation speed, etc.) on the axial force and the quality of the final shape. Similar to the study by Y. Caoqi *et al.*, they considered the plastic deformation of metal under the influence of external forces, emphasising the importance of the geometric parameters of the workpiece and tool in order to optimise the deformation process and achieve a high-quality final product. Unlike Y. Caoqi *et al.*, the presented study obtained generalised analytical expressions for the elongation coefficient and ring radius, which makes the proposed approach

universal and suitable for a wide range of geometric configurations. An innovative method for forming railway car axles using three-roll cross-screw rolling was proposed by Z. Pater *et al.* (2020). The method proposed by the authors allows complex profiles to be formed with high accuracy. In contrast to that work, the present study develops a model that describes the step-by-step reduction of the trapezoidal cross-section aspect ratio to a rectangular form, which allows control of ring geometry at intermediate stages of deformation and minimises the risk of defects.

Scientists S. Pylypaka *et al.* (2022) focused on studying the process of bending flat rings into screw conoids – conical surfaces formed by rotating a straight line around an axis at a certain angle. Like the study described above, the work of S. Pylypaka *et al.* focused on the processes of forming helical surfaces and rings using metal pressure treatment methods. Unlike S. Pylypaka *et al.*, who described the corrugation of the inner edge and the uneven wall thickness of the conoid, the presented study focuses on the uneven thickness of the ring and possible metal breaks. S. Pylypaka *et al.* used an experimental approach and numerical modelling to analyse the bending process. The authors focused on determining the optimal angles of inclination of the helix and material parameters. In contrast, the present study uses analytical modelling based on the properties of plastic deformation (constant volume and isotropy of the material). This allows analytical dependencies to be obtained for calculating the parameters of the strip and ring. Thus, the results obtained complement the research of S. Pylypaka *et al.*, offering a new approach to the formation of rings from straight strips of trapezoidal cross-section. Unlike the bending of finished rings, the proposed method allows rings to be obtained directly from the strip, which is more technological and economical. The analytical dependencies obtained in the work allow optimising the parameters of the strip and ring, which makes the presented approach more universal and accurate.

M. Pylypets *et al.* (2021) considered combined operations for the production of screw blanks, but did not focus on optimising the geometry of the strip cross-section. This work, like the presented study, is aimed at analysing metal deformation processes, but from different perspectives: M. Pylypets *et al.* (2021) focus on combined operations that include several processing stages, while the presented approach offers an analytical description of the rolling process for a trapezoidal strip. Unlike the rolling diagrams shown in Figures 2 and 4, where the sides of the ring are idealised as straight lines, in practice they will have a curved shape. This can be avoided by creating rolls with a profile that matches the cross-sectional profile of the ring after rolling. This approach was not discussed in detail in the works of L. Liang *et al.* (2021; 2022), which focused on the accuracy criteria for rolling rings with a profiled cross-section. The results obtained allow not only to calculate the parameters of the strip for forming rings, but also to optimise the profile of the rolls, which increases the accuracy and quality of the finished products.

## Conclusions

When rolling a straight strip between cylindrical or conical rolls, plastic deformation of the metal occurs, i.e. its redistribution in volume. There is no accurate analytical description of such redistribution due to the complexity of the process. This is due to the non-linearity of the deformation characteristics of the material and its interaction with the tool. The proposed analytical description of the metal mass redistribution process is based on two fundamental properties of plastic deformation: volume constancy and material isotropy. These assumptions made it possible to obtain analytical dependencies that link the geometric parameters of the initial strip with the characteristics of the formed ring. The work provides analytical expressions for the elongation coefficient  $p$  and the ring radius  $r$ , which allow the parameters of the strip for forming rings with different radii to be calculated. A model of step-by-step rolling has been developed, which

describes the gradual reduction of the aspect ratio of the trapezoidal cross-section of the strip to a rectangular one. This allows rings with uniform thickness to be obtained and the geometry to be controlled at intermediate stages of deformation, which minimises the risk of defects such as corrugation or tears. Generalised expressions for calculating the parameters of the strip and ring are proposed, which can be applied to different geometric configurations. This allows solving both the direct problem (determining the parameters of the ring for known strip dimensions) and the inverse problem (selecting the parameters of the strip to obtain a ring of a given radius). It is shown that the elongation coefficients  $p$  for different rolling cases (with cylindrical and conical rolls) do not depend on the strip width  $L_0$ , but only on the aspect ratio  $H_0$  and  $H_1$ . This simplifies calculations and allows the same analytical dependencies to be used for different types of strips. The proposed analytical description provides data for the initial adjustment of the rolls, which significantly reduces the time required for equipment preparation and reduces the cost of experimental

verification. In the future, the adjustment of the rolls can be refined experimentally, but the availability of analytical dependencies allows this process to be significantly optimised. Prospects for further research lie in the development of an analytical description of obtaining parts by rolling with a variable cross-section, as well as in studying the influence of different materials and rolling conditions on the accuracy of ring forming. This will expand the scope of application of the results obtained and increase their versatility for industrial applications.

### Acknowledgements

The authors of the article express their gratitude to the defenders of Ukraine, whose bravery and selflessness enable scientists to continue their work in their home country.

### Funding

None.

### Conflict of Interest

None.

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## Формування смуги із трапецеїдальним поперечним перерізом у кільце прокаткою між конічними валками

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**Анотація.** Для формування гвинтових поверхонь потрібне кільце, що дає змогу виготовляти їх неперервно та без зварних швів. Метою дослідження було розроблення аналітичного опису процесу деформації прямолінійної смуги у кільце в результаті її прокатки між конічними валками. Методологія дослідження ґрунтувалася на аналітичному моделюванні процесу пластичної деформації смуги із використанням припущень про ізотропність матеріалу та незмінність його об'єму. У результаті дослідження отримано аналітичні залежності між розмірами поперечного перерізу смуги до прокатки та параметрами кільця після прокатки, що дозволяють визначати оптимальні геометричні характеристики для формування кілець заданого радіуса. Розроблено узагальнені аналітичні вирази для визначення коефіцієнта подовження та радіуса кільця, які враховують зміну співвідношення сторін трапецеїдального перерізу смуги. Запропоновано модель поетапної прокатки, яка описує поступове зменшення співвідношення сторін трапецеїдального перерізу до прямокутного, що забезпечує отримання рівномірного поперечного перерізу кільця. Отримані вирази дали можливість розв'язувати як пряму, так і обернену задачі, визначати параметри вихідної смуги для формування кілець різного радіуса, прогнозувати геометрію кільця за відомими розмірами смуги, а також використовувати отримані дані для налаштування валків перед експериментальною перевіркою. Запропонована модель поетапної прокатки забезпечила рівномірний поперечний переріз кільця, що підвищує точність виготовлення гвинтових поверхонь

без зварних швів. Результати можуть бути використані у машинобудуванні для виготовлення шнеків, гвинтових поверхонь і транспортерів без зварних швів, зокрема в сільськогосподарській, харчовій, будівельній та енергетичній галузях

**Ключові слова:** пластична деформація; об'єм; ізотропний матеріал; радіус кільця; відносне подовження



UDC 635.34/.36:631.147(477.4)(292.485)

DOI: 10.31548/dopovidi/6.2025.43

## The productivity of white cabbage using organic technology in the right-bank Forest-Steppe zone of Ukraine

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**Abstract.** White cabbage (*Brassica capitata alba* L.) is one of the basic and most widely grown vegetable crops in Ukraine and worldwide. The growing demand for organic products increases the relevance of research aimed at selecting cabbage varieties and hybrids capable of ensuring high yields using organic cultivation technologies. The aim of the study was to investigate the characteristics of growth, development and yield formation of white cabbage varieties and hybrids under organic cultivation in open ground. Research on mid-season varieties and hybrids of white cabbage under organic cultivation in open ground was conducted by setting up field experiments in 2019-2023. Field and statistical research methods were used in the experimental work. The study was conducted with mid-season varieties such as 'Slava 1305', 'Slavia' and white cabbage hybrids: 'Akvarel F<sub>1</sub>', 'Megaton F<sub>1</sub>', and 'Kubok F<sub>1</sub>'. The results of the research showed that the biometric parameters of plants and products and the yield of white cabbage were significantly influenced by the biological characteristics of varieties and hybrids. The research showed that varietal characteristics influenced plant height, rosette diameter, number of leaves and leaf area. The largest leaf area of white cabbage plants in all phases of growth and development, including the phase of technical maturity, was observed in the hybrid 'Akvarel F<sub>1</sub>' – 33.9 thousand m<sup>2</sup>/ha. The highest yield during the years of research was observed in the hybrids 'Akvarel F<sub>1</sub>' and "Kubok F<sub>1</sub>", which provided an increase of 9.9 and 7.3 t/ha relative to the control. These hybrids formed the largest head weight – 1,273 g and 1,220 g, respectively. The practical value of the study lies in the fact that its results make it possible to identify the most productive varieties and hybrids of white cabbage for organic cultivation in open ground, which will contribute to increasing its yield and quality

**Keywords:** variety; hybrid; biometric indicators; organic cultivation; yield

### Suggested Citation:

Palamarchuk, I. (2025). The productivity of white cabbage using organic technology in the right-bank Forest-Steppe zone of Ukraine. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 43-52. doi: 10.31548/dopovidi/6.2025.43.

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## Introduction

Traditional white cabbage cultivation technology involves the use of high doses of mineral fertilisers and pesticides, which leads to the accumulation of nitrates in the product. This makes it necessary to switch to organic technologies, which reduce the anthropogenic load on agroecosystems and increase the environmental safety of vegetable products. In this context, it is important to scientifically substantiate the effectiveness of growing different varieties and hybrids of white cabbage using organic technology in the conditions of the Right-Bank Forest-steppe of Ukraine. The results obtained will contribute to the improvement of organic vegetable production and the expansion of the range of organic products on the market.

The study of the assortment of white cabbage is carried out in various regions of the Forest-steppe of Ukraine. According to research by I.V. Gutman *et al.* (2025), white cabbage of some of the studied hybrids showed high yield indicators. According to the results of the research, the highest yield of late-maturing white cabbage was obtained when growing the hybrids 'Jintama F<sub>1</sub>' and 'Aggressor F<sub>1</sub>', which amounted to 135.37-155.79 t/ha in 2023 and 138.66-158.4 t/ha in 2024. Considering the yield, this allows to recommend them for cultivation in the Forest-steppe zone of Ukraine. A wide range of agrotechnical measures aimed at increasing the productivity of white cabbage varieties and hybrids are used in global practice. One of the key factors for successful cultivation is sufficient soil moisture, as this crop is characterised by a high demand for water, which ensures the formation of a large leaf rosette and full head formation. Research conducted by O. Erken & M. Yildirim (2019) showed that reduced irrigation increases the content of ascorbic, lactic, tartaric and malic acids; on the other hand, reduced sugar content and total sugar content increased with full irrigation. The application of different amounts of irrigation water in the study caused changes in the growth and quality of white cabbage (*Brassica oleracea* L. *cv.* *Capitata*). Under conditions where water stress in cabbage

production exceeded 30%, a significant decrease in yield and marketability of the product was observed. The results of the study showed that different levels of soil moisture at the depth of the root system affected not only the yield but also the content of organic acids and sugars. It was possible to maintain a stable yield level when irrigation water was supplied in a volume of at least 411.2 mm. At the same time, an increased content of organic acids was observed in cabbage grown under low soil moisture conditions.

Research by S.A. Vdovenko & I.I. Palamar-chuk (2020) found that changes in weather conditions in Ukraine caused an increase in average air temperature, which in turn led to rainless periods, particularly during the growing season for vegetable crops. Thus, according to the analysis of climatic conditions in Ukraine and the yield of vegetable crops, it was found that during the period 2006-2020, the climate changed towards warming in the summer months and significant cooling in the winter, which caused a decrease in yield, negatively affecting the country's food security. Research by D. Kavaliauskaitė *et al.* (2023), conducted at the Institute of Horticulture of the Lithuanian Research Centre for Agriculture and Forestry, showed that the highest marketable yield of white cabbage reached 80.5 t/ha, and the vitamin C content was 7.80 mg per 100 g of product when granulated bird manure fertiliser was applied in autumn and mineral fertiliser in spring. After using bird manure fertiliser, the nitrate content in white cabbage heads was found to be minimal, indicating high product quality when using organic cultivation technology.

In a study by E. Kunicki *et al.* (2024), conducted in open field conditions, the effect of four biostimulants (Asahi, Optysil, Optycal and Tytanit) on the yield and chemical composition of white cabbage varieties ('Caraflex' and 'Alfredo') was studied. It was found that biostimulants did not have a significant effect on marketable yield, but all of them contributed to a significant increase in ascorbic acid content. The effect on

dry matter, sugars, phenols and antioxidant activity depended on the variety, but in general did not worsen the results compared to the control. Similar positive effects on product quality from the use of organic fertilisers were confirmed by a study by A. Turatbekova *et al.* (2024), which found that organic fertilisers had a positive effect on the quality indicators of white cabbage, including an increase in the level of essential nutrients, in particular vitamin C, total phenol content and antioxidant activity. Such improvements in quality not only increase the nutritional value of the crop, but also provide additional health benefits for consumers.

At the same time, research by V.M. Chernet-skiy & I.I. Palamarchuk (2017) on the impact of water supply on the yield of white cabbage varieties was conducted in 2011-2013. It was found that in years with insufficient water supply, cabbage yield decreased by 18.3 t/ha compared to years with sufficient moisture. In addition, in dry years, a decrease in the marketability of the crop was also noted, as evidenced by the lower weight of the heads and increased nitrate content. According to the results of studies by E.M. Kolinko & I.M. Sidorova (2023), it was found that the yield of white cabbage depended on the genotype and ranged from 128.3 t/ha ('Kepler F<sub>1</sub>') to 71.3 t/ha ('Kilastor F<sub>1</sub>'). Given these results, the aim of the study was to establish the patterns of growth and development of white cabbage varieties and hybrids using organic cultivation technology in open ground and to identify the factors that influence their productivity.

## Materials and Methods

The study, dedicated to the investigation of mid-season varieties and hybrids of white cabbage in organic cultivation in open ground, was conducted by setting up field experiments in the period 2019-2023. In accordance with the guidelines of the research methodology by A.O. Rozhkov *et al.* (2016), an experimental design was developed and measures were taken to conduct biometric measurements of white cabbage plants

at the appropriate stages and to record the yield. The study was conducted with mid-season varieties: 'Slava-1305', 'Slavia' and white cabbage hybrids: 'Akvarel F<sub>1</sub>', 'Megaton F<sub>1</sub>', and 'Kubok F<sub>1</sub>'; as they combine potentially high yields, the formation of consistently high-quality commercial products and resistance to the most common diseases without the use of chemical protection agents. The 'Slava 1305' variety was selected as the control. The experiment was repeated four times, with an area of 20 m<sup>2</sup>.

The soil type was grey forest medium loam. Vegetable peas were used as a precursor. White cabbage was grown in open ground using organic technology. The cultivation technology was generally accepted for the area, but only biological products were used for protection, in particular Lepidocide (35-70 ml/2-4 l of water against each generation of pests) and Phytocide-R (5-10 ml/10 l of water as a preventive measure). Agrotechnical measures were carried out in accordance with the requirements of the crop and the research objectives. Plant care included systematic loosening of the soil and removal of weeds. On each plot, 10 experimental plants were marked, on which phenological observations were carried out, biometric measurements were taken, etc. The beginning of each phenological phase was considered to be the time when 15% of plants entered it, and the time of the mass phase was when it occurred in 75% of plants.

Biometric measurements in the corresponding phases of plant growth and development, as well as yield accounting, were carried out on each plot. Field and statistical research methods were used in the experimental work. Phenological observations involved recording the beginning and mass phase of plants by eye. Biometric measurements were carried out to assess plant growth processes and determine morphological characteristics during the growing season. They involved determining the leaf area in dynamics, which allows assessing the intensity of photosynthetic activity and the potential for biomass accumulation, rosette diameter, plant height (using a

measuring ruler), stem thickness (with a vernier caliper) and number of leaves (by counting). The dynamics of leaf area growth were measured at the corresponding stages of growth and development of white cabbage plants, namely: at the head setting stage, the beginning of technical maturity and at the stage of technical maturity. Yield was calculated at technical maturity of heads from each plot separately by weighing. Marketable products were selected in accordance with the requirements of the current standard DSTU 7037-2009 (2010). The results obtained in the experiments were statistically processed using correlation and dispersion analyses. The experimental studies complied with institutional, national and international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992) and the Convention

on International Trade in Endangered Species of Wild Fauna and Flora (1979).

## Results

The results of the research showed that the biometric parameters of white cabbage plants were influenced by the biological characteristics of varieties and hybrids (Table 1). The highest plant height was observed in the 'Slavia' variety – 49.6 cm, which is 12.5 cm higher than the control variant and 2.0-6.1 cm higher than in other variants of the experiment. The stem thickness of the studied white cabbage variants was 1.9-2.3 cm and depended on varietal characteristics. The highest value was found in the 'Kubok F<sub>1</sub>' hybrid – 2.2 cm, which is 0.3 cm more than the 'Slava 1305' variety (control). The significance of this difference was confirmed by the results of the analysis of variance.

**Table 1.** Biometric indicators of white cabbage plants at the stage of technical maturity depending on variety under direct sowing, 2019-2023

Variety / Hybrid	Plant height, cm	Stem thickness, cm	Rosette diameter, cm	Number of leaves, pcs per plant	Leaf area, thousand m <sup>2</sup> /ha
'Slava 1305' (control)	37.1	1.9	38.7	18.5	28.6
'Slavia'	49.6	2.0	39.6	19.4	29.7
'Megaton F <sub>1</sub> '	43.5	2.0	40.9	22.6	31.6
'Akvarel F <sub>1</sub> '	47.6	2.1	41.9	23.4	33.9
'Kubok F <sub>1</sub> '	45.1	2.2	41.6	23.1	32.7
LSD <sub>0.5</sub>	1.07	0.18	0.73	1.6	0.51

**Source:** developed by the authors

The experiment showed that the diameter of the rosette in the studied hybrids was larger and ranged from 40.9 to 41.9 cm, while in the varieties this indicator ranged from 38.7 to 39.6 cm. Among all the variants of the experiment, the plants of the 'Akvarel F<sub>1</sub>' hybrid had the largest rosette diameter – 41.9 cm, which is 3.2 cm more than the control. The most leafy hybrids were 'Megaton F<sub>1</sub>', 'Akvarel F<sub>1</sub>', and 'Kubok F<sub>1</sub>', with 22.6, 23.4 and 23.1 leaves per plant, respectively. Accordingly, the highest indicator was observed in the hybrid 'Akvarel F<sub>1</sub>' – 23.4 leaves per plant, which is 4.9 leaves per plant more than the control. The largest leaf area in the experiment was observed in hybrids. The increase in this indicator in hybrids

relative to the control was 3.0-5.3 thousand m<sup>2</sup>/ha, respectively. Studies conducted to investigate the dynamics of the increase in the leaf area of white cabbage plants showed that the increase in this indicator occurred with varying intensity and depended on the variety studied and the phase of growth and development of white cabbage plants (Table 2). During the head-setting phase, the largest leaf area was observed in the 'Akvarel F<sub>1</sub>' hybrid – 0.90 m<sup>2</sup>/plant and 25.4 thousand m<sup>2</sup>/ha, which is 0.21 m<sup>2</sup> per plant and 6.0 thousand m<sup>2</sup>/ha more than the control variant, respectively. A larger leaf area relative to the control was recorded in all studied variants. The trend towards an increase in leaf area relative to the control in all

variants continued in the subsequent phases of growth and development of white cabbage. At the beginning of technical maturity, the increase in leaf area in the studied variants was 1.7-8.5 thousand m<sup>2</sup>/ha, and in the phase of technical maturity it was at the level of 1.1-5.3 thousand m<sup>2</sup>/ha. The

data in Table 2 show that the hybrids were characterised by higher assimilation surface growth activity, which had a positive effect on the productivity of white cabbage plants. A direct relationship between yield and leaf area at the technical maturity phase ( $r = 0.98 \pm 0.04$ ) was proven.

**Table 2.** Dynamics of the increase in the leaf surface area of white cabbage plants depending on the variety in seedling-free cultivation, 2019-2023

Variety / Hybrid	Head setting		Onset of technical maturity		Technical maturity	
	m <sup>2</sup> per plant	thousand m <sup>2</sup> /ha	m <sup>2</sup> per plant	thousand m <sup>2</sup> /ha	m <sup>2</sup> per plant	thousand m <sup>2</sup> /ha
'Slava 1305' (control)	0.69	19.4	0.84	23.7	0.98	28.6
'Slavia'	0.77	21.7	0.90	25.4	1.02	29.7
'Megaton F <sub>1</sub> '	0.86	24.2	1.08	30.5	1.09	31.6
'Akvarel F <sub>1</sub> '	0.90	25.4	1.14	32.2	1.17	33.9
'Kubok F <sub>1</sub> '	0.88	24.8	1.08	30.5	1.12	32.7

**Source:** developed by the authors

The marketable yield of white cabbage depended on weather conditions and the variety studied. The dry period of vegetation directly affected the formation of heads under natural water supply conditions in organic farming. The yield in the experiment was 28.6-38.5 t/ha (Table 3). The highest yields were recorded for the hybrids 'Akvarel F<sub>1</sub>' and 'Kubok F<sub>1</sub>', where the

increase relative to the control was 9.9 and 7.3 t/ha. The smallest yield increase was recorded for the 'Slavia' variety – 1.4 t/ha, and the lowest yield was recorded for the 'Slava 1305' variety (control) – 28.6 t/ha. All the variants studied had a significantly higher yield relative to the control, which was confirmed by the results of the analysis of variance.

**Table 3.** Marketable yield of white cabbage depending on the variety in seedless cultivation, 2019-2023

Variety / Hybrid	Yield, t/ha	Increase ± relative to the control	Head weight, g
'Slava 1305' (control)	28.6	–	963
'Slavia'	30.0	+1.4	975
'Megaton F <sub>1</sub> '	32.7	+4.1	1,107
'Akvarel F <sub>1</sub> '	38.5	+9.9	1,273
'Kubok F <sub>1</sub> '	35.9	+7.3	1,220
LSD <sub>0.5</sub> , t/ha	0.83	–	32.01

**Source:** developed by the authors

The amount of precipitation during the growing season of white cabbage plants in the years of the study was insufficient for high yield indicators. There was also a period of drought accompanied by high temperatures, which were not conducive to the growth, development and formation of the cabbage crop. The average weight

of heads in the experiment for the variants was 963-1273 g. The highest indicators of white cabbage head weight were obtained for the hybrids 'Akvarel F<sub>1</sub>' and 'Kubok F<sub>1</sub>', where the average weight of one head was 1,273 g and 1,220 g, respectively. Compared to the 'Slava 1305' variety (control), these hybrids had significantly higher

head weights – by 310 g and 257 g, respectively. This indicates the high yield potential of the studied hybrids, their ability to adapt to existing growing conditions and to use nutrients and moisture more efficiently. The results obtained indicate the feasibility of using the 'Akvarel F<sub>1</sub>' and 'Kubok F<sub>1</sub>' hybrids in organic farming with the possibility of obtaining the maximum possible productivity and product quality indicators. The analysis established a strong direct relationship between yield and head weight ( $r = 0.99 \pm 0.03$ ).

### Discussion

Scientists from Ukraine and other countries have conducted a series of studies on white cabbage varieties. L.M. Popova *et al.* (2019) noted that white cabbage hybrids are highly productive. The highest marketable yield was provided by the 'Globus F<sub>1</sub>' (88.69 t/ha), while for obtaining an early marketable yield, it is advisable to cultivate the hybrid 'CACM 4034 F<sub>1</sub>'. It is also important to preserve product quality during harvesting: the best indicators of product storage in the field during the harvesting period were observed in the control variant – the hybrid 'Pandion F<sub>1</sub>'. The yield of white cabbage varieties depends not least on growing conditions, in particular the moisture regime. According to research by A. Hud *et al.* (2023), when the soil was waterlogged in the late stages of growth and development, the plants experienced stress, but in the early stages of waterlogging, they recovered. Research by O.A. Sindesi *et al.* (2025) also proved that the yield of white cabbage depends on soil conditions. In particular, Copenhagen cabbage grown on sandy soils enriched with zeolite responded positively to its application, which contributed to the activation of growth processes and an increase in yield. These results also demonstrate that zeolite can be used to improve some indicators of cabbage growth. In terms of maximum plant height, when comparing the two seasons, the second growing season of cabbage showed improved plant growth on soils fertilised with zeolite. X. Mu *et al.* (2023) also found that soil mulching, a technological

element, has an impact on the yield of cabbage varieties. A comprehensive analysis of indicators by filling in the main components and functions of affiliation showed that mulching materials had the greatest effect in comprehensive assessments compared to traditional soil cultivation methods.

Scientists D. Juškevičienė *et al.* (2025) proved the positive effect of biostimulants on different varieties of white cabbage. It was found that the use of amino and humic acids affects the productivity of white cabbage. The results showed that biostimulants increase the yield of cabbage heads by 25% and 35% for the studied varieties compared to the control variant. The highest productivity among the studied varieties (72.5–78.6 t/ha) was recorded in the 'Kaminna Holova' variety in the variant using amino acids and a combination of humic and amino acids. A positive effect was also noted when treating plants with a solution of biostimulants, where an increase in vitamin C and crude protein was observed. Organic cultivation of white cabbage involves the use of organic fertilisers. Research by E. Terry-Alfonso *et al.* (2022) on vermicomposting showed that the organic fertilisers used had a positive effect, contributing to an 88% increase in yield. The inability to use chemical plant protection products in organic technologies encourages more careful control of harmful organisms and the use of all possible agrotechnical methods and biological preparations that can affect their numbers. In an experiment conducted by M. Jam & S. Fortunova (2023), damage to white cabbage by cabbage moth caterpillars was insignificant, therefore it did not require treatment with insecticides, which made it possible to obtain high-quality environmentally friendly products. O.V. Matros & O.M. Matros (2025) demonstrated in their work that in order to reduce damage to cabbage by pests, it is necessary to maintain spatial isolation, crop rotation, preserve natural buffer zones, and regularly monitor the entomofauna, taking into account climate change. According to research by A. Turatbekova *et al.* (2024), organic fertilisers have a positive effect on the quality characteristics of white

cabbage, including an increase in the level of essential nutrients such as vitamin C, an increased total phenolic content and increased antioxidant activity. O. Kuts *et al.* (2023) also found that the use of an optimal fertilisation system on white cabbage varieties, namely organic and mineral fertilisers, as well as biological fertilisation systems with a complex of microbial preparations, contributed to an increase in plant height by 22.5-31.3%, leaf rosette diameter by 21.8-32.1%, head diameter by 10.7-13.5% and head weight by 32.9-57.3%.

The results of studies by A. Lončarić *et al.* (2020) showed that the chemical composition of cabbage products primarily depends on the characteristics of the variety. The cabbage varieties studied differed in their physical and chemical composition and morphological characteristics. Six phenolic acids were identified and quantified, with sinapic acid being the dominant component (65.9-78.15 mg/kg). Aldehydes, esters, alcohols and terpenes were the main classes of organic volatile compounds present in the cabbage studied. The 'Chepinsky' cabbage variety, which had never been analysed before, contained the highest amounts of d-limonene (40.75 µg/L) and allyl isothiocyanate (1090.26 µg/L), the most important volatile compounds responsible for the taste of fresh cabbage. The results presented indicate the value of the 'Chepinsky' variety for further production and research.

The work of M. Putnik-Delic *et al.* (2023) found that the application of organic fertilisers plays an important role in the high productivity of white cabbage and kohlrabi. The use of pig and beef manure contributed to the formation of yields at the following levels: white cabbage – 32.63-49.86 t/ha, kohlrabi – 31.83-46.75 t/ha, improved plant nutrition, increased the content of essential nutrients in the soil, and created more optimal conditions for the growth and development of these crops. The application of organic fertilisers in combination with soil mulching proved to be particularly effective, contributing to moisture conservation, activation of soil microorganisms and reduction of nitrate load on products.

In the variants with the use of organic fertilisers, more uniform heads with high quality and environmental safety indicators were observed. The results obtained indicate that organic technology for growing cabbage crops makes it possible to obtain higher yields, improves the biometric indicators of products, and contributes to the preservation of natural soil fertility, which is an important factor in the sustainable development of organic vegetable growing. Research by H. Boteva *et al.* (2019), conducted using various types of biological fertilisers, showed an increase in cabbage yield (40.6 t/ha) and an improvement in the biochemical composition of the product: vitamin C content – 32.74 mg/100 g, sugar content – 5.47%, nitrates – 149 mg/kg. Thus, the yield and quality of cabbage were higher compared to the control in the variants with organic and biological fertilisers. No negative effect of organic and biological fertilisers on the shelf life of cabbage was observed. The current study showed that varieties and hybrids of white cabbage grown using organic technology differed in productivity. The most effective were the hybrids 'Akvarel F<sub>1</sub>' and 'Kubok F<sub>1</sub>', which provided the highest yield of 38.5 and 35.9 t/ha and the highest head weight of 1,273 g and 1,220 g.

## Conclusions

A study of the productivity of white cabbage grown organically and under natural moisture conditions helped to establish that varietal characteristics influence the onset of phenological phases of white cabbage plant development, as well as biometric parameters of production and yield. The results showed that the highest plant height was recorded in the 'Slavia' variety (49.6 cm), which is 12.5 cm higher than the control variant. The largest rosette diameter was found in plants of the 'Akvarel F<sub>1</sub>' hybrid (41.9 cm), with an increase of 3.2 cm relative to the control variant. The largest number of leaves was formed by plants of the 'Megaton F<sub>1</sub>', 'Akvarel F<sub>1</sub>' and 'Kubok F<sub>1</sub>' hybrids, which had 22.6, 23.4 and 23.1 leaves per plant, respectively. The largest

leaf area index was recorded in hybrids, where the increase compared to the control was 3.0-5.3 thousand m<sup>2</sup>/ha, respectively. The intensity of leaf area growth in the studied varieties varied. Greater increases in this indicator were observed in white cabbage hybrids. From the heading stage to technical maturity, the trend towards an increase in assimilation area continued. The largest leaf area in the head formation phase was observed in the hybrid 'Akvarel F<sub>1</sub>' – 0.90 m<sup>2</sup> per plant and 25.4 thousand m<sup>2</sup>/ha, where the increase relative to the control variant was 0.21 m<sup>2</sup> per plant and 6.0 thousand m<sup>2</sup>/ha, respectively. All the varieties and hybrids studied outperformed the control in this indicator.

The results of the studies showed that the yield of white cabbage in the conditions of the right-bank Forest-steppe of Ukraine was within the range of 28.6-38.5 t/ha. These indicators show a fairly high level of crop productivity, considering organic cultivation, which confirms the effectiveness of the applied technological methods and their positive impact on yield formation. Thus, the highest yield was obtained from the hybrids 'Akvarel F<sub>1</sub>' and 'Kubok F<sub>1</sub>', which provided an

increase of 9.9 and 7.3 t/ha relative to the control. The largest head weight was found in the 'Akvarel F<sub>1</sub>' and 'Kubok F<sub>1</sub>' hybrids, which weighed 1,273 g and 1,220 g, which is 310 g and 257 g more than the control, respectively. The study proves that the introduction of organic technologies for growing white cabbage is a promising direction that can preserve the environmental friendliness of products, soil quality and increase yields. Studying the yield of new varieties and hybrids of white cabbage under organic growing systems will help resolve issues related to their adaptability to stressful conditions, increase production efficiency, and provide consumers with high-quality and safe products that meet the requirements of sustainable development in the agricultural sector.

### Acknowledgements

None.

### Funding

None.

### Conflict of Interest

None.

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## Продуктивність капусти білоголової за органічної технології в умовах правобережного лісостепу України

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**Анотація.** Капуста білоголова (*Brassica capitata alba* L.) є однією з базових і найбільш вирощуваних овочевих культур в Україні та світі. Зростання попиту на органічну продукцію підсилює актуальність досліджень, спрямованих на добір сортів і гібридів капусти, здатних забезпечувати високу врожайність за органічних технологій вирощування. Метою дослідження було вивчити особливості росту, розвитку та формування врожаю сортів та гібридів капусти білоголової за органічного вирощування у відкритому ґрунті. Дослідження середньостиглих сортів та гібридів капусти білоголової за органічного вирощування у відкритому ґрунті проводили закладанням польових дослідів у 2019-2023 роках. При проведенні експериментальної роботи було використано польовий та статистичний методи досліджень. Дослідження проводили з середньостиглими сортами, такими як 'Слава 1305', 'Славія' та гібриди капусти білоголової: 'Акварель F<sub>1</sub>', 'Мегатон F<sub>1</sub>', 'Кубок F<sub>1</sub>'. Результати досліджень показали, що на показники біометричних параметрів рослин та продукції та врожайність капусти білоголової значною мірою вплинули біологічні особливості сортів та гібридів. Проведені дослідження показали, що сортові особливості здійснювали вплив на висоту рослин, діаметр розетки, кількість листків та площу листків. Найбільшу площу листків рослин капусти білоголової в усі фази росту та розвитку у тому числі і у фазу технічної стиглості відмічено у гібриду 'Акварель F<sub>1</sub>' – 33,9 тис. м<sup>2</sup>/га. Найбільша врожайність у роки досліджень була у гібридів 'Акварель F<sub>1</sub>' та 'Кубок F<sub>1</sub>', які забезпечили приріст відносно контролю 9,9 та 7,3 т/га. Дані гібриди сформували найбільшу масу головок – 1273 г та 1220 г відповідно. Практична цінність дослідження полягає в тому, що його результати дають змогу визначити найбільш продуктивні сорти та гібриди капусти білоголової для органічного вирощування у відкритому ґрунті, що сприятиме підвищенню її врожайності та якості

**Ключові слова:** сорт; гібрид; біометричні показники; органічне вирощування; урожайність



UDC 631.526.3:633.11 "324":664.72.016/(477.51)

DOI: 10.31548/dopovidi/6.2025.53

## Evaluation of winter wheat varieties based on grain quality indicators in northern Ukraine

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**Abstract.** The study was devoted to assessing the impact of hydrothermal conditions on the formation of grain quality indicators of winter wheat varieties in northern Ukraine. The relevance of the work was determined by the intensification of climatic fluctuations, which leads to a decrease in the stability of yield and grain quality, as well as the need to select varieties capable of combining high productivity and proper baking quality. The aim of the study was to identify the impact of temperature and moisture on the quality indicators of winter wheat varieties of different origins and to determine the genotypes most adapted to the conditions of the region. The study was conducted over two growing seasons (2023-2025) on eight varieties of winter wheat of Ukrainian and foreign selection. The evaluation was carried out according to the main quality indicators: weight of 1,000 grains, protein content, gluten content, vitreousness and grain nature. It was found that weather conditions significantly affected grain quality indicators. In the dry conditions of 2023/2024, grain with a higher protein content (up to 11.4%) and gluten content (up to 22.0%) was formed, while in the wetter conditions of 2024/2025, higher yields (up to 7.7 t/ha) were achieved, but the protein content decreased. The highest quality indicators were observed for the varieties 'Vezha Myronivska' and 'MIP Vyshyvanka', and the highest productivity was observed for 'MIP Valenciia' and 'Tobak'. Correlation analysis shows an inverse relationship between yield and grain quality indicators, which is exacerbated by moisture deficiency, while high

### Suggested Citation:

Prydatko, V., & Kovalyshyna, H. (2025). Evaluation of winter wheat varieties based on grain quality indicators in northern Ukraine. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 53-65. doi: 10.31548/dopovidi/6.2025.53.

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direct correlations between protein, gluten and vitreousness indicate their interdependence and the possibility of balancing quantity and quality under optimal water conditions. The practical significance of the results obtained lies in the identification of varieties capable of producing stable grain quality under different weather conditions, which can be used to improve the varietal composition of winter wheat in Ukraine and optimise agricultural technologies in the context of climate change

**Keywords:** weather conditions; gluten; protein; vitreousness; development phases

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## Introduction

Soft winter wheat has long been one of the main grain crops in Ukraine and worldwide. Among grain crops, wheat grain is distinguished by its high protein content. Grain crops provide the world's population with an average of 50-60% of their protein. The protein and gluten content in wheat grain depends largely on the variety. Its content in soft wheat grain, depending on the variety and growing conditions, can average 13-15%. Thus, studying the impact of natural factors that limit the stability of high grain quality in winter wheat varieties is becoming a dominant issue in terms of importance (Zvonar, 2020).

On a global scale, rising temperatures, precipitation deficits and increasing salinity of soils are key factors limiting wheat productivity. Climate change, especially droughts, and the spread of pests and diseases significantly reduce grain yield and quality in semi-arid regions (Holyk *et al.*, 2023). According to M.A.S. Raza *et al.* (2025), the use of zinc oxide nanoparticles (ZnO-NPs) can increase yields by up to 25%, improve grain weight and increase protein content even in the absence of moisture. Such micronutrient fertilisation technologies are considered a promising direction for adapting wheat to stressful environmental conditions. In addition to drought, another serious global problem is secondary soil salinisation. According to the results of research by M.M. Majidi *et al.* (2025), the use of synthetic hexaploid wheat (SHW) lines obtained by hybridising hard and wild forms is a new source of genetic resistance to soil salinisation. These lines are characterised by high variability, good trait inheritance and can increase tolerance to abiotic

factors, which is especially important in the context of climate change and the spread of aridisation around the world.

Grain quality is determined by a combination of morphological, physical, and biochemical properties that determine its technological purpose and baking value. The main indicators used to assess wheat grain quality include the weight of 1,000 grains, grain density, moisture content, vitreousness, protein and gluten content, and the falling number, which reflects the activity of amylase enzymes. It is the combination of these parameters that determines the suitability of grain for processing and the quality of the final product. The work of M. Helguera *et al.* (2020) summarised modern approaches to assessing grain quality in wheat breeding programmes. The authors revealed the genetic, physiological, and technological aspects of the formation of quality indicators, in particular protein content, gluten content, enzymatic activity, and structural properties of the endosperm. The study emphasised the importance of combining genomic selection methods with traditional breeding criteria to improve grain quality. This study has become an important theoretical basis for improving wheat breeding, taking into account the nutritional value and technological parameters of grain.

Modern innovative cultivation technologies demonstrate new opportunities for improving grain quality even under conditions of limited natural resources. The study by G. Albahri *et al.* (2023) focused on a variety of advanced agricultural practices, including the use of microbial biostimulants, nutrient optimisation, lighting management, and adaptation to abiotic stresses

through innovative agricultural systems. It emphasised that such approaches not only increase yields but also improve grain quality (e.g., wheat and rice) in conditions often characterised by limited or stressful resources (such as the impact of abiotic stress). R. Hasana *et al.* (2024) described the impact of alternative agricultural practices, such as regenerative or conservation methods (minimum tillage, zero tillage, mulching), on soil, crop production and the environment. The article notes that these methods are important for ensuring food security, as vital resources such as arable land and water remain limited, but at the same time, such technologies can improve the quality of primary agricultural products.

According to R.I. Topko & H.M. Kovalyshyna (2022), the production of high-quality winter wheat is becoming increasingly important, so breeding institutions must create new varieties that would ensure high grain quality even under unstable weather conditions. Foreign-bred varieties are occupying an increasingly large niche in the Ukrainian seed market, gradually displacing Ukrainian varieties. Thus, it is important to study and evaluate these varieties. Ya. Manziuk & H.M. Kovalyshyna (2022) examined the characteristics of growing foreign-bred winter wheat varieties in the Forest-Steppe zone of Ukraine. The authors noted their high productivity and good adaptation to local climatic conditions, which confirms the feasibility of expanding the use of such varieties in Ukrainian agriculture. With the help of these data, it will be possible to select the most productive and environmentally adapted seed material.

In the context of global climate change, which negatively affects the quantity and quality of crops, the variety is one of the most reliable and cost-effective factors in increasing the yield and improving the quality of winter wheat grain. Scientists note that when the average daily air temperature exceeds 20°C and the relative humidity is below 55%, shriveled, low-quality grain is formed and gluten is destroyed, which negatively affects its quality and baking properties. The optimal conditions for the accumulation

of protein and gluten in grain are daytime temperatures of 20-24°C and daylight hours of 10-12 hours (Adamenko, 2017). Along with the creation of new varieties, the preservation and reuse of the genetic potential of old wheat varieties is becoming increasingly important. N. Maravić *et al.* (2025) proved that ancient varieties and landraces maintain stable levels of protein, gluten and phenolic compounds even under climate change, making them valuable sources of genes for improving the adaptability and nutritional value of wheat.

The aim of the study was to investigate the influence of hydrothermal conditions on grain-quality indicators in winter wheat cultivars of Ukrainian breeding and those bred in Germany, France, and the Czech Republic.

## Materials and Methods

The study was conducted using a combination of methods: field, laboratory and statistical, which ensured the reliability and comprehensiveness of the results. Field studies were conducted during the 2023-2024 and 2024-2025 growing seasons. The object of the study was 8 varieties of winter wheat: 'MIP Vyshyvanka', 'Vezha Myronivska' and 'MIP Valenciia' (Myronivka Institute of Wheat named after V.M. Remeslo), 'Nosivochka' (Nosivska Selection and Research Station of the Myronivka Institute of Wheat named after V.M. Remeslo), 'Emerick' (KWS, Germany), 'Mescal' (Limagrain, France), 'Julia' (Selgen, Czech Republic), 'Tobak' (Saaten Union, Germany). The field method was used to establish experimental plots, conduct phenological observations (determining the calendar dates of the onset of the main phenophases – emergence, tillering, heading, ripening) and record yields, which made it possible to assess the dynamics of growth and development of varieties under natural conditions.

Pryluky District, in the territory of the Pidlubivka Starosta District of the Sukhopolovianska United Territorial Community near the village of Tarasivka. In 2023, the preceding crop was sunflower. Sowing was carried out on 01.10.2023, and the first seedlings were obtained on

16.10.2023, which was favoured by the dry period after sowing. The period from late June until harvest was characterised by high air temperatures. During this time, the air temperature exceeded 40°C, which resulted in the formation of small, flattened grains. Meteoblue data was used to characterise the weather conditions (hydrothermal regime) during the growing season and grain formation, which made it possible to quantitatively confirm and describe the temperature conditions. For the 2025 harvest, experimental plots were established in the southern part of the Chernihiv region, within the Pryluky district, on the territory of Malodivysia united territorial community near the village of Novyi Lad. Soybeans were the predecessor crop for the sowing of winter wheat varieties. Sowing was carried out on 29 September 2024. The beginning of germination was noted on 10 October 2023.

A laboratory method was used to assess grain quality. The study of the influence of hydrothermal conditions on grain formation and the formation of quality indicators (naturalness, protein content, raw gluten, vitreousness, falling number) was carried out using generally accepted methods (Tkachyk, 2016; Melnyk *et al.*, 2024) and in accordance with the current

standard DSTU 3768:2010 (2010). The choice of these methods was justified, since the field method ensures the representativeness of data, the laboratory method ensures the accuracy of the assessment of quality indicators, and the statistical method (mathematical data processing) is necessary to assess the reliability and validity of the results obtained. The data obtained were processed using Microsoft Excel software. Excel was used to perform basic statistical calculations, in particular to determine the arithmetic mean, standard deviation, coefficients of variation and correlation, and the least significant difference (LSD) between the indicators studied. The difference between the indicators was considered statistically significant at a significance level of  $p < 0.05$ . Experimental studies of plants, including the collection of plant material, complied with institutional, national or international guidelines. During the study, the standards of the Convention on Biological Diversity (1992) were observed).

## Results and Discussion

During the years of research, weather conditions were contrasting, especially during the period of grain formation and grain quality indicators (Table 1, Table 2).

**Table 1.** Hydrothermal indicators of the growing season for winter wheat, 2023/2024

Month	Indicators					
	Air temperature, °C			Precipitation, mm		
	Decades of the month			Decades of the month		
	I	II	III	I	II	III
2023 September	24.1	24.0	26.0	2.0	5.0	-
October	16.7	14.0	14.2	4.0	24.0	32.0
November	12.6	5.8	-1.8	29.0	21.2	39.4
December	-4.3	1.3	4.1	13.4	34.9	8.3
2024 January	-6.0	-7.2	-3.1	13.3	11.3	9.1
February	3.1	5.0	6.5	25.3	12.2	-
March	5.6	6.7	11.8	-	12.2	14.5
April	18.4	16.0	16.9	1.7	37.5	-
May	17.7	15.6	23.0	4.1	8.1	11.8
June	25.0	23.0	25.5	30.6	31.0	9.0
July	30.0	35.2	29.5	-	-	-

**Source:** developed by the authors based on own research

**Table 2.** Hydrothermal indicators of the growing season for winter wheat, 2024/2025 p.

Month	Indicators					
	Air temperature, °C			Air temperature, °C		
	Decades of the month			Decades of the month		
	I	II	III	I	II	III
2024 September	28.4	26.5	25.4	-	1.8	-
October	21.2	12.3	13.0	19.4	30.6	-
November	8.3	6.0	2.3	-	32.0	6.0
December	1.4	0.9	2.9	8.2	14.4	0.3
2025 January	4.2	1.9	5.9	12.2	1.2	0.9
February	-2.0	-8.1	-8.6	-	3.0	-
March	9.7	10.2	13.2	-	7.5	6.7
April	8.6	20.6	14.6	1.3	-	0.7
May	15.1	14.6	23.3	17.3	17.9	33.4
June	24.3	19.6	20.4	17.1	16.9	13.9
July	30.6	28.7	25.8	7.9	4.1	6.6

**Source:** developed by the authors based on own research

Weather conditions in autumn 2023 were warmer than the long-term average. In September, average daily air temperatures ranged from 24.0 to 26.0°C, which contributed to good germination and initial plant growth. However, precipitation during this month was insufficient (7.0 mm), which limited moisture accumulation in the arable soil layer. In October, the temperature dropped to 14–16°C, and moderate precipitation was observed (60.0 mm per month), which provided optimal conditions for winter crops to tiller. In November, the temperature dropped from +12.6 to -1.8°C, but sufficient precipitation (over 89 mm) ensured the replenishment of soil moisture reserves before wintering. The winter period (December–February) was characterised by unstable temperatures ranging from -7.2°C to +6.5°C. Frequent thaws could have contributed to the compaction of the snow cover and the freezing of the topsoil, but no significant damage to plants was observed. In spring (March–May 2024), average monthly temperatures rose from 5.6°C to 23.0°C. Precipitation during this period was limited (mainly 20–40 mm per month), which caused a moisture deficit during the period of plant emergence and heading. In June–July 2024, high air temperatures (up to 35°C) were observed with

low precipitation, which led to increased temperature stress and accelerated grain ripening. These conditions contributed to the formation of grain with reduced moisture content but higher protein and gluten content, which is consistent with the grain quality results obtained this year. The following growing season was characterised by higher temperatures and more uniform moisture, which created favourable conditions for the growth and productive tillering of winter crops (Table 2). In September 2024, abnormally warm weather (25.4–28.4°C) with little precipitation was observed, which somewhat complicated the initial rooting of plants. However, sufficient moisture in October (up to 50 mm) ensured the restoration of optimal conditions for autumn wheat development. The winter period (December–February) was mild, with average temperatures mostly hovering around zero or slightly above, which helped plants overwinter without significant damage. Precipitation during the winter was moderate (20–30 mm per month).

In March–April 2025, the temperature rose to 13–20°C, with little precipitation, which led to a moderate moisture deficit at the beginning of spring vegetation. However, in May, there was a significant amount of precipitation (over 68 mm),

which improved the water regime during the critical period of formation of the generative organs of winter wheat plants. In June–July 2025, high temperatures (up to 30.6°C) were observed with sufficient precipitation (an average of 15–20 mm per decade), which contributed to uniform grain filling and high yield formation, albeit with slightly reduced protein content and vitreousness. The hydrothermal conditions of both growing seasons had a significant impact on the productivity and quality of winter wheat grain. 2023/2024 was characterised by warmer but drier conditions, which contributed to the formation of grain with a high protein and gluten content, but with lower yields. In contrast, 2024/2025 was marked by a more optimal combination of temperature and moisture, which ensured a higher yield, but with a slight decrease in grain quality indicators. For both years, there is a clear trend

towards an increase in average monthly temperatures by 2–4°C compared to long-term averages, especially in the autumn and spring months. Precipitation is unevenly distributed: there is a deficit during the sowing period (September–October) and during grain filling (June–July), while in the winter months, precipitation often exceeded the norm. Such fluctuations confirm the general trend towards a shift in climatic seasons, an increase in the duration of rainless periods and more frequent occurrence of drought conditions during the critical phases of winter wheat development – emergence, tillering and heading. The study of the influence of hydrothermal conditions on grain formation and the formation of grain quality indicators of the studied varieties of winter wheat of Ukrainian and foreign selection was carried out in accordance with the generalised calendar dates of phenophases (Table 3).

**Table 3.** Phases and calendar dates of grain formation in winter wheat varieties (2024, 2025)

Grain formation phases	Grain formation dates	
	2024	2025
Grain formation	12-15.06	10-12.06
Milk ripeness	22-27.06	18-22.06
Wax ripeness	05-06.07	02-04.07
Full ripeness	10-11.07	12-14.07

**Source:** developed by the authors based on own research

An analysis of the calendar dates for winter wheat grain formation indicates a shift in the phenological phases of development to earlier dates in 2025 compared to 2024. Thus, grain formation and the phases of milk and wax ripeness began 2–4 days earlier, which is due to an increase in average air temperatures in the spring–summer period. This trend is characteristic of most regions of Ukraine. According to field observations by researchers M. Shtakal *et al.* (2022), in the Forest-Steppe zone during 2010–2021, the average daily air temperature for April–October increased by 2.2°C, and precipitation decreased by 27 mm relative to the norm, which led to an acceleration in the development of winter crops, a reduction in the grain filling period, and a decrease in soil moisture reserves during

ripening. Under these conditions, even high-yielding varieties cannot always effectively withstand the effects of weather fluctuations, in particular elevated temperatures during the grain formation phase.

Yields varied among varieties in 2024 and depended on the conditions during the grain formation period. The following varieties had significantly high, statistically reliable yield indicators: 'MIP Valenciiia' (5.6 t/ha) and 'Tobak' (5.3 t/ha). At the same time, the grain moisture content of the studied varieties was within the range of 9.2–9.5%. The average weight of 1,000 grains per variety was 46.8 g, with significantly high values for the varieties 'MIP Vyshyvanka' and 'Nosivochka' (50.5 and 48.9 g). The value of wheat grain largely depends on its protein and gluten content. High gluten

content was observed for the Ukrainian varieties 'MIPVyshyvanka' and 'Vezha Myronivska' – 22.0%, respectively. For foreign varieties – 'Emerick', 'Mescal', 'Julia', and 'Tobak' – this indicator was 20.8%, 18.4%, 21.0%, and 17.6%, respectively. The protein content

in the grain of Ukrainian varieties was also higher, ranging from 11.2% to 11.4%, while that of foreign varieties was slightly lower, ranging from 9.6% to 11.2%. The vitreousness index of the grain in all studied varieties ranged from 48% to 50% (Table 4).

**Table 4.** Grain quality indicators for Ukrainian and foreign soft winter wheat varieties (2024, 2025)

Variety	Yield, t/ha	Weight of 1,000 grains, g	Moisture, %	Grain density, g/dm <sup>3</sup>	Gluten, %	Vitreousness, %	Protein, %	Fall number, s
2024								
'Nosivochka'	3.9	<b>48.9</b>	9.3	763.0	21.0	48.0	<b>11.4</b>	<b>304.0</b>
'MIP Vyshyvanka'	3.9	<b>50.5</b>	9.2	792.0	<b>22.0</b>	50.0	11.3	<b>292.0</b>
'Vezha Myronivska'	3.9	46.5	9.2	792.0	<b>22.0</b>	48.0	11.3	<b>290.0</b>
'MIP Valenciia'	<b>5.6</b>	47.7	9.2	<b>812.0</b>	21.6	50.0	11.2	266.0
'Emerick'	4.7	43.8	<b>9.5</b>	789.0	20.8	50.0	11.2	253.0
'Mescal'	5.1	46.1	<b>9.5</b>	770.0	18.4	50.0	10.1	260.0
'Julia'	4.6	46.6	<b>9.4</b>	787.0	21.0	48.0	10.7	215.0
'Tobak'	<b>5.3</b>	44.1	<b>9.4</b>	743.0	17.6	40.0	9.6	226.0
Arithmetic mean	4.6	46.8	9.3	781.0	20.6	48.0	10.9	263.3
Standard deviation	0.7	2.3	0.1	21.4	1.7	3.4	0.7	31.7
Coefficient of variation	15.0	5.0	1.0	3.0	8.0	7.0	6.0	12.0
Coefficient of correlation	-	-0.53	0.36	-0.03	-0.56	-0.25	-0.60	-0.63
2025								
'Nosivochka'	5.6	<b>50.6</b>	12.4	766.0	16.0	32.0	9.8	271.0
'MIP Vyshyvanka'	5.4	<b>51.7</b>	12.4	766.0	16.0	32.0	9.8	280.0
'Vezha Myronivska'	6.2	47.1	12.3	793.0	<b>18.2</b>	<b>40.0</b>	<b>10.9</b>	276.0
'MIP Valenciia'	7.1	48.8	12.2	781.0	<b>17.8</b>	<b>38.0</b>	<b>10.8</b>	274.0
'Emerick'	6.9	44.6	<b>12.6</b>	<b>785.0</b>	17.0	<b>38.0</b>	<b>10.7</b>	<b>302.0</b>
'Mescal'	7.2	47.7	<b>12.6</b>	771.0	<b>18.4</b>	<b>38.0</b>	10.1	<b>297.0</b>
'Julia'	7.3	48.5	<b>12.5</b>	768.0	15.0	34.0	9.8	265.0
'Tobak'	<b>7.7</b>	45.3	12.4	769.0	15.4	36.0	10.1	270.0
Arithmetic mean	6.7	48.0	12.4	774.9	16.7	36.0	10.3	279.4
Standard deviation	0.8	2.4	0.1	10.2	1.3	3.0	0.5	13.2
Coefficient of variation	13.0	5.0	1.0	1.0	8.0	8.0	5.0	5.0
Coefficient of correlation*	-	-0.72	0.22	0.10	0.02	0.50	0.22	0.05

**Note:** \* – coefficient of variation relative to yield; values in columns that are significantly higher than the average at the 5% significance level are highlighted in bold

**Source:** developed by the authors based on own research

In 2025, the average yield of winter wheat varieties was 6.7 t/ha. High, statistically significant yield indicators were observed for the 'Tobak' variety (7.7 t/ha). The grain moisture content ranged from 12.2 to 12.6%. The average weight of 1,000 grains across varieties was 48.0 g, ranging from 44.6 g ('Emerick' variety) to 51.7 g (MIP Vyshyvanka variety). Weather conditions during the grain formation and filling period significantly affected quality indicators. The average gluten content in the varieties was 16.7%. High values for this trait were observed in the varieties 'Vezha Myronivska' (18.2%), 'Mescal' (18.4%) and 'MIP Valenciiia' (17.8%), while the lowest were found in the varieties 'Julia' (15.0%) and 'Tobak' (15.4%). The average protein content across varieties was 10.3%. High statistically significant indicators were observed in the varieties 'Vezha Myronivska' (10.9%), 'MIP Valenciiia' (10.8%) and 'Emerick' (10.7%). The average vitreousness index for all varieties was 36.0%, which is significantly lower than last year's index (48.0%). This indicator was high in the varieties

'Vezha Myronivska' (40%), 'MIP Valenciiia', 'Emerick' and 'Mescal' (38%). According to the results of research, in the favourable moisture conditions of 2025, higher plant productivity was achieved, but with a slight decrease in grain quality indicators, primarily protein, gluten and vitreousness content. In 2024, under drier conditions, on the contrary, lower yields but higher protein and raw gluten content were observed.

The results obtained (Table 5) confirm the existence of an inverse correlation between yield and grain quality indicators, in particular protein, gluten and vitreousness content. In years with higher moisture levels, an increase in yield was observed, but a decrease in protein content in the grain, while in drier conditions, on the contrary, the protein and gluten content increased, but the yield decreased. A similar trend was noted by Y. Wang *et al.* (2023), who point out that long-term selection aimed at increasing yield is often accompanied by a decrease in grain quality due to a reduction in the protein fraction as grain weight increases.

**Table 5. Interdependence between the indicators studied in 2024 and 2025**

	Yield, t/ha	Weight of 1,000 grains, g	Moisture, %	Grain density, g/dm <sup>3</sup>	Gluten, %	Vitreousness, %	Protein, %	Fall number, s
Yield, t/ha	1	-	-	-	-	-	-	-
Weight of 1,000 grains, g	-0.51/-0.71	1	-	-	-	-	-	-
Moisture, %	0.36/0.21	-0.70/-0.33	1	-	-	-	-	-
Grain density, g/dm <sup>3</sup>	-0.03/0.08	0.33/-0.51	-0.47/-0.25	1	-	-	-	-
Gluten, %	-0.56/-0.00	0.59/-0.17	-0.68/-0.11	0.80/0.67	1	-	-	-
Vitreousness, %	-0.24/0.48	0.44/-0.69	-0.13/-0.07	0.76/0.85	0.63/0.77	1	-	-
Protein, %	-0.60/0.20	0.56/-0.53	-0.57/-0.35	0.69/0.95	0.94/0.70	0.69/0.88	1	-
Fall number, s	-0.62/0.04	0.66/-0.38	-0.59/0.62	0.22/0.31	0.53/0.56	0.45/0.41	0.68/0.31	1

**Note:** the table shows data for 2024 and 2025 (2024/2025) separated by a slash

**Source:** developed by the authors based on own research

Analysis of the interdependencies between the main indicators of grain yield and quality (Table 5) confirms the existence of close correlations between the studied characteristics, which

indicates their interdependence. In 2024, there was a clear inverse correlation between yield and protein content ( $r = -0.60$ ), gluten content ( $r = -0.56$ ) and 1,000-grain weight ( $r = -0.51$ ). This

indicates that an increase in yield is accompanied by a decrease in the concentration of protein and gluten in the grain, which is a manifestation of the so-called dilution effect. A study by Y. Wang *et al.* (2023), based on a global meta-analysis, confirms the existence of a fundamental trade-off between yield and protein content in grain, despite significant improvements in both indicators due to optimisation of nitrogen nutrition. At the same time, Y. Wang *et al.* noted that grain protein content shows higher growth in years when temperatures during the ripening period are higher. In the current study, despite extremely high temperatures during grain formation, which could theoretically stimulate protein accumulation, drought was the limiting factor. Stress caused premature formation of small grains (confirmed by low 1,000-grain weight), which prevented full protein accumulation in the cells. In addition, the results obtained correlate with the conclusions of R. Koppel *et al.* (2020), who, assessing the variability of wheat quality in Northern Europe, emphasised that the variability of protein and gluten content depends most on the year (i.e. on dominant environmental factors such as the hydrothermal regime). The observations in the current study, which record a sharp manifestation of the negative “yield-quality” relationship precisely in the unfavourable, dry conditions of 2024, fully confirm this thesis, indicating that annual agroclimatic conditions were the main factor determining the relationship between productivity and quality.

At the same time, in 2025, when weather conditions were more favourable and humid, the nature of the relationship partially changed: the correlation between yield and quality indicators weakened or even became slightly positive. Thus, for protein and gluten, the coefficients were  $r=0.20$  and  $r$ =absent, respectively. This indicates that under conditions of optimal moisture and moderate temperatures, the grain formed in a more balanced manner, without a significant decrease in quality as yield increased. Among the internal relationships between quality indicators, it is worth noting the high direct correlation

between protein and gluten content ( $r = 0.94$  in 2024 and  $r = 0.70$  in 2025), which confirms their close functional interaction. There is also a strong positive correlation between vitreousness and protein content in grain ( $r = 0.69-0.88$ ), which determines the structural and mechanical quality of the endosperm. The correlation between the falling number and protein and gluten ( $r = 0.53-0.68$ ) indicates a certain stability of amylase activity within the studied varieties.

Achieving a combination of consistently high yield, resistance to adverse environmental factors and high product quality in a single variety remains an extremely challenging task in modern breeding, due to the inverse correlations between these traits. The results of the current study, which recorded negative correlations between yield and quality indicators ( $r = -0.51$  to  $-0.60$ ) in the stressful year of 2024, fully confirm this general pattern. Ukrainian scientists O.A. Demydov *et al.* (2023) in their field studies on Myronivka wheat cultivars, found that the maximum variation range of key quality indicators (sedimentation, crumb porosity, dough strength) is determined by the complex interaction of the factors “year  $\times$  predecessor” and “year  $\times$  sowing date”. This confirms that grain quality is highly variable and sensitive to environmental changes, and it is this instability that complicates the selection of varieties that combine high productivity and consistently high quality. Similarly, V.M. Pochynok & O.V. Radchenko (2011), analysing the genetic nature of protein content in grain, emphasised that this trait is controlled polygenically, but its level significantly depends on environmental conditions, in particular on temperature and precipitation during the grain filling period, which directly correlates with observations of the impact of the abnormal heat of 2024. A study by O.V. Barabolia (2008) focused on spring durum wheat also directly points to the existence of inverse relationships: an increase in sowing rates and nitrogen fertiliser doses, which increase yield and protein/gluten content, simultaneously leads to a decrease in 1,000-grain weight and specific weight. This is key

confirmation of the “dilution effect” and the negative correlation between yield and grain weight that was observed ( $r = -0.51$ ). In addition, O.V. Barbolia (2008) also noted that arid conditions during the grain formation period, regardless of the growing background, contribute to a decrease in the weight of 1,000 grains. V.V. Bazalii (2009), analysing progress in breeding for adaptability, noted that new varieties, although highly ecologically plastic, still require study under combined use of optimal and stressful conditions (such as water supply), which justifies the need for research under hydrothermal stress conditions and confirms the complexity of the breeding process. Thus, the results of the work on the negative correlation between yield and quality under stress conditions are fully consistent with the long-term conclusions of scientists and confirm the urgency of the problem of balanced selection.

Many scientists point out that the highest quality grain is formed in years with moderate or even insufficient rainfall during the filling period. This pattern is particularly relevant for Ukrainian breeding. For example, M.A. Lytvynenko (2005), analysing the potential of Ukrainian wheat, emphasised that with proper agricultural techniques and favourable but moderate moisture, the quality of domestic grain can be no worse than that of leading world producers (such as Canada). This indirectly confirms that in order to achieve high baking properties and protein concentration, preference is given not to excessive moisture, but to balanced conditions that prevent the “dilution effect”. The results of the present study were consistent with this approach in that excessive moisture is undesirable, but they revealed a critical limitation: under the conditions of heat stress that were observed in 2024, even a moderate precipitation deficit did not lead to high quality, as the dominant negative factor was heat shock, which stopped protein synthesis. At the same time, V.T. Kolyuchy and M.I. Blokhin (2007) summarise that high grain quality in the Forest-Steppe zone of Ukraine is associated precisely with periods when a moderate moisture deficit ensures

intensive protein accumulation during the filling period. This is explained by the fact that a moderate water deficit at the ripening stage can accelerate metabolic processes and the redistribution of nitrogen in the grain. The results of 2024 show that if such a deficit is accompanied by high temperatures, then instead of intensified protein accumulation, accelerated ripening occurs (formation of small, flattened grains), which leads to a low final concentration of protein and gluten.

Similar results are reported by R. Koppel *et al.* (2020), emphasising that in temperate climates, consistently high yields are achieved at the expense of reduced baking properties. Thus, the negative correlation between yield ( $r = -0.56$ ) and protein content established in the present study is consistent with international observations and confirms the need to search for varieties capable of balancing quantity and quality of production under changing climatic conditions.

Summarising the results of the studies, it can be noted that the data obtained indicate a significant influence of hydrothermal conditions on the quality and productivity of winter wheat. A pronounced dependence between the level of moisture, temperature regime and the formation of the protein-gluten complex of grain has been established. Analysis of correlation relationships confirms the interdependence of the main quality indicators that determine baking properties.

## Conclusions

As a result of two years of research (2023-2025) on eight varieties of winter wheat of Ukrainian and foreign selection, it was established that the hydrothermal conditions of the growing seasons are the dominant factor that significantly affects the formation of the quality indicators of winter wheat grain. The contrast in weather conditions between 2023/2024 and 2024/2025 made it possible to clearly differentiate varieties according to their response to stressful and optimal conditions. Under the conditions of 2023/2024, the average yield was lower (4.6 t/ha), but the grain formed with a higher protein content (10.9% on average)

and gluten content (20.6% on average). The highest quality indicators were recorded for the Ukrainian varieties 'MIP Vyshyvanka' and 'Vezha Myronivska', with a gluten content of 22.0% and a protein content of up to 11.4%. At the same time, the varieties 'MIP Valenciia' (5.6 t/ha) and 'Tobak' (5.3 t/ha) demonstrated the maximum yield under these conditions.

On the other hand, the more humid and favourable conditions of 2024/2025 ensured a significant increase in average yield to 6.7 t/ha, with the maximum indicator in the 'Tobak' variety (7.7 t/ha). However, this was accompanied by a decrease in average quality indicators: gluten content decreased to 16.7%, and protein content to 10.3%. The best quality varieties this year were 'Vezha Myronivska' (18.2% gluten, 10.9% protein) and 'Mescal' (18.4% gluten). Correlation analysis confirmed the existence of an inverse relationship between yield and quality indicators, which was particularly pronounced in the stressful year of 2024 (correlation coefficient  $r = -0.60$  for protein and  $r = -0.56$  for gluten). In the more favourable conditions of

2025, this relationship weakened significantly, indicating the possibility of balancing quantity and quality under optimal water conditions.

Based on the data obtained, the most adapted genotypes capable of producing stable grain quality for the conditions of Northern Ukraine are the varieties 'Vezha Myronivska' and 'MIP Vyshyvanka', which combine high quality indicators with sufficient productivity. The prospect for further research to expand the evaluation of winter wheat varieties in terms of grain quality in different agroclimatic zones of Ukraine and to identify genotypes that are most adapted to changing hydrothermal conditions.

### Acknowledgements

None.

### Funding

None.

### Conflict of Interest

None.

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## Оцінка сортів пшениці озимої за показниками якості зерна в умовах північної України

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**Анотація.** Дослідження присвячено оцінці впливу гідротермічних умов на формування показників якості зерна сортів пшениці озимої в умовах Північної України. Актуальність роботи зумовлена посиленням кліматичних коливань, що призводить до зниження стабільності врожайності та якості зерна, а також необхідністю добору сортів, здатних поєднувати високу продуктивність і належну хлібопекарську якість. Мета дослідження – виявити вплив температурного режиму та зволоження на показники якості зерна сортів пшениці озимої різного походження та визначити найбільш адаптовані генотипи до умов регіону. Дослідження проводили протягом двох вегетаційних періодів (2023-2025 рр.) на восьми сортах пшениці озимої української та іноземної селекції. Оцінювання здійснювали за основними показниками якості: масою 1000 зерен, вмістом білка, клейковини, скловидності та натурою зерна. Встановлено, що погодні умови значно впливали на показники якості зерна. У посушливих умовах 2023/2024 рр. формувалося зерно з підвищеним вмістом білка (до 11,4 %) і клейковини (до 22,0 %), тоді як у вологіших умовах 2024/2025 рр. забезпечувалася вища врожайність (до 7,7 т/га), але знижувався вміст білку. Найвищі показники якості відмічено для сортів 'Вежа Миронівська' та 'МІП Вишиванка', а найвищу продуктивність – 'МІП Валенсія' та 'Тобак'. Кореляційний аналіз засвідчує зворотний зв'язок між урожайністю та якісними показниками зерна, який посилюється за дефіциту вологи, водночас високі прямі кореляції між білком, клейковиною та скловидністю свідчать про їхню взаємну залежність і можливість балансування кількості та якості за оптимального водного режиму. Практичне значення отриманих результатів полягає у визначенні сортів, здатних формувати стабільну якість зерна за різних погодних умов, що може бути використано для удосконалення сортового складу пшениці озимої в Україні та оптимізації агротехнологій у контексті кліматичних змін

**Ключові слова:** погодні умови; клейковина; білок; скловидність; фази розвитку



UDC 661.162.6:635.918

DOI: 10.31548/dopovidi/6.2025.66

## The effect of growth regulators on the morphological and anatomical characteristics and yield of the tea-hybrid rose variety 'Aqua'

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### Suggested Citation:

Havrys, I., Kutovenko, V., Muliarchuk, O., Voitsekhivska, O., & Belava, V. (2025). The effect of growth regulators on the morphological and anatomical characteristics and yield of the tea-hybrid rose variety 'Aqua'. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 66-78. doi: 10.31548/dopovidi/6.2025.66.

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**Abstract.** Roses occupy a leading position among cut flowers, and the use of growth regulators activates metabolism, increases plant resistance, yield and decorative qualities of flowers. The aim was to determine the effect of various growth regulators on the development, yield formation and decorative parameters of the 'Aqua' rose variety under conditions of low-volume hydroponics in Venlo-type glass greenhouses. Kendal+, Ferti Set, Regoplant, Isabion, Gilea and Yara Vita preparations were used for the tests. The object of the study was the 'Aqua' hybrid tea rose, originally bred by the Dutch breeding company Sint Petrus Johannes Schreurs. The experiment was carried out under production conditions at the LLC 'Askania-Flora' greenhouse farm in 2023. Three foliar treatments of plants with growth-regulating substances were carried out: the first – a week after planting them in a permanent place in the greenhouse, the second – during the first budding, the third – at the beginning of mass flowering. The control variant was not sprayed with growth regulators. The research showed that the preparations Gilea and Isabion accelerated the formation of flower stalks and the timing of bending, as well as promoted faster budding and flower development. It was found that the use of Isabion increased the yield of the 'Aqua' rose variety to 267 pieces/m<sup>2</sup> (38.1 pieces/plant), which was 11% higher than the control (241 pieces/m<sup>2</sup>). Under the action of Gilea, the vase life of cut flowers reached 15 days compared to 10 days in the control. It was found that under the action of Yara Vita, the yield reached 259 pcs/m<sup>2</sup> (37.0 pcs/plant), which exceeded the control by 8%. A positive effect of the growth regulators Yara Vita, Gilea and Isabion on rose productivity, overall yield and the main decorative indicators of the flower was found. The results obtained should be taken into account when planting rose seedlings in Venlo-type glass winter greenhouses for better regulation of growth processes and increased overall yield

**Keywords:** stimulating effect; greenhouse; plant growth regulators; bud height; productivity

## Introduction

Growing cut flowers is one of the uses of greenhouse facilities, which occupies a small but stable segment of the agricultural market in Ukraine. Among the flowers grown for sale in floriculture farms, roses occupy a special place, as they are in high demand among the population. This poses a priority task for producers – to preserve the decorative qualities of flower products. At the same time, modern market requirements stipulate not only high quality and attractive appearance of flowers, but also the ability to store them for a long time after cutting, which necessitates the improvement of agricultural technologies. In particular, the use of growth regulators that can influence the physiological processes of plants, increase their resistance to stress factors and contribute to the production of products with high decorative and commercial characteristics is relevant.

Similar to the action of growth regulators, nutrients play an important role in the formation

of above-ground plant mass and determine the intensity of physiological processes. V. Gamayunova & A. Panfilova (2020) found that fertiliser application significantly increases biomass accumulation in spring barley plants, indicating a close relationship between mineral nutrition and growth processes in cultivated plants. Researchers D.N. Jaber & S.A. Mashkooor (2025) determined that spraying roses with gibberellic acid and methyl jasmonate affects the qualitative characteristics of flowers and the duration of rose flowering. Vase life and flower stalk diameter increase when plants are sprayed with a combination of gibberellic acid at a concentration of 75 and 150 mg/l with methyl jasmonate at a concentration of 200 mg/l, and the diameter of the flower increases when treated with gibberellic acid at a concentration of 75 mg/l with 100 mg/l methyl jasmonate. An increase in the number of petals is observed when spraying with gibberellic acid

at a concentration of 150 mg L<sup>-1</sup> with 100 mg L<sup>-1</sup> methyl jasmonate, while higher concentrations of both preparations lead to the opposite process. O.A. Ukrainets & V.V. Polishchuk (2024) studied the effect of different concentrations of indolyl-3-butyric and naphthylacetic acids on the process of rhizogenesis during vegetative propagation of roses. Analysis of the results showed that the use of naphthylacetic acid has a significant effect on increasing the number of primary roots on cuttings at all concentrations studied.

A study by M.A. Rasyid (2025) on the effect of concentrations (500 ppm, 1,000 ppm and 1,500 ppm) of indole-3-butyric acid on the growth rate of cuttings and the appearance of buds on roses (*Rosa centifolia* L.) found that auxin concentrations are ineffective for root growth activity and rose yield through propagation by cuttings. D. Anjelita *et al.* (2025) conducted and determined the effect of various auxin-containing preparations on the growth dynamics of rose stem cuttings. The experimental variants included three growth regulators: a commercial synthetic preparation containing 6 mg of naphthaleneacetic acid; bean sprout extract containing 6 mg of indole-3-acetic acid; and garlic extract containing 6 mg of indole-3-acetic acid. The results showed that naphthaleneacetic acid significantly increases plant height, and garlic extract combined with natural indole-3-acetic acid has a positive effect on the emergence of new shoots on cuttings. M. Zavorodnii *et al.* (2024) found that S-heterylsuccinates affect the microclonal propagation of the Lada variety of Damask rose (*Rosa damascena* Mill.). Adding this compound to the nutrient medium increases the number and length of roots in rose plants by 12%. Scientists C. Cheng *et al.* (2021) found that the opening activity of rose flowers (*Rosa hybrida*) was increased by ethylene, which specifically activated the endoreduplication of parenchyma cells at the base of the petals, leading to asymmetric growth of the petal base and, consequently, to typical flower opening. M. Thakur & R. Kumar (2020) concluded that growth regulators significantly affect yield

characteristics. The number and weight of fresh flowers per plant were significantly higher with the application of diphenylurea and kinetin compared to the control and increased the yield of fresh flowers by 56.0 and 41.6%, respectively.

According to M.H. Naeemi *et al.* (2022), improving the quality of rose flowers mainly depends on natural and chemical compounds. The growth regulators methyl jasmonate (MeJA) and brassinosteroids (BR) significantly activate protective mechanisms in plants and maintain the quality of cut flowers by increasing the level and activity of superoxide dismutase and catalase. G.A. Abdel-Wahed *et al.* (2024) investigated the effect of seaweed extract on improving the growth and flowering characteristics of *R. gallica* and *R. Chinensis*. The results showed that this preparation at a concentration of 400 ppm significantly reduces the incidence of root rot and wilting in rose plants, leading to an improvement in all growth and flowering parameters, as well as an increase in the content of photosynthetic pigments, total carbohydrates, nitrogen, phosphorus and potassium. Synthetic retardants are the main inhibitors used in floriculture. They have the ability to limit shoot growth without significantly reducing the number of leaves and leaf surface area, resulting in a compact plant habit and stable flower stalks even when light intensity is insufficient. Scientist K. Sharma (2023) summarised the results of studies on the use of growth regulators on rose crops. The author noted that such preparations contributed to increased yields, improved decorative characteristics and prolonged the shelf life of cut flowers. It was emphasised that their effectiveness depended on the type of preparation, growing conditions and plant development stages.

According to M. Yaquby *et al.* (2020), the effectiveness of growth regulators also depends on the effectiveness of other agrotechnical measures, external conditions, the condition and age of plants, and the need for strict adherence to doses, timing, and application technologies. This highlights the need for further study of growth-regulating substances on rose crops in

order to increase the adaptive potential of plants and increase crop yield and quality. There is also limited information on the effect of exogenous application of growth regulators on the growth, development and physiology of hybrid tea roses in winter greenhouse conditions.

The aim of this study was to determine how effectively plant growth regulators affect the growth, development, decorative qualities, productivity and yield of rose varieties when grown in small-volume hydroponics in modern Venlo-type glass greenhouses.

### Materials and Methods

The effect of growth regulators on physiological processes, flowering dynamics, productivity and decorative qualities of flowers was studied on plants of the 'Aqua' tea-hybrid rose variety bred by the Dutch breeding company Sint Petrus Johannes Schreurs. The experimental work was carried out at the greenhouse facility of LLC "Ascania-Flora" during 2023 in a modern Venlo-type winter greenhouse using a computerised climate control system. The research was conducted in accordance with the ethical standards set out in the Convention on Biological Diversity (1992). The 'Aqua' rose seedlings were planted in their permanent growing location in the greenhouse on 11 April 2023. The planting pattern was two rows. The rose seedlings were placed in mineral wool cubes (Grodan Plantop Rosa NG 2.0, size 100 × 100 × 65 mm). The plants were placed on mineral wool mats (Grodan Vitaflor NG 2.0, size 1000 × 235 × 75 mm). At the time of planting, the length of the shoots was 20-22 cm. Seven plants were placed per 1 m<sup>2</sup>. The substrate volume was 2.2 litres per plant.

At the beginning of cultivation, the air temperature was maintained at 8-20°C, and the air humidity was 65-75%. During the summer months, the air temperature ranged from 25 to 30°C. The length of daylight hours was of great importance for the optimal growth and development of roses. As the level of solar energy decreased or when there was high cloud cover, a plant lighting

system was turned on in the greenhouse to ensure the necessary length of daylight hours and level of light energy, which promotes the active process of photosynthesis. During the winter, lighting was provided at a level of 10,000-12,000 lux. The electric lighting mode was set for 20 hours a day, from 4 a.m. to midnight. The dark phase of photosynthesis lasted four hours. In spring, with the increase in the intensity of solar radiation and the length of daylight hours, the operating time of the electric lighting system was reduced to 16, 14, 10, etc. hours. With the transition from summer to autumn, the duration of lighting during the day was gradually increased again.

An important microclimatic parameter in growing roses in a greenhouse was the level of carbon dioxide. On average, its content in the air was maintained within 800-1,200 ppm, and on clear sunny days – up to 1,500 ppm. Watering was carried out according to the programme in automatic mode. The interval between waterings was 1-1.5 hours. The amount of nutrient solution was 2.5-6 l/m<sup>2</sup>. In closed ground conditions, the root system of the rose was rather weak, so the standard electrical conductivity value was within the range of EC = 1.2-2.2 mS/cm. In winter, the concentration of the nutrient solution was increased due to lower water consumption, and in summer it was reduced. Plant care consisted of removing suckers from the leaf axils and pruning. Before pruning, the buds were removed. The first pruning was carried out 15-20 days after planting the seedlings. Flowers were cut daily in the morning in the half-open bud phase, when the sepals were already bent back and the coloured petals were visible from above.

Six plant growth regulators were used in the study: Kendal+, Gilea, Regoplant, Ferti Set, Isabion and Yara Vita. All preparations differ in origin and basic composition. Kendal+ is a biological complex of macroelements of oligosaccharides and phytoalexins; Gilea is succinic acid, an environmentally friendly growth biostimulant; Regoplant is a growth biostimulant from a line of multicomponent preparations, a fermented

product of fungal microorganisms and ginseng root extract; Ferti Set – a combination of alginic acid, carbohydrates, betaine, phytohormones and microelements; Isabion – a set of amino acids of animal origin, macro- and microelements; Yara Vita – a balanced combination of macro- and microelements and bioactive compounds extracted from the unique *Ascophyllum nodosum* algae.

The plants were sprayed with growth regulator solutions three times: the first time – 7 days after placing the seedlings in their permanent place in the greenhouse, the second time – during the first budding, and the third time – at the beginning of mass flowering. The control variant was not sprayed with growth regulators. The concentrations recommended by the manufacturers were used to treat the plants: Kendal+

at a concentration of 25 ml per 10 l of working solution for spraying; Gilea – 200 mg/l; Regoplant – 25 ml per 10 l; Ferti Set – 0.5 l/100 l, Isabion – 10 ml/1 l of water, and Yara Vita – 20 ml of fertiliser per 5 l of water.

## Results and Discussion

The results of the studies showed that growth regulators had different effects on the dynamics of growth processes and phenological indicators of the 'Aqua' rose variety. Differences were observed in the timing of the first break, the speed of bud emergence, and the duration of flower development. The use of certain preparations accelerated the formation of generative organs, while others showed almost no difference from the control variant (Table 1).

**Table 1.** Phenological observations of rose development under the influence of plant growth regulators

Variant	Planting date	Date of first stem breaking	Duration of period, days	
			Bending to bud appearance	Flower development
No treatment (C)	11.04.23	29.04	17	21
Yara Vita		26.04	17	21
Regoplant		27.04	16	20
Gilea		26.04	15	19
Isabion		26.04	15	19
Kendal+		29.04	17	21
Ferti Set		28.04	15	19

**Note:** C – control

**Source:** developed by the authors

An important task after planting roses in a permanent place in a greenhouse is to create a powerful assimilation apparatus. This is achieved by forming productive skeletal shoots that develop from the axillary buds of the shoot's covering scales. To increase the assimilation surface, shoot bending was used. The first bends were made when buds began to appear on the shoots. Before bending, the bud was removed. The operation was carried out in the afternoon, when the turgor in the plant cells was reduced.

In the variants where Yara Vita, Gilea and Isabion preparations were used, the first bending

was carried out on 26 April, 15 days after planting the seedlings. On 27 April, bending was carried out on roses treated with Regoplant, and on 28 April, on plants treated with Ferti Set. The Kendal+ preparation did not affect the growth of rose shoots, and in this variant, the break was carried out simultaneously with the plants of the control variant. To ensure a gradual, rather than simultaneous, awakening of the buds on the plants, the operation was carried out every other day in the afternoon, when the turgor in the plant cells was reduced. Proper execution of the first pruning is one of the components of

success in increasing the flowering period. The fastest appearance of the second bud was observed when using the growth regulators Gilea, Isabion and Ferti Set. The period from breaking to bud formation lasted 15 days. Full opening of the flower was observed after another 4 days, and the total duration of its development was 19 days. The longest period from bending to bud appearance was observed in the variants with the use of Yara Vita, Kendal+ and in the control. Under these conditions, the duration of the period from planting seedlings to full flower formation was 21 days.

Yield allows to determine the feasibility of using growth regulators in production and is the main indicator of effectiveness. The first cuts of rose flower stalks using the preparations Gilea and Isabion were obtained in the third decade of May. However, their effect on plants in the last two months (October-November) was insignificant. It can be assumed that the stimulating effect of growth regulators decreased and plant metabolism slowed down. Determination of the total yield of the 'Aqua' rose variety revealed that the growth regulators Isabion, Gilea and Yara Vita had a significant impact on this indicator (Table 2).

**Table 2.** Rose yield depending on plant growth regulator

Variant	Total yield, pcs/m <sup>2</sup>	Productivity	
		pcs/plant	% to control
No treatment (C)	241	34.4	-
Yara Vita	259	37.0	+8
Regoplant	252	36.1	+5
Gilea	261	37.3	+8
Isabion	267	38.1	+11
Kendal+	248	35.4	+3
Ferti Set	250	35.7	+4
LSD <sub>0.5</sub>	15.4	2.3	-

**Note:** C – control

**Source:** developed by the authors

The growth regulator Isabion had the highest stimulating effect on the total number of flower stalks and exceeded the control indicator by 26 pieces/m<sup>2</sup>, making it the most effective among the options studied. The preparations Gilea and Yara Vita also demonstrated a significant increase in the total yield, creating an increase of 20 and 18 pieces/m<sup>2</sup> over the control. A positive effect on the increase in flowering shoots was also noted with the use of the growth regulators Kendal+, Regoplant and Ferti Set. However, their effect was weaker and did not differ significantly from the control option.

The productivity indicator of a single plant is particularly valuable in the production of flower and decorative products, as it allows to predict the yield per area, compare the impact of microclimatic factors, agrotechnical measures, green

operations, etc. on a single plant. Tracking productivity at the level of an individual plant makes it possible to determine the uniformity of bushes, lack of nutrition or the impact of stress, which is especially important when growing flowers, where each plant must be aesthetically attractive. In the current study, a significant increase in productivity was obtained with the use of the Isabion preparation – 38.1 pcs/m<sup>2</sup>, which is 3.7 pcs/m<sup>2</sup> higher than the control indicator and amounts to 11%. The use of Gilea and Yara Vita preparations increased the formation of flower stalks on the plant by 2.9 and 2.6 pcs/m<sup>2</sup>, which exceeded the control variant by 8%.

An increase in productivity by 3-4 flower stalks is of practical importance. Since the plant density is 7 plants per 1 m<sup>2</sup>, this gives 21-32 additional marketable cuttings per m<sup>2</sup>, which significantly

increases profits. The other variants showed a tendency to increase productivity, but the difference from the control was within the margin of error. The growth regulator Kendal+ had the weakest effect on the productivity of a single plant – 3%.

According to a study by K. Sharma (2023), the application of naphthylacetic acid at a concentration of 25 mg/l increased the length of shoots and flower yield on Damask rose plants. Also, according to B. Muradi *et al.* (2023), cytokinins are used to enhance cell division and tissue differentiation, and they cause active shoot formation *in vivo*. In addition, scientists

A. Porcher *et al.* (2021) claim that cytokinin preparations cause rose bud growth not only in light but also in darkness, which explains the active formation of flower stalks in early spring when using isabion, which contains amino acids in combination with auxin-like and cytokinin-like forms. During studies of the decorative qualities of 'Aqua' rose flowers, it was found that the growth regulators used significantly affected a number of important indicators. In particular, they changed the height of the bud, the diameter of the open flower, the number of petals and the shelf life of cut flowers in a vase (Table 3).

**Table 3.** Decorative indicators of rose flowers of the 'Aqua' variety under the influence of growth regulators

Variant	Bud height, cm	Diameter of open flower, cm	Number of petals, pcs.	Vase life, days
No treatment (C)	6.7	9.6	43.6	10
Yara Vita	7.3	10.9	51.4	14
Regoplant	6.9	10.2	48.8	12
Gilea	7.4	11.2	50.7	15
Isabion	7.1	10.7	47.4	12
Kendal+	6.5	9.7	45.2	10
Ferti Set	7.0	10.3	50.3	11
LSD <sub>05</sub>	0.6	1.0	5.6	-

**Note:** C – control

**Source:** developed by the authors

The product Gilea had a significant effect on bud height. The diameter of the open flower significantly exceeded the control variant when using Gilea, Yara Vita and Isabion – by 17, 14 and 11%, respectively. The double-floweredness (number of petals) was significantly influenced by Yara Vita, Gilea and Ferti Set. Under their influence, double-floweredness increased by 7.8, 7.1 and 6.7 petals, respectively. The vase life of the flower stalk in the cut was most prolonged by the preparation Gilea – by 5 days compared to the plants in the control variant. The main component of this growth regulator is succinic acid. This result coincides with the data of S. Kazaz *et al.* (2019), who evaluated the effect of various organic acids on the storage of cut roses. Succinic acid (SUA) at a concentration of 100 mg/l

significantly prolonged the vase life of flowers to 13.07 days compared to 8.53 days in the control. In general, Gilea and Yara Vita demonstrated the most comprehensive effect on decorative characteristics. These preparations simultaneously ensured an increase in flower size, an increase in the number of petals, and a significant prolongation of vase life, which makes them promising for use in industrial rose cultivation.

Studies conducted by A. Alzuhairi & A. Al-Sheikly (2022) and A. Zahid *et al.* (2022) confirm the data obtained in the current study on a significant improvement in rose flowering parameters as a result of treatment with plant growth regulators. Spraying with gibberellic acid (200 mg/l) accelerated the appearance of flowering buds, increased their number, increased the length of

the pedicel, the diameter of the flower and the shelf life of the cut flower. The study by L. Hong *et al.* (2021) analysed the morphogenesis of rose flowers under the influence of growth regulators during the induction of flowering of vegetative shoots *in vitro*, which reveals the mechanism of action of some growth-regulating substances. Flower development goes through three stages: apical meristem of the shoot → floral meristem → flower bud. During the transition to the floral meristem, the levels of cytokinins, auxins, and gibberellins increase, indicating their key role in triggering flowering. It has been proven that a combination of gibberellic acid – 0.5 mg/l, 1-naphthylacetic acid – 0.1 mg/l and 6-benzylaminopurine – 2.5-3.0 mg/l induces flower meristem *in vitro*, which subsequently develops into a flower bud. The most common method of applying gibberellic acid preparations is spraying the entire plant or its individual parts, or applying drops of suspension to buds and floral primordia. Under the influence of gibberellins on rose plants, R. Gupta & S. Chakrabarty (2013) observed elongation of shoots, increased terry and brightness of colour, increased size of inflorescences and accelerated flowering times. In the current study, an increase in shoot height and acceleration of bud formation were similarly noted, in particular with the use of the preparations Gilea and Isabion, which confirms the stimulating effect of gibberellins on the generative development of plants. Scientists N. Zieslin & R. Ben-Zaken (1992) found that gibberellin preparations stimulate the growth of structures that had formed prior to treatment. Thus, to increase the size of inflorescences, enhance their colour and terry, treatment is carried out when all elements of the flower are fully formed, and to change the flowering time, when all parts of the flower are formed but the buds are still green. The results obtained in the current study confirm this conclusion: the maximum effect was observed when the treatment was carried out in the phase of complete flower formation, which manifested itself in a reduction of the “bending-bud appearance” period to

15 days. According to I. Havris *et al.* (2019), the products Bionur and Bud accelerated the development of rose flower stalks, reduced the duration of the bud emergence and flower development periods, and also affected plant productivity and the decorative properties of the flower. In the current experiment, similar patterns were observed: the preparations Isabion and Gilea contributed to faster development of flower stalks and an increase in decorative indicators (flower diameter, number of petals, vase life), which is consistent with the literature data. Thus, the results of the current study confirm the conclusions of previous authors and demonstrate a similar effect of gibberellin preparations on the growth, development and decorative properties of roses, although the degree of effectiveness of individual agents (Gilea, Isabion, Yara Vita) was more pronounced in greenhouse conditions of small-volume hydroponics.

Auxin-like substances are used in the vegetative propagation of roses and other flowers to intensify the rhizogenesis of cuttings (Oliynyk, 2017). The most widely used substances for this purpose are heteroauxin,  $\beta$ -indolylbutyric and  $\alpha$ -naphthylacetic acids, as well as vitamins B1 and C. For the treatment of cuttings, aqueous solutions of preparations are prepared, as well as powders and, based on powders, pastes. The concentrations of different substances vary for different crops. S. Ghosh *et al.* (2017) determined the effect of plant growth regulators on stem cuttings of Damask rose (*Rosa damascena* Mill.). It was found that the use of such preparations increased the survival rate and shortened the germination period of cuttings. A similar trend was observed in the current study: the use of auxin-based preparations ensured faster root system formation, which had a positive effect on the further development of plants and an increase in the number of flower stalks.

Thus, the results of the observations confirm the conclusions of S. Ghosh *et al.* (2017) about the stimulating effect of auxins on the process of rhizogenesis and rooting of cuttings, which is an important stage for the successful vegetative propagation of roses.

The results obtained indicate that the use of growth regulators had a significant effect on both the phenological indicators and the productivity and decorative characteristics of the 'Aqua' rose variety. The highest efficiency was achieved with the preparations Gilea, Isabion and Yara Vita, which contributed to an increase in yield, an increase in flower size and number of petals, as well as a significant prolongation of the vase life of cut products. Other preparations had a less pronounced but still positive effect compared to the control. Thus, the use of growth regulators can be considered a promising direction for optimising rose cultivation technology in low-volume hydroponics. The set of data obtained formed the basis for formulating generalised conclusions of the study.

### **Conclusions**

After planting the 'Aqua' rose seedlings, the main task of agricultural technology was to form bushes with productive skeletal shoots developing from axillary buds. The assimilation surface was created by bending the shoots in the afternoon, after removing the buds, when the turgor of the cells was reduced. The use of growth regulators accelerated the first bending. The most effective preparations were Gilea, Isabion and Yara Vita – bending was carried out 15 days after planting.

Observation of the phenological phases of growth and development of rose plants showed that the shortest period from breaking to bud appearance (15 days) was observed in the variants with the use of Gilea, Isabion and Ferti Set. Complete flower formation in these variants took 19 days. The longest period between breaking and bud appearance was observed with the use of Yara Vita and Kendal+ – 21 days. The first flower cuttings were obtained in the third decade of May with the use of Gilea and Isabion. The growth regulator Isabion had the greatest effect on the yield and productivity of rose flower stalks per m<sup>2</sup> – +26 pieces (38.1 pieces/m<sup>2</sup>, +11% from the control), Gilea – +20 pieces, Yara Vita – +18 pieces. The weakest effect was from the use of Kendal+ (+3%). A noticeable effect of growth regulators was also

noted on the decorative qualities of the flower. The height of the bud increased significantly with the use of the growth regulator Gilea. The diameter of the flower increased by 17% (Gilea), 14% (Yara Vita), 11% (Isabion) compared to the control. The number of petals increased with the use of Yara Vita (+7.8), Gilea (+7.1) and Ferti Set (+6.7). Vase life increased by 5 days (Gilea) and 4 days (Yara Vita).

Thus, the greatest stimulating effect on growth and phenological parameters, productivity and decorative characteristics was shown by the plant growth regulators Gilea and Isabion, as well as, to some extent, Yara Vita. Other preparations (Kendal+, Regoplant, Ferti Set) had a weaker or local effect. The use of growth-regulating substances allows to shorten the period of shoot development and budding, increase the yield and decorative properties of roses, and extend the life of cut flowers.

Based on the results obtained, it is promising to study the mechanisms of action of growth regulators on roses in different phenological phases. In particular, it is advisable to evaluate the long-term effects of growth stimulants on plant metabolism in the late period (October-November), since previous observations have shown a decrease in the stimulating effect during this period. Investigate the interaction of various growth-regulating substances with each other and with growing conditions (temperature, lighting, nutrition) to create practical protocols for improving the productivity and quality of flowers. These areas will deepen the understanding of the role of stimulants in regulating rose growth and flowering and optimise production technologies for commercial greenhouses.

### **Acknowledgements**

None.

### **Funding**

None.

### **Conflict of Interest**

None.

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## Вплив застосування рістрегулюючих речовин на морфо-анатомічні показники та урожайність троянди чайно-гібридного типу сорту 'Аква'

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**Анотація.** Троянда посідає провідне місце серед квітів на зріз, а застосування регуляторів росту забезпечує активацію метаболізму, підвищує стійкість рослин, урожайність та декоративність квіток. Мета полягала у визначенні дії різних регуляторів росту на процеси розвитку, формування врожаю та декоративні параметри троянди сорту 'Аква' за умов малооб'ємної гідропоніки у скляних теплицях типу Venlo. Для випробувань використано препарати Кендал+, Ферті Сет, Регоплант, Ізабіон, Гілею та Яра Віта. Об'єктом дослідження були рослини чайно-гібридної троянди сорту 'Аква', оригіномом якої є нідерландська селекційна компанія Sint Petrus Johannes Schreurs. Експеримент реалізовано у виробничих умовах тепличного господарства ТОВ «Асканія-Флора» впродовж 2023 року. Здійснено триразову позакореневу обробку рослин розчинами рістрегулюючих речовин: перше – через тиждень після висаджування їх на постійне місце в теплицю, друге – в період першої бутонізації, третє – на початку масового цвітіння. Контрольний варіант регуляторами росту не обприскували. В результаті досліджень встановлено, що препарати Гілея та Ізабіон прискорювали формування квітконосів і терміни проведення залому, а також сприяли швидшій появі бутонів та розвитку квітки. Виявлено, що застосування Ізабіону підвищило урожайність троянди сорту

‘Аква’ до 267 шт./м<sup>2</sup> (38,1 шт./рослину), що на 11 % перевищувало контроль (241 шт./м<sup>2</sup>). За дії Гілеї вазостійкість зрізаних квіток досягала 15 діб проти 10 діб у контролі. З’ясовано, що за дії Яра Віта урожайність досягала 259 шт./м<sup>2</sup> (37,0 шт./рослину), що перевищувало контроль на 8 %. Виявлено позитивний вплив рістрегулюючих речовин Яра Віта, Гілея та Ізабїон на продуктивність троянди, загальну урожайність і основні декоративні показники квітки. Одержані результати варто враховувати під час висаджування саджанців троянди у скляних зимових теплицях типу Venlo для кращого регулювання ростових процесів і підвищення загальної врожайності

**Ключові слова:** стимулююча дія; теплиця; регулятори росту рослин; висота бутона; продуктивність



UDC 630\*24:582.946.1(477.46)

DOI: 10.31548/dopovidi/6.2025.79

## Causes and trends in pathological damage to common ash

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**Abstract.** Intensive dieback of *Fraxinus excelsior* L. in the Forest-Steppe zone of Ukraine has led to a significant deterioration in the sanitary condition of ash stands and increased the risk of losing their ecosystem stability, making this issue particularly urgent amid the simultaneous influence of pathogens and the activation of a complex of stem-damaging insect species. This study aimed to analyse the ecological role of common ash in forest ecosystems, as well as to assess the sanitary condition and causes of ash stand decline, particularly the impact of *Hymenoscyphus fraxineus* and *Agrilus planipennis*. The study employed methods of analytical review, synthesis of previous research, critical comparison of results obtained by different authors, and logical-structural generalisation to identify key patterns in the development of pathological processes in ash stands. It was established that common ash supports intensive nutrient cycling due to litterfall with a high content of calcium and magnesium, and the rapid mineralisation of organic matter, which supports soil fertility and understorey development. According to the analysed studies, more than 950 associated species have been recorded, including 44 obligate and 62 highly specialised organisms, demonstrating the ash's importance for biodiversity. The loss of ash reduces the rate of organic matter mineralisation and disrupts the nitrogen and carbon balances in the soil. Analysis of the sanitary condition in the Right-Bank Forest-Steppe of Ukraine showed that the most severely affected stands are located in the Cherkasy and Vinnytsia regions, where the area of dieback exceeds 600 ha. The main degradation factors include infections caused by *Hymenoscyphus fraxineus*,

### Suggested Citation:

Babych, Yu., & Puzrina, N. (2025). Causes and trends in pathological damage to common ash. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 79-92. doi: 10.31548/dopovidi/6.2025.79.

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damage by *Agrilus planipennis*, bacterial diseases, and the combined influence of saprotrophic fungi. The results also indicate a strong relationship between the intensity of damage and tree age, stand density, and soil type, which enables the identification of zones at the greatest risk

**Keywords:** *Fraxinus excelsior* L.; *Hymenoscyphus fraxineus*; *Agrilus planipennis*; forest ecosystems; sanitary condition of forests

## Introduction

Forest dieback and degradation are complex processes driven by multiple factors, including long-term weakening of forest stands, stressful weather conditions, and subsequent infestation by pests and pathogens. Climate change, prolonged droughts, and unstable groundwater levels play a key role. As a result, trees become less resilient, and stress factors trigger their decline, which is further accelerated by the activity of harmful organisms. V. Tkach & O. Kobets (2025) examined the impact of climate change and military aggression on Ukraine's forests, revealing significant degradation of forest ecosystems. They highlighted how these factors, alongside environmental stressors, exacerbate forest health deterioration and lead to biodiversity loss. The study emphasised the need for urgent conservation efforts and sustainable management practices to mitigate further damage.

From the late 20<sup>th</sup> to the early 21<sup>st</sup> century, a significant deterioration in the sanitary condition of many tree species was recorded. These changes were caused by the combined influence of abiotic, biotic, and anthropogenic factors that together reduced the resistance of trees to pests and pathogens (Polk *et al.*, 2022). Since the 2000s, the issue of widespread ash dieback has become widely discussed both in scientific circles and among forestry professionals in Ukraine and beyond. According to T. Pyvovar *et al.* (2023), the degradation of ash stands has affected large parts of Europe, including the Baltic states, Poland, Norway, Sweden, Germany, Austria, Switzerland, and several other countries.

I. Matsiakh & V. Kramarets (2020) noted that the primary weakening factor is the invasive fungus

*Hymenoscyphus fraxineus*, which causes ash dieback disease. Symptoms include necrotic bark lesions, disruption of the conductive system, and rapid mortality of trees of various ages. Weakened trees are easily colonised by secondary infections, such as *Armillaria* spp. and *Pseudomonas* spp., which accelerate their death. Additional pressure is exerted by invasive phyllophagous insects, among which a prominent pest is the ash leaf-curl aphid *Prociphilus fraxinifolii*. Scientists J.P. George *et al.* (2024) have established that these insects reduce photosynthetic activity and further weaken tree resistance to diseases. The spread of harmful organisms is facilitated by intensive international trade in planting material and climate change, which enable their movement and acclimatisation.

C. Fritsch *et al.* (2024) noted that under the influence of anthropogenic changes, drought, and fragmentation of forest stands, the area of dieback centres continues to expand, the condition of ash stands continues to deteriorate, and their age structure is shifting. The combination of these factors highlights the need for more detailed research on pathological processes and the development of effective measures to preserve common ash in natural and artificial ecosystems.

However, despite significant progress in studying ash dieback pathology, several important knowledge gaps remain. In particular, region-specific factors of ash stand degradation under current climate change conditions are still insufficiently explored. The interactions between *Hymenoscyphus fraxineus* and secondary pests and pathogens in Eastern European forests, especially in Ukraine, also remain poorly assessed.

In addition, there is a notable lack of long-term monitoring data on the extent of ash dieback, which would allow accurate tracking of the dynamics and intensity of these processes. The study aimed to examine the ecological importance of common ash within forest ecosystems and evaluate the health of ash stands, focusing on the factors contributing to their decline, particularly the influence of *Hymenoscyphus fraxineus* and *Agrilus planipennis*.

The article utilised scientific sources, including publications in peer-reviewed journals, monographs, and conference proceedings. The main information databases used for source retrieval were Google Scholar, Scopus, ResearchGate, Web of Science, as well as university electronic archives and open-access resources of international scientific organisations. The selection of sources was based on the principles of scientific novelty, reliability, relevance, representativeness, and thematic correspondence to the

issue of pathological ash dieback. The analysis included studies addressing the aetiology, distribution, influence of biotic and abiotic factors, and conservation measures for *Fraxinus excelsior* L. stands.

### Ecological role of common ash (*Fraxinus excelsior* L.) in forest ecosystems

Common ash (*Fraxinus excelsior* L.) plays a key role in the functioning and stability of European forest ecosystems. As noted by A. Kamińska *et al.* (2025), ash stands have the ability to stabilise the microclimate, regulate soil moisture and fertility, and serve as a habitat for numerous plant and animal species (Fig. 1). Owing to its rapid nutrient cycling and the high content of base cations in its litter, ash supports favourable conditions for the development of the herb layer and soil microbiota. Its root system contributes to soil structure formation, while leaf litter enriches the soil with nutrients, ensuring efficient mineralisation of organic matter.



**Figure 1.** Common ash plantations

**Source:** authors' photo

The degradation of ash due to dieback has significant ecological consequences: crown density decreases, light availability and the microclimate of the understorey change, conditions for herbaceous and bryophyte species deteriorate, and the biogeochemical balance becomes disrupted. Such changes can potentially lead to reduced biodiversity and the loss of ecosystem

services. Although ash can grow in various soil types and climatic conditions, optimal conditions for its growth are usually observed on moist, clay-rich soils that provide sufficient moisture and nutrients. Therefore, it is most often found along forest edges, riverbanks, and lake shores (Vasaitis & Enderle, 2017). From an ecological perspective, ash also plays an important role in maintaining

biodiversity. According to R. Mitchell *et al.* (2014), 953 species associated with ash have been recorded in the United Kingdom, of which 44 are obligate – completely dependent on this tree species – and 62 are highly associated. These organisms include fungi, mosses, lichens, and invertebrates. This demonstrates the wide ecological connections of ash and its irreplaceable role in supporting various trophic levels of forest communities.

A distinctive feature of ash is its formation of mycorrhizal associations with a relatively narrow range of fungi (Vasaitis & Enderle, 2017). However, due to the low specificity of these associations, the loss of ash is unlikely to cause a drastic decline in fungal diversity, though it may affect the overall balance within soil microbial communities. Research by T. Riutta *et al.* (2012) shows that changes in stand structure, particularly the loss of ash, influence the activity of micro- and macro-organisms, altering litter decomposition dynamics and the carbon cycle. According to S. Fuchs *et al.* (2024), an important ecological function of ash is its contribution to nutrient cycling. Ash leaf litter is characterised by high calcium and magnesium content and a low carbon-to-nitrogen ratio. Ash sheds its leaves while they are still green, facilitating the rapid return of nitrogen, phosphorus, potassium, and sulphur to the soil and increasing soil fertility. Ash roots also decompose faster and contain more nutrients compared with other broadleaf species (Agan *et al.*, 2020). Due to the activity of microorganisms and invertebrates, the mineralisation of organic residues proceeds more intensively, ensuring a more complete nutrient cycle and preventing excessive accumulation of litter (Vasaitis & Enderle, 2017).

The replacement of ash by tree species with more acidic and slowly decomposing litter, such as beech or oak, may lead to reduced nutrient availability and a decline in plant species with high soil-fertility requirements. This, in turn, affects the species composition and functioning of the ecosystem, decreasing the diversity of ground vegetation (Langer, 2017). Studies by

T. Hultberg *et al.* (2020) confirm that the loss of ash results in noticeable changes in soil processes and nutrient cycling. The authors found that the disappearance of ash reduces the rate of organic matter mineralisation, disrupts the nitrogen and carbon balances, and influences the formation of soil microbial communities. J. George *et al.* (2022) noted that due to its combination of characteristics of both a pioneer species and a stable component of forest stands, ash can participate in both primary and secondary succession, serving as an important intermediary element in forest regeneration and the formation of forest structure. In mature forests, ash often grows within mixed stands, where it may be a dominant species or a component of communities with beech (*Fagus sylvatica*), pedunculate and sessile oak (*Quercus robur*, *Q. petraea*), small-leaved and large-leaved lime (*Tilia cordata*, *T. platyphyllos*), birch (*Betula pubescens*), alder (*Alnus glutinosa*, *A. incana*), and other species. Thus, common ash is one of the key tree species that shape ecological balance, trophic interactions, and biogeochemical processes within European forest ecosystems. Its dieback and gradual decline may have long-term consequences for forest structure, productivity, and biodiversity, necessitating the development of strategies for the conservation and restoration of this species.

As shown by J. Richter *et al.* (2025), ash-dominated forests are characterised by higher understorey species diversity compared with communities dominated by beech or alder. Under ash canopies, favourable light and moisture conditions develop, supporting numerous shade-tolerant and moisture-dependent herbaceous species. The authors analysed over 1,900 phytosociological descriptions from northern Germany, creating an ecological baseline prior to the onset of widespread ash dieback. The results demonstrated that an increasing proportion of ash in the upper canopy is directly associated with higher understorey diversity. They identified 43 vascular plant species closely associated with ash forests, including

*Veronica montana*, *Paris quadrifolia*, *Sanicula europaea*, and *Carex sylvatica*, among others. Many of these species have limited distributions and are sensitive to changes in microclimate and light conditions, which makes them highly vulnerable to ash decline. Research by K. Turczański (2020) confirms that common ash (*Fraxinus excelsior* L.) is an important component of European forest ecosystems, significantly influencing soil conditions, understorey dynamics, and biocoenotic stability. The authors found that soil acidity (pH) and organic matter content are directly linked to the intensity of ash defoliation. In areas with more neutral pH and higher organic matter content, trees exhibited better physiological condition, and the understorey supported greater plant species diversity. The results indicate that ash strongly affects soil processes – through its litter, root system, and chemical composition of its leaf litter, it regulates soil fertility and the growth conditions of other plants. Changes in ash condition (particularly due to dieback) lead to alterations in understorey structure, reduced species diversity, and deterioration of soil trophic status.

Thus, *Fraxinus excelsior* L. performs a multi-functional ecological role in forests, including maintaining soil fertility and chemical balance, supporting understorey diversity, and influencing the structure and stability of forest ecosystems. From an ecological standpoint, the loss of ash leads to changes in forest structure, decreased soil fertility, and the disappearance of associated microorganisms, fungi, and invertebrates. Its weakening or death may trigger long-term shifts in trophic chains and the structure of biogeocoenoses.

### Early detection and geographic spread of ash dieback

The first cases of ash dieback were recorded in 1990 in Poland (Kowalski, 2006). This disease, known as ash dieback (ADB), is caused by the pathogen *Hymenoscyphus fraxineus* and has spread extensively across Europe over the past 30 years, advancing at a rate of 30 to 70 km per year. Ascospores of *Hymenoscyphus fraxineus* were

found up to 100 metres ahead of the disease front, indicating that the fungus often becomes well established in an area long before visible symptoms appear (Orton *et al.*, 2018). ADB affects European ash throughout its natural range; the pathogen has been detected in the United Kingdom and Ireland since 2012 (Coker *et al.*, 2019). Long-term studies have shown that *Hymenoscyphus fraxineus* may have been present in Poland as early as the 1960s, where the disease went unnoticed due to a lack of knowledge about its symptoms.

Despite significant media attention, monitoring, and responses to suspected signs of decline, the causes of the disease's spread remain incompletely determined (Kirisits *et al.*, 2010; Chandler *et al.*, 2016). At early stages, the disease is difficult to detect because it can easily be confused with several other tree disorders (Vasaitis & Enderle, 2017). Studies by R. Becker *et al.* (2020) conducted in the United Kingdom revealed that some trees already exhibited pronounced ADB symptoms before 2012. Using dendrochronological methods, it was established that the infection of these trees could have occurred in 2004–2005. Initial cases of ADB may be highly localised or spread slowly due to factors such as mating-type incompatibility, environmental conditions, and the isolation of ash trees in agricultural landscapes. The lack of targeted ADB surveys before 2012 and low public awareness of the disease's symptoms resulted in its spread remaining undetected for a long time.

Ash leaves become infected by ascospores that are dispersed by wind. The fungus then enters through the petiole and branches into the tree tissues. Repeated infections result in rapid crown dieback, often leading to tree death within a few years. Detecting new infections is difficult because the damage can remain unnoticed for up to three years. Symptoms of infection include wilting of branches in the upper crown and vertical bark cracks (Liziniwicz *et al.*, 2022). Norwegian researchers O. Díaz-Yáñez *et al.* (2020) have been monitoring the spread of *Hymenoscyphus fraxineus* in southwestern Norway since 2008 and

estimated its spread rate at 51 km per year. They also recorded a 74% increase in ash mortality after the disease became widespread. Although the proportion of ash in Norway's national inventory plots is very low, these results indicate a high invasiveness of the pathogen, which does not depend on host density. Alternative hosts were identified – living or dead trees of several species, including *Acer platanoides*, *Alnus* spp., *Betula* spp., *Corylus avellana*, *Fagus sylvatica*, *Juniperus communis*, *Picea abies*, *Pinus sylvestris*, *Populus tremula*, *Prunus* spp., *Quercus* spp., *Salix* spp., and *Sorbus aucuparia*. This knowledge may contribute to the conservation of biodiversity associated with ash.

In 2022, European scientists published a large-scale study on ash dieback across Europe. They identified 407 sites in 27 countries where ash occurs. The study period covered 1987-2020. It was found that between 1987 and 2000, tree mortality was moderate and sporadically recorded in Spain, France, Romania, Slovakia, Italy, Lithuania, and Moldova. Between 2000 and 2010, mortality accelerated mainly in Poland, Lithuania, Belarus, Sweden, Denmark, Germany, the Czech Republic, and some regions of France. Although mortality remained low (1%-3%) in most countries, significant losses (10%-100%) were recorded at certain sites in Poland and Sweden. Between 2010 and 2020, mortality increased sharply across Europe. The most striking example is southern Scandinavia, where all ash trees died at eight of the surveyed sites (Marçais *et al.*, 2022).

German researchers S. Fuchs *et al.* (2024) conducted a study showing that ash dieback, caused by the fungus *Hymenoscyphus fraxineus*, is closely linked to environmental conditions and stand structure. Based on observations of 1,365 trees across eleven monitoring sites throughout Germany, they found that the most severe damage occurred on wet hydromorphic soils, in stands with high ash density, and among younger and thinner trees. Moreover, severe basal infections proved to be a reliable indicator of probable tree mortality. The study also indicated that despite a general trend of stand decline, the condition of individual

trees improved at six of the eleven sites, suggesting the natural selection of more resistant genotypes. The researchers concluded that, to preserve ash, it is necessary to avoid establishing pure ash stands, especially on waterlogged sites, and instead focus on selecting less susceptible populations and promoting mixed stands. The study by M. McMullan *et al.* (2018) on the spread of ash dieback in Europe significantly expanded understanding of the disease's dynamics and the factors influencing its progression. The authors emphasised that *Hymenoscyphus fraxineus* had spread across much of the continent before it was officially identified as the causative agent of ash dieback.

A. Agan *et al.* (2020) highlighted that the intensity of tree infection depends on the biological characteristics of ash, particularly tree age, and site conditions, which determine its vulnerability. These findings confirm that the population dynamics of the pathogen are complex, and the scale of its spread requires long-term monitoring and the development of adaptive management strategies. Studies within the German FraxForFuture programme demonstrated that disease severity depends on site conditions, stand structure, and tree age. Ash dieback was more pronounced in areas with high tree density and on hydromorphic (wet) soils, whereas large, dominant trees were more resistant to the pathogen. This indicates selective natural survival – the gradual persistence of more tolerant individuals may form the basis for species recovery.

In many countries, measures have been implemented to limit the spread of ash dieback and to preserve ash trees. These measures include the felling and burning of infected trees, the establishment of plantations using genetically clean seed material, monitoring the spread of the disease, and conducting scientific research to identify effective control methods (Nielsen *et al.*, 2022). Key measures to control the spread of the disease and conserve ash trees include:

- mechanical control methods;
- identification and selection of genetically resistant trees;

- monitoring programmes for disease spread;
- development of new, effective control strategies.

European countries have implemented national research programmes on ash dieback, focusing on various aspects of the biology and ecology of this disease. Only through the shared utilisation and synthesis of existing knowledge has it been possible to achieve a comprehensive understanding of ash dieback and develop modern practical recommendations for the sustainable management of *Fraxinus* stands in Europe. These measures have been carried out using an innovative interdisciplinary approach involving plant pathologists, breeders, and foresters (McKinney *et al.*, 2011; Lenz *et al.*, 2016).

In addition to ADB, another significant threat to *Fraxinus excelsior* L. is the Emerald Ash Borer (EAB) (*Agrilus planipennis*), an invasive insect from Asia that kills ash trees (Fig. 2). EAB was first detected in the United States in south-eastern Michigan in 2002. According to estimates by the U.S. Department of Agriculture, EAB had been present in the region since the 1990s (Petter *et al.*, 2020).



**Figure 2.** General appearance of the EAB

**Source:** David Cappaert, Michigan State University

In the United States, the response to EAB has been coordinated by the Animal and Plant Health Inspection Service (APHIS), the U.S. Forest Service, and the Agricultural Research Service. Key measures have included biological control, monitoring using traps, and the search for effective methods to regulate pest populations. Research has focused on identifying biocontrol agents, particularly parasitic wasps, and assessing the

ecological and economic impacts of the EAB spread (Jonsson & Thor, 2012).

Ash dieback is an example of a serious pathogen-induced disease that reduces the commercial value of timber and has negative consequences for forestry and forest conservation. The scale of biological invasions has increased significantly, with no signs of this trend slowing down. It is evident that greater efforts are required to control and prevent the introduction of new pests and pathogens. However, the global scale of spread and the anthropogenic movement of plants and plant products pose challenges to the effectiveness of such measures.

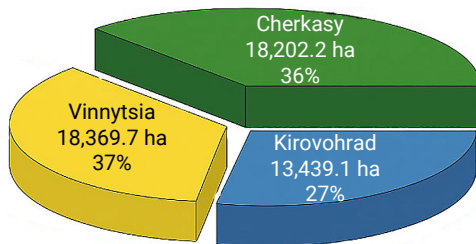
Since the 1990s, ash mortality has sharply increased and has already reached catastrophic levels in northern Europe, where most trees had disappeared from study sites by the end of 2020. Research provides critical information on the impact of *Hymenoscyphus fraxineus* (ash dieback, ADB) and the EAB (*Agrilus planipennis*) on ash stands. Scientific data indicate the widespread occurrence of both ADB and EAB in Europe and Ukraine. ADB has been recorded across significant parts of Europe, including the United Kingdom, Ireland, Spain, Sweden, and Ukraine (Davydenko *et al.*, 2022). Researchers have emphasised the difficulty of early disease detection, as symptoms may be inconspicuous or easily confused with other tree pathologies. This complicates the delineation of disease spread and the implementation of necessary control measures, highlighting the high potential for further pathogen expansion into new regions. The importance of systematic monitoring and the application of appropriate control measures is strongly emphasised.

### **Sanitary conditions and causes of degradation of *Fraxinus excelsior* L. stands in the Right-Bank Forest-Steppe of Ukraine**

According to the State Forest Resources Agency of Ukraine, common ash plantations cover an area of 151.6 thousand hectares, which accounts for 2.4% of the forested lands of the forest fund. In particular, in

the Cherkasy region, ash plantations occupy 12 thousand hectares (Meshkova, 2019). During the 1980s and 1990s, before the outbreak of the epidemic in the early 2000s, foresters consciously aimed to increase the proportion of ash in many regions of Europe.

According to the National Forest Inventory (n.d.), the total area of ash (*Fraxinus excelsior* L.) stands in the Right-Bank Forest-Steppe of Ukraine is 50,011 ha. The largest share is in Vinnytsia Region, with 18,369.7 ha, accounting for 37% of the total area. A slightly smaller but comparable area is found in the Cherkasy Region, with 18,202.2 ha, or 36%. The smallest area of ash stands is in the Kirovohrad Region, where they occupy 13,439.1 ha (27%) (Fig. 3).

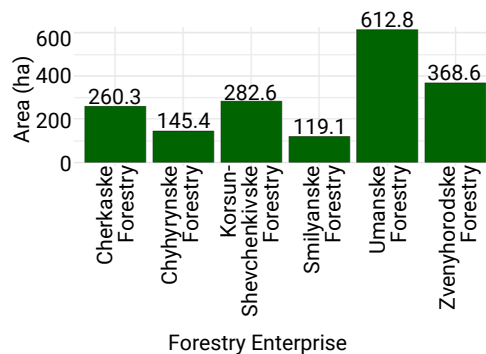


**Figure 3.** Area of common ash (*Fraxinus excelsior* L.) stands in the Right-Bank Forest-Steppe of Ukraine

**Source:** compiled by the authors based on National Forest Inventory (n.d.)

The first signs of ash dieback in Ukraine were observed in 2010. Prior to 2010, limited attention was paid to the sanitary condition of ash stands in Ukraine, as there were no reports of wilting symptoms. Morphological signs of ash dieback were recorded in the eastern part of the country and included uneven bud break, isolated shoot necroses, discolouration of the wood, and premature leaf fall (Davydenko *et al.*, 2019). Figure 4 shows the area of ash dieback in the Cherkasy Region in 2024. The chart illustrates the areas of dieback of common ash stands across various forestry enterprises in the region. The largest dieback areas are recorded in Umanske Forestry (612.8 ha) and Zvenyhorodske Forestry (368.6 ha), indicating the

most critical situation. Other enterprises also show considerable degradation (260-280 ha), while the lowest values are observed in Chyhyrnske and Smilianske forestries. Overall, the chart demonstrates an uneven yet widespread pattern of ash dieback throughout the region. This confirms that the dieback of common ash has a large-scale and regionally extensive character, affecting substantial areas in all forestry enterprises. The most severe levels of degradation indicate a critical condition of ash stands and highlight the need for urgent restoration and protection measures.



**Figure 4.** Ash dieback area in the Cherkasy Region in 2024, according to forest protection enterprises

**Source:** compiled by the authors based on K. Davydenko *et al.* (2019)

The main causes of common ash dieback include (Matsiakh & Kramarets, 2014): the ash dieback pathogen (*Hymenoscyphus fraxineus*); EAB (*Agrilus planipennis*); damage by other pests; and bacterial infections. Ash dieback (*Hymenoscyphus fraxineus*) is one of the most serious diseases threatening the survival of European ash (*Fraxinus excelsior* L.) across Europe. According to a study by R. Vasaitis & R. Enderle (2017) conducted in the Left-Bank Forest-Steppe of Ukraine (Sumy and Kharkiv regions), the disease was widespread in both natural and artificial stands. The intensity of infection was found to depend on site conditions, stand age, density, site quality class, and the proportion of ash in the stand

composition. A.N. Drogvalenko *et al.* (2019) reported that the highest frequency of infection occurred in stands up to 80 years old, with 40%-70% ash composition and a density greater than 0.5. The disease was most prevalent in fertile, fresh site conditions, which are also the most favourable for ash growth. These findings indicate the high prevalence of ash dieback and the complex interaction of factors influencing disease development, underscoring the need for further research to develop effective measures for the conservation of ash stands in Ukraine. These results are consistent with findings that affected trees host a significantly higher diversity of pathogenic and saprotrophic fungi, which intensify necrotic processes and accelerate wood degradation. Researchers also note the presence of a wide range of accompanying pathogenic fungi (including *Venturia fraxini*, *Alternaria alternata*, *Cytospora* spp., and *Aureobasidium pullulans*), which exacerbate the damage and accelerate tissue mortality.

Research by V. Meshkova *et al.* (2021) found that ash dieback is often associated with butt rots and, to some extent, bark beetle infestations, with the most dangerous fungi being *Hymenoscyphus fraxineus*, *Cytospora* spp., and *Diplodia* spp. The authors concluded that the weakening of ash trees is a complex process caused by the interaction of multiple pathogens and pests. The study established that ash dieback in Ukraine results from the combined impact of the fungal pathogen *Hymenoscyphus fraxineus* and the invasive insect *Agrilus planipennis*. *Hymenoscyphus fraxineus* causes necrosis, shoot dieback, and gradual tree weakening, while *Agrilus planipennis* destroys the conductive tissues of the trunk, leading to rapid dieback. The research highlighted that the interaction of these two factors significantly accelerates the degradation of ash stands.

In 2019, the EAB was detected for the first time in Ukraine, in the Luhansk Region. However, the variability of larval developmental stages and the presence of exit holes indicate that the pest likely entered the region as early as 2017 or even earlier, probably from the nearby Voronezh region

of Russia. Within two years, EAB had spread over 300 km westward from the initial detection site, affecting hundreds of *Fraxinus excelsior* L. and *Fraxinus pennsylvanica* trees. By the end of 2021, the pest had been recorded across most of Luhansk and in parts of the Kharkiv Region. In 2022, EAB was observed in parks in Kyiv, and by 2023, it had spread to large areas of the Kharkiv Region. Field studies in 2022-2023 were significantly limited due to ongoing hostilities. It is likely that EAB entered Ukraine via wooden crates used for transporting goods. Since then, the pest has spread in all directions, establishing itself in eastern Ukraine by 2019 and showing potential for further expansion westward toward Europe (Meshkova *et al.*, 2024).

N. Puzrina *et al.* (2025) conducted a detailed study of ash responses to EAB infestation, paying particular attention to morphological changes in buds as an early diagnostic criterion. The authors found that larval damage to conductive tissues disrupts nutrient flow to apical buds, resulting in underdevelopment, premature drying, and asymmetric bud break. These symptoms correspond to other signs of infestation and confirm that bud condition changes can serve as an early and reliable indicator of the onset of invasion. H. Mazurchuk *et al.* (2025) showed that rapid, critical ash dieback is the result of a complex interaction between *Agrilus planipennis*, stem-boring insects, and fungal and bacterial pathogens, which has a significant impact, particularly on larger-diameter trees. The study by I.M. Kulbanska (2015) found that tubercular disease is the most widespread and harmful infectious disease of ash (*Fraxinus excelsior* L.), caused by the bacterium *Pseudomonas syringae* pv. *savastanoi*. The research reported that the disease manifests as tumour formation on trunks and branches, followed by cracking and cambium death, which significantly weakens the trees. The pathogen affects trunks, shoots, inflorescences, and samaras, while leaves remain resistant even under artificial infection. Isolation of bacteria from affected organs confirmed their pathogenicity, and the detection of

*Pseudomonas* in ash gall wasps suggests a possible role of insects in spreading the infection.

Ash dieback in Ukraine is driven by the combined effects of the invasive fungus *Hymenoscyphus fraxineus*, the EAB, and associated pathogens. Damage is mostly observed in stands of particular age and composition, while climatic stresses further exacerbate degradation. This situation indicates a significant deterioration of the sanitary condition of ash forests and underscores the urgent need for measures to preserve and restore them. The only effective countermeasure against ash dieback is the implementation of sanitary thinning.

### Conclusions

Ash (*Fraxinus excelsior* L.) provides significant ecosystem services and plays a key role in maintaining biodiversity. Analysis of ash distribution shows that the largest areas of ash forests in the Forest-Steppe are concentrated in Vinnytsia and Cherkasy regions, where they occupy an important share of the forest estate. However, since 2010, there has been a rapid increase in the area affected by dieback, accompanied by characteristic symptoms such as uneven bud break, shoot necroses, and premature leaf fall. The key factors driving degradation are the invasive fungus *Hymenoscyphus fraxineus* and the EAB (*Agrilus planipennis*). The impact of these organisms is complex: *Hymenoscyphus fraxineus* causes necrotic lesions and weakens trees, while *Agrilus planipennis* destroys vascular tissues, accelerating their death. Additional complicating factors include bacterial infections, such as ash tubercular disease, and associated fungal pathogens (including *Cytospora*, *Diplodia*, *Venturia*, and others), which form complex infection syndromes. The most vulnerable are stands up to 80 years old, growing in fertile, moist conditions, with a high proportion of ash in the canopy.

European studies also confirm the severity of the threat posed by ADB and the EAB. *Hymenoscyphus fraxineus* spreads both through airborne ascospores and via the movement of infected planting material, while EAB rapidly expands its range in various countries. Data from Austria, Poland, Scandinavia, and other regions indicate a continuous increase in infection intensity, the emergence of new foci, and negative consequences for forest ecosystem stability. The loss of ash leads to a deterioration of forest structure, reduced biodiversity, and weakened ecosystem functions. Thus, the degradation of ash stands results from the combined effects of invasive pathogens, pests, and climatic stresses. Existing data confirm the critical condition of ash forests in the Right-Bank Forest-Steppe of Ukraine and many European regions, highlighting the urgent need for conservation measures, monitoring, and management of invasions.

Future research prospects include expanding knowledge of ADB and EAB interactions under different ecological conditions, establishing long-term monitoring programmes, identifying resistant ash genotypes, studying the impact of climate change on pathogen spread, and developing integrated strategies for the protection and restoration of ash ecosystems. These research priorities will determine the effectiveness of future measures for ash conservation in Ukraine and Europe.

### Acknowledgements

None.

### Funding

None.

### Conflict of Interest

None.

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## Причини та тенденції патологічних уражень ясена звичайного

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**Анотація.** Інтенсивне всихання *Fraxinus excelsior* L. у Лісостепу України призвело до значного погіршення санітарного стану ясеневих насаджень і підвищило ризик втрати їхньої екосистемної стабільності, що робить цю проблему особливо актуальною на тлі одночасного впливу патогенів та активізації комплексу стовбурових комах. Метою дослідження було проаналізувати екологічну роль ясена звичайного в лісових екосистемах, а також оцінити санітарний стан і причини всихання ясеневих насаджень, зокрема вплив *Hymenoscyphus fraxineus* та *Agrilus planipennis*. У дослідженні застосовано методи аналітичного огляду, синтезу попередніх досліджень, критичного порівняння результатів різних авторів та логіко-структурного узагальнення для виявлення основних закономірностей розвитку патологічних процесів у ясеневих деревостанах. Встановлено, що ясен звичайний забезпечує інтенсивний колообіг поживних речовин завдяки підстилці з високим вмістом кальцію та магнію і швидкою мінералізацією органічної речовини, що сприяє підтриманню родючості ґрунтів та розвитку підліску. Згідно з аналізом досліджень, зафіксовано понад 950 видів асоційованих організмів, серед яких 44 облігатних та 62 високоспеціалізованих, що демонструє значущість ясена для біорізноманіття. Втрата ясена знижує швидкість мінералізації органічної речовини та порушує баланс азоту й вуглецю в ґрунті. Аналіз санітарного стану ясеневих насаджень Правобережного Лісостепу України показав, що найбільш уражені насадження знаходяться в Черкаській та Вінницькій областях, де площа всихання перевищує 600 га. Основними факторами деградації є інфекції *Hymenoscyphus fraxineus*, пошкодження *Agrilus planipennis*, бактеріальні захворювання та комплексний вплив сапротрофних грибів. Результати також вказують на тісний зв'язок інтенсивності ураження з віком дерев, густотою насаджень і типом ґрунту, що дозволяє прогнозувати зони найбільшого ризику

**Ключові слова:** *Fraxinus excelsior* L.; *Hymenoscyphus fraxineus*; *Agrilus planipennis*; лісові екосистеми; санітарний стан лісів



## ***Chlorella vulgaris* microalgae as a potential source of vitamins B<sub>12</sub> and D<sub>2</sub>**

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**Abstract.** The research relevance is determined by the need to summarise current knowledge on the mechanisms of vitamin B<sub>12</sub> and D<sub>2</sub> accumulation in *Chlorella vulgaris* and the factors determining their biochemical variability in microalgae cultures. The study aimed to systematise literature data for 2020-2025 and identify key factors regulating sterol metabolism and vitamin profile in *Chlorella vulgaris*. The study was based on targeted information search, content analysis, critical comparison, and qualitative synthesis of previous scientific works. Based on the analysis of sources, the study demonstrated that the type of nutrient medium determined the level of micronutrients in the biomass of *Chlorella vulgaris*. Mineral media provided stable growth but did not contain cobalamin (vitamin B<sub>12</sub>), while organic substrates – liver extract, whey, permeates, and anaerobic digestates demonstrated a higher potential for cell enrichment due to natural coenzymes, proteins and trace elements. Cobalt ions (Co<sup>2+</sup>) were shown to promote greater accumulation of cobalamin in biomass, while magnesium (Mg<sup>2+</sup>), zinc (Zn<sup>2+</sup>), iron (Fe<sup>2+</sup>/Fe<sup>3+</sup>) and copper (Cu<sup>2+</sup>) were substantial cofactors for enzymes and transport systems that influenced sterogenesis. Regarding vitamin D<sub>2</sub> (ergocalciferol), the literature confirmed the decisive role of ultraviolet radiation in the B range (UV-B), which initiated the photoconversion of ergosterol. The intensity and spectral composition of light determined the rate of sterol accumulation, while salinity, nitrogen or phosphorus deficiency, and other stress factors contributed to increased lipidation and changes in the sterol pool. The study determined that in the stationary phase of the culture, lipid and sterol fractions accumulated more

### **Suggested Citation:**

Bortsiukh, V., & Golub, N. (2025). *Chlorella vulgaris* microalgae as a potential source of vitamins B<sub>12</sub> and D<sub>2</sub>. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 93-113. doi: 10.31548/dopovidi/6.2025.93.

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intensively than during the period of active cell division. A generalised analysis of sources showed that the most effective enrichment of *Chlorella vulgaris* with vitamins B<sub>12</sub> and D<sub>2</sub> is achieved by combining organic nutrient media, an optimised lighting spectrum with a UV-B component, and a controlled mineral composition of the medium

**Keywords:** : cobalamin; ergocalciferol; organic extracts; light spectrum; ergosterol; metal ions; stress factors

## Introduction

Vitamin B<sub>12</sub> ensures the synthesis of deoxyribonucleic acid (DNA), normal functioning of the nervous system and blood formation processes, while vitamin D<sub>2</sub> participates in the regulation of calcium metabolism, maintains bone tissue health and performs an immunomodulatory function. Available epidemiological data indicate the prevalence of deficiencies in these micronutrients among various population groups, creating a need for affordable and plant-based sources of vitamins (Peshuk *et al.*, 2024). Regarding vitamin D<sub>2</sub>, a key limitation is that its formation in microalgae depends on the availability of sterol precursors and photochemical conditions, which vary significantly in natural environments. Thus, the determination of the potential of *Chlorella* as a source of both vitamins is a relevant scientific task that combines the issues of food security and optimisation of biotechnological production.

To outline the nutritional potential of microalgae, O.V. Kozhemiaka & L.V. Peshuk (2023) described *Chlorella* as a valuable component of health and functional products. The study emphasised its pronounced biological activity and potential for the creation of food supplements. To summarise biotechnological approaches to the intensive cultivation of *Chlorella*, V. Grishko *et al.* (2024) systematised cultivation regimes, performance parameters and areas of practical application. Technological factors that determine the quality of biomass in industrial and laboratory conditions were outlined. To further analyse the vitamin composition of microalgae, J.A. Coronado-Reyes & J.C. González-Hernández (2023) summarised the sources of

micronutrients in different types of algae. The study highlighted the key features of vitamin profile formation and the importance of microalgae as alternative nutrient resources. In the direction of studying the interaction of microalgae with microbial communities, M. Ribeiro *et al.* (2023) investigated lactobacilli from *Chlorella* photobioreactors as potential producers of vitamin B<sub>12</sub>. The study established the ability of certain strains to synthesise cobalamin and outlined the prospects for their use in food technology.

Emphasising bioactive properties, R.A. Hamouda *et al.* (2022) evaluated the antioxidant and antitumour effects of *Chlorella vulgaris* extracts. The study demonstrated significant activity of the biomass in a series of biochemical tests, confirming its functional potential. In the context of the need to control nutrient stability, M. Islam (2024) analysed the consistency of vitamin B<sub>12</sub> content in commercial microalgae powders. Differences between product batches were recorded, and their possible impact on the nutritional value of the products was assessed. Within the framework of analysing the interaction of microalgae with soil microbial systems, C. Liu *et al.* (2025) showed that a synthetic microalgal community stimulated pepper growth on mineral substrates. The study also demonstrated an enrichment of root endophytic bacteria and fungi, which accompanied an increase in plant biomass. In the context of studying bioremediation processes, D. Zhao *et al.* (2025) established the effectiveness of a consortium of microalgae and bacteria in removing Cu<sup>2+</sup> ions from water systems. In addition, the physiological reactions of the co-culture were described, and

an Artificial Neural Network (ANN) model for predicting detoxification efficiency was constructed.

Concerning the role of mineral nutrition, M. Peng *et al.* (2025) determined changes in the growth of *Corethron sp.* under the influence of nitrogen, phosphorus, iron, and silicon. A simultaneous increase in lipid content and transformation of the fatty acid profile depending on the combinations of nutrients was demonstrated. Emphasising light regulation of metabolism, S. Maulana *et al.* (2025) evaluated the responses of *Navicula sp.* to red and blue light. The results demonstrated changes in lipid, protein, carbohydrate, and pigment composition depending on the spectral mode. Within the framework of bioinformatic generalisation, A. Voshall *et al.* (2021) compared sterol pathways in green algae. Key enzymatic steps separating ergosterol and phytosterol biosynthesis pathways were identified. In the spectrum of functional applications, A.R. Mendes *et al.* (2024) systematised data on the chemical components of *Chlorella vulgaris*. A wide range of bioactive effects were noted, determining its potential for food, medical and feed technologies. Therefore, a comprehensive analysis of the presented research suggests that varying the composition of the nutrient medium (organic extracts, metal ions, fixed C/N ratio) with controlled lighting parameters (including ultraviolet light) may be an effective strategy for increasing the nutritional value of *Chlorella vulgaris* biomass.

The study aimed to analyse current scientific approaches to enriching *Chlorella vulgaris* biomass with vitamins B<sub>12</sub> and D<sub>2</sub> based on the determination of various factors (lighting, nutrient medium composition) that influence the synthesis and accumulation of these metabolites. The objectives of the study were to determine the conditions that influence the accumulation of vitamins B<sub>12</sub> and D<sub>2</sub> in *Chlorella vulgaris*, to characterise changes in the sterol profile under different light and nutrient regimes, and to evaluate the role of organic substrates in modifying the biochemical parameters of the culture.

## Materials and Methods

The study was conducted in the format of a narrative literature review, within which a systematic review and qualitative analysis of publications devoted to the mechanisms of vitamin B<sub>12</sub> accumulation, sterol metabolism regulation, and vitamin D<sub>2</sub> biosynthesis in *Chlorella vulgaris* was conducted. The initial stage of the information search covered 412 publications found using complex key queries in the Scopus, Web of Science, PubMed, SpringerLink, Wiley Online Library, ScienceDirect, MDPI, and Google Scholar databases. After filtering by publication year (2020-2025), completeness of experimental data, availability of quantitative indicators, and relevance to the areas of photovitaminogenesis, sterogenesis, and *Chlorella cultivation* conditions, 136 sources were retained. At the content filtering stage, works that duplicated results, contained contradictory or unclear data, did not have a quantitative part, were predominantly descriptive, and all Russian-language publications were removed; a total of 87 materials were rejected. The final review corpus consisted of 21 full-text sources that met the thematic, methodological, and quality criteria.

Search queries were formed using combined keywords covering various aspects of the topic: *Chlorella vulgaris*, ergosterol, vitamin D<sub>2</sub>, vitamin B<sub>12</sub> accumulation, UV-induced conversion, light spectra, salinity stress, metal cofactors, nitrogen limitation, lipid accumulation, photobioreactor optimisation, and microalgae sterols.

The selected materials were summarised through a structural analysis of the experimental conditions, which included lighting parameters (intensity, spectral composition, photoperiod), temperature regimes, pH of the environment, salinity, concentrations of macro- and microelements, addition of organic substrates (liver extracts, whey, permeate, amino acid additives), ultraviolet (UV) irradiation regimes – Ultraviolet A (UVA, long-wave UV radiation), Ultraviolet B (UVB, medium-wave UV radiation) and Ultraviolet C (UVC, short-wave UV radiation), as well as stress factors, in particular the presence of heavy metals, oxidative stress, salinity of the environment,

nitrogen or phosphorus deficiency and their ratio. The analysis covered various cultivation systems: fermenters, stirred photobioreactors, tubular systems, thin-film installations and biofilm structures.

The results of different authors were compared using a set of integrative parameters, which formed a consistent model of factors that regulate the biochemical composition of *Chlorella vulgaris*. These parameters included: (1) vitamin B<sub>12</sub> content depending on the presence of microbial consortia, sources of organic nitrogen, and cultivation conditions; (2) ergosterol profile at different light intensities, growth phases, and ion composition of the medium; (3) vitamin D<sub>2</sub> content after photochemical conversion of ergosterol under the action of different ranges of UV irradiation; (4) lipid metabolism, in particular changes in triglycerides, polyunsaturated fatty acids and membrane lipids; (5) the influence of mineral factors Mg<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>2+</sup>/Fe<sup>3+</sup>, Cu<sup>2+</sup> as cofactors of sterogenesis enzymes; (6) the influence of salinity, which has been considered a trigger for lipid and sterol accumulation; (7) spectral dependence of growth and biochemical composition, especially with regard to blue and red light, which modulate lipid and pigment synthesis in different ways; (8) the effect of organic stimulants, such as liver extracts containing a complex of vitamins (in particular B<sub>12</sub>) and microelements capable of increasing biomass yield with enriched elemental composition. Generalisation was achieved by comparing data between studies and identifying common patterns, such as the relationship between the content of cobalamin and ergosterol in microalgae biomass and the light spectrum, the influence of metal ions on the synthesis and accumulation of corresponding metabolites, the role of UV in the formation of D<sub>2</sub>, the influence of growth phases on lipid dynamics and the transition of metabolism to a stress regime.

## Results

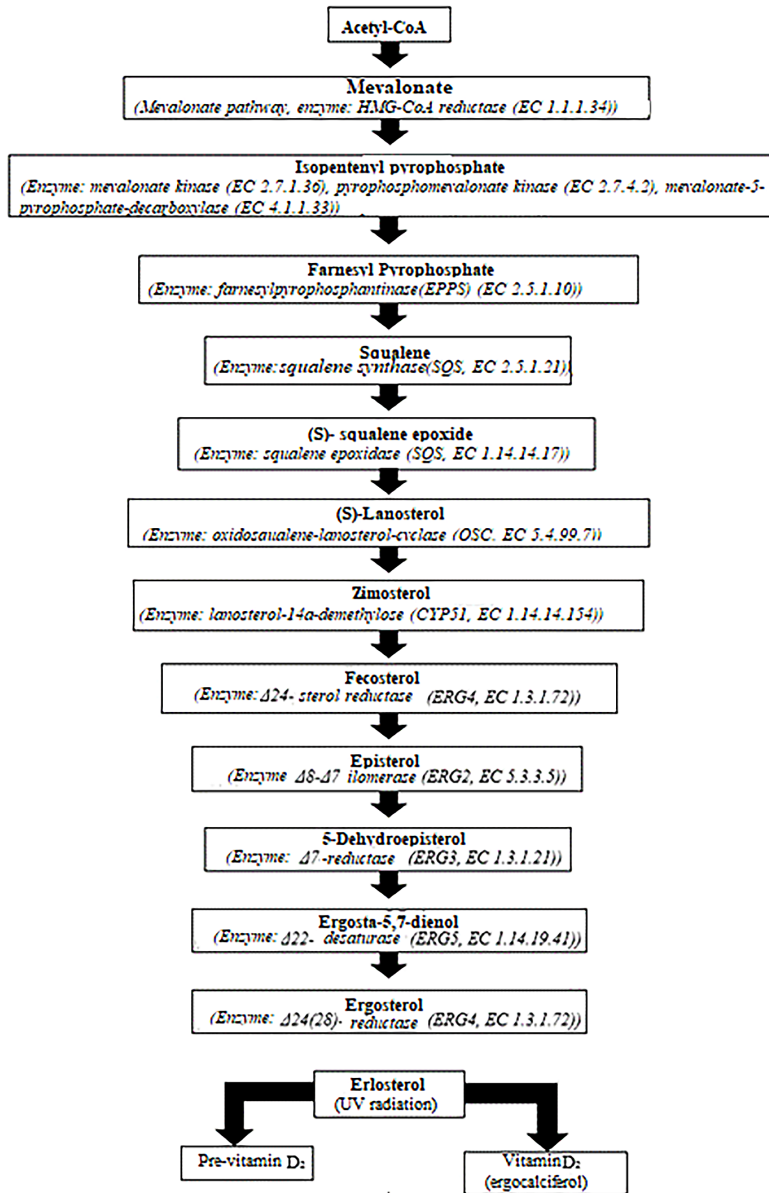
### Vitamin D<sub>2</sub>: Biosynthesis, influence of factors, peculiarities of microalgae cultivation

The biosynthesis of vitamin D<sub>2</sub> in microalgae is noteworthy due to the specific mechanisms of

ergocalciferol formation. A. Voshall *et al.* (2021) showed that in *Chlorella*, vitamin D<sub>2</sub> is formed from its own sterols, in contrast to vitamin B<sub>12</sub>, which microalgae do not synthesise. The study determined that the process involves two key steps: first, sterogenesis, during which ergosterol is formed via the mevalonate pathway (cycloartenol→lanosterol→ergosterol); second, photochemical conversion, in which ergosterol is converted to pre-D<sub>2</sub> under the action of UV light, which is then isomerised to ergocalciferol. Researchers emphasise that it is the photochemical conversion of sterols that is the critical phase determining the final yield of vitamin D<sub>2</sub> in green algae. A simplified diagram of the biosynthesis (according to the Kyoto Encyclopedia of Genes and Genomes (KEGG)) of vitamin D<sub>2</sub> in *Chlorella vulgaris* is shown in Figure 1. Under the influence of ultraviolet light, ergosterol is photochemically converted to previtamin D<sub>2</sub>, which is further isomerised to ergocalciferol (vitamin D<sub>2</sub>). The biosynthesis of ergosterol in microalgae is influenced by the growth phase, light intensity and spectrum, salt and metal content in the environment, temperature, pH, and subsequent UV irradiation, which converts ergosterol into vitamin D<sub>2</sub>. In this context, C. Carlberg (2022) considered the photoproduction of vitamin D in a broader evolutionary framework, emphasising the role of UV-dependent sterol conversions as an adaptive mechanism that explains the variability of metabolic responses in different groups of photosynthetic organisms. C. Der *et al.* (2024) emphasised the multifunctionality of sterols in cellular signalling processes and showed that their synthesis is significantly modified under the influence of environmental stress factors, which is consistent with data on the sensitivity of microalgae to light and chemical cultivation parameters. O.H. Abdelkarim *et al.* (2025) emphasised the species diversity of microalgae as sources of sterols and demonstrated that the level of ergosterol formation is determined by a combination of lighting, medium composition and physiological state of cultures, confirming the key role of environmental

conditions in the regulation of sterol metabolism. The study demonstrated that the ability of microalgae to produce sterols significantly depends on their species and cultivation conditions, including light regime, which shapes their different potential for vitamin D<sub>2</sub> photoproduction. M.F. Leung &

P.C.K. Cheung (2021) summarised experimental data on UV-induced vitamin D<sub>2</sub> synthesis in fungi and provided quantitatively confirmed examples of a sharp increase in its content after irradiation, emphasising the universality of the photochemical mechanism among different eukaryotic groups.



**Figure 1.** Ergocalciferol biosynthesis by *Chlorella* microalgae

**Source:** compiled by the authors based on M. Durdakova *et al.* (2024), Kyoto Encyclopedia of Genes and Genomes (n.d.)

T. Zhang & J. Long (2025) determined that the intensity and spectral composition of light directly determine the level of lipids and sterols in green microalgae. The researchers showed that increased light intensity stimulates lipidation in *Chlorella vulgaris*, increasing the proportion of lipids from approximately 19.5% to 25.6% of dry weight (+31%), while in *Chlorella sorokiniana*, blue light provides an accumulation of about 34% lipids within 10 days of cultivation. The study emphasised that varying light intensity and cultivation parameters accelerate the formation of lipid and sterol fractions, confirming the light dependence of the anabolic pathways of green algae. A.N.M. Azhan *et al.* (2025) showed that the spectral quality of light, in particular blue radiation, modulates the synthesis of fatty acids and chlorophyll, creating favourable conditions for increased lipid formation in *Chlorella sorokiniana*, which is consistent with the results regarding the potential for sterol accumulation and photobiological regulation of lipid metabolism. Trace elements, particularly divalent metals, acted as cofactors in the enzymatic reactions of the sterol pathway, as magnesium (Mg<sup>2+</sup>) ensured stable enzyme function and supported biomass growth, which indirectly enhanced ergosterol accumulation, and the results of Y. Feng *et al.* (2021) showed that varying concentrations of available trace elements, including Mg<sup>2+</sup>, directly affected the intensity of lipid metabolism, highlighting their regulatory function in sterogenesis.

Zinc (Zn<sup>2+</sup>) is a cofactor of numerous dehydrogenases and other enzymes involved in sterol biosynthesis; it participates in the sequential conversion of squalene to lanosterol and further to ergosterol, i.e., it directly affects the key stages of this metabolic pathway (Kyoto Encyclopedia of Genes and Genomes, n.d.). Iron (Fe<sup>2+</sup>/Fe<sup>3+</sup>) is substantial in the functioning of enzymes that ensure cellular respiration and energy metabolism, creating conditions for the stable course of sterol pathways. Copper (Cu<sup>2+</sup>) in low concentrations can activate several oxidases and maintaining

the overall metabolic tone of the cell, which also creates the conditions for enhanced ergosterol synthesis. This is indicated by the results of A.L. Souza & G.J. Patti (2021), who demonstrated that changes in the availability of transition metals restructure the cell's metabolome and affect the activity of metal-dependent enzymes. This shift in metabolic flows enhances reactions associated with lipid and sterol synthesis, thus consistent with data on the importance of trace elements in the regulation of sterologenes. For example, in yeast, where the mechanisms have been studied in detail, the addition of zinc increased the carbon flux towards ergosterol by +28.6%. The presence of Zn<sup>2+</sup> and Cu<sup>2+</sup> enhanced sterol synthesis even in the presence of inhibitors, while Fe deficiency reduced sterol levels (Souza & Patti, 2021). A similar mechanism of action is expected in microalgae. At the same time, it should be emphasised that an excess of any of these metals has a toxic effect on microalgae cells, reduces their viability and can inhibit enzyme activity; therefore, the selection of trace element concentrations is critical for optimising cultivation conditions and increasing ergosterol yield (Feng *et al.*, 2021).

G. Salbitani & S. Carfagna (2021) showed that forms of nitrogen can significantly affect structural and energy metabolism in microalgae: ammonium, as a more "desert" substrate for the cell, accelerates nitrogen assimilation and affects the balance between protein and lipid synthesis. The study also demonstrated that under conditions of variable nitrogen availability, cells switch to increased accumulation of energy and structural lipids, which is consistent with a possible increase in sterologenes. Such metabolic changes confirm that nitrogen compounds are one of the key regulators that determine the level of sterol formation in microalgae. The increase in ergosterol content under conditions of moderate salinity is also confirmed by data from H.E.A. Ali *et al.* (2021), determining that the combination of light and salt stress in *Chlorella vulgaris* cultures activated lipid synthesis,

enhanced the accumulation of sterol fractions, and was accompanied by an increase in the level of secondary metabolites, including carotenoids and antioxidants. Thus, moderate salt stress acts as an effective trigger for increased accumulation of ergosterol in microalgae. The optimal parameters for *Chlorella* growth, a temperature of 25-30°C and a pH of  $\approx 7$ , are critical for the activity of sterol pathway enzymes, which is consistent with the findings of A.E. Kholif & O.A. Olafadehan (2021), demonstrating that

*Chlorella vulgaris* was highly sensitive to temperature and pH shifts, which directly affected its chemical composition and metabolic processes. The study noted that changes in the physicochemical parameters of the environment can significantly affect the synthesis of lipids, proteins, and secondary metabolites, which indirectly indicates the potential variability of the sterol profile. A generalised description of the influence of certain factors on sterol biosynthesis in microalgae is given in Table 1.

**Table 1.** The influence of certain factors on sterol biosynthesis in *Chlorella* microalgae

Factor	Condition	Effect
Lighting intensity	Increase in sensitivity	Increase in lipid content in <i>C. vulgaris</i> cells from $\sim 19.5\%$ to $\sim 25.6\%$ ( $\approx +31\%$ ).
Visible light spectrum	Blue light (450-470 nm)	On the 10 <sup>th</sup> day of cultivation, <i>C. sorokiniana</i> cells showed an increase in lipid content of $\approx 34.06\%$ compared to other spectra.
Zn <sup>2+</sup> ions	Addition of Zn <sup>2+</sup> ions to the culture medium	The addition of Zn <sup>2+</sup> ions to the yeast culture medium led to a 28.6% increase in ergosterol content. A similar effect is also expected for <i>Chlorella</i> microalgae.
Growth phase	Phase 2 (stationary)	The ergosterol content in the stationary phase of <i>C. sorokiniana</i> cultivation increased by 22.8% compared to the active growth phase.

**Source:** compiled by the authors based on A.E. Kholif & O.A. Olafadehan (2021), A.L. Souza & G.J. Patti (2021), H.E.A. Ali *et al.* (2021), T. Zhang & J. Long (2025), A.N.M. Azhan *et al.* (2025)

Therefore, to increase ergosterol biosynthesis and vitamin D<sub>2</sub> content in *Chlorella* biomass, it is advisable to cultivate cells in a stationary growth phase using stress factors (salinity), provide lighting with blue spectrum elements, maintain optimal temperature and pH, and add microdoses of Zn and Fe.

#### **Vitamin B<sub>12</sub>: Accumulation mechanism, influence of factors, peculiarities of microalgae cultivation**

The *Chlorella* is considered a potential source of vitamin B<sub>12</sub>, but the presence of this micronutrient depends on the activity of bacterial symbionts and the form of the vitamin that accumulates, while S.P. van den Oever & H. Mayer (2022) demonstrated that microalgae-based products contained predominantly non-bioavailable forms of cobalamin rather than its inactive analogues, which are unable to perform physiological functions in the human body. The study also noted that the formation

of the active form of B<sub>12</sub> depends on the presence of specific bacterial producers, and their absence in artificial cultivation conditions explains the minimal level of this metabolite in *Chlorella*. This approach demonstrates that the microbial environment with which algae interact is central to B<sub>12</sub> accumulation, rather than the algae themselves. A comparative characterisation of vitamin B<sub>12</sub> content (true and substitutes) in different types of microalgae is presented in Table 2.

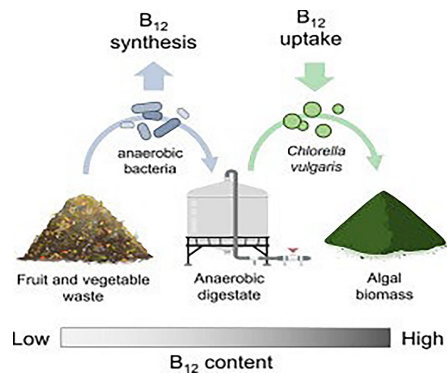
Following Table 2, *Chlorella* grown in natural conditions contains significant amounts of true vitamin B<sub>12</sub> ( $\sim 100$  mcg/100 g) and a few of its analogues ( $< 10$  mcg/100 g). It has a positive effect on B<sub>12</sub> levels in the body. Artificially cultivated *Chlorella*, especially under sterile conditions, contains no or very little vitamin B<sub>12</sub>, so it is not an effective source of it. Other microalgae, such as *Spirulina* and *AFA*, are high in B<sub>12</sub> analogues, which may even impair the absorption of this vitamin.

**Table 2.** Comparative table of B<sub>12</sub> content in microalgae

Microalgae genus	True B <sub>12</sub> (µg/100 g)	Analogue of B <sub>12</sub> (mcg/100 g)	The effect of B <sub>12</sub> contained in microalgae on B <sub>12</sub> status in the human body
<i>Spirulina</i>	~35	~120	negative
<i>AFA (Aphanizomenon flos-aquae)</i>	~30	~570	negative
Nori (red algae)	varies	varies	not determined
<i>Chlorella</i> (natural habitat)	~100	<10	positive
<i>Chlorella</i> (artificially cultivated, fermented)	–	–	neutral

**Source:** compiled by the authors based on Vitamin B<sub>12</sub> in algae: *Spirulina*, *Chlorella* and nori (n.d)

In contrast to bacteria, microalgae, in particular *Chlorella vulgaris*, are not capable of autonomous synthesis of cobalamin, since their genome lacks the genes for biosynthesis of this coenzyme (Peshuk *et al.*, 2024). However, they can accumulate and store B<sub>12</sub> that enters the environment from exogenous sources. In natural ecosystems, *Chlorella* obtained vitamin B<sub>12</sub> through symbiosis with producer bacteria, which secreted biologically active forms of cobalamin (methylcobalamin and adenosylcobalamin) into the environment, which were absorbed by algae cells through specialised transport systems. M. Peng *et al.* (2025) demonstrated that the availability of macro- and microelements significantly modified these metabolic processes, affecting the overall efficiency of vitamin absorption. This effect is explained by the synergy of metabolisms: bacteria provide cobalamin, and microalgae create conditions for their growth by releasing oxygen and organic substrates. Thus, the study proved that co-cultivation of *Chlorella* with bacteria significantly increases the content of biologically active B<sub>12</sub> in biomass. The summary by B. Nowruzi *et al.* (2022) demonstrates that the interaction between algae and bacteria is multi-level: bacteria not only supply vitamin B<sub>12</sub>, but also regulate the signalling pathways and metabolic activity of algae. The study noted that the exchange of nutrients, the release of phytohormones, and the stabilisation of the microenvironment create conditions in which both groups of organisms function more efficiently. This interdependence accounts for a higher level of cobalamin in co-cultures compared to microalgae monocultures (Fig. 2).

**Figure 2.** Simplified scheme of B<sub>12</sub> accumulation in microalgae biomass

**Source:** compiled by the authors based on K.P.Papadopoulos *et al.* (2023)

In addition to symbiotic relationships with producer microorganisms, a significant factor is the presence of metal ions in the environment, which can affect the level of B<sub>12</sub> biosynthesis by bacteria, the bioavailability of the vitamin, and the efficiency of its accumulation by *Chlorella* cells. Cobalt (Co<sup>2+</sup>) is the central atom in the structure of cobalamin. The addition of cobalt ions to the environment stimulates both the synthesis of B<sub>12</sub> by bacteria (precoryne formation) and the accumulation of the vitamin in microalgae (when co-cultured with producer bacteria) (Kyoto Encyclopedia of Genes and Genomes, n.d.). Some experiments have shown that optimal concentrations (0.01–0.1 mg/L) significantly increase the content of active forms of vitamin B<sub>12</sub> methylcobalamin, 5'-deoxyadenosylcobalamin and hydroxycobalamin in biomass (an increase of 7–12% compared to the

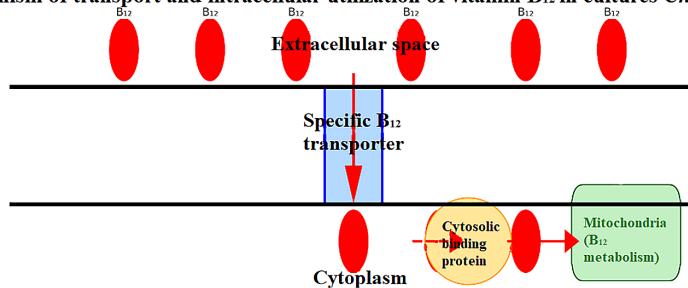
control). X. Ji *et al.* (2021) further emphasised the significance of cobalamin in “algae-bacteria” systems: the authors showed that the presence of an external source of vitamin B<sub>12</sub> alters the expression of proteins associated with nutrient uptake and intercellular interaction. Under the influence of cobalamin, the consortium demonstrated increased metabolic activity, which was reflected in better nitrogen and phosphorus uptake and more active algae growth. This effect indicates that even minor changes in coenzyme availability can significantly affect the functioning of mixed cultures and the accumulation of B<sub>12</sub> in algal biomass.

Zinc (Zn<sup>2+</sup>), magnesium (Mg<sup>2+</sup>) and iron (Fe<sup>2+</sup>/Fe<sup>3+</sup>) acted as cofactors for enzymes involved in vitamin B<sub>12</sub> metabolism and transport, while iron additionally influenced the efficiency of electron transport during photosynthesis, which enhanced cobalamin uptake by cells, and L.M. González-González & L.E. De-Bashan (2021) showed that changing the mineral composition of the environment in mixed “microalgae-bacteria” systems directed metabolic flows towards more intensive formation of secondary metabolites, emphasising the significance of micronutrient interactions. The results demonstrated that a balanced supply of metal ions not only maintains enzymatic activity but also

enhances cooperation between bacterial cobalamin producers and algal cells, contributing to more efficient accumulation of vitamin B<sub>12</sub> in biomass.

Excessive concentrations of heavy metals (Cu<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>) inhibited *Chlorella* growth and reduced vitamin B<sub>12</sub> absorption efficiency due to oxidative stress, while controlled addition of micronutrients, especially cobalt, could serve as a tool to increase cobalamin content in microalgae biomass. In this context, R.P. Singh *et al.* (2023) demonstrated that different types of abiotic stress, particularly metal-induced and osmotic stress, significantly modified the metabolic activity of microalgae, enhancing the accumulation of lipids and secondary metabolites at moderate metal concentrations, but causing metabolic disorders and loss of biosynthetic potential at toxic levels. B<sub>12</sub> transporters, homologous to bacterial carrier proteins (BtuB, BtuFCD), function in the cell membranes of microalgae. They ensure the active transport of cobalamin across the membrane, after which the vitamin is deposited in the cytoplasm in the form of methyl- and adenosylcobalamin. The presence of these systems explains the ability of *Chlorella* to rapidly absorb exogenous B<sub>12</sub> within a few hours after its addition to the medium (Fig. 3).

#### Mechanism of transport and intracellular utilization of vitamin B<sub>12</sub> in cultures *Chlorella*



**Figure 3.** Mechanism of B<sub>12</sub> transport into *Chlorella* cells

**Source:** compiled by the authors based on C. Nef *et al.* (2022), N. Zhao *et al.* (2024)

It is worth noting that in most eukaryotic cells (including algae), vitamin B<sub>12</sub> metabolism is not uniform and occurs in several parts of the cell. In microalgae, particularly *Chlorella*, the situation is

more complex. Studies show that B<sub>12</sub>-dependent methionine synthase (METH) may be present in the cytoplasm and chloroplasts. At the same time, no evidence of B<sub>12</sub>-dependent methylmalonyl-CoA

mutase, characteristic of animals, was found in many green algae. This means that *Chlorella* most likely uses B<sub>12</sub> mainly in the cytoplasm and chloroplasts, rather than in the mitochondria (Nef *et al.*, 2022). The *Chlorella vulgaris* can be cultivated on different types of nutrient media: mineral (BG-11, Chu-10, WC, LC Oligo), organic (whey, food processing waste, meat extracts) and mixed (combinations of mineral and organic components). The choice of medium determines the growth rate, chemical composition of cells and accumulation of vitamin B<sub>12</sub>. Classic mineral media provide macro- and microelements (nitrogen, phosphorus, magnesium, potassium, iron, and microelements), but do not contain vitamin B<sub>12</sub>. For species that depend on cobalamin, it must be added separately, for example, in the form of an f/2 vitamin solution. This is critical if the culture is maintained under axenic conditions, i.e., it is a pure culture without any accompanying microorganisms, in particular B<sub>12</sub>-producing bacteria. In such cases, microalgae cannot obtain the vitamin from an external source other than artificially added (Singh *et al.*, 2023).

Different media have different effects on growth and metabolism. For *Chlorella vulgaris*, the study demonstrated that LC Oligo and Chu-10 media provide higher biomass productivity and alter the carbohydrate-to-lipid ratio compared to WC, where growth is less intense. Thus, in semi-continuous cultivation, the specific growth rate is 0.84 d<sup>-1</sup> in LC Oligo medium, 0.79 d<sup>-1</sup> in Chu-10, and only 0.62 d<sup>-1</sup> in WC, i.e., biomass productivity in LC Oligo is almost 35% higher than in WC. The BG-11 medium is one of the most common for phototrophic microalgae cultivation due to its optimal content of macroelements and microelements, which ensures stable growth and high biomass concentration (Ampofo & Abbey, 2022). In contrast, Tris-Acetate-Phosphate (TAP) medium contains organic carbon in the form of acetate, which ensures growth of *Chlorella* in a mixotrophic mode, combining photosynthesis with the use of an external source of organic matter. This often leads to increased growth rates and changes in biomass composition, including greater lipid accumulation, although TAP contains virtually no vitamin B<sub>12</sub> (Singh *et al.*, 2023).

The ability of *Chlorella* to assimilate ammonium, nitrate and urea is well known; ammonium is often the most energy-efficient source of N when pH is controlled, while urea or nitrate is better for some strains. The correct selection of N form increases growth rate and protein content. Some studies indicate that urea can maintain higher productivity and a more stable pH than nitrate, which indirectly improves the physiological condition of cells (Papadopoulos *et al.*, 2023). The transition to organic or waste-based substrates (whey, permeates, anaerobic digestates, pig manure permeates, etc.) often increases growth (mixo-/heterotrophy) and paves the way for the intake of exogenous B<sub>12</sub> through associated microbiota or vitamin content in the streams (Ali *et al.*, 2021). For *Chlorella*, the study demonstrated that whey and permeates can increase biomass yield relative to controls in several modes, provided that trace elements are corrected. Thus, when 10% whey was added to the mineral nutrient medium in fed-batch mode, biomass growth increased to 17.2 ± 1.3 g/L, although the lipid fraction decreased to 20.5 ± 0.3%, which still exceeds the control values (mineral medium). In mixed media, “pig manure permeate + whey” (instead of BG-11) achieves comparable or higher productivity due to the high content of N, P and organic carbon; this also significantly reduces the cost of media (González-González & De-Bashan, 2021).

Anaerobic digestates are another effective source of nutrients and B<sub>12</sub>. Studies have shown that even when vegetable and fruit waste is used as a substrate for fermentation, a significant amount of cobalamin is produced, which can be effectively accumulated by *Chlorella vulgaris*. With this approach, the vitamin B<sub>12</sub> content in the biomass reaches 10.6 µg/g dry weight, which significantly exceeds the level in standard mineral media (Feng *et al.*, 2021). Additionally, the concentration of B<sub>12</sub> in the digestate reached 13.8 µg/L at the time of inoculation, and on day 13, *Chlorella* absorbed approximately 26% of the vitamin present in the medium. The study also noted that ammonia toxicity caused by high pH is a major growth

limiter and can be overcome by adjusting the pH to increase productivity (Papadopoulos *et al.*, 2023).

The use of beef liver extract is notable. This product is one of the richest natural sources of vitamin B<sub>12</sub> (up to 60-80 µg/100 g). The use of liver extract as a component of the medium provides not only a supply of cobalamin, but also proteins, peptides and trace elements that further stimulate growth. Patent developments since the 1980s have noted that the combination of liver extract with optimal spectral lighting promotes the intensive accumulation of B<sub>12</sub> in *Chlorella* cells (Der *et al.*, 2024). Since *Chlorella vulgaris* is unable to synthesise cobalamin, the most common approach to its enrichment is to add organic substrates containing this vitamin to the culture medium. Studies from the last decade (2015-2025) show that organic extracts (meat, milk, food waste) can significantly increase B<sub>12</sub> levels in biomass while stimulating cell growth (Salbitani & Carfagna, 2021). Animal liver, particularly beef liver, is one of the most concentrated natural sources of vitamin B<sub>12</sub>, with a content of 60-80 µg/100 g of product (Durdakova *et al.*, 2024). Therefore, liver extracts are most often used in biotechnological experiments. Their advantage lies not only in their high cobalamin content, but also in the presence of proteins, peptides, iron and trace elements, which further stimulate the growth of *Chlorella vulgaris* (Zhang & Long, 2025).

As early as 1980-1990, a method for using liver extract to enrich *Chlorella* with vitamin B<sub>12</sub> was patented in Japan (Kholif & Olafadehan, 2021). The patent documents emphasised that the technological approach of combining such an extract with controlled spectral lighting (blue range) provided a high level of vitamin accumulation. Modern studies have confirmed that the introduction

of liver extract into the medium can significantly increase the content of biologically active B<sub>12</sub> in *Chlorella vulgaris* biomass, as well as increase the dry matter yield compared to mineral media (Souza & Patti, 2021). Therefore, the liver is a particularly promising substrate for microalgae cultivation. In addition to liver, milk whey, soy protein hydrolysates, food waste extracts, and anaerobic digestates are used as nutrient media (Mendes *et al.*, 2024). They may also contain traces of cobalamin synthesised by microbial communities and serve as sources of readily available nitrogen and organic carbon. However, these sources are inferior to liver in terms of B<sub>12</sub> content.

Thus, the use of organic extracts, in particular liver extracts, for the cultivation of *Chlorella vulgaris* has a dual effect: enrichment of biomass with biologically active vitamin B<sub>12</sub>, which is substantial for the creation of functional foods (especially for vegans and vegetarians); rational use of food and meat industry waste, which is in line with modern concepts of bioeconomics. Liver extract is one of the richest organic sources of vitamin B<sub>12</sub>; therefore, its use in the nutrient medium ensured a higher content of cobalamin in the biomass of *Chlorella vulgaris* compared to other organic and mineral substrates. Light is one of the key regulatory factors of *Chlorella vulgaris* growth and metabolism. The intensity, duration and spectral composition of light determine photosynthetic efficiency, pigment content and the rate of metabolite biosynthesis, including vitamin B<sub>12</sub>. In general, increasing light intensity stimulates the rate of photosynthesis and biomass accumulation up to a certain limit, after which photosaturation and photodestructive processes occur. The optimal light intensity for *Chlorella vulgaris* varies depending on the spectrum, cultivation conditions and type of medium (Table 3).

**Table 3.** Light intensity ranges and their effect on *Chlorella* growth

Illuminance range, µmol · m <sup>-2</sup> · s <sup>-1</sup>	Biological effect
~80	Beginning of noticeable growth
80-150	Efficient area: stimulation of growth and biomass accumulation
>150	Possible photo suppression (especially >200)

**Source:** compiled by the authors based on A.E.P. Cunha *et al.* (2023); B.M. de Brito *et al.* (2023)

Continuous lighting (24/0) stimulates more intensive growth but is sometimes accompanied by a decrease in photosynthetic efficiency. An alternative is to use 16/8 or 12/12 (light/dark) modes, which mimic natural daily cycles and can promote more stable metabolism (Bialevich *et al.*, 2022). Wavelength (spectral composition) is a critical parameter for metabolism. Blue light (450-470 nm) induces intense absorption by chlorophyll *a* and promotes increased photosynthetic activity. Studies show that under blue light, *Chlorella* accumulates more protein and vitamin B<sub>12</sub> (Voshall *et al.*, 2021). Red light (650-680 nm) activates photosystem II and stimulates carbohydrate biosynthesis, increasing biomass yield (Liu *et al.*, 2025). Green light (520-550 nm) penetrates the photobioreactor, improving cell illumination

in dense cultures. It is less effective for pigment biosynthesis but can stabilise the photosynthetic process in mixed lighting (Gao *et al.*, 2023). A combination of blue and red light is often found to be most effective for the growth of *Chlorella vulgaris*, as it provides a balance between photosynthetic productivity and metabolite biosynthesis. Patent and experimental studies have shown that the potential technology of adding a source of B<sub>12</sub> (e.g., liver extract) in combination with blue lighting can increase the accumulation of cobalamin in *Chlorella* cells (Ashaari *et al.*, 2024). This confirms the substantial role of spectral conditions in regulating vitamin biosynthesis and transport. A general description of the factors and their influence on the accumulation of vitamin B<sub>12</sub> by *Chlorella* microalgae is provided in Table 4.

**Table 4.** The influence of certain factors on the accumulation of B<sub>12</sub> in *Chlorella* microalgae

Factor	Conditions/examples	Effect on B <sup>2+</sup>
Symbiosis with bacteria	Co-cultivation with <i>Propionibacterium freudenreichii</i>	Increase in B <sub>12</sub> content in biomass several times over (exact figures were not provided by S. Maulana <i>et al.</i> (2025))
Metal ions	Addition of Co <sup>2+</sup> (0.01-0.1 mg/l)	7-12% increase in B <sub>12</sub> in biomass compared to control (Ji <i>et al.</i> , 2021)
	Zn <sup>2+</sup> , Mg <sup>2+</sup> , Fe <sup>2+</sup> /Fe	Indirect stimulation of B <sub>12</sub> (enzyme cofactors) absorption (González-González & De-Bashan, 2021)
	Excess of Cu <sup>2+</sup> , Ni <sup>2+</sup> , Pb <sup>2+</sup>	Decreased <i>Chlorella</i> growth and reduced B <sub>12</sub> accumulation (Singh <i>et al.</i> , 2023)
Environment type	BG-11, Chu-10, WC, LC Oligo	Mineral media do not contain B <sub>12</sub> ; therefore, the vitamin must be added externally (Ricky <i>et al.</i> , 2022)
	TAP (mixotrophy, acetate)	Higher height and lipids, but low B <sub>12</sub> levels (Ricky <i>et al.</i> , 2022)
	Organic/waste substrates (whey, permeates)	Increased biomass yield, exogenous B <sub>12</sub> intake via associated microbiota (Kashyap <i>et al.</i> , 2025)
Anaerobic digestates	Substrate – vegetable and fruit waste	B <sub>12</sub> content in biomass up to 10.6 µg/g dry weight; in the medium – 13.8 µg/l; absorption ~26% in 13 days (Kolesovs & Semjonovs, 2023)
Organic extracts	Beef liver extract (60-80 mcg/100 g B <sub>12</sub> )	Significant increase in B <sub>12</sub> and biomass; the patent describes the method but does not provide numerical data (Ashaari <i>et al.</i> , 2024)
Light (intensity and spectrum)	80-150 µmol·m <sup>-2</sup> ·s <sup>-1</sup>	Optimal intensity for growth; above – photostress (Cunha <i>et al.</i> , 2023)
	12/12 mode (light/dark)	Stable metabolism, improved balance (Bialevich <i>et al.</i> , 2022)
	Blue light (450-470 nm)	Increased protein and B <sub>12</sub> content (Kamolrat <i>et al.</i> , 2023)
	Red light (650-680 nm)	Increased protein and carbohydrate content (Gao <i>et al.</i> , 2023)
	Mixed blue + red	Most effective for growth and B <sub>12</sub> (Gao <i>et al.</i> , 2023)

**Source:** compiled by the authors

Thus, the accumulation of vitamin B<sub>12</sub> in *Chlorella vulgaris* largely depends on external factors. The most effective strategies are the

use of organic extracts, anaerobic digestates and co-cultivation with cobalamin-producing bacteria, while optimal lighting conditions and

the presence of metal ions act as additional regulators of this process.

### Potential biotechnological applications of *Chlorella* microalgae

Enriched with vitamin B<sub>12</sub>, *Chlorella vulgaris* biomass has a wide range of potential applications in the food, feed, and pharmaceutical industries, as well as in the bioeconomy. In the food industry, *Chlorella vulgaris* is already used as a “superfood” containing high levels of protein, essential amino acids, fatty acids, pigments and trace elements. Biomass enriched with vitamin B<sub>12</sub> can be used as a basis for functional products such as drinks, protein bars or dietary supplements, especially for people on vegan diets who lack natural sources of cobalamin (Peshuk *et al.*, 2024). In addition, *Chlorella* can be a source of ergocalciferol (vitamin D<sub>2</sub>), which is formed from ergosterol, a natural cell sterol, under the influence of UV radiation. The additional D<sub>2</sub> content in the biomass enhances the functional value of the products by supporting normal calcium metabolism, bone health and immune function (Hamouda *et al.*, 2022). In animal husbandry, the use of microalgae not only provides animals with an additional source of nutrients, but also improves their growth, immunity and reproductive functions. Enriched with B<sub>12</sub> and D<sub>2</sub>, *Chlorella* helps to increase the content of cobalamin and ergocalciferol in meat, milk and eggs, reduces the need for synthetic premixes, and is also a valuable component in fish farming and aquaculture, where microalgae are a natural part of the diet (Abdelkarim *et al.*, 2025).

In pharmaceuticals and medicine, *Chlorella vulgaris*, which contains biologically active forms of vitamin B<sub>12</sub> and ergocalciferol, can be used to prevent and treat deficiencies of these vitamins, which are a global problem and are associated with anaemia, bone metabolism disorders, cognitive dysfunction and an increased risk of cardiovascular disease. Enriched microalgae biomass is considered a basis for the creation of functional preparations capable of supporting the nervous system, blood formation and bone health. In

addition, *Chlorella* exhibits antioxidant, immunomodulatory, and detoxification properties, which expand its therapeutic potential (Selvaraj, 2024). Equally relevant is the use of microalgae in ecology and economics. The use of by-products from the food and meat industries, in particular liver extracts, as components of nutrient media for the cultivation of *Chlorella vulgaris*, combines the utilisation of organic waste with the production of biomass enriched with vitamin B<sub>12</sub> (Zhang & Long, 2025). This approach reduces production costs and environmental impact while providing high-quality raw materials for food, pharmaceutical and feed purposes, which correlate with the principles of sustainable development.

### Discussion

The results showed that vitamin D<sub>2</sub> formation in *Chlorella* is a multi-component process in which ergosterol synthesis and its subsequent photochemical transformations under the action of ultraviolet light were central. An increase in light intensity and a change in the spectrum were accompanied by an increase in the lipid pool by about one-third, which simultaneously increased the potential for sterol accumulation and created favourable conditions for the formation of D<sub>2</sub>. Salt stress also had a significant effect on this process: the addition of 300 ppm NaCl in combination with the late growth phase resulted in a 28-43% increase in ergosterol, reflecting the adaptive restructuring of cells under osmotic stress. The action of microelements was also substantial: changes in the concentrations of Mg<sup>2+</sup>, Zn<sup>2+</sup> and Fe<sup>2+</sup>/Fe<sup>3+</sup> modified the activity of enzymes involved in the sterol pathway, promoting the formation of ergosterol. Maintaining optimal temperature conditions (25-30°C) and neutral pH further stabilised sterol metabolism and maintained its intensity.

Among the studies investigating the ability of microalgae to accumulate cobalamin under various conditions, the most notable were those by B. Xu *et al.* (2021) and A. Tzachor *et al.* (2024). B. Xu *et al.* determined that *Chlorella* can absorb

vitamin B<sub>12</sub> more efficiently in mixed cultures: the combination of microalgae with fungi and the presence of organic substrate and available cobalt ions in the environment significantly enhanced the accumulation of active forms of cobalamin in cells. The study also emphasised that the concentration of trace elements and the type of environment determined whether microalgae could absorb B<sub>12</sub> in a biologically available form. A study by A. Tzachor *et al.* demonstrated that light regimes have a significant effect on the accumulation of active vitamin B<sub>12</sub>. The researchers showed that the combination of light intensity, spectrum, and culture growth stage determined how effectively cells were able to accumulate B<sub>12</sub>. Compared to these studies, the present study covered a wider range of conditions that influenced the enrichment of *Chlorella* biomass with active forms of cobalamin: cobalt availability, type of medium, interaction with microorganisms, growth phase, salinity level, light intensity, and the presence of moderate stress factors. This approach provided a more comprehensive overview of the parameters that ensure the maximum content of bioavailable B<sub>12</sub> in microalgae biomass.

Within the scope of studies devoted to biologically active components of microalgae, L. Klamann *et al.* (2023) and A.W. Almutairi *et al.* (2021) examined factors influencing the metabolism and accumulation of key compounds. The study by L. Klamann *et al.* analysed the conditions under which phototrophic and mixotrophic cultures of different species of Lemnaceae can accumulate bioavailable vitamin B<sub>12</sub>. The study demonstrated that cobalamin levels increased sharply in the presence of available micronutrients, organic carbon, and stable interaction with producer bacteria. Although the model objects differed from *Chlorella*, the study outlined the main conditions necessary for the accumulation of active forms of B<sub>12</sub>. This idea is consistent with the results of analysis by A.W. Almutairi *et al.* on the lipid reactions of *Chlorella vulgaris* at elevated salinity. The results confirmed that stressful conditions can activate metabolic pathways, but the authors

did not analyse the absorption or accumulation of cobalamin. The present study complements these findings by incorporating the conditions that determine the uptake and accumulation of B<sub>12</sub>, the role of trace elements, light, bacterial symbionts, and environmental characteristics.

In the field of research on alternative sources of nutrients and conditions for the accumulation of vitamin B<sub>12</sub> by microalgae, the results of S. Sultana *et al.* (2023) and F. Bunbury *et al.* (2022) were substantial. S. Sultana *et al.* demonstrated that not all marine bacteria that produce cobalamin release it into the environment in quantities sufficient for assimilation by microalgae. The study emphasised that the availability of active forms of B<sub>12</sub> significantly depends on the bacterial strain and environmental characteristics, which explains the limited accumulation of this vitamin in microalgae under artificial cultivation conditions. The results of F. Bunbury *et al.* supplemented this observation: the study found that effective B<sub>12</sub> uptake by microalgae is only possible with close and prolonged contact with producing bacteria. The study demonstrated that the stability of the interaction and the availability of metabolically active forms of cobalamin determine the actual level of its accumulation in cells. Compared to these sources, this study covered a wider range of factors, as it included an assessment not only of the bacterial supply of B<sub>12</sub>, but also of the role of trace elements, salt and light triggers, and environmental conditions that affect the further accumulation of the vitamin – blocks that were not considered in the aforementioned studies.

Within the scope of studies that intersected with the topic of the biochemical quality of microalgae, the most relevant in terms of content were the studies by E. Barber-Lluch *et al.* (2021) and C.Y. Tong *et al.* (2025). E. Barber-Lluch *et al.* showed significant variability in vitamin B<sub>12</sub> concentrations in the coastal waters of the north-western Pyrenean shelf, which was determined by hydrological conditions and the activity of microbial communities. The study emphasised that the unstable availability of cobalamin in the natural

environment directly affects the ability of microalgae to accumulate biologically active forms of B<sub>12</sub>. C.Y. Tong *et al.* demonstrated that co-cultivation of *Chlorella sorokiniana* with bacteria that overproduce vitamins resulted in synergistic biomass growth, increased lipid content, and more efficient uptake of B<sub>12</sub> into cells. The study determined that bacterial producers can compensate for cobalamin deficiency in the environment and create conditions under which microalgae accumulate significantly more vitamin than under autotrophic cultivation. Compared to these studies, the study provided a broader analytical context, as it additionally covered the influence of light regimes, salinity, metal cofactors, nitrogen sources, and growth stages on the accumulation of B<sub>12</sub> and sterols. Aspects such as regulated availability of micronutrients, UV-dependent sterol transformations, and stress factors were not considered in the studies by E. Barber-Lluch *et al.* and C.Y. Tong *et al.*, which renders the presented review more comprehensive.

The analytical field of research was expanded by A. Ahirwar *et al.* (2024) and S. Vogeler *et al.* (2021). A. Ahirwar *et al.* considered fermentation approaches to increasing the nutritional value of algal biomass but did not analyse the accumulation of vitamin B<sub>12</sub>, the role of metals, the conditions for sterol synthesis, or the photochemical formation of D<sub>2</sub>. S. Vogeler *et al.* studied the distribution of B<sub>12</sub> in the tissues of bivalve molluscs to evaluate natural sources of micronutrients, but the mechanisms of B<sub>12</sub> accumulation by microalgae, the effect of trace elements, salinity, and light regimes were not evaluated. Compared to these studies, the presented research provided a broader picture of the processes, as it combined the mechanisms of B<sub>12</sub> and D<sub>2</sub> synthesis, the sterol pathway, UV conversion of ergosterol, the influence of Mg<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>2+</sup>/Fe<sup>3+</sup>, and the addition of organic substrates. This demonstrated the factors regulating the biochemical composition of *Chlorella*, which previous studies had not covered.

Within the scope of studies on light regimes, stress factors and metabolic changes in microalgae,

the most relevant were those by B.A. Pessi & O. Bernard (2024) and K. Gopalakrishnan *et al.* (2024). B.A. Pessi & O. Bernard modelled the dynamics of metabolic flows in the “microalgae-bacteria” symbiosis, identifying the conditions that stimulated lipid synthesis under the action of regulators, in particular biotin, but the work did not cover the sterol pathway and the photochemical formation of vitamin D<sub>2</sub>. K. Gopalakrishnan *et al.* analysed the co-cultivation of microalgae and bacteria in wastewater to increase biomass productivity, but the influence of salinity, the role of trace elements and the accumulation of ergosterol were not considered. In contrast to these approaches, the presented study exceeded the scope of assessment of growth parameters and covered the full range of biochemical processes: ergosterol synthesis, its modulation by metals, the response of cells to different light spectra and salinity, as well as the photoconversion of ergosterol to vitamin D<sub>2</sub>. This breadth of coverage formed a holistic view of the mechanisms of sterol and calciferol accumulation, which was not presented in the aforementioned studies. A summary of the results showed that this work formed a comprehensive multifactorial model of the conditions that determined the accumulation of cobalamin by microalgae, from interaction with bacterial producers to the role of organic substrates, trace elements, lighting and stress factors, offering a much broader and deeper analytical approach than previous studies.

## Conclusions

A summary of the literature data shows that *Chlorella vulgaris* biomass is a promising source of functional nutrients, in particular vitamins B<sub>12</sub> and D<sub>2</sub>. Due to the lack of endogenous synthesis systems, the accumulation of cobalamin in microalgae cells depends on its external supply; therefore, the type of nutrient medium and the availability of exogenous sources of vitamin B<sub>12</sub> are substantial. Organic media (liver extract, whey, food processing waste) provide a significantly higher B<sub>12</sub> content in microalgae biomass compared to classic mineral media (BG-11, Chu-10, WC). Light

conditions are an additional regulator: blue light stimulates photosynthetic activity and transport systems, promoting better vitamin absorption. A substantial factor is the presence of metal ions in the medium, in particular cobalt, which is part of cobalamin, and other trace elements that indirectly affect the overall metabolism of *Chlorella* microalgae and the accumulation of vitamin B<sub>12</sub>.

Vitamin D<sub>2</sub> synthesis in *Chlorella vulgaris* is based on the photochemical conversion of ergosterol under the action of UV radiation, which confirms the analogy with the mechanisms in fungi. The yield of ergocalciferol depends on light parameters, nitrogen nutrition, the C:N ratio, and the state of the photosynthetic apparatus. Metal ions (Mg, Fe, Zn) are essential cofactors for sterol metabolism, while excess Cu or Zn can induce oxidative stress and alter the sterol balance. Stress factors are an additional regulator of productivity. Increased salinity stimulates metabolic restructuring and promotes lipid accumulation, which can also affect the sterol pool associated with D<sub>2</sub> synthesis. At the same time, the growth prophase (logarithmic stage) is critical for the active synthesis of biomass and metabolites, while in the stationary growth stage, the accumulation of lipids and certain vitamins is higher, which must be considered in the optimisation of cultivation conditions.

Thus, effective enrichment of *Chlorella vulgaris* biomass with vitamins B<sub>12</sub> and D<sub>2</sub> is possible when several factors are combined: the use of organic media containing exogenous cobalamin; an optimal light spectrum (blue and UV-B ranges); controlled mineralisation of the medium, incorporating metal ions; and the application of stress conditions that stimulate the accumulation of lipids and sterol precursors. This creates the possibility of simultaneously obtaining biomass enriched not only with B<sub>12</sub>, but also with D<sub>2</sub>, which increases its functional value for food, pharmaceutical and nutraceutical products. Further research should address optimisation of the cultivation conditions of *Chlorella vulgaris* to increase the production of vitamins B<sub>12</sub> and D<sub>2</sub>, improving light and mineral regimes, and clarifying the key biochemical mechanisms of their accumulation.

### Acknowledgements

None.

### Funding

None.

### Conflict of Interest

None.

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## Мікродорості *Chlorella vulgaris* як перспективне джерело вітамінів B<sub>12</sub> та D<sub>2</sub>

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**Анотація.** Актуальність даного дослідження обумовлена необхідністю узагальнити сучасні знання щодо механізмів накопичення вітамінів B<sub>12</sub> і D<sub>2</sub> у *Chlorella vulgaris* та факторів, що визначають їхню біохімічну варіативність у культурах мікродоростей. Метою роботи було систематизувати дані літератури за 2020-2025 роки та визначити ключові чинники, що регулюють стерольний метаболізм і вітамінний профіль *Chlorella vulgaris*. Дослідження базувалося на методах цільового інформаційного пошуку, контент-аналізу, критичного порівняння та якісного синтезу результатів попередніх наукових робіт. На основі аналізу джерел було показано, що тип живильного середовища визначає рівень мікронутрієнтів у біомасі *Chlorella vulgaris*. Мінеральні середовища забезпечували стабільний ріст, але не містили кобаламіну (вітаміну B<sub>12</sub>), тоді як органічні субстрати – печінковий екстракт, молочна сироватка, пермеати та анаеробні дигестати – демонстрували вищий потенціал збагачення клітин завдяки природним коферментам, білкам і мікроелементам. Показано, що іони кобальту (Co<sup>2+</sup>) сприяли більшому накопиченню кобаламіну в біомасі, а магній (Mg<sup>2+</sup>), цинк (Zn<sup>2+</sup>), залізо (Fe<sup>2+</sup>/Fe<sup>3+</sup>) і мідь (Cu<sup>2+</sup>) виступали важливими кофакторами ферментів та транспортних систем, які впливали на стерогенез. Щодо вітаміну D<sub>2</sub> (ергокальциферолу), літературні дані підтвердили вирішальну роль ультрафіолетового випромінювання діапазону В (UV-B), яке ініціювало фотоконверсію ергостеролу. Інтенсивність та спектральний склад освітлення визначали швидкість накопичення стеролів, тоді як солоність, дефіцит азоту чи фосфору та інші стресові чинники сприяли посиленню ліпідогенезу й змінам стерольного пулу. Встановлено, що у стаціонарній фазі культури ліпідні та стерольні фракції накопичувалися інтенсивніше, ніж у період активного поділу клітин. Узагальнений аналіз джерел показав, що найбільш ефективно збагачення *Chlorella vulgaris* вітамінами B<sub>12</sub> і D<sub>2</sub> досягається за умови поєднання органічних живильних середовищ, оптимізованого спектра освітлення з UV-B компонентом та контрольованого мінерального складу середовища

**Ключові слова:** кобаламін; ергокальциферол; органічні екстракти; спектр світла; ергостерол; іони металів; стресові фактори



UDC 636.4.083.37:591.5:502.174(477)

DOI: 10.31548/dopovidi/6.2025.114

## Assessment of the behavioural performance of growing pigs under different enrichment strategies in the context of implementing animal welfare regulations in Ukraine

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**Abstract.** The strengthening of animal welfare requirements in Ukraine necessitates the adaptation of pig housing technologies to European integration standards. The study aimed to assess the impact of different environmental enrichment strategies on the play behaviour of growing pigs under industrial conditions. The experiment was conducted on four groups of pigs, each consisting of 22 individuals, aged 28 days at the start, with identical feeding and housing conditions. Enrichment objects were introduced before the start of video monitoring to ensure animal adaptation. Behavioural observations were carried out in three periods: days 29-35, 50-56, and 70-76. Data analysis was performed using two-way analysis of variance with fixed factors “group” and “day,” as well as their interaction “group × day.” The results showed that the “group” factor accounted for 51.7% of the variation in the first period, and in periods 2 and 3, it reached 99% ( $P < 0.001$ ). The duration of play behaviour in the control group ranged from 6.7 to 12.6 minutes, while in the group with a cotton rope, it was up to 42.7 minutes; in the group with bundles of packing paper, up to 65.2 minutes; and in the group with bottles partially filled with grain, up to 76.2 minutes. The results obtained for all periods of the study indicate that the “group” factor explained 78.0% of the total variation in play behaviour, while the “experiment period” explained only 5.0%, and unorganised factors accounted for 14.5%, indicating the clear dominance of enrichment type over time-

### **Suggested Citation:**

Chentsov, M., & Lykhach, A. (2025). Assessment of the behavioural performance of growing pigs under different enrichment strategies in the context of implementing animal welfare regulations in Ukraine. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 114-127. doi: 10.31548/dopovidi/6.2025.114.

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related changes. Hierarchical variance analysis confirmed that in all groups, both the period and the day of the experiment had a highly significant impact (all  $P < 0.001$ ), but the proportion of variation due to unorganised factors was highest in the growing piglets of the control group, at 53.6%. These data suggest a significant stimulatory effect of environmental enrichment on pig activity. The advantage of the enriched groups demonstrates the potential for improving the behavioural and emotional components of animal welfare. It is emphasised that the implementation of accessible and effective enrichment strategies is of practical importance for modernising pig housing in Ukraine in line with welfare requirements

**Keywords:** ethical standards; European integration; behaviour; pigs; sustainable development; technology

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## Introduction

In modern animal husbandry, the issue of animal welfare is becoming increasingly important, both from an ethical perspective and in terms of economic and production considerations. This is particularly relevant in intensive pig farming, where limited space, standardised feeding and housing systems often prevent the expression of natural species-specific behavioural responses. Scientific studies by M. Delsart *et al.* (2020), L.A. Boyle *et al.* (2021), and K. Bucková *et al.* (2022) unanimously indicate that the implementation of environmental enrichment strategies promotes the increase of active behaviours such as exploratory behaviour, object manipulation, and play interaction, while simultaneously reducing stress, aggressive, or stereotypical behaviours in pigs.

According to Council Directive 2008/120/EC (European Union, 2008), pigs “must have constant access to a sufficient number of objects that can be provided for appropriate research and manipulation” to improve their welfare. Numerous studies by J. Li *et al.* (2020) have shown that in the absence of stimuli in pig housing, environmental enrichment with materials such as straw bedding or peat can reduce destructive behaviour and, conversely, enhance play interactions. A review by I.G. Colditz *et al.* (2024) highlights that, particularly during the juvenile phases of animal development, the environment plays a crucial role in shaping adaptive abilities and behavioural plasticity. The works of S. Pietroseoli & C. Tang (2020), H.-S. Mun *et al.* (2023) emphasised that the presence of auditory effects,

chewing objects, digging, or manipulation responds to the internal motivation of pigs to explore their environment and engage in social interactions.

In the context of animal husbandry legislation and practice, it is important to note that European Union countries have long implemented standards for pig housing conditions, taking animal welfare into account, including requirements for the presence of enrichment materials or objects (European Union, 2021). This holds particular significance for Ukraine: within the framework of its European integration obligations, and in preparation for the implementation of modern standards at the farm level, it has been announced that, starting from January 1, 2026, animal welfare regulations will be mandatory on all farms (Order of the Ministry for Development of Economy..., 2021). Thus, Ukrainian pig farming is on the threshold of significant transformations – both technological and behavioural. In the field of pig behaviour, environmental enrichment serves as both a technological tool and an indicator of welfare. Scientific studies by A. Lykhach *et al.* (2022) confirmed that the introduction of objects such as ropes, bottles filled with grain, hanging tree branches, or paper stimulation can contribute to an increase in the duration of play behaviour, a decrease in aggression, improved social interaction, and fewer injuries (such as those to the skin or tail). The study by L. Luo *et al.* (2020) showed that pigs in enriched environments had a lower frequency of aggressive contacts after regrouping.

The relevance of this approach for Ukraine is amplified by several factors: first, consumer interest in animal welfare, and second, Ukrainian producers, planning or already engaged in exports to EU countries, face the demand to comply with stricter standards. Therefore, research related to piglet behaviour in the context of environmental enrichment becomes of strategic importance: not only as a scientific contribution but also as a practical recommendation for the modernisation of the Ukrainian pig farming industry. Despite the availability of scientific data, there are several gaps that are relevant to Ukraine. Among them is the insufficient research on the play behaviour of piglets during the growing phase in the context of enrichment implementation, especially considering Ukrainian climatic, technological, and farm conditions. Thus, in the context of Ukrainian practice and science, the task is to develop a methodology that takes into account species-specific piglet behaviour, uses adapted enrichment objects, and evaluates their impact under industrial housing conditions. The next logical step is empirical research that will allow for a quantitative assessment of changes in piglet behaviour depending on the types of enrichment, as well as their impact on welfare and productivity.

In connection with the above, the aim of the study was to assess the impact of different environmental enrichment strategies on the duration and nature of play behaviour in growing piglets under industrial housing conditions, considering

the future implementation of animal welfare regulations in Ukraine.

## Materials and Methods

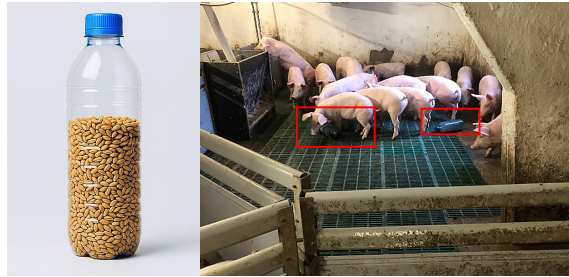
Animal handling in the experiment fully complied with international and national ethical standards: European legislation (European Union, 2008; 2010) and Ukrainian regulations (Order of the Ministry for Development of Economy..., 2021). The study protocol was approved by the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine (30 November 2023, protocol No. 008). Experimental research was conducted in 2023 at a farm in Ukraine – the private leased enterprise “Victoria” in the Bashtanka district of Mykolaiv region. Four groups of piglets participated in the study, with the maternal breed being Large White sows and the paternal breed being PIC 337 boars: Group I was the control group, and Groups II, III, and IV were the experimental groups, each consisting of 22 individuals. The age of the piglets at the start of the experiment was 28 days. Piglets in Group I (the control group) were kept without enrichment objects; Group II was provided with a twisted cotton rope with a diameter of 16 mm (Fig. 1), which was hung on the pen fence; Group III had 1-litre plastic bottles, filled 50% with grain (Fig. 2), with 4 bottles per pen, rotated weekly; Group IV had packaging paper with a density of 80 g/m<sup>2</sup> (Fig. 3), twisted into bundles, which was replaced twice daily.



**Figure 1.** Twisted cotton rope, hung on the pen fence for the piglets in the experimental Group II

**Note:** the enrichment object for the growing piglets is highlighted by a red oval

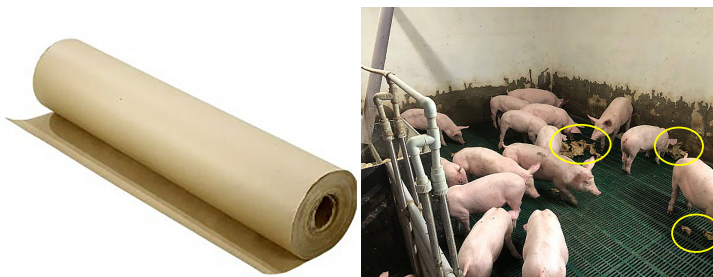
**Source:** authors' development



**Figure 2.** Plastic bottles, filled 50% with grain, for the piglets in the experimental Group III

**Note:** the enrichment objects for the growing piglets are highlighted by yellow circles

**Source:** authors' development



**Figure 3.** Packaging paper, twisted into bundles, for the piglets in the experimental Group IV

**Note:** the enrichment objects for the growing piglets are highlighted by yellow circles

**Source:** authors' development

Enrichment objects were provided to the experimental groups of piglets throughout the day before the start of video monitoring (Day 0) to allow the animals to adapt to the objects by the following morning. The objects (Figs 2 and 3) were placed on the floor in the cleanest part of the pen, at a sufficient distance from the walls to ensure full access. The hanging object (Fig. 1) was positioned on the pen fence in such a way that the piglets could manipulate it from all sides. The piglets were housed in pens with solid slatted polymer flooring, with an area of 0.4 m<sup>2</sup> per head. Feed was delivered to automatic feeders via rope-and-pulley conveyors. One automatic feeder was installed for every two adjacent pens, as well as two automatic drinking cups in each pen. Feeding in all experimental groups was the same and was carried out according to detailed feeding standards, taking into account the physiological

characteristics of the animals; the feeding type was dry (Ibatulin, 2017).

Until the 42<sup>nd</sup> day of life, the piglets were fed ad libitum with a full-feed prestarter compound feed from "Cehavekorm" LTD Ukraine. From the 42<sup>nd</sup> to the 49<sup>th</sup> day, they were gradually switched to a starter compound feed produced at their own feed mill based on cereal crops, with the addition of "Cehavit pig concentrate starter" from "Cehavekorm" LTD Ukraine. Nutritional value: crude protein content – 182.700 g/kg, metabolisable energy – 3,164.800 kcal/kg. Feed composition (%): wheat – 45.000; barley – 25.000; "Cehavit pig concentrate starter" – 30.000 (quality certificate according to DSTU 4508:2005 (2005)).

The ventilation system in the room with negative pressure was implemented using exhaust fans and intake valves. Air was supplied through the valves, mixed with the existing air, and then

extracted by the fan. The microclimate was regulated by computer equipment. The microclimate parameters complied with the Departmental standards for technological design (Ministry of Agrarian Policy of Ukraine, 2005). Microclimate parameters: air temperature – 22.0-24.0°C; relative humidity – 60.0-80.0%; air velocity – 0.20-0.60 m/s. During the early stages of piglet growth, heating was provided by infrared heaters suspended at a height of 1.2 m. The room was heated by a pyrolysis boiler with a capacity of 100 kW and a two-pipe system. Manure removal from the room was carried out by vacuum-gravity method – as it accumulated under the slatted baths, but no less frequently than once every two weeks. The piglets were delivered to the section every Thursday, immediately after weaning from the sows and weighing. They stayed there for 7 weeks until reaching 77 days of age, after which they were re-weighed and sent for fattening.

The behavioural acts of growing pigs in the experimental groups, depending on the type of enrichment object, were visualised through video monitoring using Boblov KJ21 video recorders (China) (with a resolution of 1920×1080 (Full HD), providing a full view of the objects. Recording was conducted from 8:30 AM to 4:30 PM during full daylight, as pigs virtually do not manipulate enrichment objects during the dark hours. Behavioural observations were carried out through video analysis, focusing on play behaviour, which was defined as any directed action towards the enrichment object, performed deliberately and involving contact by the piglet's snout, head, limbs (pushing, hitting, rubbing), or mouth (chewing, biting, pulling, tearing, shaking). Behavioural sampling was conducted

over four continuous one-hour periods, evenly distributed throughout the daylight hours (9:00-10:00, 11:00-12:00, 13:00-14:00, 15:00-16:00) (Ladyka & Khmelnychiy, 2023). Since the frequency of play behaviour in growing piglets from different groups (depending on the type of enrichment object) was analysed in three time periods – 29-35 days (period 1), 50-56 days (period 2), and 70-76 days (period 3) – the analysis was conducted in three stages.

In the first stage, the strength of the effect of the group and the day of the experiment on the variability of the frequency of play interactions in growing piglets was investigated using a two-way analysis of variance algorithm (with fixed factors). In the second stage, the strength of the effect of the group and the experiment period (all data for separate days were combined) on the variability of the frequency of play interactions in growing piglets was determined using a two-way analysis of variance algorithm (with fixed factors). In the third stage, the strength of the effect of the period and the day of the experiment (within each separate period) on the variability of the frequency of play interactions in growing piglets for each of the three groups was determined using a hierarchical two-way analysis of variance algorithm (with fixed factors). All calculations were performed using STATISTICA v. 7.0 software.

## Results and Discussion

The results of the first stage of the study indicate a significant impact of the factors being investigated. Table 1 presents the results of the two-way analysis of variance on the effect of the group and the day of the experiment on the duration of play behaviour in growing piglets during period 1.

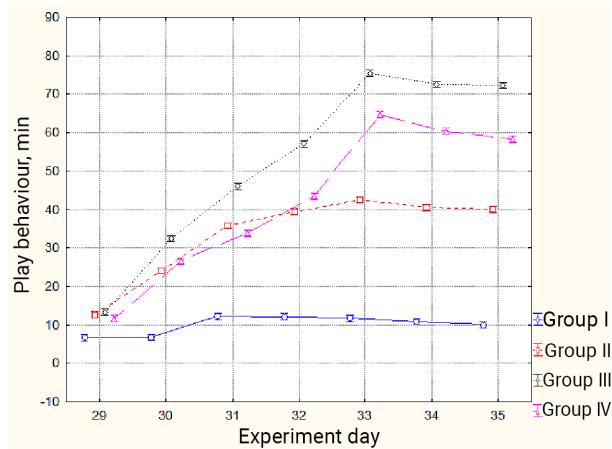
**Table 1.** Results of the two-way analysis of variance on the effect of the group and the day of the experiment on the duration of play behaviour in growing piglets (period 1)

Source of variance	SS	df	MS	F	P	h <sup>2</sup> , %
Group (A)	153,442.9	3	51,147.6	13,784.8	<0.001	51.7
Day of experiment (B)	101,599.1	6	16,933.2	4,563.7	<0.001	34.2
A × B	39,557.0	18	2,197.6	592.3	<0.001	13.3
Error	2,181.7	588	3.7			0.7
Total	296,780.7	615				100.0

**Source:** authors' development

The significant effects of the factors “group” and “day of the experiment”, as well as their interaction, were established (in all cases:  $P < 0.001$ ). The strongest effect was observed for the factors “group”

(51.7%) and “day of the experiment” (34.2%). Figure 4 shows the arithmetic mean values of the duration of play behaviour in growing piglets depending on the group and the day of the experiment during period 1.



**Figure 4.** Arithmetic mean values ( $\pm 95\%$  CI) of the duration of play behaviour in growing piglets depending on the group and the day of the experiment during period 1

**Note:** CI – confidence interval

**Source:** authors' development

The obtained estimates of the average duration of play behaviour gradually increased during period 1, reaching their peak on days 33-35. This was particularly pronounced for the animals in the three experimental groups (II, III, and IV). There were also clear intergroup differences. The lowest estimates of the duration of play behaviour (6.7-12.2 minutes) were noted for the piglets in the control Group I. Significantly higher estimates were obtained for the animals in experimental Group II (with maximum values during period 1 of 40.0-42.5 minutes), and finally, for the animals in Groups III and IV, the maximum values during period 1 reached 75.4 and 64.6 minutes, respectively, as visualised on day 33 of the experiment.

The next stage of the analysis (period 2) confirmed the trends, but with new emphases. Table 2 presents the results of the two-way analysis of variance on the effect of the group and the day of the experiment on the duration of play behaviour in growing piglets during period 2. Although a significant effect of both main factors and their interaction was also established (in all cases:  $P < 0.001$ ), nearly all the variability in the duration of play behaviour in piglets during this period was associated with intergroup differences – the effect of the “group” factor accounted for 99.0%. Figure 5 shows the arithmetic mean values ( $\pm 95\%$  CI) of the duration of play behaviour in growing piglets depending on the group and the day of the experiment during period 2.

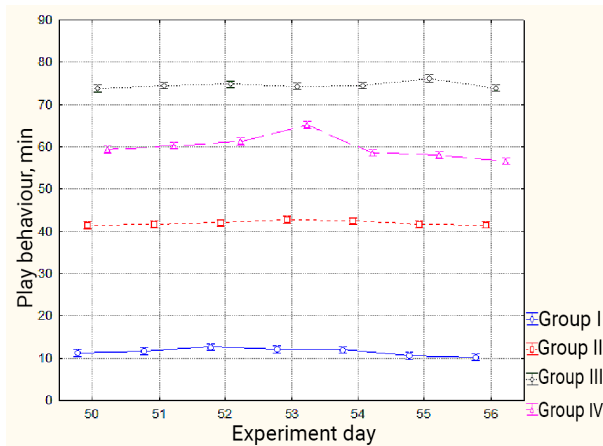
**Table 2.** Results of the two-way analysis of variance on the effect of the group and the day of the experiment on the duration of play behaviour in growing piglets (period 2)

Source of variance	SS	df	MS	F	P	$h^2$ , %
Group (A)	341,171.3	3	113,724.8	30,379.3	<0.001	99.0
Day of experiment (B)	487.9	6	81.3	21.7	<0.001	0.1

Table 2. Continued

Source of variance	SS	df	MS	F	P	h <sup>2</sup> , %
A×B	754.8	18	41.9	11.2	<0.001	0.2
Error	2,201.2	588	3.7			0.6
Total	344,615.1	615				100.0

Source: authors' development



**Figure 5.** Arithmetic mean values ( $\pm 95\%$  CI) of the duration of play behaviour in growing piglets depending on the group and the day of the experiment during period 2

Note: CI – confidence interval

Source: authors' development

During the specified period, there were very slight fluctuations in the duration of play behaviour in piglets from different groups, against the backdrop of significant differences in the estimates for each group. Again, the lowest estimates of the average duration of play behaviour during period 2 were observed for the animals in the control Group I (10.2-12.6 minutes), with higher estimates for the animals in experimental Group II (41.5-42.7 minutes), even higher estimates for the animals in experimental Group IV (56.6-65.2 minutes), and finally, the highest estimates were visualised for the animals in experimental Group III (73.8-76.2 minutes). It is noteworthy that for the piglets in experimental Group II, these day-to-day fluctuations did not have statistical significance ( $F=1.45$ ;  $P>0.05$ ) and were very weak (although statistically significant) for the animals in experimental Group III. In contrast, among the piglets in control Group I

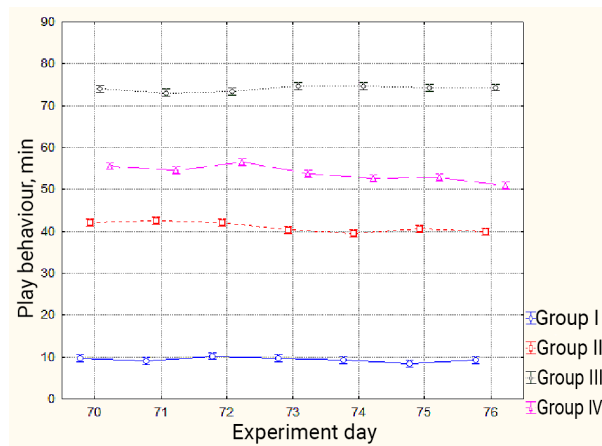
and experimental Group IV, the average estimates of the duration of play behaviour during period 2 initially increased (reaching their maximum on days 52 and 53, respectively), but then decreased.

Table 3 presents the results of the two-way analysis of variance on the effect of the group and the day of the experiment on the duration of play behaviour in growing piglets during period 3. Once again, as in the previous experiment period, a significant effect of both main factors and their interaction was established (in all cases:  $P<0.001$ ), but nearly all the variability in the duration of play behaviour in piglets during this period was also associated with intergroup differences – the effect of the “group” factor accounted for 99.1%. Figure 6 shows the arithmetic mean values ( $\pm 95\%$  CI) of the duration of play behaviour in growing piglets depending on the group and the day of the experiment during period 3.

**Table 3.** Results of the two-way analysis of variance on the effect of the group and the day of the experiment on the duration of play behaviour in growing piglets (period 3)

Source of variance	SS	df	MS	F	P	h <sup>2</sup> , %
Group (A)	339,694.8	3	113,231.6	26,880.0	<0.001	99.1
Day of experiment (B)	281.6	6	46.9	11.1	<0.001	0.1
A × B	463.1	18	25.7	6.1	<0.001	0.1
Error	2,476.9	588	4.2			0.7
Total	342,916.5	615				100.0

Source: authors' development



**Figure 6.** Arithmetic mean values ( $\pm 95\%$  CI) of the duration of play behaviour in growing piglets depending on the group and the day of the experiment during period 3

Note: CI – confidence interval

Source: authors' development

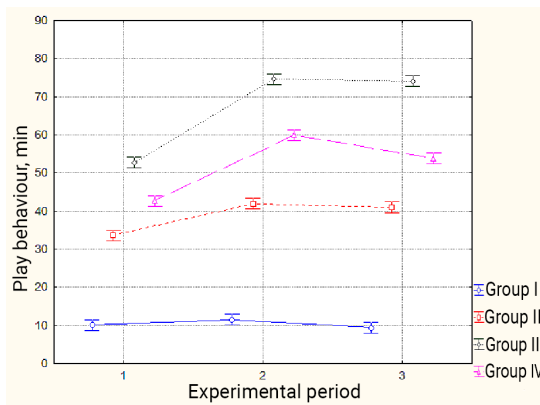
As in the previous experimental period, during period 3, very slight fluctuations in the duration of play behaviour in piglets from different groups were observed, against the backdrop of significant differences in the estimates for each group. The relationship between the estimates for animals in different groups remained the same as during period 2. Notably, for the animals in control Group I and experimental Group III, these day-to-day fluctuations did not have statistical significance (in both cases:  $P > 0.05$ ). To identify long-term trends, an analysis of the effect of the factors “group” and “experiment period” was conducted. When considering the variability of play behaviour duration over a larger time scale

(in terms of individual periods), the previously established patterns remained, with a more significant influence of the “group” factor (the effect size was 78.0%) compared to the “experiment period” factor (5.0%). The relatively large proportion attributable to the effect of unorganised factors (14.5%) indicates a certain level of variability in the raw data obtained within each experiment period (Table 4). Overall, the duration of play behaviour in growing piglets, regardless of group membership, demonstrates an increase in estimates during period 2, with either the achieved level being maintained or slightly decreasing (among animals in control Group I and experimental Group IV) during period 3 (Fig. 7).

**Table 4.** Results of the two-way analysis of variance on the effect of the group and the experiment period on the duration of play behaviour in growing piglets

Source of variance	SS	df	MS	F	P	h <sup>2</sup> , %
Group (A)	807,631.8	3	269,210.6	3,295.06	<0.001	78.0
Experiment period (B)	51,542.2	2	25,771.1	315.43	<0.001	5.0
A × B	26,677.2	6	4,446.2	54.42	<0.001	2.6
Error	150,003.3	1,836	81.7			14.5
Total	1,035,854.6	1,847				100.0

Source: authors' development



**Figure 7.** Arithmetic mean values ( $\pm$  95% CI) of the duration of play behaviour in growing piglets depending on the group and the experiment period

Note: CI – confidence interval

Source: authors' development

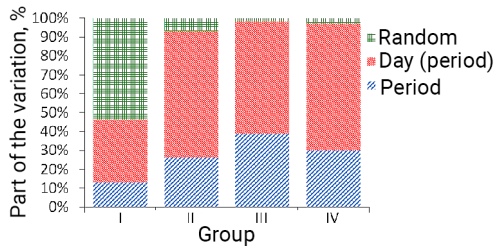
Additional hierarchical analysis allowed for a deeper assessment of the effect size of each time level. Table 5 presents the results of the hierarchical two-way analysis of variance on the effect of the period and the day of the experiment on the duration of play behaviour in growing piglets. Overall, a highly significant effect was found for both the experimental periods (in all cases:

$P < 0.001$ ) and the day of the experiment within individual periods (in all cases:  $P < 0.001$ ) for the animals in all four groups. More interestingly, the relationship between the influence of these two time scales on the variability in the duration of play behaviour in growing piglets was established among the animals in the control and experimental groups (Fig. 8).

**Table 5.** Results of the hierarchical two-way analysis of variance ( $F$ ;  $P$ ) on the effect of the period and the day of the experiment on the duration of play behaviour in growing piglets

Group	Experiment period (A)	Day of experiment within the period (B(A))
I Control	53.28 (<0.001)	15.23 (<0.001)
II Experimental	870.72 (<0.001)	246.60 (<0.001)
III Experimental	5,407.95 (<0.001)	915.48 (<0.001)
IV Experimental	2,839.99 (<0.001)	708.71 (<0.001)

Source: authors' development



**Figure 8.** Estimates of the effect size of the period and the day of the experiment (within individual periods) on the duration of play behaviour in growing piglets

**Source:** authors' development

For the animals in control group I, nearly half (53.6%) of the variability in the duration of play behaviour was caused by unorganised factors, i.e., related to interindividual differences. For the animals in the control group, the effect size of differences between individual experimental periods was only 13.0%, while for the animals in the experimental groups, the corresponding estimate was 2-3 times higher. Finally, for the animals in control group I, the effect size of differences between individual days of the experiment (within periods) was higher, at 33.40%, while for the animals in the experimental groups, the corresponding estimate was almost twice as high. Therefore, it can be concluded that the nature of the variability in the duration of play behaviour was more influenced by the presence of the enrichment object in pig housing during the growing phase, rather than the type of enrichment object itself.

The results of the conducted study, which revealed a significant effect of enrichment objects on the duration of play behaviour in piglets, are fully consistent with current views on the importance of the environment in shaping animal behavioural responses. Specifically, the differences observed between the experimental groups, recorded during all observation periods, confirm that the type and availability of enrichment materials determine the level of play activity in growing piglets. J.-M. Beaudoin *et al.* (2019) demonstrated that cleanliness, novelty, and the

physical characteristics of enrichment objects critically influence the maintenance of pig interest. In the present study, this was particularly evident for the groups with bottles filled with grain and paper balls, which were regularly updated, stimulating play activity. N. Casal-Plana *et al.* (2017) also emphasised that the use of natural additives combined with enrichment objects positively influences both behaviour and weight gain in the studied groups of pigs. In turn, E. Fàbrega *et al.* (2019) indicated that even under unfavourable climatic conditions, an enriched environment contributes to the improvement of the physiological and productive parameters of pigs. This allows the effect of enrichment to be viewed as a universal tool for enhancing their welfare.

In turn, J. Lee *et al.* (2025) demonstrated that the introduction of enrichment has the greatest impact during the early period of weaning piglets from sows. The increased duration of play behaviour observed in the current study can be explained by a reduction in stress due to the ability to fulfil natural needs for exploration and manipulation. L. Luo *et al.* (2022) also confirm that enrichment enhances the resilience of piglets to social stressors, such as regrouping. The practical significance of the current study is supported by the works of A. Lykhach *et al.* (2022) and R.F. Oliveira *et al.* (2023), who prove that even simple and inexpensive materials, such as plastic bottles filled with grain or wood shavings, are effective behavioural enrichment tools. These reduce aggression levels and maintain animal activity. This is particularly important in the context of current requirements for pig welfare in industrial farming conditions. At the same time, the choice of inexpensive yet effective enrichment objects can be a cost-effective solution for both small and large pork producers.

K. Steinerová *et al.* (2024) emphasised that play behaviour is a biomarker of a positive emotional state, and its presence correlates with physiological indicators of pig welfare. The data from the current study regarding the stable increase in play activity in the experimental groups support this statement. Additionally, as noted by

K.C. Smith & M.K. Pierdon (2024), environmental enrichment contributes to a reduction in the frequency of skin injuries, which is an indirect indicator of decreased aggressive behaviour within the group. Thus, the introduction of enrichment objects can be seen not only as an element of welfare but also as a tool for reducing stress factors and preventing cannibalism. In period 1, it was found that the “group” factor accounted for 51.7% of the variation in play behaviour, “day of the experiment” – 34.2%, and their interaction – 13.3%. In subsequent periods, the effect of the day decreased, while the effect of the group reached up to 99%. The analysis between periods also confirmed the dominant role of the type of enrichment (78% variation). This indicates a trend towards the stabilisation of behavioural responses after adaptation to new conditions, when the piglets had already become accustomed to the presence of objects and were actively using them. Play behaviour in the current study also demonstrated high informativeness in the context of assessing the emotional state of the animals. This is confirmed by the correlation of results between periods, where positive dynamics in play activity were observed in the experimental groups throughout the entire observation period. Meanwhile, in the control group, play remained at a consistently low level, which may indicate background stress and a lack of motivation for social or object-related interaction.

Environmental enrichment not only increases animal activity but also stabilises their behaviour over time. This makes it possible to recommend the regular use of enrichment objects in piglet housing systems as a means of improving welfare domains and reducing stress manifestations. The choice of enrichment type is crucial: objects that can be explored, moved, or destroyed generate more interest and longer play activity. Therefore, considering the specific behaviour of growing piglets when selecting enrichment is critically important. The experience of foreign studies by N. Alvarez-Hernandez *et al.* (2023) has shown that a combination of olfactory, tactile, and visual stimuli

has the greatest effect. Furthermore, an important aspect of future research is the development of assessments of the economic efficiency of enrichment, in order to balance the needs for animal welfare with the commercial interests of farms.

In conclusion, the current study makes a new contribution to the field of pig behaviour, confirming that quality enrichment can become an effective strategy for the behavioural and emotional support of animals in industrial conditions. The obtained data suggest the need for improving pig housing systems with the mandatory inclusion of enrichment components, which will not only enhance their welfare but also improve the overall efficiency of pork production.

## Conclusions

Summarising the results of the obtained data, a number of key conclusions can be formulated, reflecting the importance of environmental enrichment in shaping the play behaviour of growing piglets and defining the potential directions for practical application of the findings. The study established that the factor “group” has the greatest impact on the duration of play behaviour in growing piglets. Specifically, in period 1, the effect size of this factor was 51.7% of the total variation ( $P < 0.001$ ), while in periods 2 and 3, it accounted for 99.0% and 99.1%, respectively ( $P < 0.001$ ), indicating its dominant significance. The effect of the “day of the experiment” factor was moderate only in period 1, where it explained 34.2% of the variation ( $P < 0.001$ ), and significantly decreased in subsequent periods – only 0.1% in periods 2 and 3, indicating the animals’ adaptation to the environmental conditions and the stabilisation of behavioural activity. The interaction of the factors “group × day” had a statistically significant but smaller effect – 13.3% in period 1, 0.2% in period 2, and 0.1% in period 3 (all  $P < 0.001$ ), indicating a moderate impact of daily fluctuations against the backdrop of the stable influence of the type of enrichment.

The lowest average duration of play behaviour was observed in the animals of control group I (ranging from 6.7 to 12.6 minutes in

different periods), while in experimental group II with the rope, the duration increased to 42.7 minutes, in group IV with paper bundles it increased to 65.2 minutes, and the highest values were recorded in group III with grain-filled bottles – up to 76.2 minutes on days 33-53 of the experiment. The summarised results for all periods of the study demonstrate that the “group” factor explained 78.0% of the total variation in play behaviour, while the “experiment period” accounted for only 5.0%, and unorganised factors accounted for 14.5%, indicating a clear dominance of the type of enrichment over time-related changes. Hierarchical variance analysis confirmed that in all groups, there was a highly significant effect of both the period and the day of the experiment (all  $P < 0.001$ ), but the proportion of variation explained by unorganised factors was highest in the growing piglets of control group I – 53.6%, whereas in the experimental groups, it significantly decreased.

Thus, environmental enrichment significantly enhances the play activity of piglets, serves as a means of reducing stress and aggressive

behaviour, and is also a reliable behavioural indicator of animal welfare in intensive farming systems. The prospects for future research lie in expanding experimental models to account for individual characteristics of piglets (temperament, gender, genetic origin), including physiological welfare indicators (cortisol levels, immune markers), as well as long-term monitoring of enrichment effectiveness. An important area of research is also the durability of the enrichment effect over time, including the dynamics of decreasing interest in enrichment objects.

### Acknowledgements

The authors would like to express their gratitude to the owner and the team of the private leased enterprise “Victoria” for providing the base for scientific research.

### Funding

None.

### Conflict of Interest

None.

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## Оцінка ігрової поведінки поросят на дорощуванні за різних стратегій збагачення в контексті імплементації правил благополуччя тварин в Україні

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**Анотація.** Посилення вимог до благополуччя тварин в Україні вимагає адаптації технологій утримання свиней до стандартів європейської інтеграції. Дослідження було спрямоване на оцінку впливу різних стратегій збагачення середовища на ігрову поведінку поросят на дорощуванні в промислових умовах. Експеримент проведено на чотирьох групах поросят по 22 голови, віком 28 днів на початку, за умов однакової годівлі та утримання. Об'єкти збагачення вводились перед початком відеомоніторингу для забезпечення адаптації тварин. Поведінкові спостереження здійснювались у три періоди: 29-35-а, 50-56-а та 70-76-а доба. Аналіз проведено методом двофакторного дисперсійного аналізу з фіксованими факторами «група» та «доба», а також їх взаємодією «група × доба». Результати експерименту показали, що фактор «група» пояснював 51,7 % варіації в періоді 1, а вже у періодах 2 і 3 досягав 99 % ( $P < 0,001$ ). Тривалість ігрової поведінки у контрольній групі становила 6,7-12,6 хв, тоді як у групі з бавовняною мотузкою – до 42,7 хв, у групі з купками пакувального паперу – до 65,2 хв, а у групі з пляшками, наполовину наповнені зерном – до 76,2 хв. Отримані результати за всі періоди дослідження свідчать, що фактор «група» пояснював 78,0 % загальної варіації ігрової поведінки, тоді як «період експерименту» – лише 5,0 %, а неорганізовані фактори – 14,5 %, що вказує на чітку перевагу впливу типу збагачення над часовими змінами. Ієрархічний дисперсійний аналіз підтвердив, що в усіх групах мав місце високовірогідний вплив як періоду, так і доби експерименту (усі  $P < 0,001$ ), проте частка варіації за неорганізованих факторів найвища у поросят на дорощуванні I контрольної групи – 53,6 %. Ці дані свідчать про значний стимулюючий ефект збагачення середовища на активність поросят. Перевага груп із збагаченням демонструє можливість підвищення поведінкових та емоційних складників благополуччя. Підкреслено, що впровадження доступних і ефективних стратегій збагачення має практичну значущість для модернізації утримання поросят в Україні з урахуванням вимог благополуччя

**Ключові слова:** етичні стандарти; євроінтеграція; поведінка; свині; сталий розвиток; технологія



UDC 636.4:591.133:579.61:636.085

DOI: 10.31548/dopovidi/6.2025.128

## The effect of a phytogetic preparation on productivity, biochemical blood status, and intestinal microbiota of replacement gilts

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**Abstract.** With the increasing use of antibiotics in pig farming, there is a growing need for alternative solutions to support animal health and productivity, which underscores the importance of studying the effectiveness of phytogetic additives in pig feeding. The study is dedicated to evaluating the impact of a phytogetic feed additive based on dried garlic and caraway on productivity, metabolic status, and the microbiota of the large intestine of replacement gilts of the (LW × L) genotype. The experiment was conducted under production conditions on two groups of animals (n = 80), which received either the basic diet without the additive (control) or the feed additive at a dose of 0.10% of the compound feed mass (experimental group). Starting from the 17<sup>th</sup> week of growth, the experimental group of gilts showed a significant advantage in live weight ( $p \leq 0.001$ ) and average daily gains ( $p \leq 0.01$ ), which persisted until the 28<sup>th</sup> week of age. The overall animal survival rate was 95% compared to 85% in the control group, indicating an improvement in physiological condition and stress resistance. Biochemical serum indicators showed an increase in albumin content, protein coefficient, calcium, phosphorus, and iron, while a decrease in  $\gamma$ -glutamyltransferase and indirect bilirubin was observed, reflecting optimisation of protein, mineral, and pigment metabolism, and a hepatoprotective effect. Bacteriological analysis revealed an increase in the proportion of *Lactobacillus amylovorus*, *L. reuteri*, *Clostridium butyricum*, and *Eubacterium rectale*, and a decrease in *Staphylococcus aureus*, *S. epidermidis*, and *Streptococcus suis*, indicating restoration of microbial

### Suggested Citation:

Bevz, N., & Lykhach, V. (2025). The effect of a phytogetic preparation on productivity, biochemical blood status, and intestinal microbiota of replacement gilts. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 128-143. doi: 10.31548/dopovidi/6.2025.128.

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balance, increased production of short-chain fatty acids, and colonisation resistance of the intestine. It was established that the phytogetic additive used exhibits a combined stimulating effect, simultaneously influencing the microbiota, metabolism, and physiological adaptation of the animals

**Keywords:** biochemical blood indicators; immunomodulation; sustainable pig farming; large intestine; phytobiotic

## Introduction

Modern pig farming faces the challenge of increasing production efficiency while the use of antibiotics and synthetic growth stimulants continues to rise. In the context of transitioning to antibiotic-free technologies, an important direction is the use of natural additives that promote stable growth and development of young animals, strengthen the immune system, and improve the animals' adaptive capabilities. One such innovative solution is the use of phytogetic feed additives, which contain biologically active plant-derived components with antimicrobial, antioxidant, and anti-inflammatory effects.

Modern pig farming sets high demands for production efficiency, biosecurity, and animal health, while simultaneously reducing the use of antibiotics and synthetic growth stimulants. J. Wang *et al.* (2024) conducted a literature review examining phytogetic feed additives as natural alternatives to antibiotics in animal husbandry. The authors analysed numerous publications demonstrating the effectiveness of phytogetic additives in improving animal health and productivity. They also highlighted the potential of these additives to reduce the use of synthetic growth stimulants and antibiotics in livestock farming. According to N.L. Bevz & V.Ya. Lykhach (2024), in the context of transitioning to antibiotic-free production technologies, actively supported at both the European Union level and in Ukraine, the use of natural products in pig feeding has become particularly important. In their studies, R. Faustov *et al.* (2022) proved that the introduction of phytogetic preparations into the diet of fattening pigs ensures stable growth and development of young animals, strengthens the immune system,

and enhances the adaptive capabilities of the organism. As noted by J. Lee *et al.* (2024), one of the promising directions for such solutions is the use of phytogetic feed additives, or phytobiotics, which contain biologically active plant-derived compounds – essential oils, phenolic substances, flavonoids, terpenoids, alkaloids, and other components with antimicrobial, antioxidant, and anti-inflammatory effects.

In the works of V.O. Alieksiev (2025), it was noted that interest in phytogetic feed additives for different animal species has significantly increased. Phytogetic preparations, as evidenced by the data of N. Xiao *et al.* (2025), have shown the ability to positively affect digestion, stimulate enzyme secretion, stabilise intestinal microbiota, improve nutrient absorption, regulate immune response, and reduce oxidative stress levels. According to S. Biswas & I.H. Kim (2023), the introduction of phytogetic components into pig feeding contributes to increased average daily weight gain, improved feed conversion, reduced frequency of gastrointestinal disorders, and maintenance of stable physiological condition. These effects are due to the presence of essential oils and extracts containing substances with pronounced biological properties – carvacrol, thymol, allicin, limonene, etc., which exhibit a wide range of antimicrobial activity against pathogenic bacteria and fungi.

As noted by T.Yu. Mykhailenko & M.Yu. Sychov (2022), among the variety of phytogetic additives, significant attention is drawn to products based on garlic (*Allium sativum* L.) and caraway (*Carum carvi* L.), which have a synergistic effect in normalising intestinal microbiota, stimulating digestion, activating enzymatic processes,

and increasing the body's resistance. It has been proven that the inclusion of garlic extract or powder in the diet of piglets contributes to improved growth rates, reduced cholesterol levels in serum, increased antioxidant activity, and normalisation of glucose and urea levels. The addition of caraway, in turn, enhances the secretory activity of digestive glands, stimulates appetite, inhibits the growth of pathogenic microbiota, and promotes the colonisation of beneficial lactobacilli in the intestine. Despite the study by M. Rabelo-Ruiz *et al.* (2021) on the use of phytogenic additives in pig feeding, the issue of their effectiveness in replacement young stock remains insufficiently studied. Most scientific works focus on the weaning or fattening period, while data on the impact of such additives on gilts during the growing period until their first insemination is limited. This period is crucial as active growth processes, formation of the reproductive organs, and physiological maturity occur, requiring optimal support for metabolic processes and the immune system. The reaction of replacement gilts' bodies to phytogenic stimulants in terms of biochemical blood indicators and changes in the microbiota composition of the large intestine, which plays a key role in maintaining metabolic homeostasis, is also insufficiently explored.

M. Walter *et al.* (2025) highlighted an important modern trend of applying a comprehensive approach to evaluate the effectiveness of feed additives, which includes not only productivity indicators but also physiological, biochemical, and microbiological parameters. This approach aligns with the modern concept of "gut health" – an integrated understanding of digestive tract health as the foundation for effective growth and productivity of animals. Q. Tang *et al.* (2025) demonstrated that studying the relationship between microbiota composition, metabolic processes, and immune system status allows for the development of new approaches to feeding technologies aimed at preventing stress conditions and enhancing the body's natural resistance. V. Ivanov *et al.* (2023) expressed the opinion that conducting

such studies in real farm conditions, where factors such as microclimate, housing technology, animal genetic traits, and practical feeding aspects affect the results, holds significant scientific value. This ensures practical relevance and increases the importance of the obtained results for production. Furthermore, the use of modern laboratory methods ensures the reliability and accuracy of the data, meeting current international standards for experimental work in veterinary science and livestock production technologies.

Therefore, the relevance lies in justifying the effectiveness of using a phytogenic feed additive in the feeding of replacement gilts, which contributes to the optimisation of metabolic processes, maintenance of stable microbiota, and increased productivity without the use of antibiotics.

The aim of the study was to determine the impact of phytobiotic on productivity, biochemical blood indicators, and intestinal microbiota of replacement gilts during the growing period until their first insemination.

## Materials and Methods

The study was conducted in 2023-2025 under the conditions of the privately leased enterprise "Victoria" in the Bashtanka district of Mykolaiv region. A total of 160 clinically healthy replacement gilts of a crossbred combination of Large White × Landrace (LW × L) from the breeding company "PIC" (UK) were used in the experiment. At 11 weeks of age, animals with a live weight of 32.02-32.62 kg were divided into two groups of 80 individuals each based on the analog method. Throughout the experiment, all experimental gilts received standard basic diets. Prior to the start of the scientific and production experiment, an acclimatisation period lasting 7 days (from 11 to 12 weeks of age) was conducted. The control group (I) received the basic diet (BD) without any additional feed additives. The experimental group (II) received an additional natural growth stimulator, "Imunochasnyk", at a dose of 1,000 g/ton of compound feed from the 12<sup>th</sup> to the 28<sup>th</sup> week of growth, according to the manufacturer's

recommendations. The animals were fed specialised compound feeds for replacement gilts of periods I and II, manufactured in the farm's own feed mill. The diets were formulated based on the feeding strategies adopted by the farm and the methodological recommendations of genetic and feed companies (PIC – genetic company, n.d.).

Composition of “Imunochasnyk” (per 100 g of product): dried garlic (*Allium sativum* L) – 95 g, caraway (*Carum carvi* L) – 5 g, manufactured by order of “Eagle Trading LLC” (Ukraine), manufacturer: Argano Organics LLC, Unit 2 Industrial Estate, Saharanpur (India). The feed additive “Imunochasnyk” is a plant-based growth stimulator based on essential oils. Its composition includes essential oils of garlic and caraway at concentrations according to the manufacturer's guidelines. The use of this additive helps to stabilise digestion processes, promote the formation of healthy intestinal microbiota, enhance growth intensity, and improve the functional activity of the animals' immune system.

To ensure the adequacy of the diets in terms of nutrient content, premixes produced by LLC “Tsekhave Ukraine” were used. The experimental feed additive was incorporated directly into the compound feed during its manufacture at the feed mill when formulating the respective diets. Replacement gilts were fed a complete diet at a rate of 2.52 kg of feed per head per day with the following nutritional value: crude protein content of 155.0 g/kg and metabolisable energy of 12.36 MJ/kg. The composition of 1 kg of the complete feed included the following ingredients (%): maize (35), wheat (21), barley (10), wheat bran (12), sunflower meal (10), soybean meal (7), Cehavit sow premix replacement (5). Feed was provided ad libitum. Watering of the experimental animals was carried out using nipple drinkers at a ratio of one drinker per 10 animals.

Housing conditions complied with production standards and technological regulations for the agro-industrial complex (Ministry of Agrarian Policy of Ukraine, 2005). Replacement pigs were housed in groups of 20 animals per pen with a

concrete slatted floor, providing a floor area of 1.0-1.65 m<sup>2</sup> per animal. Veterinary and preventive measures for animals of all experimental groups were carried out according to a unified scheme approved at the farm. Microclimate parameters in the housing facility for the experimental animals were ensured by the operation of a negative ventilation system, which included an axial exhaust fan installed in the ceiling section of the building and air inlet valves located in the walls of the facility. Coordination of the ventilation system components was performed using microprocessor-controlled equipment, which ensured automatic maintenance of the specified microclimate parameters. Manure removal from the facility was carried out using a vacuum-gravity system of periodic operation, which involved manure channels located beneath the entire pen area and a network of pipelines for transporting manure effluent to manure collectors situated outside the livestock building. Feeding, watering, housing conditions, animal care, and veterinary and preventive measures during the experiment were conducted in compliance with the requirements of current European and national legislation of Ukraine on the welfare of farm animals during their housing (Order of the Ministry..., 2021).

The productive performance of replacement gilts during the growing period until first insemination was assessed using a set of indicators, including live weight (kg) and average daily gain (g). In addition, herd survival rate and the proportion of culled animals during the specified period were determined in accordance with generally accepted methodological approaches (Voloshchuk, 2014; Ibatulin & Zhukorskyi, 2017; Ladyka & Khmelnychiy, 2023). Biochemical blood analyses of replacement gilts from the experimental and control groups at the end of the growing period, at 190 days of age, were conducted to evaluate the functional status of the liver and kidneys, protein, carbohydrate and mineral metabolism, as well as enzymatic activity of the organism. Blood samples were collected in the morning before feeding by puncture of the

jugular vein from five clinically healthy animals in each group. Blood samples were collected into tubes containing a clot activator, followed by centrifugation to obtain serum. Biochemical analyses were performed in the independent laboratory of LLC "Expert Centre 'Biolaits'" using an automatic biochemical analyser ERBA XL-640 (Erba, Czech Republic) and appropriate certified reagents. The obtained results were compared with reference (normative) values for pigs of the corresponding age (Voloshchuk, 2014; Ibatulin & Zhukorskiy, 2017; Ladyka & Khmelnychiy, 2023).

At the final stage of the scientific and production experiment, the effect of the feed additive "Imunochasnyk" on the quantitative and species composition of the microbiota of the large intestine of replacement gilts was investigated. Faeces from the experimental gilts served as the material for studying the microbial composition; samples were collected from 10 animals in each group onto a clean substrate, after which they were transferred into sterile plastic containers and sent to the independent laboratory of LLC "Expert Centre 'Biolaits'". Microbiological analysis of faecal samples was conducted for the quantitative and qualitative determination of microorganisms through their identification using MALDI-TOF MS. The experimental research protocol was approved by the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine in accordance with Good Clinical Practice (GCP) requirements regarding the

protection and humane treatment of experimental animals, No. 039/2025 dated 26 June 2025.

The obtained research results were processed using statistical methods (Kramarenko *et al.*, 2019) with the use of computer technology and the STATISTICA v.7.0 software package (StatSoft Inc., USA), in accordance with the methodological approaches described by I.I. Ibatulin & O.M. Zhukorskiy (2017) and V.I. Ladyka & L.M. Khmelnychiy (2023).

## Results and Discussion

The study of productive performance of replacement gilts of the (LW × L) genotype showed that at the initial stages of rearing (11-12 weeks of age), the live weight of animals in both groups was almost identical (32.02-32.62 kg), indicating homogeneity of the initial experimental material (Table 1). During this period, no significant differences were observed between the control (I) and experimental (II) groups, and culling due to injuries or diseases was minimal (0-2.5%). Starting from the 17<sup>th</sup> week of rearing, a significant advantage of the experimental group in live weight was established (66.22 kg) compared with the control group (61.40 kg,  $p \leq 0.001$ ). Accordingly, average daily gains in animals receiving the feed additive were significantly higher, amounting to 817.14 g versus 731.43 g in the control group ( $p \leq 0.01$ ), which indicated a stimulating effect of the additive on the growth intensity of the young animals.

**Table 1.** Productive traits of replacement gilts ( $n = 80$ ),  $\bar{X} \pm S_{\bar{X}}$

Trait	Group / Age	I (control)	II (experimental)
11 weeks			
Live weight, kg		32.62 ± 0.248	32.02 ± 0.330
12 weeks			
Live weight, kg		36.80 ± 0.434	37.62 ± 0.404
Culling (injuries, diseases), %		0	2.5
17 weeks			
Live weight, kg		61.40 ± 0.412	66.22 ± 0.384***
Average daily gain, g		731.43 ± 4.562	817.14 ± 3.632**
Culling (injuries, diseases), %		5.0	2.5
22 weeks			
Live weight, kg		88.16 ± 0.282	95.22 ± 0.314***

**Table 1. Continued**

Trait	Group / Age	I (control)	II (experimental)
22 weeks			
Average daily gain, g		764.57 ± 4.082	828.57 ± 3.420***
Culling (injuries, diseases, other), %		7.9	0
26 weeks			
Live weight, kg		<b>108.20 ± 0.308</b>	<b>117.80 ± 0.296***</b>
Average daily gain, g		715.71 ± 3.502	806.43 ± 2.786***
Culling (injuries, diseases, other), %		0	0
28 weeks			
Live weight, kg		118.64 ± 0.282	128.52 ± 0.226***
Average daily gain, g		745.71 ± 4.40	765.71 ± 3.30**
Culling (injuries, diseases, other), %		2.9	0
Survival rate, %		85.0	95.0

**Note:** \*\* –  $p \leq 0.01$ ; \*\*\* –  $p \leq 0.001$

**Source:** authors' development

At 22 weeks of age, the differences between the groups persisted and even intensified: the live weight of gilts in the experimental group reached 95.22 kg, which significantly exceeded the control by 7.06 kg ( $p \leq 0.001$ ). Average daily gain during this period was also higher, amounting to 828.57 g compared with 764.57 g in the control group ( $p \leq 0.001$ ). No cases of culling were recorded in the experimental group, whereas in the control group it reached 7.9%, indicating a positive effect of the additive on animal health and viability. By 26 weeks of age, the advantage of the experimental group was maintained: the live weight of gilts reached 117.80 kg, which was 9.6 kg higher than in the control group ( $p \leq 0.001$ ). Average daily gain was also significantly higher (806.43 g versus 715.71 g,  $p \leq 0.001$ ). At this stage, no culling of animals was observed in either group. At the end of the growing period (28 weeks), the difference between the groups remained substantial. The live weight of gilts in the experimental group reached 128.52 kg, exceeding the control value (118.64 kg) by 9.88 kg

( $p \leq 0.001$ ). Average daily gains remained high, amounting to 765.71 g compared with 745.71 g in the control group ( $p \leq 0.01$ ). Overall survival in the experimental group was 95.0%, whereas in the control group it was 85.0%, confirming an improvement in physiological status and a reduction in stock losses due to the use of the investigated phyto-genic feed additive. Thus, inclusion of the additive in the diet of replacement gilts at a dose of 0.10% of feed weight ensured increased growth intensity, improved average daily gains, and higher survival rates. This indicates its effectiveness as an immunostimulatory and growth-promoting agent in the system of rearing replacement pigs.

Biochemical indicators of blood serum reflect the state of metabolism, the functional activity of organs, and the response of the animal organism to dietary factors. Analysis of the obtained data demonstrated that pigs in the experimental group receiving the phyto-genic feed additive exhibited positive changes in a number of metabolic indicators compared with the control group (Table 2).

**Table 2. Biochemical blood indicators of replacement gilts when using the phyto-genic feed additive ( $n = 5$ ),  $\bar{X} \pm S_x$**

Indicator	Reference range	Group		Difference II vs I
		I (control)	II (experimental)	
Albumins (ALB), g/L	25-45	22.34 ± 0.821	25.33 ± 0.621	2.99*
Alkaline phosphatase (ALP), U/L	30-150	258.37 ± 21.533	280.23 ± 20.819	21.86

Table 2. Continued

Indicator	Reference range	Group		Difference II vs I
		I (control)	II (experimental)	
Alanine aminotransferase (ALT), U/L	12-54	63.82 ± 3.961	69.76 ± 8.471	5.94
Alpha-amylase (AMY), U/L	≤3500	2766.97 ± 351.098	3353.01 ± 399.331	586.04
Aspartate aminotransferase (AST), U/L	12-42	51.80 ± 4.754	66.19 ± 10.437	14.39
Calcium (Ca), mmol/L	2.0-3.0	2.41 ± 0.043	2.58 ± 0.035	0.17*
Cholesterol (CHOL), mmol/L	1.1-3.4	2.50 ± 0.063	2.56 ± 0.138	0.06
Creatinine (CRE), μmol/L	100-208	70.74 ± 3.992	83.54 ± 5.241	12.80
Direct bilirubin (DBIL), μmol/L	0-5.3	0.26 ± 0.063	0.27 ± 0.030	0.01
Gamma-glutamyltransferase (GGT), U/L	10-60	31.31 ± 5.210	25.84 ± 5.045	-5.47
Glucose (GLUC), mmol/L	2.5-8.1	5.25 ± 0.177	5.76 ± 0.363	0.51
Phosphorus (P), mmol/L	1.5-3.2	3.59 ± 0.122	3.70 ± 0.108	0.11
Iron (Fe), μmol/L	15-38	18.85 ± 1.643	28.72 ± 3.831	9.87*
Magnesium (Mg), mmol/L	0.74-1.32	0.89 ± 0.021	0.96 ± 0.028	0.07
Total bilirubin (TBIL), μmol/L	0.1-6.84	2.44 ± 0.090	2.33 ± 0.039	-0.11
Total protein (TP), g/L	50-85	55.81 ± 3.408	56.19 ± 2.201	0.38
Globulins (GLB), g/L	20-35	33.46 ± 3.569	30.86 ± 1.952	-2.60
Protein coefficient	0.8-1.2	0.72 ± 0.086	0.84 ± 0.051	0.12
De Ritis index (AST/ALT)	0.8-1.3	0.80 ± 0.032	0.92 ± 0.058	0.12
Ca/P ratio	1.2-1.8	0.68 ± 0.037	0.70 ± 0.032	0.02
Indirect bilirubin (IBIL), μmol/L	1.7-8.6	19.38 ± 1.429	8.94 ± 0.914	-10.44**

Note: \*\* –  $p \leq 0.01$ ; \*\*\* –  $p \leq 0.001$

Source: authors' development

**Protein metabolism.** The albumin content in the experimental group amounted to 25.33 g/L, which corresponded to the physiological range (25-45 g/L) and exceeded the control by 2.99 g/L. The values in the control group were below the lower limit of the norm. This indicated activation of protein synthesis in the liver and improvement of plastic processes. Total protein content in animals of both groups remained within the physiological range (50-85 g/L); however, a tendency towards a slight increase was observed in the experimental gilts (56.19 g/L versus 55.81 g/L). The globulin content in the experimental group decreased (30.86 g/L compared with 33.46 g/L in the control), which resulted in an increase in the protein coefficient from 0.72 to 0.84 – a value that reached the optimal range (0.8-1.2) in the experimental animals. This indicated a more balanced ratio between albumin and globulin synthesis, reflecting a stable immunobiochemical status.

**Enzymatic activity.** The activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in pigs of both groups exceeded the

reference ranges (12-54 and 12-42 U/L, respectively), which may be associated with intensive protein metabolism during the period of active growth. At the same time, in the experimental group the ALT level was higher (69.76 U/L), and AST exceeded the control by 14.39 U/L, which resulted in an increase in the de Ritis index to 0.92 (within the physiological range of 0.8-1.3). This indicated the absence of pathological changes in hepatocytes and good adaptation of the liver to the action of the phytogenic additive. Alkaline phosphatase (ALP) activity in the experimental group increased by 21.86 U/L, which was likely associated with intensified bone tissue growth processes. Gamma-glutamyltransferase (GGT) activity in animals of the experimental group decreased (25.84 versus 31.31 U/L), indicating a reduced hepatic load. Alpha-amylase activity remained within the normal range (up to 3,500 U/L) and showed a tendency to increase (3,353.01 U/L), which may indicate stimulation of pancreatic enzymatic activity.

**Carbohydrate metabolism.** The glucose concentration in the blood serum of pigs in both groups remained within the physiological range (2.5-8.1 mmol/L) and was slightly higher in the experimental animals (5.76 versus 5.25 mmol/L), which may be a consequence of more efficient nutrient utilisation when the experimental feed additive was included in the diet.

**Mineral metabolism.** The calcium and phosphorus contents in both groups were at adequate levels; however, in the experimental group an increase in calcium concentration to 2.58 mmol/L was observed, along with a slight increase in phosphorus to 3.70 mmol/L. Despite this, the Ca/P ratio remained below the optimal range (0.70 compared with the recommended 1.2-1.8), indicating a certain imbalance in mineral metabolism typical of young animals during periods of intensive growth. The magnesium content remained within the physiological range (0.96 mmol/L).

**Pigment metabolism indicators.** The levels of total and direct bilirubin remained within the physiological range, whereas the concentration of indirect bilirubin in the experimental group (8.94  $\mu\text{mol/L}$ ) decreased compared with the control group (19.38  $\mu\text{mol/L}$ ), indicating a reduction in haemolysis processes and stabilisation of the functional state of the liver.

**Other metabolic indicators.** Creatinine levels in animals of both groups were below the reference

values (83.54 versus 70.74  $\mu\text{mol/L}$ ), which may be associated with age-related growth characteristics and a lower level of muscle metabolism compared with adult sows. The iron concentration in the experimental group significantly exceeded that of the control group (28.72 versus 18.85  $\mu\text{mol/L}$ ), indicating improved haematopoietic processes under the influence of the phytogetic additive. Cholesterol levels remained stable within the physiological range (2.56 mmol/L), indicating the absence of disturbances in lipid metabolism.

The obtained results demonstrated that the use of the phytogetic additive in the feeding of replacement gilts had a positive effect on protein, carbohydrate, and mineral metabolism, contributed to the stabilisation of hepatic enzymatic activity, and promoted improved protein synthesis and metabolic adaptation of the animals during the growing period.

Bacteriological analysis of samples from the large intestine of replacement gilts revealed the presence of a diverse commensal microbiota, represented mainly by Gram-positive cocci and rods of the genera *Streptococcus*, *Lactobacillus*, *Enterococcus*, and *Clostridium*. In the control group, facultative anaerobes predominated (*Streptococcus alactolyticus*, *Enterococcus faecalis*, *Staphylococcus* spp.), whereas in the experimental group acid-producing and butyrate-producing bacteria prevailed (*Lactobacillus amylovorus*, *L. reuteri*, *Clostridium butyricum*, *Eubacterium rectale*) (Table 3).

**Table 3.** Changes in the species composition of the dominant microbiota of the large intestine of replacement gilts under the influence of the phytogetic feed additive, %

Taxon	Group, n = 10 animals		Description
	I (control)	II (experimental)	
<i>Streptococcus alactolyticus</i>	100	100	Dominant commensal of the large intestine; ferments carbohydrates and maintains a stable pH environment.
<i>Lactobacillus amylovorus</i>	40	90	Lactic acid producer; competitively suppresses opportunistic microbiota.
<i>Lactobacillus reuteri</i>	30	70	Produces reuterin; reduces adhesion of <i>E. coli</i> and <i>Staphylococcus</i> spp.
<i>Enterococcus faecalis</i>	60	50	Permanent commensal; excessive levels are associated with inflammatory processes.
<i>Staphylococcus aureus</i>	40	10	Opportunistic pathogen; inhibited by phytogetic phenolic compounds.
<i>Staphylococcus epidermidis</i>	30	10	Reduced carriage indicates improved hygienic status of the intestinal mucosa.

Table 3. Continued

Taxon	Group, n = 10 animals		Description
	I (control)	II (experimental)	
<i>Streptococcus suis</i>	30	10	Potentially pathogenic; reduced carriage indicates a preventive effect.
<i>Clostridium butyricum</i>	20	40	Butyrate producer; improves epithelial regeneration and energy metabolism.
<i>Eubacterium rectale</i>	10	30	Produces butyrate; involved in stabilisation of the intestinal barrier function.
<i>Escherichia coli</i> (non-pathogenic strains)	30	15	Reduced putrefactive processes and proteolytic activity.

Source: authors' development

The presence of *Streptococcus alactolyticus* in 100% of samples from both groups confirms its role as a permanent dominant in the large intestine of pigs. As reported by H. Wang *et al.* (2023), *S. alactolyticus* contributes to carbohydrate fermentation and the production of short-chain fatty acids (SCFAs), which are essential for the energy supply of enterocytes. In the experimental group, the proportion of lactobacilli increased more than twofold. This may be attributed to the phytogenic additive containing garlic and caraway extracts, which likely stimulated the growth of lactic acid bacteria due to the presence of biologically active compounds with prebiotic properties. The observed reduction in the prevalence of *S. aureus*, *S. epidermidis*, and *S. suis* (by 3-4 times) may be associated with the antimicrobial activity of essential oils that disrupt the cell walls of Gram-positive bacteria. In addition, *Lactobacillus reuteri* produces reuterin, which exhibits bacteriostatic activity against *Staphylococcus* and *E. coli*, explaining the lower carriage rate of opportunistic microorganisms in the experimental group. *Clostridium butyricum* and *Eubacterium rectale* were detected in 40% and 30% of experimental samples, respectively, which was twice as high as in the control group. Butyrate is a key metabolite for nourishing the colonic epithelium and regulating apoptosis and anti-inflammatory responses. The increased abundance of these bacteria indicates activation of anaerobic fermentation and normalisation of microbial homeostasis.

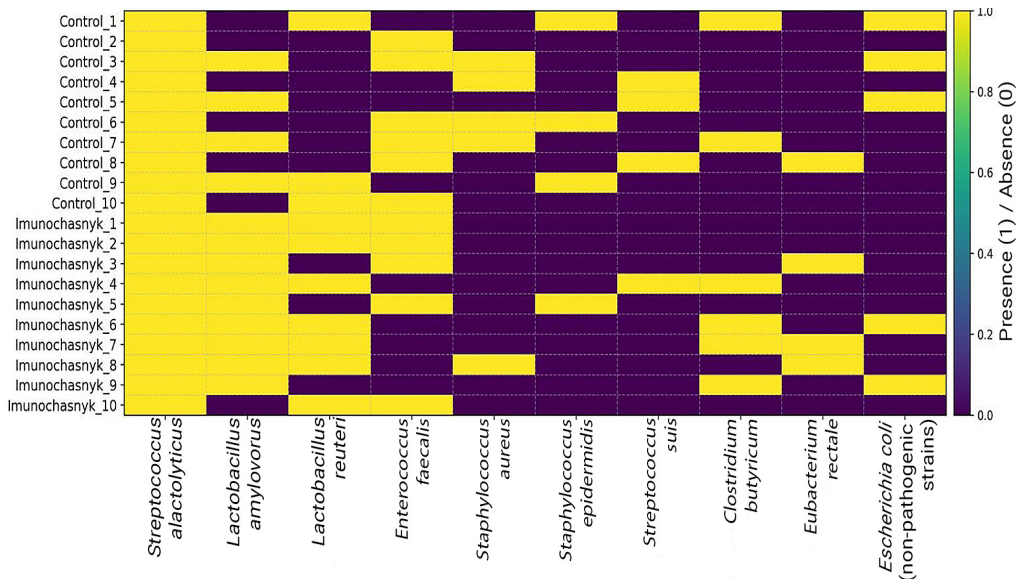
The reduction in the prevalence of *Escherichia coli* in the experimental group to 15%

reflects a positive effect of the phytogenic additive on the balance of the intestinal ecosystem. It is evident that an increased abundance of *Lactobacillus* spp. and butyrate-producing bacteria is associated with suppression of *E. coli* growth, a reduction in putrefactive processes, and improved nutrient utilisation. The complete repertoire of the presence/absence of microbiota taxa in intestinal samples from the experimental replacement gilts is presented in Figure 1. The obtained results demonstrated a classical pattern of microbial balance shift from facultative anaerobes towards obligate fermenters, which ensure a stable environment in the large intestine. This direction of change corresponds to contemporary concepts regarding the mechanism of action of phytogenic feed additives, namely stimulation of probiotic microbiota, inhibition of pathogenic microorganisms, and enhancement of intestinal colonisation resistance.

The obtained results of the study confirmed a significant positive effect of the experimental phytogenic feed additive on the productivity, metabolic status, and intestinal microbiota of replacement gilts. Starting from the 17<sup>th</sup> week of rearing, animals receiving the additive showed a significant advantage in live weight and average daily gains compared with the control group ( $p \leq 0.001$ ), which persisted until the end of the experiment. At 28 weeks of age, animals in the experimental group demonstrated an average live weight of 128.52 kg compared with 118.64 kg in the control group, while average daily gains amounted to 765.71 g versus

745.71 g, respectively ( $p \leq 0.01$ ). In addition, survival rate in the group of gilts receiving the experimental phytobiotic reached 95%, whereas in the control group this indicator did not exceed 85%. Similar results have been reported in contemporary scientific reviews. For example, M. Madesh *et al.* (2025) emphasised that the use of phytogetic feed additives in pig production contributes to improved productivity, nutrient utilisation, and feeding efficiency through modulation of the microbiota and stimulation of

digestive enzymatic activity. Likewise, M. Mahboubi (2019) and H.A. Cho *et al.* (2024) demonstrated that inclusion of phytogetic mixtures in the diets of fattening pigs increased average daily gains and reduced stress-induced physiological responses. Thus, the results of the present experiment are consistent with current evidence indicating that phytogetic additives exert a combined stimulatory effect by simultaneously influencing the microbiota, metabolism, and overall physiological status of animals.



**Figure 1.** Presence/absence of species-level microbiota taxa in the experimental groups of replacement gilts

**Source:** authors' development

Biochemical blood serum indicators reflected an improvement in metabolic processes and the functional state of the liver in replacement gilts receiving the experimental feed additive. An increase in albumin content (25.33 g/L) combined with a decrease in globulins resulted in an increase in the protein coefficient to 0.84, indicating optimisation of protein synthesis and a better balance between plastic and immune processes (Li *et al.*, 2021). In the present study, ALT and AST activities in animals of the experimental group exceeded those of the control group but remained within

the physiological range, while the de Ritis index (0.92) indicated the absence of hepatocyte damage. An increase in alkaline phosphatase (ALP) activity by 21.86 U/L reflected intensification of growth processes and bone tissue remodelling, whereas a decrease in gamma-glutamyltransferase (GGT) to 25.84 U/L indicated a reduced hepatic load and improved detoxification function of the liver. Similar "liver-safe" profiles under phytogetic feeding strategies were described by F. Chen *et al.* (2024), who reported that phytogetic additives and essential oils in pigs improved

antioxidant status without inducing pathological shifts in hepatic enzyme activity; under certain feeding regimens, an increase in plasma antioxidant enzymes was also observed without adverse effects on AST or ALT. Comparable trends were reported by A.O. John (2024) and V.G. Papatziros *et al.* (2024), who documented improvements in haematological and enzymatic parameters in pigs receiving phytogenic feed additives.

The blood glucose concentration in the experimental animals (5.76 mmol/L) was slightly higher than in the control group (5.25 mmol/L), indicating more efficient carbohydrate utilisation and a more stable energy balance. Higher serum levels of calcium (2.58 mmol/L) and phosphorus (3.70 mmol/L) also reflected improved mineral metabolism and active growth processes. The increased iron concentration (28.72 versus 18.85  $\mu\text{mol/L}$ ) indicated stimulation of erythropoiesis and enhanced oxygen transport, thereby supporting more intensive growth of the animals. At the same time, the reduction in indirect bilirubin levels (8.94 versus 19.38  $\mu\text{mol/L}$  in the control group) indicated stabilisation of liver functional status and a decrease in haemolysis processes. Similar changes were described by C.N. Shili *et al.* (2021), who emphasised that the state of the intestinal microbiota directly influences hepatoprotective function, hepatic enzyme activity, and overall metabolism in pigs. The biochemical results of the present study are consistent with the findings of E.R. Grela *et al.* (2013) and M. Mohammadi & I.H. Kim (2018) regarding the effects of phytogenic mixtures on oxidative stress and inflammatory markers in pigs. For example, supplementation of garlic powder combined with oregano essential oil in weaned piglets improved productivity without signs of systemic inflammation or adverse changes in oxidative stress biomarkers at an optimal dose (0.4%), whereas overdosing negated the beneficial effect, highlighting the importance of appropriate inclusion technology and dose titration. This aligns well with the concept of the present mixture at a dose of 0.1% as a “mild” modulator rather than an aggressive antimicrobial agent.

The microbiome shifts observed in the present study exhibited a clear functional vector: in the experimental group, the proportion of acid-producing and butyrate-generating taxa increased (in particular *Lactobacillus amylovorus*, *L. reuteri*, *Clostridium butyricum*, and *Eubacterium rectale*), whereas in the control group facultative anaerobes predominated (*Streptococcus alactolyticus*, *Enterococcus faecalis*, *Staphylococcus* spp.). The direction of these changes fits well within the contemporary model of the “feed – microbiota – metabolism axis”, whereby stimulation of microbial carbohydrate fermentation in the large intestine enhances the production of short-chain fatty acids (SCFAs), which nourish colonocytes, stabilise the intestinal barrier, and reduce systemic inflammation. Experimental studies by F. Wan *et al.* (2024) have demonstrated that phytogenic strategies and butyrate-oriented approaches modify the pig gut microbiome, increase SCFA production, and improve anti-inflammatory and antioxidant indices.

As shown by the results of the study, the application of *Lactobacillus reuteri*, particularly the LR1 strain, may serve as an effective means of improving intestinal barrier function in pigs. The high abundance of this strain in the experimental group indicates its role in modulating the microbiota of the large intestine, specifically by increasing the proportion of *Lactobacillus* spp., which has a beneficial effect on epithelial integrity. Similar findings have been reported in other studies, notably by B. Yang *et al.* (2022), who demonstrated that the use of *L. reuteri* LR1 improved the caecal microbiome and promoted restoration of intestinal barrier function in piglets. This effect may contribute to a reduction in the carriage of opportunistic pathogenic bacteria, such as enterobacteria and staphylococci, through the production of reuterin – a natural antimicrobial metabolite. In addition, the increased concentration of *Lactobacillus reuteri* in the experimental group of pigs confirms the ability of this strain to reduce adhesion of *Escherichia coli* and *Staphylococcus* spp., thereby improving

the hygienic status of the intestinal mucosa and reducing the level of pathogenic microorganisms in the intestinal environment.

The increased proportion of butyrate-producing bacteria (*Clostridium butyricum*, *Eubacterium rectale*) provides a mechanistically convincing explanation for the biochemical shifts observed in the present study. Butyrate is the primary “fuel” for colonic enterocytes, modulates gene expression, exhibits anti-inflammatory properties, and is associated with improved metabolic efficiency. In pigs, enhancement of endogenous butyrate production through dietary approaches – such as supplementation with protected butyrate, prebiotics, or microbiome-targeted phytogetic mixtures – has been linked to reduced pro-inflammatory cytokine levels and improved antioxidant responses (Wan *et al.*, 2024). This is consistent with the ALP/GGT profile observed in the present study, as well as with improved indicators of energy metabolism (glucose), mineral metabolism (Ca, P), and erythropoiesis (Fe). In the current experiment, such effects may have been among the factors contributing to increased live weight and improved blood parameters, as also reported by J.O. Alagbe *et al.* (2024).

The role of garlic and caraway essential oils in the phytogetic feed additive also includes a selectively antimicrobial component: phenolic compounds (carvacrol and thymol), as well as sulphur-containing garlic compounds, particularly allicin, inhibit *Staphylococcus aureus*, *S. epidermidis*, and *Streptococcus suis* while not disrupting beneficial anaerobic consortia. Studies in pigs have already demonstrated that combinations of essential oils (oregano, mint, thyme, etc.) restructure the microbial profile in a direction favourable for productivity without compromising safety based on key biomarkers (Duarte & Kim, 2022). This provides additional support for the present observation of a reduced prevalence of *Escherichia coli* (from 30% to 15%) and a decline in staphylococci in the experimental group.

In summary, the positive changes in productivity and metabolic status observed in replacement

gilts receiving the experimental phytogetic can be attributed to the systemic effects of phytogetic components, which simultaneously regulate microbiota composition, enzymatic activity, protein and mineral metabolism, and the antioxidant status of the organism. This is consistent with the concept of the “feed – microbiota – metabolism axis”, according to which a stable and balanced microbiota promotes more efficient energy utilisation, supports liver function, reduces systemic inflammation, and enhances animal productivity. Thus, the inclusion of the phytogetic additive “Imunochasnyk” in the diet of replacement gilts may be considered an effective means of biological modulation of metabolism, improvement of health status, and enhancement of growth intensity under industrial production conditions.

## Conclusions

The results of the study confirm that the inclusion of a phytogetic feed additive in the diet of replacement gilts of the (LW × L) genotype at a dose of 0.10% of feed weight exerted a pronounced positive effect on productivity, metabolic status, and the microbiota of the large intestine. Starting from the 17th week of rearing, animals in the experimental group demonstrated a significant advantage in live weight and average daily gains ( $p \leq 0.001$ ), which persisted until the end of the study; by 28 weeks of age, live weight exceeded the control by 9.88 kg, while herd survival reached 95%, which was 10% higher than in the control group. The experimental feed additive thus exhibited growth-promoting and preventive effects, improving the viability of replacement gilts. Biochemical indicators reflected optimisation of protein and mineral metabolism, increased albumin levels and protein coefficient, and reduced GGT and indirect bilirubin concentrations, indicating a hepatoprotective effect and improved metabolic adaptation. In addition, increased calcium, phosphorus, and iron levels indicated activation of bone formation and erythropoiesis, while elevated glucose concentration reflected a more stable energy balance.

Bacteriological investigations demonstrated that the use of the investigated feed additive induced a shift in the microbiota towards beneficial anaerobic microorganisms. In the experimental group, the proportions of *Lactobacillus amylovorus*, *L. reuteri*, *Clostridium butyricum*, and *Eubacterium rectale* increased, whereas the abundance of potentially pathogenic *Staphylococcus* spp. and *Streptococcus suis* decreased by 3-4 times. Such microbial reconfiguration enhances the production of short-chain fatty acids, particularly butyrate, which improves intestinal barrier function and antioxidant status.

Overall, the experimental additive can be considered an effective and safe immunostimulatory agent for young pigs, exerting its effects through the “feed – microbiota – metabolism axis” mechanism and ensuring improved growth performance, maintenance of hepatic homeostasis, enhanced intestinal colonisation resistance, and

better overall physiological status of the animals. Further studies should focus on determining the optimal dosage and duration of supplementation for different pig breeds, investigating the dynamics of immunological and antioxidant markers, and developing models of sustainable pig production based on the use of natural phytogenic stimulators.

### Acknowledgements

The authors express their gratitude to the owner and staff of the privately leased enterprise “Victoria” for providing the facilities and resources for the scientific research.

### Funding

None.

### Conflict of Interest

None.

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## Вплив фітогенного препарату на продуктивність, біохімічний статус крові та мікробіоту кишківника ремонтних свинок

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**Анотація.** В умовах збільшення використання антибіотиків у свинарстві зростає потреба в альтернативних рішеннях для підтримки здоров'я та продуктивності тварин, що обумовлює важливість дослідження ефективності фітогенних добавок у годівлі свиней. Дослідження присвячене оцінці впливу фітогенної кормової добавки, створеної на основі висушеного часнику та кмину, на продуктивність, метаболічний статус і мікробіоту товстого кишківника ремонтних свинок генотипу (ВБ × Л). Експеримент проведено у виробничих умовах на двох групах тварин ( $n = 80$ ), які отримували основний раціон без добавки (контроль) або з додаванням кормової добавки у дозі 0,10 % від маси комбікорму (дослід). Починаючи з 17-го тижня вирощування, у свинок дослідної групи спостерігалось достовірне переважання за живою масою ( $p \leq 0,001$ ) та середньодобовими приростами ( $p \leq 0,01$ ), яке зберігалось до 28-тижневого віку. Загальна збереженість тварин становила 95 % проти 85 % у контролі, що свідчить про покращення фізіологічного стану та стійкості до стресу. Біохімічні показники сироватки крові засвідчили підвищення вмісту альбумінів, білкового коефіцієнта, кальцію, фосфору та заліза при одночасному зниженні  $\gamma$ -глутамілтрансферази і непрямого білірубину, що відображає оптимізацію білкового, мінерального та пігментного обмінів і гепатопротекторний ефект. Бактеріологічний аналіз показав зростання частки *Lactobacillus amylovorus*, *L. reuteri*, *Clostridium butyricum* і *Eubacterium rectale* та зниження *Staphylococcus aureus*, *S. epidermidis* і *Streptococcus suis*, що свідчить про відновлення мікробного балансу, підвищення продукції коротколанцюгових жирних кислот і колонізаційної резистентності кишківника. Встановлено, що використана фітогенна добавка проявляє комбінований стимулюючий ефект, одночасно впливаючи на мікробіоту, обмін речовин і фізіологічну адаптацію тварин

**Ключові слова:** біохімічні показники крові; імунотулювання; статє свинарство; товстий кишківник; фітобіотик



UDC 639.2:621.824

DOI: 10.31548/dopovidi/6.2025.144

## Key areas of decarbonisation in the maritime industry

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**Abstract.** The study aimed to assess the environmental impacts of alternative fuels in the maritime sector, particularly their potential for global warming, ocean acidification, eutrophication and marine toxicity. To this end, a comparative analysis of different fuels was conducted, including liquefied natural gas, green methanol products, ammonia, biofuels and traditional heavy fuel oil. The results showed that liquefied natural gas, despite its lower CO<sub>2</sub> emissions, had a high climate impact due to methane leaks, with a global warming potential of 0.18 to 0.22 kg CO<sub>2</sub>-eq/MJ. Green biofuels, particularly methanol, had the lowest global warming potential (0.016-0.020 kg CO<sub>2</sub>-eq/MJ), but their direct CO<sub>2</sub> emissions during combustion remained high. Ammonia, as a carbon-free fuel, reduced CO<sub>2</sub> emissions but produced significant amounts of nitrous oxide (N<sub>2</sub>O), which has a significant impact on the climate balance. The ocean acidification potential for heavy fuel oil was 0.18 kg SO<sub>2</sub>-eq/MJ, and for ammonia, 0.10 kg SO<sub>2</sub> eq/MJ. Eutrophication analysis showed that ammonia and nitrous oxide emissions significantly increase nitrogen levels in marine ecosystems, which can cause algae growth. The toxicity of different fuels showed that ammonia has the greatest potential to harm marine organisms, even at low concentrations. The practical significance of the results is determined by the need for a comprehensive assessment of the environmental impact of the transition to alternative fuels in shipping, which can be used by maritime transport authorities, environmental agencies, seaports, and companies involved in the development and implementation of technologies for decarbonising shipping and reducing the environmental impact of maritime activities

**Keywords:** alternative fuels; environmental impact; global warming; acidification potential; ammonia; methanol

### Suggested Citation:

Savchuk, Ye. (2025). Key areas of decarbonisation in the maritime industry. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 144-157. doi: 10.31548/dopovidi/6.2025.144.

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## Introduction

Maritime transport is one of the main sources of global greenhouse gas emissions, which significantly affects the planet's climate stability. It contributes not only to increased CO<sub>2</sub> concentrations in the atmosphere, but also to the release of other harmful substances such as nitrogen oxides (NO<sub>x</sub>), sulphur (SO<sub>x</sub>) and particulate matter (PM), which negatively affect air quality and marine ecosystems. In addition, water pollution and ocean acidification are among the main factors disrupting biogeochemical cycles, particularly the carbon and nitrogen cycles, which threaten marine life. This necessitates the urgent adaptation of the maritime industry to new environmental standards, in particular the decarbonisation of the maritime sector. Under these conditions, a review of the literature was used to compare the results of different research approaches and derive consistent guidelines for practice.

D.M. Reshetkov & N.L. Pavlova (2022a) reviewed key initiatives to reduce greenhouse gas emissions in seaports. The study noted the significance of renewable energy sources and alternative fuels in reducing the environmental impact of maritime transport. The study examined initiatives involving the installation of technologies to reduce CO<sub>2</sub> emissions and the transition to more sustainable energy sources. In another paper, D.M. Reshetkov & N.L. Pavlova (2022b) examined global initiatives related to the decarbonisation of maritime transport, in particular the introduction of alternative fuels such as liquefied natural gas (LNG) and biofuels, and their role in reducing greenhouse gas emissions. The study noted that national and international organisations are already working to create a regulatory framework for such initiatives. The significance of digitalisation and automation of processes in the maritime sector for effective decarbonisation was considered by B. Kormych & T. Averochkina (2024). The study noted that the use of digital technologies can be used for the effective management of seaports and the reduction of CO<sub>2</sub> emissions, which is a substantial step in the decarbonisation process.

A systematic review of technological and operational measures in shipping aimed at achieving the International Maritime Organisation's 2050 targets was conducted by G. Mallouppas & E.A. Yfantis (2021). The study examined in detail alternative fuel technologies, such as methanol and ammonia, as well as innovative technologies, including rotary sails and wind-assisted propulsion, to reduce CO<sub>2</sub> emissions and other pollutants. E. Kostidi & D. Lyridis (2024) emphasised ports as substantial elements of maritime decarbonisation. The study conducted a bibliometric review that identified promising areas for reducing emissions in ports, such as the use of renewable energy sources and technologies to optimise energy consumption. An overview of the role of alternative fuels in the maritime industry, highlighting opportunities and technological limitations (e.g., methane leaks in LNG), was provided by V.A. dos Santos *et al.* (2022). The study emphasised the potential of biofuels and ammonia as fuels in shipping, as well as their ability to reduce greenhouse gas emissions compared to traditional fuels.

R. Bhattacharyya *et al.* (2023) examined the role of nuclear energy in the decarbonisation of the maritime industry. The study analysed the possibility of using nuclear energy to reduce CO<sub>2</sub> emissions in maritime transport and outlined the prospects and challenges for its integration into modern ships. Studying port decarbonisation strategies, A. Raihan *et al.* (2025) noted that one of the main trends is the use of low-carbon technologies, including hybrid systems and wind assistance, to reduce CO<sub>2</sub> emissions in port areas. The study noted the relevance of the integration of these technologies with modern port management systems. T. Ma *et al.* (2025) analysed modern research trends in decarbonisation of the maritime industry, focusing on emerging technologies such as the use of hydrogen fuels, biofuels and electrification of maritime transport. The scientists also proposed a structural analysis of decarbonisation trends, in particular, the use of various types of alternative fuels. A bibliometric study by

G. Xiao *et al.* (2025), which covered the use of digital technologies in shipping for decarbonisation, revealed a significant increase in publications on digital twins, blockchain systems and the Internet of Things (IoT). The study noted that despite the high interest in digital tools, their impact on actual CO<sub>2</sub> emissions remains rather limited.

Decarbonisation of the maritime sector is an integral part of combating climate change and achieving sustainable development. A review of research and initiatives has shown that decarbonising maritime transport requires a comprehensive approach that includes the use of alternative fuels, the introduction of innovative technologies such as wind-assisted propulsion and hybrid systems, and the application of digital technologies for energy management and emissions reduction. These initiatives have the potential to reduce the negative impact of maritime transport on the climate, ocean acidification and marine ecosystems, as well as to improve the maritime industry's resilience to environmental challenges.

The study aimed to analyse the main directions of decarbonisation of maritime transport using alternative fuels and innovative technologies, in particular their impact on ecosystems and climate change. To achieve this goal, the following tasks were set: to analyse the environmental effects of using various alternative fuels on global warming, ocean acidity and biogeochemical cycles; to investigate the toxic effects of new fuels on marine organisms, in particular fish, shrimp and phytoplankton; to assess the risks associated with the environmental and climate impacts of new technologies in maritime transport.

## Materials and Methods

A study of the environmental impact of using alternative fuels in the global maritime industry was conducted for the period 2000-2025 using several environmental assessment methods to determine the impact of different types of fuel on the environment and marine ecosystems. To achieve this goal, Life Cycle Assessment (LCA) and Ecological Risk Assessment (ERA) methods were

used, as well as methods that assess the impact on marine ecosystems. All methods were aimed at a comparative analysis of the environmental characteristics of fuels such as liquefied natural gas (LNG), methanol, ammonia, biofuels and traditional heavy fuel oil.

To assess the environmental impact of alternative fuels, the LCA method was used, which made it possible to determine the overall environmental impact of fuel at all stages of its life cycle, from raw material extraction to fuel use on board a ship. For each type of fuel, the boundaries of the system were defined, covering the stages of extraction, transportation, storage, consumption and emissions associated with its combustion. One MJ of energy obtained from fuel combustion was chosen as the unit of operation. The inventory collected data on greenhouse gas emissions such as CO<sub>2</sub>, CH<sub>4</sub>, NO<sub>x</sub>, SO<sub>x</sub>, NH<sub>3</sub>, as well as particulate matter (PM). Primary data on emissions from liquefied natural gas and traditional fuels were obtained from SEA\LNG Limited & Society for Gas as a Marine Fuel Limited (2019), N. Pavlenko *et al.* (2020). The analysis of emissions from green methanol and ammonia was based on data from H. Güleroğlu & Z. Yumurtacı (2025) and O. Guyon *et al.* (2025). Additional information on the pollutant profile is provided by studies by J. Hansson *et al.* (2019), R. Laursen *et al.* (2022), and V. Dulière *et al.* (2020). These data assessed the impact of different types of fuel on environmental categories such as global warming (GWP100), ocean acidification, eutrophication, toxicity to biota, and climate change. The environmental impact assessment was conducted using specialised SimaPro software, which was used to model the fuel life cycle and calculate the total impact on the environment.

The acidification potential of the ocean was assessed based on modelling the transformation and atmospheric deposition of the main acid-forming compounds, sulphur oxides (SO<sub>x</sub>) and nitrogen oxides (NO<sub>x</sub>). For each type of fuel, the contribution to acidification was calculated based on SO<sub>x</sub> emissions (for heavy fuel oil and

other carbon fuels) and  $\text{NO}_x$  emissions (for ammonia and fuels with high combustion temperatures). The quantitative assessment of acidification potential was expressed in  $\text{SO}_2$  equivalents per unit of energy ( $\text{kg SO}_2\text{-eq/MJ}$ ) according to standard LCA protocols (Hansson *et al.*, 2019; Campbell *et al.*, 2021). The direct impact on seawater pH from the discharge of alkaline effluents from exhaust gas treatment systems (scrubbers) was assessed separately, using monitoring and modelling data on the chemical composition of effluents (Stripple & Zhang, 2019).

The ERA method was used to analyse the toxicity of fuel and its combustion products, such as nitrous oxide ( $\text{N}_2\text{O}$ ) for ammonia and methanol, and methane for LNG. ERA involved identifying hazards arising from fuel use and determining the impact of toxic substances on marine ecosystems, particularly plankton, fish and benthic organisms. The pathways of exposure were determined through emissions to the atmosphere, which enter the ocean and biota through the deposition of toxic compounds. The ERA also included determining the probability of environmental impacts such as changes in ocean pH, toxicity to organisms and disruption of biodiversity. As a result, pollution levels for different types of fuel were calculated, and the potential threat to marine ecosystems was assessed.

Various methods were used to assess the impact of alternative fuels on marine ecosystems, including monitoring changes in water pH, toxic effects on marine organisms (plankton, fish, benthic organisms), and assessment of the impact on biodiversity. pH change was assessed based on ocean acidification potential, which was determined through  $\text{SO}_x$  emissions for heavy fuel oil and  $\text{NO}_x$  emissions for ammonia (Campbell *et al.*, 2021). The impact of emission cleaning technologies, in particular scrubbers, on the marine environment was analysed separately, with data obtained from the source (Stripple & Zhang, 2019). For each fuel type, the analysis examined how emissions of these pollutants alter ocean acidity and how this affects marine ecosystems. The toxic effects on

marine organisms were also assessed using  $\text{LC}_{50}$  (lethal concentration for 50% of organisms) and  $\text{EC}_{50}$  (concentration at which 50% of organisms experience development or growth) indicators. The effects on fish, shrimp, oysters and other marine organisms were assessed, as well as the consequences for phytoplankton (Miller *et al.*, 1990). In addition, secondary pollutants such as nitrates formed as a result of ammonia combustion, which have a chronic impact on marine ecosystems, in particular on algae growth and biodiversity decline, were studied (Wang *et al.*, 2022).

Standard statistical analysis was used to process the data obtained, in particular t-tests to compare the average values between different types of fuel and their environmental impacts. The level of statistical significance was assessed using p-values, where the significance level  $\alpha$  was set at 0.05. To analyse variations and compare environmental effects depending on fuel type, appropriate statistical methods were used to accurately assess the difference in impacts and identify the most environmentally efficient fuels for the maritime industry.

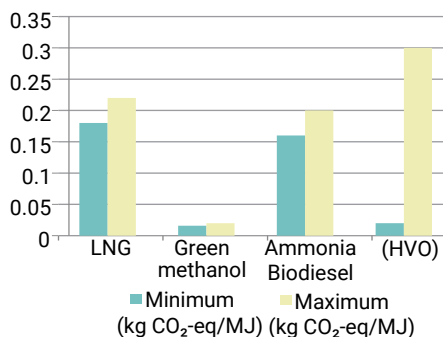
## Results

### Global warming potential (GWP100) of different types of fuel

An analysis of the global warming potential of alternative marine fuels revealed significant differences between fuels due to the specific nature of their emissions throughout their entire life cycle. For LNG, the study determined that despite lower direct carbon dioxide emissions during combustion compared to traditional fuel oil, the climate advantage was largely offset by methane leaks. The global warming potential over a 100-year horizon (GWP100) for LNG ranged from 0.18 to 0.22  $\text{kg CO}_2\text{-eq/MJ}$ . This range was due to the high global warming potential of methane, which is 28-36 times higher than that of  $\text{CO}_2$ , and varied depending on the level of leaks during production, transportation and use (SEA\LNG Limited & Society for Gas as a Marine Fuel Limited, 2019; Pavlenko *et al.*, 2020).

The “Green” methanol, obtained from renewable raw materials, showed significantly lower values. Its GWP100 was estimated to range from 0.016 to 0.020 kg CO<sub>2</sub>-eq/MJ for methanol produced from renewable energy sources such as wind or solar energy. However, direct CO<sub>2</sub> emissions from fuel combustion in marine engines remained high, indicating the need for compensation through a closed carbon cycle to achieve climate neutrality (Güleroğlu & Yumurtacı, 2025; Guyon *et al.*, 2025).

Ammonia, being a carbon-free fuel, was characterised by zero direct CO<sub>2</sub> emissions. However, the study identified a key problem related to incomplete combustion, which led to the formation of nitrous oxide (N<sub>2</sub>O). Since the global warming potential of N<sub>2</sub>O exceeds that of CO<sub>2</sub> by 265–298 times, even minor emissions have a significant impact on the overall balance. In the absence of optimised engines and exhaust gas cleaning systems, the total GWP100 for ammonia could reach 0.16–0.20 kg CO<sub>2</sub>-eq/MJ. As for biodiesel (HVO) and other biofuels, their net CO<sub>2</sub> balance was close to zero, as confirmed by life cycle analysis data. However, GWP100 values showed a significant range from 0.02 to 0.30 kg CO<sub>2</sub>-eq/MJ (Guyon *et al.*, 2025). This variability was directly related to the type of feedstock used for production and the effects of indirect land use changes, which could significantly increase the overall greenhouse footprint (Fig. 1).



**Figure 1.** Comparison of average GWP100 values for different alternative marine fuels

**Source:** compiled by the author based on N. Pavlenko *et al.* (2020), O. Guyon *et al.* (2025), H. Güleroğlu & Z. Yumurtacı (2025)

Thus, the analysis of global warming potential (GWP100) for promising types of marine fuel revealed significant heterogeneity in their climate impact. Despite the rejection of carbon, all alternative fuels showed significant compromises. For LNG, methane leaks were the main factor; for ammonia, it was nitrous oxide formation; and for biofuels, it was high dependence on the raw material base and the consequences of land use changes. Even “green” methanol, which had the lowest scores, required further consideration of direct CO<sub>2</sub> emissions. The results confirmed that switching to any of the fuels considered is not an unconditional solution for mitigating the climate impact of shipping and requires a comprehensive assessment of all greenhouse gases throughout the entire life cycle.

#### **Acidification potential of the ocean and eutrophication potential**

A detailed analysis of the potential for ocean acidification revealed complex interactions between various pollutants produced by different types of fuel and technologies. For traditional heavy fuel oil with high sulphur content, the main mechanism of acidification was emissions of sulphur oxides (SO<sub>x</sub>). As a result of photochemical reactions in the atmosphere, SO<sub>x</sub> was oxidised to sulphur trioxide (SO<sub>3</sub>), which, interacting with atmospheric moisture, formed sulphuric acid aerosols (H<sub>2</sub>SO<sub>4</sub>). Further atmospheric leaching of these aerosols by rainfall led to the entry of hydrogen ions (H<sup>+</sup>) and sulphate ions (SO<sub>4</sub><sup>2-</sup>) into the surface layers of the ocean, lowering their pH. The quantitative assessment of the acidification potential for heavy fuel oil was 0.18 kg SO<sub>2</sub>-eq/MJ, confirming its significant contribution to global acidification (Hansson *et al.*, 2019).

When using carbon-free fuels, in particular ammonia, the profile of pollutants changed significantly. Although SO<sub>x</sub> emissions were completely absent, the main driver of acidification was nitrogen oxide (NO<sub>x</sub>) emissions, which were formed during high-temperature combustion in internal combustion engines. In the atmosphere,

$\text{NO}_x$  oxidised to nitrogen dioxide ( $\text{NO}_2$ ), which reacted with hydroxyl radicals ( $\text{OH}\cdot$ ) to form nitric acid ( $\text{HNO}_3$ ). This acid, settling on the ocean surface, dissociated with the release of  $\text{H}^+$  ions and nitrate ions ( $\text{NO}_3^-$ ). Calculations showed that the acidification potential for ammonia reached 0.10 kg  $\text{SO}_2$ -eq/MJ, which rendered it comparable in impact to low-sulphur fuels, but with a different chemical mechanism (Laursen *et al.*, 2022).

The impact of scrubber technology (exhaust gas cleaning systems) was studied separately. The study determined that when an open scrubber system was used to remove  $\text{SO}_x$  from

flue gases, pollutants were transferred from the gas phase to the water phase. The discharge of these alkaline effluents, containing sulphites, sulphates and heavy metals, directly into the marine environment caused sharp local changes in pH. In contrast to the global phenomenon of acidification from atmospheric deposition, this effect was localised, creating areas of chemical stress in port waters and shipping lanes where massive discharges of wastewater occurred. The concentration of pollutants in effluents could exceed background values by tens of times (Dulière *et al.*, 2020) (Table 1).

**Table 1.** Comparative indicators of acidification potential and main chemical mechanisms of impact

Influence source	Primary chemical agent	Reaction product	Acidification potential (kg $\text{SO}_2$ -eq/MJ)
Heavy fuel oil	$\text{SO}_x$	$\text{H}_2\text{SO}_4$	0.18
Ammonia	$\text{NO}_x$	$\text{HNO}_3$	0.10
Scrubbers (drains)	Sulphites/Sulphates	Alkaline effluents (local pH increase)	Not measured in $\text{SO}_2$ -eq. (direct chemical pollution)

**Source:** compiled by the author based on H. Stripple & Y. Zhang (2019), V. Dulière *et al.* (2020), M. Campbell *et al.* (2021)

The assessment showed that the potential for ocean acidification from maritime activities was shaped by several interrelated pathways. Traditional fuel oil remained the main source of acidification through  $\text{SO}_x$  emissions, while the transition to ammonia shifted this impact to  $\text{NO}_x$  emissions with similar harmful effects. At the same time, scrubber technology used to reduce atmospheric emissions created an additional burden on the marine environment through the direct discharge of chemically active effluents. Thus, various options for decarbonising ship energy did not eliminate the problem of acidification, but only transformed its manifestations, creating both global and localised threats to marine ecosystems.

A detailed analysis of the eutrophication potential of marine activities revealed complex dynamics of nutrient inputs into marine ecosystems, particularly using new types of fuel. The most significant source of nitrogen pollution was found to be direct emissions of ammonia ( $\text{NH}_3$ ) when used as a primary fuel. Unreacted ammonia

entered the atmosphere, where it underwent transformation and transport over long distances. Further dry and wet deposition of nitrogen compounds on the ocean surface led to excessive input of bioavailable nitrogen into the marine environment. A quantitative assessment within the life cycle analysis showed that the eutrophication potential for ammonia was 0.065 kg  $\text{PO}_4$ -eq/MJ, which exceeded similar indicators for traditional carbon fuels. An additional contribution to eutrophication processes was made by gaseous emissions of nitrous oxide ( $\text{N}_2\text{O}$ ), which was formed as a by-product of ammonia combustion in ship engines. After entering the atmosphere,  $\text{N}_2\text{O}$  underwent photochemical transformations to form nitrate compounds, which subsequently settled on the ocean surface. This process posed a particular threat to open water areas where the natural nutrient content was a limiting factor for phytoplankton growth. Extrapolation of the data showed that even at a concentration of  $\text{N}_2\text{O}$  in emissions of 0.3% of the total volume, its

contribution to the overall eutrophication potential could reach 15-20% (Miller *et al.*, 1990).

A systematic study of the impact of ship discharges revealed their role as a significant source of local pollution. Analysis of ballast water composition showed the presence of nitrogen compounds in concentrations of 2.5-4.8 mg/l and phosphorus in concentrations of 0.3-0.7 mg/l. In areas with

intensive shipping, this led to an increase in nitrate concentrations in coastal waters by 25-40% compared to background values (Miller *et al.*, 1990). The impact was particularly noticeable in the waters of large ports, where the accumulation of nutrients from effluents stimulated the growth of microscopic algae, in particular representatives of the genera *Alexandrium* and *Pseudo-nitzschia* (Table 2).

**Table 2.** Comparative characteristics of eutrophication sources from marine activities

Influence source	Influence mechanism	Primary components	Eutrophication potential	Spatial scale of impact
Ammonia (NH <sub>3</sub> ) emissions	Atmospheric precipitation	Nitrogen compounds	0.065 kg PO <sub>4</sub> -eq/MJ	Regional (up to 500 km)
Nitrous oxide (N <sub>2</sub> O) emissions	Atmospheric transformation and precipitation	Nitrate compounds	0.010-0.015 kg PO <sub>4</sub> -eq/MJ	Global
Ballast water	Direct source	N, P, organic compounds	3.2-5.8 kg PO <sub>4</sub> -eq./equivalent ballast water	Local (ports and coastline)
Domestic waste	Direct source	N, P, organic matter	0.8-1.2 kg PO <sub>4</sub> -eq/day per vessel	Local (ports and coastline)

**Source:** compiled by the author based on D.C. Miller *et al.* (1990)

A study of the eutrophication potential of marine activities revealed the complex nature of the impact of various sources of nitrogen pollution. The use of ammonia as an alternative fuel created stable channels for the entry of bioavailable nitrogen into marine ecosystems through atmospheric deposition. At the same time, nitrogen oxide emissions and direct discharge of ship waste created an additional burden on coastal and ocean ecosystems. The results showed that the transition to nitrogen-containing fuels without adequate emission treatment and waste disposal systems could lead to a significant increase in eutrophication processes, especially in regions with intensive shipping.

#### Marine toxicity potential and comparative analysis of the environmental footprint of innovative technologies

Research into the marine toxicity potential of alternative fuels and related emissions has revealed varying levels of threat to marine organisms. Experimental data on the acute toxicity of methanol to aquatic organisms showed LD<sub>50</sub> values for fish species of 2,500-5,000 mg/L over

a 96-hour exposure period. For invertebrates, particularly shrimp of the genus *Artemia*, the LC<sub>50</sub> value was 7,800-12,000 mg/L. Calculations showed that a spill of 100 tonnes of methanol in the enclosed waters of the port could create local concentrations exceeding 1,500 mg/L, posing a threat to benthic organisms. Ammonia showed significantly higher toxicity compared to methanol. For most fish species, lethal LC<sub>50</sub> concentrations ranged from 0.2 to 2.5 mg/L after 96 hours of exposure. Sensitive invertebrates, such as crab and oyster larvae, showed lethal effects at concentrations as low as 0.5-1.2 mg/L. Modelling of a large-scale ammonia spill of 500 tonnes showed that under conditions of limited water circulation, toxic concentrations could persist in a 5-metre-thick layer of water for 24-48 hours, creating a zone of complete marine organism mortality over an area of up to 5 km<sup>2</sup>. Atmospheric deposition of nitrate compounds formed as a result of NO<sub>x</sub> emissions had toxic effects of a different type. Chronic exposure to nitrates at concentrations of 25-40 mg/l caused developmental disorders in fish larvae and a decrease in phytoplankton diversity. Experiments have shown

that nitrate concentrations above 50 mg/l inhibit the growth of macroalgae and coral polyps. In areas with intensive shipping, an increase in

background nitrate concentrations to 15-20 mg/l was observed, which could cause sublethal effects in sensitive species (Wang *et al.*, 2022) (Table 3).

**Table 3. Toxicity parameters of various pollutants for marine organisms**

Contaminant	Test organism	Toxicity value	Value	Exposure duration
Methanol	Fish ( <i>Oncorhynchus mykiss</i> )	LC <sub>50</sub>	4.500 mg/l	96 hours
Methanol	Shrimp ( <i>Artemia salina</i> )	LC <sub>50</sub>	9.500 mg/l	48 hours
Ammonia	Fish ( <i>Cyprinodon variegatus</i> )	LC <sub>50</sub>	0.8 mg/l	96 hours
Ammonia	Oyster larvae ( <i>Crassostrea gigas</i> )	LC <sub>50</sub>	1.1 mg/l	48 hours
Nitrates	Fish larvae ( <i>Danio rerio</i> )	EC <sub>50</sub> (development disorders)	45 mg/l	72 hours
Nitrates	Phytoplankton ( <i>Skeletonema costatum</i> )	EC <sub>50</sub> (growth impairment)	60 mg/l	96 hours

**Source:** compiled by the author based on Y. Wang *et al.* (2022)

The assessment of marine toxicity potential revealed significant differences in the level of threat posed by different types of fuel and associated pollutants. Ammonia proved to be the most toxic, with a direct lethal effect on marine organisms even at low concentrations. Methanol showed moderate toxicity but posed a threat in large-scale spills. Atmospheric deposition of nitrates had a chronic effect, manifested in impaired development of marine organisms and changes in the structure of plankton communities. The results showed that the transition to alternative fuels required consideration not only of their greenhouse characteristics, but also of the potential consequences for marine ecosystems in the event of accidents and long-term operational impacts.

Research into the environmental effectiveness of innovative technologies has demonstrated significant potential for reducing anthropogenic pressure on marine ecosystems. The implementation of aerodynamic and hydrodynamic improvements, in particular Flettner rotors and anti-fouling systems (ALS), has resulted in fuel consumption reductions of 8-15%, depending on the type of vessel and operating conditions. On transatlantic routes, an average reduction in CO<sub>2</sub> emissions of 12% was recorded, which amounted to approximately 1,500 tonnes per year for a standard-sized vessel. SO<sub>x</sub> emissions were reduced proportionally by 10-14% and NO<sub>x</sub> emissions by 8-11%, which

directly affected ocean acidification potential indicators. In addition, there was a 9-13% reduction in particulate matter (PM) emissions, which is relevant for maintenance of air quality in coastal areas. Hybrid energy systems demonstrated high efficiency in areas with special environmental requirements. When using rechargeable batteries for manoeuvring within ports and coastal areas, there was a sharp reduction in NO<sub>x</sub> emissions by 94-98% compared to traditional diesel engine operation. Particulate matter emissions (PM<sub>2.5</sub> and PM<sub>10</sub>) were reduced by 89-95%, significantly improving air quality in port cities. Analysis of data from large container ships showed that the use of hybrid systems for 1,200 hours per year prevented emissions of approximately 5.8 tonnes of NO<sub>x</sub> and 0.9 tonnes of particulate matter per ship. In addition, a reduction in underwater noise levels of 12-15 dB was recorded, which reduced acoustic stress for marine life (Carjova *et al.*, 2025) (Table 4). An analysis of the environmental footprint of innovative technologies has demonstrated their significant effectiveness in reducing anthropogenic impact on the marine environment. Aerodynamic and hydrodynamic improvements have led to an overall reduction in all categories of emissions through reduced fuel consumption, while hybrid energy systems have demonstrated maximum effectiveness in the most vulnerable coastal areas. The results indicate that combining

these technologies with low-carbon fuels can provide a synergistic effect in the decarbonisation of the maritime industry, minimising several types of environmental impact simultaneously.

**Table 4. Comparative effectiveness of innovative technologies in reducing emissions**

Technology	CO <sub>2</sub> emission reduction (%)	NO <sub>x</sub> emission reduction (%)	SO <sub>x</sub> emission reduction (%)	PM emission reduction (%)	Most efficient area
Flettner rotors	10-15	8-12	10-14	9-13	Open sea, areas with stable winds
ALS systems	8-12	7-10	8-12	8-11	High-speed sailing conditions
Hybrid systems	100 (in power supply mode)	94-98	95-99	89-95	Port waters, coastal areas

**Source:** compiled by the author based on K. Carjova *et al.* (2025)

## Discussion

The results of the study confirmed the heterogeneity of the potential of various alternative fuels in reducing greenhouse gas emissions, which requires a comprehensive approach to their use in maritime transport. In particular, the use of liquefied natural gas has demonstrated advantages in reducing CO<sub>2</sub> emissions compared to traditional fuel oil, but the significant role of methane leaks, which greatly increase the potential for global warming, requires additional measures to minimise this risk. This aspect is consistent with the findings of V. Koilo (2024), who emphasised the need to ensure technological excellence in reducing methane emissions when using LNG to achieve effective decarbonisation of the maritime industry.

Analysis of “green” methanol obtained from renewable energy sources has shown a significantly lower impact on global warming, as confirmed by studies such as those by V.J. Jimenez *et al.* (2022), highlighting the advantages of renewable energy sources in the context of reducing emissions in maritime transport and improving the energy efficiency of ships. However, as noted in the study, the high dependence on CO<sub>2</sub> emissions from methanol combustion requires further attention to compensation through a closed carbon cycle, which is critical to achieving climate neutrality. As for ammonia, despite its near-zero CO<sub>2</sub> emissions, the presence of nitrous oxide (N<sub>2</sub>O) as a combustion by-product significantly affects its climate potential. This observation is

consistent with the study by E. Ejder *et al.* (2024), highlighting the need to develop technologies that reduce N<sub>2</sub>O emissions in ammonia engines for effective decarbonisation of maritime transport. The researchers emphasised that ensuring proper control of incomplete combustion and improving the efficiency of dual-fuel engines is a step towards reducing the negative environmental impact of ammonia. Biofuels, in particular Hydrotreated Vegetable Oil (HVO), have the least impact on the climate, but significant variations in GWP100 from 0.02 to 0.30 kg CO<sub>2</sub>-eq/MJ indicate that efficiency depends on the type of raw material. This correlates with the findings of a study by M. Lind *et al.* (2023), which noted the importance of developing standards for assessing the life cycle of biofuels to reduce their negative impact on the climate and marine ecosystems. The study highlighted that the incorporation of all stages of the biofuel life cycle would reduce their negative impact.

Decarbonising the maritime sector is a complex and multifaceted process that requires the integration of innovative technologies and strategies to reduce emissions at all stages, from fuel production to its consumption in shipping. Studies such as that by J.M.M. Prados *et al.* (2024) highlight the significance of strategies aimed at reducing CO<sub>2</sub> emissions and supporting a stable transition to a sustainable maritime industry by 2050. The results of the study on the potential of various alternative fuels for decarbonising

the maritime industry support current scientific views on the need to develop and apply the latest technologies in the maritime sector. The study by D.R. Cunha *et al.* (2025) highlights the key challenges for decarbonising ports and the maritime industry, noting the significance of integrating renewable energy sources and alternative fuels into maritime transport to achieve sustainable development. The authors also highlighted the need to develop appropriate infrastructure and legislative support for the successful implementation of these initiatives.

Y. Wang & L.A. Wright (2021) examined the economic, technological and political challenges associated with the introduction of alternative fuels in the maritime sector. In particular, the study noted that the use of fuels such as methanol, ammonia and hydrogen has environmental potential but requires significant investment in technology and infrastructure. This is consistent with the findings of the current study, which states that when switching to alternative fuels, both positive and negative environmental impacts must be considered, including toxic effects and global warming potential. The use of hydrogen technologies for energy conservation in the maritime sector, which is one of the promising strategies for decarbonisation, has been studied by R. Jayabal (2025). The study also emphasised the significance of developing the appropriate infrastructure for hydrogen technologies, which reduces CO<sub>2</sub> emissions and contributes to the sustainable development of the industry. According to this, hydrogen is a key element of future efforts to combat climate change in the maritime sector, which is consistent with the results of a study that highlights the importance of hydrogen fuels in reducing global warming. The study by A.I. Ibokette *et al.* (2024) examines the regulatory and operational challenges of implementing zero-emission technologies in the US maritime industry. In particular, it highlights the need for improved legal regulations and government support to ensure effective decarbonisation. These results are consistent with conclusions regarding

the need to improve regulatory aspects for the integration of new technologies and fuels into maritime transport, which will ensure a more efficient decarbonisation process.

The significance of achieving sustainable development goals, in particular Goal 13 (climate action), through decarbonisation of the maritime sector, was discussed by P.C. Ezinna *et al.* (2021). The study emphasised the need to integrate environmental standards and modern technologies that will help reduce emissions and improve the environmental status of marine ecosystems. This is consistent with the findings of this study, which highlights the need to develop low-carbon technologies for maritime transport. Z.Y. Song *et al.* (2025) explored the concept of “green maritime logistics corridors” and their potential for decarbonising maritime transport. This new solution involves optimising shipping through the introduction of green technologies and the sharing of environmental practices. Based on the results of this study, it is possible to confirm the importance of developing such initiatives to reduce emissions and create sustainable logistics routes. Solutions for decarbonising international shipping, in particular, policy recommendations and technological solutions that can reduce CO<sub>2</sub> emissions, are discussed in the study by Z. Wan *et al.* (2018). The study emphasised that effective decarbonisation of maritime transport requires the introduction of innovative technologies and active cooperation with international organisations to create standards and mechanisms for financing such initiatives. This is consistent with the findings of the presented study, which indicate that decarbonisation of maritime transport requires not only technical changes but also changes in the regulatory framework to ensure an effective transition to alternative fuels and reduce emissions.

The study by A. Romano & Z. Yang (2021) provided an overview of the state of decarbonisation in shipping for the period 2000-2020, noting progress in the use of alternative fuels such as LNG, biofuels and ammonia. The researchers also emphasise the significance of integrating

decarbonisation strategies with other aspects of the maritime economy, such as economic efficiency and safety. This correlates with the results of this study, which shows that the transition to alternative fuels must be ensured not only technologically, but also economically, in terms of reducing the cost of transport services. Initiatives to improve the energy efficiency of ships and reduce CO<sub>2</sub> emissions were considered in a study by M. Issa *et al.* (2022). The researchers emphasised the importance of technologies that reduce the energy consumption of ships, such as energy recovery systems and efficient energy management systems. The results of the presented study confirm that to achieve a significant reduction in emissions, it is necessary to use both new types of fuel and efficient energy systems. An analysis of the potential for reducing greenhouse gas emissions using antifouling coatings in maritime transport was conducted by A. Farkas *et al.* (2021). The researchers noted that these technologies can reduce water resistance, leading to lower fuel consumption and, accordingly, lower CO<sub>2</sub> emissions. This supports the findings of this study, which emphasises the need to integrate various technological innovations, such as the use of new coatings and rotary sails, to achieve comprehensive decarbonisation.

In summary, it is possible to state that decarbonisation of the maritime sector requires a comprehensive approach that encompasses both the introduction of new technologies and the improvement of the regulatory framework and infrastructure. The transition to alternative fuels such as methanol, ammonia and hydrogen, as well as the integration of green technologies and digital solutions, are necessary steps to reduce emissions and improve the environmental health of marine ecosystems.

### Conclusions

Research into the environmental impacts of alternative fuels in the maritime sector has shown that different fuels have significant impacts on the environment, including global warming,

ocean acidification, eutrophication and toxicity to marine ecosystems. An analysis of the global warming potential (GWP100) for different types of fuel revealed significant variability: for LNG, GWP100 ranged from 0.18 to 0.22 kg CO<sub>2</sub>-eq/MJ, which was due to methane leaks, which have a global warming potential 28-36 times higher than CO<sub>2</sub>. At the same time, green methanol obtained from renewable energy sources had significantly lower GWP100 values, ranging from 0.016 to 0.020 kg CO<sub>2</sub>-eq/MJ, but direct CO<sub>2</sub> emissions from its combustion remained high. Ammonia, as a carbon-free fuel, showed virtually zero direct CO<sub>2</sub> emissions, but produced significant amounts of nitrous oxide (N<sub>2</sub>O), which increased its global warming potential to 0.16-0.20 kg CO<sub>2</sub> eq/MJ.

Regarding ocean acidification, the acidification potential for heavy fuel oil was 0.18 kg SO<sub>2</sub>-eq/MJ, which was due to sulphur oxide (SO<sub>x</sub>) emissions. For ammonia, this figure was lower at 0.10 kg SO<sub>2</sub>-eq/MJ, but the acidification problem was offset by NO<sub>x</sub> emissions, which formed nitric acid (HNO<sub>3</sub>). Eutrophication analysis showed that ammonia emissions caused excessive bioavailable nitrogen entering marine ecosystems. The eutrophication potential for ammonia was 0.065 kg PO<sub>4</sub>-eq/MJ, which exceeded similar indicators for traditional carbon fuels. In terms of toxicity, ammonia was found to be the most toxic to marine organisms, with an LC<sub>50</sub> for fish of 0.8 mg/L and for oyster larvae of 1.1 mg/L. Methanol, in spills of 100 tonnes, could create local concentrations exceeding 1,500 mg/l, posing a threat to benthic organisms. Innovative technologies such as the Flettner rotor and hybrid energy systems have shown significant environmental benefits, including a 12% reduction in CO<sub>2</sub> emissions, a 94-98% reduction in NO<sub>x</sub> emissions in port waters, and an 89-95% reduction in particulate matter emissions. This highlights the significance of the latest technologies in the reduction of the environmental footprint of shipping.

The results obtained showed that switching to alternative fuels in maritime transport is not an unconditional solution for mitigating environmental

impacts but requires a comprehensive assessment of all fuel life cycle factors. Further research should address the optimisation of technologies for cleaning emissions of ammonia and nitric oxide, as well as reducing methane leaks when using LNG. Incorporating all aspects of the impact of alternative fuels will help to develop strategies for the effective decarbonisation of the maritime industry.

None.

None.

None.

## Acknowledgements

## Funding

## Conflict of Interest

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## Основні напрямки декарбонізації морської галузі

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**Анотація.** Метою дослідження було оцінити екологічні наслідки використання альтернативних видів палива в морській галузі, зокрема їхній потенціал щодо глобального потепління, підкислення океанів, евтрофікації та морської токсичності. Для цього було проведено порівняльний аналіз різних видів палива, включаючи зріджений природний газ, зелену метанолову продукцію, аміак, біопалива та традиційний важкий мазут. Результати показали, що зріджений природний газ, незважаючи на знижені викиди CO<sub>2</sub>, мав високий кліматичний вплив через витоки метану, з потенціалом глобального потепління від 0,18 до 0,22 кг CO<sub>2</sub>-екв./МДж. Зелені біопалива, зокрема метанол, мали найнижчий потенціал глобального потепління (0,016-0,020 кг CO<sub>2</sub>-екв./МДж), але їхні прямі викиди CO<sub>2</sub> під час спалювання залишалися високими. Амміак, як безвуглецеве паливо, знижував викиди CO<sub>2</sub>, однак утворював значні кількості закису азоту (N<sub>2</sub>O), що суттєво впливає на кліматичний баланс. Потенціал підкислення океану для важкого мазуту становив 0,18 кг SO<sub>2</sub>-екв./МДж, а для аміаку – 0,10 кг SO<sub>2</sub>-екв./МДж. Аналіз евтрофікації показав, що викиди аміаку та закису азоту значно підвищують рівень азоту в морських екосистемах, що може викликати розвиток водоростей. Токсичність різних палив продемонструвала, що аміак має найбільший потенціал для шкоди морським організмам, навіть за низьких концентрацій. Практична значимість результатів полягає в необхідності комплексної оцінки екологічних наслідків при переході до альтернативних палив у судноплаванні, що може бути використано органами управління морським транспортом, екологічними агентствами, морськими портами, а також компаніями, які займаються розробкою і впровадженням технологій для декарбонізації судноплавання та зниження екологічного впливу морської діяльності.

**Ключові слова:** альтернативні види палива; екологічний вплив; глобальне потепління; потенціал підкислення; аміак; метанол



UDC 634.11:631.526.32

DOI: 10.31548/dopovidi/6.2025.158

## Plasticity and stability of quantitative productivity traits of apple cultivars under the conditions of Podillia

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**Abstract.** Apple was one of the leading fruit crops worldwide, ranking third in production volumes after bananas and watermelons, and the continuously increasing demand for apples determined the need to improve cultivars with high productivity and adaptability. The aim of the study was to evaluate apple cultivars in terms of ecological plasticity in order to identify promising genotypes for breeding programmes. The results of cultivar testing of apple of Ukrainian and foreign breeding (orchard established in 2017, planting scheme 2.5 × 4.5 m, rootstock MM106) were presented based on yield and average fruit weight. Five apple cultivars ('Free Gold', 'Juliet', 'Luna', 'Red Topaz', 'Rosela') selected from 27 introduced genotypes tested during 2021-2024 were evaluated. The highest values of environmental indices, both for apple yield and fruit weight, were recorded in 2023 and amounted

### Suggested Citation:

Tarnavska, K., Babiy, S., & Venediktov, O. (2025). Plasticity and stability of quantitative productivity traits of apple cultivars under the conditions of Podillia. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 158-169 doi: 10.31548/dopovidi/6.2025.158.

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to 1.80 and 6.15, respectively. The lowest environmental indices were observed in 2022 and 2024, when the average yields were 0.28 and 1.3 t ha<sup>-1</sup>, respectively. This analysis demonstrated the strong influence of annual climatic fluctuations on cultivar productivity and emphasised the need to consider genotype adaptability in breeding programmes. Based on mathematical processing of yield and average fruit weight data, regression equations and ecological plasticity indices were derived. It was established that, in terms of fruit yield, the cultivars 'Rosela' and 'Juliet' belonged to the intensive type and were characterised by high sensitivity to changes in environmental conditions, with regression coefficients of 2.743 and 0.921, respectively. For average fruit weight, a high level of ecological plasticity was demonstrated by the cultivars 'Rosela', 'Luna', and 'Free Gold', whereas 'Juliet' belonged to the neutral type. Assessment of ecological stability showed that, for yield, 'Free Gold', 'Luna', and 'Red Topaz' were the most stable cultivars. The cultivar 'Juliet' was relatively stable, although it did not exhibit a high yield level. The highest average yield over the years was recorded for 'Rosela', which was simultaneously the least stable. For average fruit weight, 'Free Gold' and 'Luna' proved to be stable cultivars

**Keywords:** yield; fruit weight; attractiveness; flavour quality; adaptability parameters; environmental index

## Introduction

Apple (*Malus domestica* Borkh.) was one of the most important fruit crops worldwide and a key component of horticulture in Ukraine. The high nutritional value of the fruits, versatility of use, long period of productive fruiting, and stable market demand ensured the strategic importance of apple orchards for the economy of the agricultural sector. Ukrainian orchards traditionally cultivated apple as a major industrial and food crop, which was an important factor in ensuring food security and the development of the processing industry.

Under current conditions of global climate change, fruit crop production was subjected to significant challenges. Increases in average annual temperatures, the occurrence of abnormal temperature fluctuations in spring and autumn, uneven distribution of precipitation, and the growing frequency of extreme weather events directly affected the structure of the apple vegetation cycle, pollination efficiency, fruit set formation, and fruit growth. M. Deori *et al.* (2024) considered that these factors had a substantial impact on fruit productivity and quality, which was manifested in pronounced interannual variability of yield even in well-established cultivars. In this context, understanding the mechanisms of adaptive responses of cultivars to changing

environmental conditions and their ability to maintain productivity under variable conditions was of particular importance.

According to R.E. Sestras & A.F. Sestras (2023), adaptability parameters, in particular ecological plasticity and stability of productive traits, were regarded as key characteristics determining the suitability of a cultivar for cultivation under specific geographical and climatic conditions. Plasticity defined the ability of a cultivar to change the level of expression of economically important traits in response to variations in environmental conditions, whereas stability reflected the variability of these traits across different growing years. High plasticity could be desirable under favourable conditions, as it allowed the maximum realisation of potential productivity. At the same time, low stability could lead to substantial fluctuations in yield, which was undesirable for predictable production. Conversely, excessively stable cultivars could be less responsive and fail to exploit their productive potential in favourable years (Kryvoshapka *et al.*, 2020).

In their study, X. Li *et al.* (2023) demonstrated that a range of mathematical approaches was applied to assess plasticity and stability, including regression analysis, environmental indices,

variation parameters, and other statistical methods. These approaches allowed an objective evaluation of cultivar response types to changing environmental conditions, the identification of genotypes with high adaptability, and the prediction of their performance under climatic uncertainty. In modern fruit crop breeding, comprehensive assessment based on such parameters was an important component in the selection of genotypes for commercial cultivation. Podillia represented an area characterised by high variability of hydrothermal conditions during the growing season. I.S. Sadovskyi & V.M. Matsievskiy (2023) demonstrated that weather conditions within a single year could vary from favourable for the formation of generative organs to stressful, creating serious challenges for the adaptability of apple cultivars. Under such conditions, intensive cultivars could exhibit unpredictable changes in yield, which emphasised the need for regional evaluation of cultivars based on adaptability parameters.

Although numerous cultivars of foreign breeding were being introduced in Ukraine, reliable data on their plasticity and stability under the conditions of Podillia remained insufficient or fragmented. The study by G. Locatelli *et al.* (2022) focused mainly on absolute indicators of yield, fruit weight, or product quality, without a comprehensive analysis of the adaptive responses of cultivars to changes in growing conditions. Such limitations in scientific information restricted the possibilities for forming an optimal cultivar assortment for intensive horticulture, especially in the context of increasing climatic risks. Ukrainian researchers K.P. Tarnavska & T.M. Kovalenko (2022) emphasised the need for multi-year evaluation of apple cultivars based on adaptability parameters, including ecological plasticity and productivity stability. This approach made it possible not only to identify the most promising cultivars for specific conditions but also to establish a foundation for breeding programmes aimed at developing genotypes with an optimal combination of productivity, fruit quality, and adaptive reliability. The practical significance

of their research lay in the ability to recommend specific cultivars for commercial cultivation under conditions of variable abiotic factors, which had a positive effect on production stability and the economic efficiency of horticulture.

Thus, the study of plasticity and stability of quantitative productivity traits of apple cultivars under the conditions of Podillia was a scientifically substantiated and practically important task. It allowed the integration of phenotypic evaluation results with regional environmental conditions, ensured breeding-relevant selection of cultivars, and enabled the development of recommendations for optimising the cultivar assortment for intensive apple cultivation under conditions of a changing climate.

The aim of the study was to evaluate the plasticity and stability of productivity and fruit weight of apple cultivars and to identify valuable genotypes among them as source material for breeding for adaptability, as well as cultivars promising for intensive orchards.

## Materials and Methods

The experimental part of the study on ecological parameters of apple productivity was conducted in the research orchard of the Department of Cultivar Testing and Technologies of Fruit and Berry Crop Cultivation of the Podillia Horticultural Research Station of the Institute of Horticulture of the National Academy of Agrarian Sciences (NAAS) of Ukraine, located in Vinnytsia District, Vinnytsia Region, during 2021-2024. The territory of the experimental plots of the Podillia Horticultural Research Station belonged to the Dniester Upland and the second geomorphological region of the Zhmerynka Upland, which resulted in the formation of grey podzolised medium loamy soils. The soil cover of the experimental plots was represented by grey forest heavy loamy soil. According to agrochemical analyses, the humus content was low, amounting to 2.1% (Tyurin method), while the content of easily hydrolysable nitrogen ranged from 3.4 to 5.4 mg eq. per 100 g of soil (Cornfield method). A pronounced deficiency of

potassium was observed, with its content in the humus–eluvial horizon reaching only 12–14 mg eq. per 100 g of soil. The content of available phosphorus was at a medium level, amounting to 10–12 mg eq. per 100 g of soil. The depth of the humus–eluvial horizon did not exceed 25–30 cm, with a sharp transition to the illuvial horizon. The soil colour was grey, and a morphologically distinct eluvial horizon was absent, which was considered the main diagnostic feature. The plough layer was loose, with a bulk density of 1.32 g cm<sup>-3</sup>, whereas the subsoil layer was compacted and granular. The transitional horizon occurred at a depth of 42–70 cm; it was weakly illuvial, almost devoid of humus, brownish-grey in colour, compacted, and nut-shaped in structure. Overall, the physicochemical properties of the soil at the experimental site were typical of those found across large areas of Podillia. Despite certain limitations of grey forest soils, they were suitable for the cultivation of perennial fruit plantations.

The territory where the experimental field was located was characterised by a mild temperate continental climate according to agroclimatic indicators. Winters were prolonged but mild, while summers were warm. During the years of the study, weather conditions in the region were unstable, with dry years alternating with wetter ones. The most favourable weather conditions for apple yield formation during the study period were observed in 2023. The hydrothermal conditions in that year, particularly during critical stages of the growing season, were characterised by moderate precipitation. In April, 81.9 mm of precipitation was recorded, which was sufficient for the formation of generative organs and the initial stage of fruit growth. Moisture supply in June and July was fully satisfactory for the course of all physiological processes in plants, amounting to 39.6 and 68.1 mm of precipitation, respectively. The temperature regime during the tree growing season in 2023 was close to optimal for the formation of high apple yields. The average daily air temperatures in May, June, and July were 15.3, 19.6, and 21.4°C, respectively.

The least favourable weather conditions were recorded in 2022 and 2024. The hydrothermal conditions of the growing season in these years were specific and insufficiently favourable, particularly during the stages of formation and pollination of generative organs. In 2022, apple flowering occurred under conditions of critical soil moisture deficit during the first and second ten-day periods of May. Precipitation in May was recorded only in the third ten-day period, amounting to 35.3 mm, which represented 50% of the long-term average. The average daily air temperature during this month was 15.4°C, corresponding to the norm, whereas the minimum dropped to +2.0°C. Similar weather conditions were observed in 2024. The average daily air temperature in April exceeded the norm by 2.4°C and amounted to 12.1°C, while the minimum decreased to -1.2°C (20 April). In May, minimum night-time temperatures dropped to +1.1°C. Such weather conditions during the flowering period had a significant effect on pollination of fruit tree flowers and substantially reduced fruit set.

For the experiment, five apple cultivars were selected from a total collection of 27 introduced cultivars based on high indicators of marketable fruit quality and organoleptic evaluation of taste (Forster *et al.*, 2021). The studied apple cultivars were 'Free Gold', 'Juliet', 'Luna', 'Red Topaz', and 'Rosela'. The orchard was established in 2017 with a planting scheme of 2.5 × 4.5 m on MM106 rootstock. Three replications were provided for each cultivar. The trees were maintained according to standard intensive horticulture technology, including pruning, fertilisation, and protection against pests and diseases (Delgado *et al.*, 2021; Chaploutskyi *et al.*, 2024; Szpyrka *et al.*, 2025). All studies conducted at the Podillia Horticultural Research Station of the Institute of Horticulture of the NAAS of Ukraine complied with the requirements of the Convention on Biological Diversity (1992), which regulates the use of biological resources and ensures biodiversity conservation.

For a comprehensive evaluation of the cultivars, modern methods for determining the

plasticity and stability of economically valuable traits were applied:

► the environmental index ( $I$ ), which was determined as an indicator reflecting the deviation of yield in a particular year from the mean value across all years of observation (Mather & Jinks, 1982; Kang, 1998);

► the regression coefficient ( $b_i$ ), which indicated the average response of a cultivar to changes in environmental conditions, characterised its plasticity, and allowed prediction of changes in the studied trait within a range of environmental conditions ( $b_i < 0.75$  – neutral type;  $0.75 \leq b_i \leq 1.25$  – moderately sensitive;  $b_i > 1.25$  – highly sensitive) (Yan & Kang, 2003; Grando, 2020);

► stability variances ( $S_i$ ) and deviations from the mean variance ( $S_i^2 - S_{\text{average}}^2$ ), which allowed the stability of yield and fruit weight traits to be assessed independently of weather fluctuations (Crossa *et al.*, 2022).

Statistical data processing was performed using MS Excel and Statistica 12, which corresponded to modern methodological approaches for regression and variation analysis (Crossa *et al.*, 1988; Yan & Kang, 2003). Regression line graphs and stability histograms were constructed to visually represent the behaviour of the cultivars under variable environmental conditions.

## Results and Discussion

The average yield of the studied apple cultivars over four years amounted to 1.73 t ha<sup>-1</sup> (Table 1). The most favourable year in terms of conditions for yield formation was 2023, which was confirmed by the maximum environmental index ( $I_{2023} = 1.80$ ). In contrast, 2022 was characterised by the poorest conditions ( $I_{2022} = -1.45$ ), which resulted in a sharp decline in average yield to 0.28 t ha<sup>-1</sup>. Such fluctuations indicated a substantial influence of weather factors on the realisation of the productive potential of the cultivars.

**Table 1.** Adaptability parameters of apple cultivars based on yield

Cultivar	Yield, t ha <sup>-1</sup>				Average (2021-2024)	Regression coefficient, $b_i$	Stability variance, $S_i^2$	$S_i^2 - S_{\text{average}}^2$
	2021	2022	2023	2024				
'Free Gold'	0.8	0.1	1.5	1.7	1.03	0.343	0.5	-3.1
'Juliet'	2.4	0.8	3.6	1.0	1.95	0.921	1.7	-2.0
'Luna'	0.8	0.1	2.2	1.2	1.08	0.610	0.8	-2.9
'Red Topaz'	2.5	0.3	1.5	0.8	1.28	0.383	0.9	-2.8
'Rosela'	2.5	0.1	8.8	1.8	3.30	2.743	14.5	10.8
<b>Mean</b>	1.80	0.28	3.52	1.30	1.73	1.00	3.7	0.0
<b>Environmental index (I)</b>	0.08	-1.45	1.80	-0.43				

**Note:** orchard established in 2017; planting scheme 2.5×4.5 m; rootstock MM106

**Source:** developed by the authors

The highest average yield over the study period was recorded for the cultivar 'Rosela' (3.30 t ha<sup>-1</sup>), which significantly exceeded the overall mean value. At the same time, this cultivar was characterised by the highest stability variance ( $S_i^2 = 14.5$ ) and a positive deviation from the mean variance ( $S_i^2 - S_{\text{average}}^2 = 10.8$ ), indicating low ecological stability and a high dependence on environmental conditions. Regression coefficients showed that the cultivars 'Rosela' ( $b_i = 2.743$ )

and 'Juliet' ( $b_i = 0.921$ ) were characterised by increased ecological plasticity, actively responding to improvements in growing conditions. Such cultivars were considered suitable for intensive production systems under a high level of agricultural inputs. In contrast, the cultivars 'Free Gold', 'Luna', and 'Red Topaz' ( $b_i < 0.75$ ) belonged to the neutral type, which indicated a limited response to changes in environmental conditions and relatively stable yield performance.

Assessment of yield stability showed that the most stable cultivars were 'Free Gold' ( $S_i^2 = 0.5$ ), 'Luna' ( $S_i^2 = 0.8$ ), and 'Red Topaz' ( $S_i^2 = 0.9$ ); however, they were characterised by lower average yield levels. This indicated a trade-off between

stability and productivity, which was typical for perennial fruit crops. The graphical representation of ecological stability based on yield was presented in Figure 1 using the deviation from the mean variance ( $S_i^2 - S_{average}^2$ ).

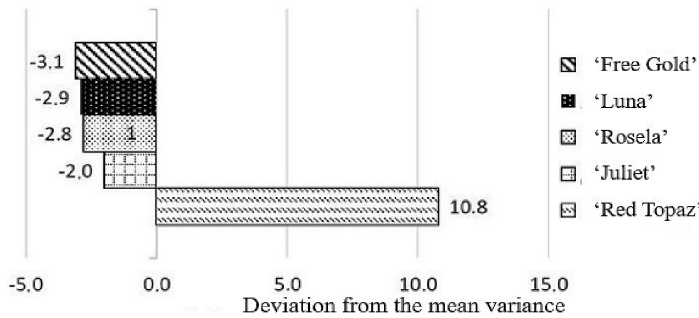


Figure 1. Yield stability of apple cultivars

Note: average for 2021-2024

Source: developed by the authors

The average fruit weight over the years of the study amounted to 149.45 g (Table 2). The most favourable years for the formation of large fruit size were 2023 and 2024, as indicated

by the high environmental indices ( $I_{2023} = 6.15$ ;  $I_{2024} = 5.75$ ). The least favourable year was 2021 ( $I_{2021} = -9.45$ ), which negatively affected the average fruit weight values.

Table 2. Adaptability parameters of apple cultivars based on average fruit weight

Cultivar	Average fruit weight, g				Average (2021-2024)	Regression coefficient, $b_i$	Stability variance, $S_i^2$	$S_i^2 - S_{average}^2$
	2021	2022	2023	2024				
'Free Gold'	140	155	148	173	154.0	1.217	198.0	-327.9
'Juliet'	125	190	140	150	151.3	0.462	772.9	247.0
'Luna'	120	120	135	153	132.0	1.697	246.0	-279.9
'Red Topaz'	175	130	180	120	151.3	-1.055	939.6	413.7
'Rosela'	140	140	175	180	158.8	2.679	472.9	-53.0
<b>Mean</b>	140.0	147.0	155.6	155.2	149.45	1.00	525.9	0.0
<b>Environmental index (I)</b>	-9.45	-2.45	6.15	5.75				

Note: orchard established in 2017; planting scheme 2.5 × 4.5 m; rootstock MM106

Source: developed by the authors

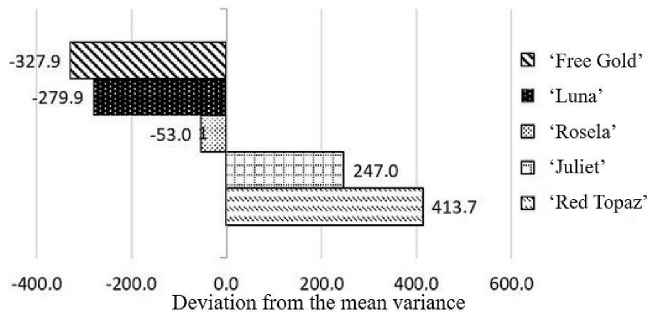
Based on average fruit weight, the cultivar 'Rosela' stood out with the highest value (158.8 g), whereas the cultivars 'Free Gold' and 'Juliet' showed values close to the overall average (154.0 and 151.3 g, respectively). High values of the regression coefficient for the cultivars 'Rosela' ( $b_i = 2.679$ ), 'Luna' ( $b_i = 1.697$ ), and 'Free Gold'

( $b_i = 1.217$ ) indicated their high ecological plasticity with respect to fruit weight. This implied that under improved environmental conditions these cultivars were able to substantially increase fruit size. At the same time, the cultivar 'Juliet' ( $b_i = 0.462$ ) exhibited a neutral response to changes in growing conditions, while 'Red Topaz' showed

a negative regression coefficient ( $b_i = -1.055$ ), indicating a non-standard response and a potential decrease in fruit weight in favourable years.

In terms of stability parameters, the most uniform fruit weight was formed by the cultivars 'Free Gold' ( $S_i^2 = 198.0$ ) and 'Luna' ( $S_i^2 = 246.0$ ). At the same time, the cultivars 'Juliet' and 'Red Topaz'

were characterised by high variance values (772.9 and 939.6, respectively), indicating considerable variability of the trait and lower predictability of results. The graphical representation of ecological stability of the cultivars based on average fruit weight was presented in Figure 2 using the deviation from the mean variance ( $S_i^2 - S_{\text{average}}^2$ ).



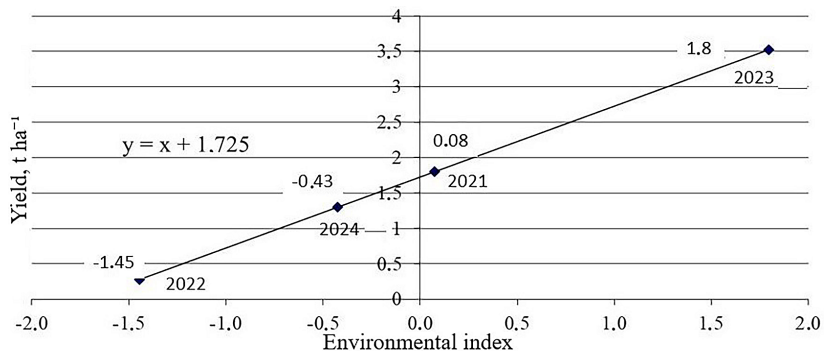
**Figure 2.** Stability of the average fruit mass of apple varieties

**Note:** average for 2021-2024

**Source:** developed by the authors

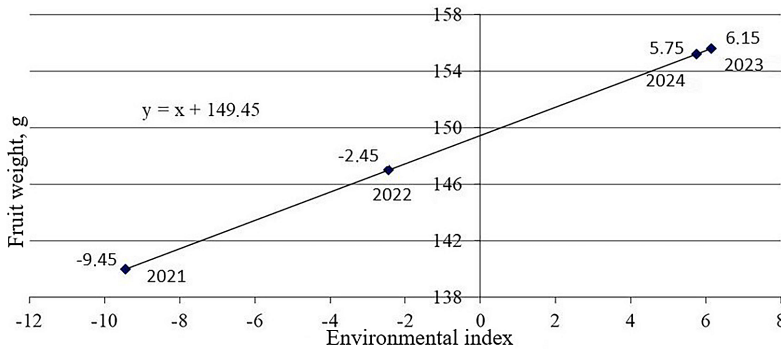
The slope of the regression lines provided additional information on the adaptive responses of apple cultivars to changes in environmental conditions. As the angle of inclination of the regression line increased, cultivar sensitivity to the variability of ecological factors also increased. The study established that the regression lines for yield and average fruit weight had nearly identical slopes, which confirmed the presence of a direct correlation between these

economically valuable traits. The calculated regression coefficients, representing the tangent of the angle of inclination, made it possible to construct graphs for the visual assessment of ecological plasticity of the cultivars. The corresponding graphical representations of the regression lines for yield and average fruit weight were presented in Figures 3 and 4, providing a clear illustration of the adaptive characteristics of the studied cultivars.



**Figure 3.** Regression line and ecological plasticity indices of apple cultivar yield in relation to changes in environmental conditions (2021-2024)

**Source:** developed by the authors



**Figure 4.** Regression line and ecological plasticity indices of average fruit weight of apple cultivars in relation to changes in environmental conditions (2021-2024)

**Source:** developed by the authors

Based on mathematical processing of yield and average fruit weight data, regression equations were derived, namely  $y_1 = x_1 + 1.725$  and  $y_2 = x_2 + 149.45$ , on the basis of which the ecological plasticity index was determined. A comprehensive analysis of ecological plasticity and stability parameters indicated that the cultivar 'Rosela' combined a high potential for yield and fruit weight but was characterised by low stability, which limited its use under conditions of an unstable climate. The cultivars 'Free Gold' and 'Luna' demonstrated an optimal balance between stability and adaptability, making them promising for cultivation across different agroecological zones. The obtained results confirmed the appropriateness of using regression analysis parameters for the justified selection of apple cultivars in breeding programmes and commercial production.

The obtained research results confirmed the decisive role of the "genotype × environment" interaction in the formation of productivity of apple cultivars, which was consistent with the concepts of adaptive breeding of fruit crops proposed by H. Muranty *et al.* (2015) and C. Ma *et al.* (2024). These studies stated that the conditions of Podillia were characterised by pronounced interannual variability of hydrothermal factors, which determined differentiated cultivar responses to changes in the ecological background and required a comprehensive assessment of plasticity and stability of economically valuable traits. In the course

of the present study, it was established that the weather conditions of 2023 were the most favourable for the formation of yield and average fruit weight, as reflected by the maximum values of the environmental index. The optimal combination of temperature regime and moisture supply during critical phases of organogenesis contributed to a more complete realisation of the genetic potential of the cultivars, which was consistent with the results reported by P. Pfeleiderer *et al.* (2021) and C. Jena & K. Pramanik (2025) obtained in temperate climate zones. In contrast, 2022 and 2024 were characterised by abiotic stresses during the flowering period, which negatively affected pollination, fruit set, and final yield. The use of regression coefficients ( $b_i$ ) allowed clear differentiation of cultivars according to the type of ecological response. The cultivar 'Rosela' demonstrated a high level of ecological plasticity for yield, which was expressed by a sharp increase in productivity in favourable years and pronounced fluctuations in unfavourable ones. According to T. Kondratenko & P. Kondratenko (2020), such a response type was typical of intensive genotypes that were advisable to cultivate under conditions of a high agricultural background and the possibility of regulating the water regime.

The cultivars 'Free Gold', 'Luna', and 'Red Topaz' belonged to the neutral type in terms of yield, with  $b_i < 1$ , indicating a weaker dependence on weather variability. Such genotypes were more

stable over time and were recommended for areas of risk-prone horticulture, where ensuring a predictable level of yield was of particular importance (Berven *et al.*, 2025). Similar patterns were reported in the studies of G. Locatelli *et al.* (2022) concerning the stability of fruit crops in Central and Eastern Europe. Assessment of stability based on the variance of deviations from regression ( $S^2$ ) showed that cultivars with high plasticity generally exhibited lower stability. In particular, the cultivar 'Rosela' had the highest  $S^2$  values for yield, which confirmed the inverse relationship between the intensity of the genotypic response and the stability of trait expression. A similar pattern has been widely described in breeding studies by G. Sanford *et al.* (2021) and is considered typical for perennial crops.

Analysis of the regression lines for yield and average fruit weight showed a similar pattern of slope, indicating the presence of a correlation between these traits. This supported the position of S. Knapp & M.G.A. van der Heijden (2018) on the necessity of a comprehensive assessment of cultivar productivity, as selection based solely on a single trait could lead to undesirable trade-offs. In modern breeding programmes, multifactorial approaches combining the evaluation of  $G \times E$  interactions, plasticity, and stability are increasingly applied. The obtained results confirmed the suitability of using the environmental index, regression coefficients, and stability parameters as effective tools for assessing the adaptive potential of apple cultivars. Such an approach allowed not only the justified recommendation of cultivars for production but also the identification of directions for their use in breeding, either as donors of stability or of potential productivity.

Thus, the research results indicated that under the conditions of Podillia the most promising cultivars were those combining moderate ecological plasticity with high stability of expression of economically valuable traits, which ensured the adaptation of orchards to current climatic challenges and increased the efficiency of commercial horticulture.

## Conclusions

As a result of the four-year cultivar evaluation of apple cultivars, a substantial influence of interannual variability of hydrothermal conditions on the formation of yield and average fruit weight under the conditions of Podillia was established. The most favourable conditions for the realisation of the productive potential of the cultivars were formed in 2023, which was confirmed by the maximum environmental indices for the traits "yield" ( $I = 1.80$ ) and "average fruit weight" ( $I = 6.15$ ), as well as by the highest average productivity values over the study period. It was determined that, in terms of fruit yield, the cultivars 'Rosela' ( $b_i = 2.743$ ) and 'Juliet' ( $b_i = 0.921$ ) were characterised by increased ecological plasticity and an intensive type of response to environmental conditions, which ensured high yield levels in favourable years but was accompanied by reduced stability. The cultivars 'Free Gold', 'Luna', and 'Red Topaz' belonged to the neutral type of ecological response and provided a relatively stable level of productivity under different growing conditions. The highest average yield during the study was recorded for the cultivar 'Rosela' ( $3.30 \text{ t ha}^{-1}$ ); however, it proved to be the least stable for this trait ( $S^2 = 14.5$ ), indicating a high dependence of its productivity on favourable weather conditions.

In terms of average fruit weight, a high level of ecological plasticity was established for the cultivars 'Rosela' ( $b_i = 2.679$ ), 'Luna' ( $b_i = 1.697$ ), and 'Free Gold' ( $b_i = 1.217$ ), which determined their ability to form large fruits in years with optimal growing conditions. The most stable cultivars for this trait were 'Free Gold' ( $S^2 = 198.0$ ) and 'Luna' ( $S^2 = 246.0$ ), whereas 'Juliet' ( $S^2 = 772.9$ ) and 'Red Topaz' ( $S^2 = 939.6$ ) were characterised by considerable variability of this trait. A comprehensive assessment of plasticity and stability of yield and average fruit weight allowed the conclusion that, under the conditions of Podillia, the optimal combination was moderate ecological plasticity with high stability of trait expression, which ensured predictable productivity and reduced production risks. Based on the set of adaptability

parameters, the cultivars 'Free Gold' and 'Luna' showed the greatest practical and breeding value, as they combined yield stability with an adequate level of marketable fruit quality. The obtained results confirmed the appropriateness of using the environmental index, ecological plasticity coefficients, and stability parameters for the selection of promising apple genotypes and could be applied in breeding programmes and in the formation of cultivar assortments for the Podillia region.

For an objective assessment of the potential of new apple tree genotypes in the conditions of Podillia, it is advisable to use a comprehensive

set of adaptability indicators, including environmental indices, ecological plasticity and stability coefficients, which allows improving the efficiency of variety selection at the early stages of varietal testing.

### Acknowledgements

None.

### Funding

None.

### Conflict of Interest

None.

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## Пластичність і стабільність кількісних ознак продуктивності сортів яблунь в умовах Поділля

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**Анотація.** У світі яблуня є однією з провідних плодкових культур, що займає третє місце за обсягами виробництва після бананів та кавунів, і постійно зростаючий попит на яблука обумовлює необхідність вдосконалення сортів з високими показниками продуктивності та адаптивності. Метою дослідження була оцінка сортів яблуні за показниками екологічної пластичності для визначення перспективних генотипів для селекційних програм. Наведено результати сортовивчення яблуні української та зарубіжної селекції (насадження 2017 року, схема 2,5 × 4,5 м, підщепа ММ106) за показниками врожайності та середньої маси плодів. Проведено оцінювання п'яти сортів яблуні ('Фрі Голд', 'Джульєт', 'Луна', 'Ред Топаз', 'Розела'), відібраних із 27 інтродукованих генотипів, що проходили сортовивчення у 2021-2024 рр. Найвищі значення індексів середовища, як за врожайністю яблунь, так і масою плодів, були відмічені в 2023 році, і відповідно були – 1,80 та 6,15. Найменші індекси середовища відмічені у 2022 р. і 2024 р., середня врожайність в якій відповідно становила 0,28 та 1,3 т/га. Такий аналіз демонструє високий вплив річних коливань кліматичних факторів на продуктивність сортів та підкреслює необхідність врахування адаптивності генотипів у селекційних програмах. За результатами математичної обробки показників врожайності плодів і середньої маси плоду виведено рівняння регресії та індекси екологічної пластичності. Встановлено, що за врожайністю плодів сорти 'Розела' і 'Джульєт' є сортами інтенсивного типу, які характеризуються високою чутливістю до змін умов середовища, коефіцієнти регресії відповідно становлять 2,743 і 0,921. За середньою масою плоду високий рівень екологічної пластичності демонструють сорти 'Розела', 'Луна' та 'Фрі Голд', тоді як сорт 'Джульєт' належить до нейтрального типу. Оцінка екологічної стабільності сортів показала, що за врожайністю найбільш стабільними були 'Фрі Голд', 'Луна' та 'Ред Топаз'. Сорт 'Джульєт' виявився відносно стабільним, хоча він не відзначився високим рівнем врожайності. Найвищу врожайність у середньому за роками показав сорт 'Розела', який одночасно є найменш стабільним. За середньою масою плоду стабільними виявилися сорти 'Фрі Голд' та 'Луна'

**Ключові слова:** врожайність; маса плоду; привабливість; смакові якості; параметри адаптивності; індекс середовища



UDC 631.31:631.547:631.55(474.4)(292.485)

DOI: 10.31548/dopovidi/6.2025.170

## The importance of alfalfa and its growth processes during harvesting phases in the conditions of the right-bank Forest-Steppe

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**Abstract.** The aim of the study was to investigate the impact of harvesting dates and fertilization levels on the height of alfalfa (*Medicago sativa*) plants in the conditions of the Right-bank Forest-Steppe. The research was conducted at the experimental field of Vinnytsia National Agrarian University with variants of different sowing rates (6.0-8.0 million seeds/ha) and four levels of mineral nutrition. In the sowing year, the lowest height of alfalfa plants was observed at the sowing rate of 8.0 million seeds/ha (63.4-64.4 cm), which increased to 64.4-66.3 cm at the sowing rate of 6.0 million seeds/ha. Complex fertilizers contributed to an increase in plant height from 0.9-1.9 cm to 1.0-2.7 cm compared to the control. It was found that plants, under stress conditions due to high air temperature and insufficient moisture at the root system depth, could not effectively absorb mineral fertilizers from the soil, which led to a reduction in growth rates. In the second year of vegetation, considering the hydrothermal conditions of the summer period, an impact on the growth processes of alfalfa was observed. This affected the plant height during both the cuts and the harvesting phases. After three cuts at the

### Suggested Citation:

Hetman, N., Danyliuk, B., & Kovalenko, V. (2025). The importance of alfalfa and its growth processes during harvesting phases in the conditions of the right-bank Forest-Steppe. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 170-180. doi: 10.31548/dopovidi/6.2025.170.

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budding stage, the average plant height reached 54.3-54.5 cm, regardless of the sowing rate and mineral nutrition level. The results followed a sinusoidal pattern: the tallest plants were at the first cut (73.7-73.8 cm), with the shortest height during the second cut (41.4-41.8 cm), and a further increase to 47.7-47.9 cm at the third cut. At the beginning of flowering, the tallest plants reached 85.7-86.0 cm, while at subsequent cuts, made 40-41 days later, a decrease in height was observed: 43.9-44.4 cm at the second cut and 54.3-55.2 cm at the third cut. The average plant height at the sowing rates was 61.4-61.8 cm. Thus, alfalfa formed three full cuts during the growing season, with an average height of 54.3-54.5 cm. The tallest plants reached 76.8-85.8 cm with the application of mineral fertilizers at the dose of  $N_{35}P_{90}K_{150} + S_{23.5}Ca_{30}Mg_{20}$ , while the average values were 55.7-62.7 cm at a sowing rate of 6.0 million seeds/ha. The results of the study can be used to optimize the agrotechnology of alfalfa cultivation in the conditions of the Forest-Steppe

**Keywords:** *Medicago sativa*, budding, beginning of flowering, sowing rate, fertilizers

## Introduction

Perennial leguminous grasses are one of the priority areas for the development of forage production and agriculture, as the basis for organic production of plant raw materials for the preparation of various types of animal feed. They play an environmental role in agro-landscapes according to their morphological and genetic characteristics, contribute to soil fertility enhancement and improve its structure, which is particularly relevant in the current conditions of agricultural development. Therefore, significant attention should be paid to perennial leguminous grasses, especially the most widespread and productive crop – alfalfa, which, with strict adherence to cultivation techniques, is capable of fully realizing its genetic potential during prolonged use of the sward (Zhang & Wang, 2025). Breeding programs aimed at improving alfalfa focus on increasing yield and persistence, which are key factors for adaptation to different agroecosystems (Medina et al., 2025).

Alfalfa (*Medicago sativa* L.), known as the “queen of forage crops”, was recognised as the most important perennial forage legume crop cultivated worldwide due to its comprehensive ecological adaptability, high nutritional value, and ability to fix nitrogen. According to W. Wan et al. (2022), fertiliser application not only increased alfalfa yield by 19.2%, but also improved its quality through an increase in crude protein content by

7.7% and a reduction in acid-detergent fibre content by 2.9%. This confirmed the importance of optimising fertilisation systems to achieve maximum productivity. Y. Wang et al. (2024) established that the level of nitrogen fertilisation significantly influenced nitrogen use efficiency, yield, and quality of alfalfa across different cuts. It was noted by the researchers that improper nitrogen application led to reduced nitrogen use efficiency and even adverse effects on yield and forage quality. These findings underlined the importance of optimising nitrogen fertiliser rates. Drought tolerance in alfalfa was considered a critically important characteristic in the context of global climate change.

In the study by N. Hetman et al. (2025), the role of alfalfa in the greening of agricultural production was investigated, particularly its capacity to enrich soils with nitrogen and improve their physico-chemical properties. The importance of utilising alfalfa to reduce soil erosion and land degradation, as well as to decrease reliance on chemical fertilisers, was emphasised by the authors. It was demonstrated in the work that alfalfa could serve as a key component of sustainable agriculture, contributing to environmentally safe production. V. Greveniotis et al. (2024) conducted research on the yield stability and main qualitative characteristics of alfalfa, including plant height, number of nodes, green mass yield, and dry matter

yield. Statistical analysis revealed significant differences, indicating a high genotype-by-year interaction, which underscored the importance of adapting varieties to specific growing conditions. The study by I.S. Varol *et al.* (2024) established that alfalfa is sensitive to drought in terms of green mass yield, while showing tolerance to water stress in terms of hay yield and protein content, which is significant for developing cultivation strategies under limited water supply. The research by Y. Liu *et al.* (2018) demonstrated that a decrease in yield due to water scarcity was observed in the studied varieties, while crude protein content was reduced as a result of drought stress. Early harvesting provided higher yields, confirming the importance of optimising mowing dates.

The uniqueness of the crop also lies in its ability to prevent the salinisation of irrigated land. Its roots draw moisture from deeper soil layers, thereby lowering the groundwater level and interrupting the upward flow of water from the lower soil horizons (Tyshchenko & Tyshchenko, 2014). According to U.M. Karbivska (2020), around 150-200 kg/ha of nitrogen accumulates in the roots and plant residues of alfalfa, making it an important element in the system of organic farming. Despite the substantial volume of research, the issue of the impact of harvesting dates in combination with different levels of mineral nutrition on the growth processes of forage alfalfa in the Right-Bank Forest-Steppe remains insufficiently studied. S.F. Antoniv *et al.* (2023) highlighted the agroecological importance of alfalfa as the basis of high-protein fodder in the Forest-Steppe and Steppe regions of Ukraine; however, the issue of optimising harvesting dates depending on the developmental phase of the plants requires further study. Given the scientific data provided and the conflicting results regarding the combined effect of harvesting dates and mineral nutrition levels, there is a need for an in-depth study of the growth processes of forage alfalfa under specific soil and climatic conditions. Special attention should be given to evaluating the formation of plant height depending on the harvesting phases, which is of significant practical

importance for optimising cultivation technology. The aim of this study was to establish the patterns of plant height formation in forage alfalfa depending on harvesting phases and fertilisation levels.

## Materials and Methods

The research was conducted at the experimental field of Vinnytsia National Agrarian University, focused on the productivity of agricultural crops, including forage alfalfa, during the period from 2024 to 2025. The soil cover of the experimental site is represented by grey forest soils, which, based on morphological characteristics, occupy an intermediate position between light and dark grey soils. According to agrochemical surveys, the arable layer of soil (0-30 cm) has the following physicochemical properties: humus content (according to Tyurin) was 2.69%, alkaline-hydrolysed nitrogen (according to Kornfield) – 81 mg/kg, available phosphorus and exchangeable potassium (according to Chirikov) were 218 and 72 mg per 1 kg of soil, respectively; available sulphur (S) 5.3 mg/kg, available copper (Cu) 0.29, available zinc (Zn) 0.45, available manganese (Mn) 14.6 mg/kg, and the pH of the salt extract was 6.1.

The climatic conditions of the Right-Bank Forest-Steppe zone are heterogeneous, characterised by warm summers and moderately cold winters. During the research period (2024-2025), in the second year of forage utilisation in 2025, the hydrothermal conditions were characterised by an increase in the average monthly air temperature from 19.5°C to 21.5°C, with atmospheric precipitation from June to August amounting to 87 mm. Mineral fertilisers were applied during pre-sowing cultivation according to the experimental variants, which differed in their supply of essential nutrients and accompanying macro- and micro-elements. The control variant involved the application of mineral fertilisers at the rate of  $N_{35}P_{90}K_{90}$  with the addition of sulphur ( $S_{3.5}$ ). In the experimental variants, increased nutrient levels were applied with the same rate of nitrogen and phosphorus ( $N_{35}P_{90}$ ), but with a gradual increase in potassium dosage to  $K_{120}$  and  $K_{150}$ , along with the addition of sulphur, calcium, and magnesium in the corresponding

amounts. This approach allowed the evaluation of the effect of different levels of complex mineral nutrition on the growth processes of forage alfalfa under identical agro-technical conditions. The variety of forage alfalfa 'Radoslava', created at the Institute of Feed and Agriculture of Podillia of the National Academy of Agrarian Sciences, and included in the State Register of Plant Varieties of Ukraine, was sown in the experiment. This variety is tolerant to increased soil acidity (pH of the salt solution 5.0-5.5). The 'Radoslava' variety is characterised by increased drought tolerance, resistance to Fusarium wilt, and productive longevity in both pure and mixed swards.

For the assessment of the growth stages of alfalfa plants, the BBCH scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) was used. Statistical data processing was carried out using analysis of variance, with the determination of the confidence interval of the arithmetic mean at the 5% significance level. The research was conducted in accordance with ethical standards, particularly within the framework of the Convention on Biological Diversity (1992).

## Results and Discussion

It was established that in the sowing year, under conditions of adequate soil moisture in April 2024 at seeding depth and an average daily air temperature of 12.0°C, the inter-phase period "sowing-emergence" lasted 10 days. Subsequent growth processes were primarily determined

by the biological characteristics of alfalfa and weather conditions. In May, active development of leaf-stem mass was observed, and the beginning of flowering phase (BBCH code 60) occurred 68-69 days after full emergence. Growth and development processes of alfalfa occurred under conditions of coordinated interaction of soil and climatic factors, such as moisture, heat, light, and soil fertility. Regardless of the fertilisation background, the lowest height of alfalfa plants was observed at a sowing rate of 8.0 million viable seeds/ha, measuring 63.4-64.4 cm, which increased to 64.4-66.3 cm at a sowing rate of 6.0 million/ha. With the application of complex fertiliser, an increase in plant height from 0.9-1.9 cm to 1.0-2.7 cm was observed compared to the control.

Research demonstrated that in the second year of life (2025), under conditions of unstable moisture supply and gradual temperature increase throughout the vegetation period, alfalfa cultivar 'Radoslava' responded to environmental conditions through changes in plant height across cuts, which were conducted at two timings at the budding phase and at the beginning of flowering. Across three cuts at the budding phase, the average plant height reached 54.3-54.5 cm, regardless of sowing rates and mineral nutrition level. The obtained values exhibited a sinusoidal pattern, with the highest values observed at the first cut, reaching 73.7-73.8 cm, the lowest at the second cut at 41.4-41.8 cm, followed by subsequent growth to 47.7-47.9 cm in the third cut, which was formed in August (Table 1).

**Table 1.** Effect of sowing rates and fertilisation on alfalfa plant height at the budding phase,  $M \pm m^*$

Fertilisation	Sowing rate, million seeds/ha	Cuts			Average
		1	2	3	
$N_{35}P_{90}K_{90} + S_{3.5}$ (control)	6.0	71.1 ± 8.01	40.6 ± 6.18	46.5 ± 7.89	52.7 ± 7.36
	8.0	73.2 ± 5.45	41.4 ± 6.20	47.8 ± 5.96	54.1 ± 5.87
$N_{35}P_{90}K_{120} + S_{13.5}Ca_{15}Mg_{10}$	6.0	73.4 ± 4.67	41.5 ± 5.22	48.3 ± 6.55	54.4 ± 5.48
	8.0	73.6 ± 5.41	41.6 ± 4.05	47.6 ± 8.39	54.3 ± 5.95
$N_{35}P_{90}K_{150} + S_{23.5}Ca_{30}Mg_{20}$	6.0	76.8 ± 3.03	42.0 ± 5.40	48.4 ± 6.36	55.7 ± 4.93
	8.0	74.4 ± 4.04	42.3 ± 2.17	48.4 ± 5.98	55.0 ± 4.06
Average	6.0	73.8 ± 5.23	41.4 ± 5.60	47.7 ± 6.93	54.3 ± 5.92
	8.0	73.7 ± 4.97	41.8 ± 4.14	47.9 ± 6.78	54.5 ± 5.29

**Note:** \* –  $M \pm m$  – confidence interval of the arithmetic mean at the 5% significance level

**Source:** developed by the authors

In the current study, complex fertilisation also positively influenced linear plant parameters, although the effect was less pronounced under drought stress conditions. The obtained results were consistent with data from W. Wan *et al.* (2022), who established that fertiliser application increased alfalfa yield by an average of 19.2%. Specifically, the best linear parameters were obtained at a sowing rate of 6.0 million viable seeds/ha, measuring 76.8 cm with  $N_{35}P_{90}K_{150} + S_{23.5}Ca_{30}Mg_{20}$  at the first cut harvest, which decreased in the second and third cuts to a range of 42.0-48.4 cm. The average values across three cuts were 55.7 cm, which was 3.0 cm higher than with the application of mineral fertilisers at a dose of  $N_{35}P_{90}K_{90} + S_{3.5}$ . Similar patterns were observed by Y. Wang *et al.* (2024), who established that nitrogen fertilisation level significantly influenced nitrogen use efficiency and alfalfa yield

across different cuts. The current study also revealed a differentiated effect of fertilisation depending on the cut and harvesting phase.

Hydrothermal conditions of the vegetation period facilitated regrowth and formation of the third cut of alfalfa forage at the budding phase on 20 August and at the beginning of flowering in the first decade of September (04.09), with forage cutting conducted 37 and 41 days after the second cut, respectively. According to biological characteristics, alfalfa is classified as a long-day crop; therefore, during shortening of daylight hours with increased temperature regime and uneven distribution of atmospheric precipitation, plant height decreased at the budding phase, measuring 46.5-48.4 cm, with the highest values at maximum fertilisation of 48.4 cm. Concurrently, plant height increment during the vegetation period increased compared to the first and second cuts to 1.25-1.31 cm/day (Table 2).

**Table 2.** Average daily plant height increment of alfalfa across cuts, depending on sowing rates and fertilisation at the budding phase, cm/day

Fertilisation	Sowing rate, million seeds/ha	Cuts			Average
		1	2	3	
$N_{35}P_{90}K_{90} + S_{3.5}$ (control)	6.0	1.02	1.07	1.25	1.11
	8.0	1.06	1.08	1.29	1.14
$N_{35}P_{90}K_{120} + S_{13.5}Ca_{15}Mg_{10}$	6.0	1.05	1.09	1.30	1.15
	8.0	1.05	1.09	1.29	1.14
$N_{35}P_{90}K_{150} + S_{23.5}Ca_{30}Mg_{20}$	6.0	1.09	1.10	1.31	1.17
	8.0	1.06	1.11	1.31	1.16
Average	6.0	1.05	1.09	1.29	1.14
	8.0	1.05	1.10	1.29	1.15

**Source:** developed by the authors

Research by Z. Zhou *et al.* (2024) confirmed that drought stress represents a significant environmental challenge that impedes alfalfa growth and yield worldwide. Z. Zhou *et al.* identified drought as a major challenge limiting alfalfa production and threatening food security. Water deficit causes damage to plants by disrupting various physiological processes such as carbon assimilation, cellular hydration, and leaf gas exchange. In the current study, a decrease in plant growth rates during the summer period was also observed due to insufficient moisture supply,

particularly in the second cut. When determining plant height increment across cuts, their stability between variants was revealed in the first cut, followed by subsequent growth in the second and third cuts to 1.07-1.31 cm/day. Y. Shen *et al.* (2013) established that cutting height significantly influenced shoot regrowth and alfalfa yield, which was confirmed by current observations regarding differentiated increment across cuts. P. Álvarez-Vázquez *et al.* (2020) investigated alfalfa productivity depending on regrowth age in the spring period, identifying optimal harvesting

timings for yield maximisation. Plant height increment rates increased with application of maximum mineral fertiliser dose from 1.29 to 1.31 cm/day, regardless of sowing rate, with average experimental values of 1.29 cm/day. Observations demonstrated that upon reaching the beginning of flowering phase, the highest plant

height was obtained in the first cut at 85.7-86.0 cm, whilst during the vegetation period, in the formation of subsequent cuts conducted on average after 40-41 days, a decrease was observed: in the second cut to 43.9-44.4 cm and in the third cut to 54.3-55.2 cm, with average values by sowing rates of 61.4-61.8 cm (Table 3).

**Table 3.** Alfalfa plant height across cuts depending on sowing rates and fertilisation at the beginning of flowering phase,  $M \pm m^*$

Fertilisation	Sowing rate, million seeds/ha	Cuts			Average
		1	2	3	
$N_{35}P_{90}K_{90}+S_{3.5}$ (control)	6.0	85.4 ± 5.71	43.0 ± 4.83	50.6 ± 5.12	59.7 ± 5.22
	8.0	87.6 ± 4.85	43.6 ± 5.12	52.4 ± 3.88	61.2 ± 4.95
$N_{35}P_{90}K_{120}+S_{13.5}Ca_{15}Mg_{10}$	6.0	86.8 ± 6.57	44.3 ± 3.59	54.4 ± 4.00	61.8 ± 4.72
	8.0	85.4 ± 5.72	44.4 ± 4.38	54.5 ± 5.67	61.4 ± 5.26
$N_{35}P_{90}K_{150}+S_{23.5}Ca_{30}Mg_{20}$	6.0	85.8 ± 8.11	44.5 ± 3.44	57.8 ± 3.07	62.7 ± 4.87
	8.0	84.2 ± 7.66	45.2 ± 4.00	58.7 ± 3.87	62.7 ± 5.18
Average	6.0	86.0 ± 6.80	43.9 ± 3.95	54.3 ± 4.06	61.4 ± 4.94
	8.0	85.7 ± 6.08	44.4 ± 4.50	55.2 ± 4.47	61.8 ± 5.13

**Note:** \* –  $M \pm m$  – confidence interval of the arithmetic mean at the 5% significance level

**Source:** developed by the authors

It was demonstrated that across experimental variants, the tallest plants were observed at a sowing rate of 8.0 million/ha with mineral fertiliser at a dose of  $N_{35}P_{90}K_{90} + S_{3.5}$ , measuring 87.6 cm. Under conditions of insufficient moisture supply, plants exhibited decreased nutrient absorption from the soil, particularly with increased mineral fertiliser doses, which influenced height parameters of 84.2-85.8 cm. These results confirmed data from Y. Liu *et al.* (2018), who established that early harvesting ensured higher yield. The current study also revealed that the first cut was characterised by the highest linear plant parameters regardless of fertilisation variant. It was established that in the third cut, plants on average reached a height of 50.6-58.7 cm, or were 4.1-10.3 cm taller compared to values at the budding phase. Concurrently, average daily plant height increment was 1.23-1.43 cm/day. Y. Song *et al.* (2019) described in detail the mechanisms of alfalfa response to abiotic stress, including morphological, physiological, and molecular adaptations. Under the conditions

examined in the current study, a differentiated plant response to hydrothermal conditions was also observed, depending on growth and development phase. When comparing the obtained data with the budding phase, it could be concluded that over 7 days, height increased by 12.0-13.2 cm. Changes in parameters were explained by increased average daily plant increment to 1.71-1.88 cm/day, depending on sowing rates and fertilisation. Research by Y. Sun *et al.* (2022) confirmed that application of nitrogen and phosphorus fertilisers significantly influenced agronomic characteristics of alfalfa, particularly plant height and growth intensity. The obtained data indicated intensive plant height growth upon reaching the beginning of flowering phase compared to budding, regardless of fertilisation level. The trend of increasing plant height increment was maintained across experimental variants (Table 4). In the current study, substantial variability of parameters was also observed depending on weather conditions of the vegetation period. Results from Y. Feng *et al.* (2022) indicated a negative

correlation between alfalfa yield and crude protein content. This emphasised the importance of optimising harvesting timings, which was confirmed by current study data regarding the differentiated influence of harvesting phase on linear plant parameters. Comprehensive research by Y. Feng *et al.* also demonstrated that average alfalfa dry matter yield in China was  $11.18 \pm 6.69$  t/ha

with an average crude protein content of  $19.05 \pm 2.87\%$ . The authors established a negative correlation between yield and protein content, indicating the necessity of balancing quantitative and qualitative parameters when optimising cultivation technologies. V. Greveniotis *et al.* (2024) in their research on alfalfa yield stability revealed significant genotype-by-year interaction.

**Table 4.** Average daily plant height increment of alfalfa across cuts depending on sowing rates and fertilisation at the beginning of flowering phase, cm/day

Fertilisation	Sowing rate, million seeds/ha	Cuts			Average
		1	2	3	
$N_{35}P_{90}K_{90} + S_{3.5}$ (control)	6.0	1.08	1.08	1.23	1.13
	8.0	1.11	1.09	1.27	1.16
$N_{35}P_{90}K_{120} + S_{13.5}Ca_{15}Mg_{10}$	6.0	1.10	1.11	1.33	1.18
	8.0	1.08	1.11	1.32	1.17
$N_{35}P_{90}K_{150} + S_{23.5}Ca_{30}Mg_{20}$	6.0	1.09	1.11	1.41	1.20
	8.0	1.06	1.13	1.43	1.21
Average	6.0	1.09	1.10	1.32	1.17
	8.0	1.08	1.11	1.35	1.18

**Source:** developed by the authors

Research by M. Daud *et al.* (2025) emphasised that alfalfa drought tolerance was relatively high compared to other forage crops due to a deep root system that could reach 1.5-4 m. This was confirmed by observations from the current study: despite unfavourable hydrothermal conditions, alfalfa cultivar 'Radoslava' formed three full cuts across the studied growth and development phases. With available hydrothermal resources during the alfalfa vegetation period in 2025, three full cuts were obtained at the budding phase and at the beginning of flowering. On average, plants reached a height within the range of 54.3-61.8 cm with plant leafiness at 53.0-54.5%, depending on growth and development phase and the factors studied. It was established that the effect of studied factors was stable and ensured the best linear plant parameters of 62.7 cm at the beginning of flowering phase under conditions of alfalfa sowing at both sowing rates and use of complex fertiliser with increased macro and micro-element levels. Height was 3.0 cm (5.0%) higher than the control with application

of  $N_{35}P_{90}K_{90} + S_{3.5}$ . The difference between average plant height values across growth and development phases was 7.0 cm; that is, plant height from the budding phase to the beginning of flowering increased by 7 cm.

Results obtained in the study were consistent with principles presented in works by V. Petrychenko *et al.* (2018), where alfalfa was considered as a stabilising factor in forage production intensification due to high productivity and forage quality. The established dependence of plant height on harvesting phases confirmed the feasibility of a differentiated approach to forage cutting timings, which influenced the formation of its structure. This was explained by research results from V.F. Petrychenko & H.P. Kvitko (2010), according to which the highest protein proportion was concentrated in alfalfa leaves, buds, and flowers, whilst stems were characterised by significantly lower nutritional value, which substantiated the advantages of harvesting at budding and beginning of flowering phases. Research by G. Bélanger *et al.* (2020) confirmed that harvesting

alfalfa at the early budding phase compared to the beginning of flowering reduced dry matter yield by 2.03 t/ha; however, it increased total digestible nutrient concentration by 44 g/kg, indicating the necessity of optimising harvesting timings depending on the target purpose of forage. Research by H. Wang *et al.* (2023) demonstrated that alfalfa populations exhibited significant genetic diversity and capacity for local adaptation, ensuring their suitability to various agroecological conditions and prolonged productive utilisation of forage. Results obtained from the current study confirmed the determining role of weather conditions in the formation of alfalfa growth processes, which was consistent with conclusions by M.H. Kvitko (2017), obtained for conditions of the Right-Bank Forest-Steppe on grey forest soils. The variability of plant height across cuts and harvesting phases established in the experiment was explained by fluctuations in temperature regime and moisture supply throughout the vegetation period, which significantly influenced the intensity of growth and forage regrowth. Under conditions of elevated temperatures and moisture deficit, a decrease in growth rates was observed, which confirmed the high sensitivity of alfalfa to hydrothermal factors, regardless of mineral nutrition level. Thus, the obtained data supplemented results from previous research and indicated the necessity of accounting for weather conditions when interpreting the effects of agrotechnical measures on alfalfa growth and development.

The obtained experimental data demonstrated a stable effect of studied factors on alfalfa growth processes under conditions of the Right-Bank Forest-Steppe. The identified patterns of plant height changes depending on harvesting phases and mineral nutrition levels indicated the determining role of cutting timings in the formation of linear forage parameters. Under the influence of hydrothermal conditions of the vegetation period, a differentiated plant response to fertilisation was observed, which determined the variability of growth parameters across cuts.

## Conclusions

It was established that despite challenging hydrothermal conditions of the Right-Bank Forest-Steppe (increase in average monthly air temperature from 19.5 to 21.5°C and insufficient atmospheric precipitation of 87 mm during June-August), during the vegetation period, alfalfa cultivar 'Radoslava' formed three full cuts at the budding phase and at the beginning of flowering, which indicated its high adaptability to growing conditions. On average across three cuts, plant height reached 54.3-54.5 cm at the budding phase and 61.4-61.8 cm at the beginning of flowering phase. The tallest plants were observed in the first cut, measuring 76.8-85.8 cm with application of mineral fertilisers at a dose of  $N_{35}P_{90}K_{150}+S_{23-5}Ca_{30}Mg_{20}$ . Average plant height values were 55.7-62.7 cm at a sowing rate of 6.0 million viable seeds/ha. The difference between harvesting phases was 7.0 cm, which confirmed the feasibility of harvesting at the beginning of flowering phase to obtain maximum vegetative mass.

It was demonstrated that complex mineral fertilisation contributed to plant height increment from 0.9-1.9 cm to 1.0-2.7 cm compared to the control. Average daily plant height increment was 1.05-1.17 cm/day at the budding phase and 1.13-1.21 cm/day at the beginning of flowering phase, which indicated more intensive growth at later harvesting. The highest average daily increment was observed in the third cut, measuring 1.25-1.31 cm/day. It was established that under drought stress conditions, plants were unable to effectively absorb mineral fertilisers from the soil, which resulted in decreased growth rates, particularly in the second cut (41.4-44.4 cm). This indicated the necessity of accounting for hydrothermal conditions when planning fertilisation systems and adjusting fertiliser application rates depending on moisture supply. Prospects for further research lie in studying the influence of different fertilisation systems on dry matter yield and qualitative composition of alfalfa forage production (protein content, fibre, metabolisable energy), as well as investigating crop water consumption

and moisture use efficiency under conditions of climate change in the Right-Bank Forest-Steppe. None.

## Funding

## Acknowledgements

None.

## Conflict of Interest

None.

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## Значення люцерни посівної та ростові процеси за фазами збирання в умовах Лісостепу правобережного

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**Анотація.** Метою роботи було вивчення впливу строків збирання та рівнів удобрення на висоту рослин люцерни посівної в умовах Лісостепу правобережного. Дослідження проводилися на дослідному полі Вінницького національного аграрного університету за варіантами з різними нормами висіву (6,0-8,0 млн/га) та чотирма рівнями мінерального живлення. У рік сівби найменша висота рослин люцерни посівної була за нормою висіву 8,0 млн/га схожих насінин (63,4-64,4 см), що зросла до 64,4-66,3 см при нормі висіву 6,0 млн/га. Комплексне добриво сприяло приросту висоти рослин від 0,9-1,9 см до 1,0-2,7 см порівняно з контролем. Встановлено, що рослини, перебуваючи в стресових умовах через підвищену температуру повітря і недостатність вологи на глибині кореневої системи, не змогли ефективно споживати мінеральні добрива з ґрунту, що призвело до зниження темпів приросту. На другий рік вегетації, з огляду на гідротермічні умови літнього періоду, спостерігалось впливання на ростові процеси люцерни. Це позначилося на висоті рослин як за укосами, так і за фазами збирання травостою. За три укоси у фазі бутонізації середня висота рослин досягала 54,3-54,5 см, незалежно від норм висіву та рівня мінерального живлення. Результати мали синусоїдальний вигляд: найвища висота рослин була за першого укосу – 73,7-73,8 см, з найменшою висотою при скошуванні другого укосу – 41,4-41,8 см, з подальшим зростанням до 47,7-47,9 см у третьому укосі. У фазі початку цвітіння найбільша висота рослин досягала 85,7-86,0 см, а на наступних укосах, що проводилися через 40-41 добу, спостерігалась знижена висота: у другому укосі 43,9-44,4 см і в третьому укосі 54,3-55,2 см. Середня висота рослин за нормами висіву складала 61,4-61,8 см. Таким чином, люцерна посівна за період вегетації сформувала три повноцінних укоси, із середньою висотою 54,3-54,5 см. Найбільші рослини досягали 76,8-85,8 см при внесенні мінеральних добрив у дозі  $N_{35}P_{90}K_{150} + S_{23,5}Ca_{30}Mg_{20}$ , а середні показники становили 55,7-62,7 см при сівбі 6,0 млн/га. Результати досліджень можуть бути використані для оптимізації агротехнологій вирощування люцерни посівної в умовах Лісостепу

**Ключові слова:** *Medicago sativa*, бутонізація, початок цвітіння, норма висіву, добрива

**НАУКОВІ ДОПОВІДІ НАЦІОНАЛЬНОГО УНІВЕРСИТЕТУ  
БІОРЕСУРСІВ І ПРИРОДОКОРИСТУВАННЯ УКРАЇНИ**

*Науковий журнал*

**Том 21, № 6. 2025**

Заснований у 2005 р. Виходить 6 разів на рік

**Відповідальний редактор:**  
В. Постої

**Адреса видавництва:**

Національний університет біоресурсів і природокористування України

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<https://scireports.com.ua/uk>

**SCIENTIFIC REPORTS OF THE NATIONAL UNIVERSITY  
OF LIFE AND ENVIRONMENTAL SCIENCES OF UKRAINE**

*Scientific Journal*

**Volume 21, No. 6 . 2025**

Founded in 2005. Published 6 times a year

**Managing editor:**

V. Postoi

**Publisher's address:**

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

Tel.: +38(044)-258-42-63

E-mail: [sr@scireports.com.ua](mailto:sr@scireports.com.ua)

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